

Chapter 1

An outlook on world biofuel production and its implications for the animal feed industry

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ABSTRACT

Many countries have adopted policies that support expanded production and use of liquid biofuels for transportation. These policies are intended to enhance domestic energy security, spur economic development and reduce emissions of greenhouse gases (GHG) and other pollutants. Biofuel policies, along with changing energy market fundamentals, have contributed to a significant increase in global biofuel production in recent years. While considerable research and development is under way to commercialize new types of biofuel and feedstocks, the two primary biofuels produced globally today – ethanol and biodiesel – are predominantly derived from agricultural commodities, such as grain, sugar and oilseeds. The use of certain feedstocks for biofuels production also results in the co-production of animal feed. Globally, these animal feed co-products are growing in volume and importance. The increased use of agricultural commodities for biofuels is generally expected to contribute to marginally higher costs for certain livestock and poultry feeds, though the impacts are shown by the literature to be modest in nature and there are offsetting effects. Increased substitution of co-products for traditional feedstuffs in feed rations helps mitigate potential input cost increases faced by livestock and poultry producers. Further, increased agricultural productivity and output has ensured that the global supply of crops available for non-biofuels uses has continued to grow in the long term. Growth in the use of agricultural commodities for biofuels is expected to continue in the next 10 years, but with growth rates slowing in key producing countries as government-imposed limits on grain use for biofuels are reached and new non-agricultural feedstocks are commercialized.

INTRODUCTION – THE CASE FOR EXPANDING BIOFUEL PRODUCTION

The confluence of several economic, geopolitical and environmental factors in recent years has stimulated increased global interest in advancing the production and consumption of liquid biofuels for transportation. Historically, interest in biofuels has been primarily driven by national desires to enhance energy security and reduce dependency on fossil fuels. Through stimulation of demand for agricultural commodities, biofuels have also been promoted as a means of enhancing rural economic development and increasing farm income. More recently, however, biofuels have been endorsed as a key component of national and international strategies to reduce greenhouse gas (GHG) emissions and mitigate potential climate change effects. As seen in Figure 1, these factors have contributed to a significant increase in global biofuels production in recent years, with world output growing nearly five-fold between 2001 and 2009 (U.S. EIA, 2010).

Government policy

In an effort to decrease fossil fuel use, stimulate economic development and reduce GHG emissions, many national

governments have enacted policies in recent years that support increased domestic production and use of biofuels. For example, Brazil mandates the minimum level of ethanol that must be blended with petrol. Brazil previously provided subsidies to ensure the price of ethanol was below the price of petrol and required the nation's largest petroleum company to purchase increasing amounts of ethanol (Hofstrand, 2009). Both Brazil and Argentina also have established programmes requiring that biodiesel be blended into petroleum diesel at specified levels. In the United States, Congress established a Renewable Fuel Standard (RFS) in 2005 requiring that petroleum refiners blend increasing volumes of renewable fuels, including biofuels like ethanol and biodiesel. The RFS was modified and expanded in the Energy Independence and Security Act of 2007, requiring petroleum refiners to use 136 billion litres (36 billion gallons) of renewable fuels annually by 2022. The United States also provides fuel excise tax credits, which were scheduled to expire on 31 December 2011, to petrol and diesel fuel blenders who blend ethanol and biodiesel. In the European Union, various member states have established mandates and provided fuel excise

MAIN MESSAGES

- Biofuels policies, along with changing energy market fundamentals, have contributed to a significant increase in global biofuel production in recent years.
- The two primary biofuels produced globally today – ethanol and biodiesel – are predominantly derived from agricultural commodities, such as grain, sugar and oilseeds.
- The increased use of agricultural commodities for biofuel is generally expected to contribute to marginally higher feed prices for livestock and poultry producers, though the impacts are shown by the literature to be modest in nature.
- Increased substitution of co-products for traditional feedstuffs in feed rations helps mitigate potential input cost increases faced by livestock and poultry producers.
- Increased agricultural productivity and output has ensured that the global supply of crops available for non-biofuel uses has continued to grow over the long term.
- Growth in the use of agricultural commodities for biofuel production is expected to continue in the next 10 years, but growth rates are expected to slow in key producing countries as government-imposed limits on grain use for biofuels are reached and new non-agricultural feedstocks are commercialized.

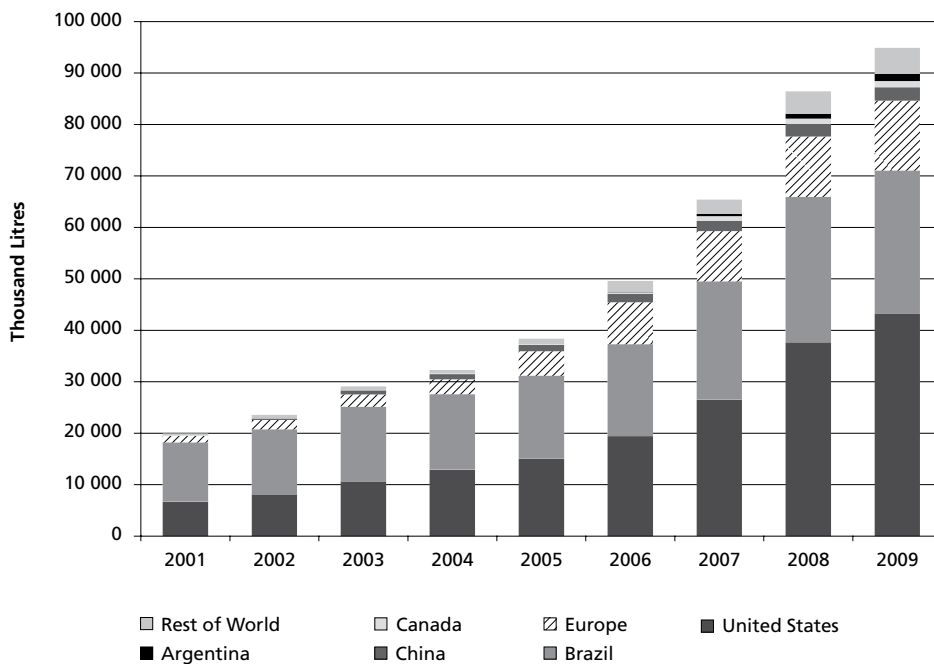
tax exemptions to encourage biofuels use. Additionally, a 2003 European Commission (EC) directive called for member states to ensure biofuels represented 2 percent of petrol and diesel fuel consumption by 2005 and 5.75 percent by 2010. A 2009 EC directive established that 10 percent of energy used for transportation in the European Community by 2020 must derive from renewable sources, such as biofuels. Many other countries, including Canada, China, India, Japan and South Africa,

have in recent years enacted blending requirements or other policies supporting biofuels production and use (Nylund *et al.*, 2008).

Energy market factors

While government policy has played an important role in stimulating growth in global biofuels production and consumption, demand for biofuels also has been accelerated by global economic and energy market forces. Declining

FIGURE 1
2001–2009 global biofuels production by nation or region



Source: U.S. EIA, 2010

global crude oil productive capacity coupled with growing demand, particularly in developing nations, has led to higher crude oil prices in recent years. As such, biofuels from a variety of feedstocks have become more economically competitive with petroleum-based fuels. Long-term energy supply and demand forecasts generally indicate sustained increases in world crude oil prices (U.S. EIA, 2011), suggesting improved economic competitiveness for biofuels. If global crude oil prices remain at historically elevated levels, and if feedstock prices decline from the weather-related highs of 2010/2011, biofuel production in many countries could exceed the volumes specified by national policies and directives based purely on its economic competitiveness with petroleum-based fuels (Hayes, 2008).

COMMON BIOFUELS, FEEDSTOCKS AND CO-PRODUCTS

Two biofuels – ethanol (ethyl alcohol) and biodiesel from fatty acid methyl esters – account for the vast majority of global biofuel production and use today. These biofuels are made today primarily from agricultural commodities, such as grain and sugar (ethanol) and vegetable oil (biodiesel). Significant research and development efforts are under way to commercialize new biofuels (e.g. butanol) and new feedstocks (e.g. cellulosic agricultural residues, municipal solid waste, algae, etc.) (Solomon, Barnes and Halvorsen, 2007). However, these “next generation” feedstocks and biofuels are unlikely to be produced in quantity in the short term according to most projections (U.S. EIA, 2011). Further, the co-products from many of these new feedstocks are not likely to have applications in the animal feed market, at least initially. Thus, the primary focus of this paper is on current ethanol and biodiesel feedstocks and the co-products that result from common processing methods.

Ethanol feedstocks and processes

Ethanol is a petroleum petrol replacement produced today mainly from grains and sugar cane. Other less common feedstocks include sugar cane and beet molasses, sugar beets, cassava, whey, potato and food or beverage waste. In 2010, approximately 87 billion litres (23 billion gallons) of ethanol were produced, with the United States, Brazil, and the European Union accounting for 93% of this output (RFA, 2011a).

Grains

Grains such as maize, wheat, barley and sorghum are common feedstocks for ethanol production, and to a lesser extent are also rye, triticale, sorghum [milo] and oats. The grain ethanol process is generally the same for all of these grain feedstocks, though there are some slight differences and the co-product characteristics vary somewhat depending on the grain used.

Two processes are primarily used to make ethanol from grains: dry milling and wet milling. In the dry milling process, the entire grain kernel typically is ground into flour (or “meal”) and processed without separation of the various nutritional component parts of the grain. The meal is slurried with water to form a “mash”. Enzymes are added to the mash, which is then processed in a high-temperature cooker, cooled and transferred to fermenters where yeast is added and the conversion of sugar to ethanol begins. After fermentation, the resulting “beer” is transferred to distillation columns where the ethanol is separated from the residual “stillage”.

The stillage is sent through a centrifuge that separates the solids from the liquids. The liquids, or solubles, are then concentrated to a semi-solid state by evaporation, resulting in condensed distillers solubles (CDS) or “syrup”. CDS is sometimes sold direct into the animal feed market, but more often the residual coarse grain solids and the CDS are mixed together and dried to produce distillers dried grain with solubles (DDGS). In the cases where the CDS is not re-added to the residual grains, the grain solids product is simply called distillers dried grain (DDG). If the distillers grain is being fed to livestock in close proximity to the ethanol production facility, the drying step can be avoided and the product is called wet distillers grain (WDG). Because of various drying and syrup application practices, there are several variants of distillers grain (one of which is called modified wet distillers grain), but most product is marketed as DDGS, DDG or WDG.

Some dry-mill ethanol plants in the United States are now removing crude maize oil from the CDS or stillage at the back end of the process, using a centrifuge. The maize oil is typically marketed as an individual feed ingredient or sold as a feedstock for further processing (e.g. for biodiesel production). The co-product resulting from this process is colloquially known as “oil extracted” DDGS or “de-oiled” DDGS. These co-products typically have lower fat content than conventional DDGS, but slightly higher concentrations of protein and other nutrients.

A very small number of dry-mill plants also have the capacity to fractionate the grain kernel at the front end of the process, resulting in the production of germ, bran, “high-protein DDGS” and other products (RFA, 2011b). In some cases, ethanol producers are considering using the cellulosic portions of the maize bran as a feedstock for cellulosic ethanol. The majority of grain ethanol produced around the world today comes from the dry milling process.

In the wet milling process, shelled maize is cleaned to ensure it is free from dust and foreign matter. Next, the maize is soaked in water, called “steepwater”, for between 20 and 30 hours. As the maize swells and softens, the steepwater starts to loosen the gluten bonds with the maize, and begins to release the starch. The maize goes on

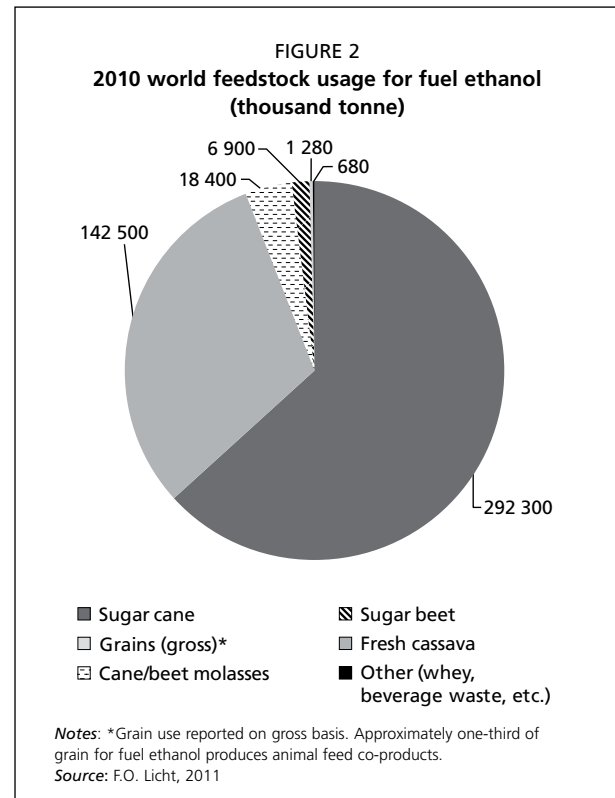
to be milled. The steepwater is concentrated in an evaporator to capture nutrients, which are used for animal feed and fermentation. After steeping, the maize is coarsely milled in cracking mills to separate the germ from the rest of the components (including starch, fibre and gluten). Now in a form of slurry, the maize flows to the germ separators to separate out the maize germ. The maize germ, which contains about 85 percent of the maize's oil, is removed from the slurry and washed. It is then dried and sold for further processing to recover the oil. The remaining slurry then enters fine grinding. After the fine grinding, which releases the starch and gluten from the fibre, the slurry flows over fixed concave screens which catch the fiber but allow the starch and gluten to pass through. The starch-gluten suspension is sent to the starch separators. The collected fibre is dried for use in animal feed.

The starch-gluten suspension then passes through a centrifuge where the gluten is spun out. The gluten is dried and used in animal feed. The remaining starch can then be processed in one of three ways: fermented into ethanol, dried for modified maize starch, or processed into maize syrup. Wet milling procedures for wheat and maize are somewhat different. For wheat, the bran and germ are generally removed by dry processing in a flour mill (leaving wheat flour) before steeping in water.

In 2010, an estimated 142.5 million tonne of grain was used globally for ethanol (F.O. Licht, 2011), representing 6.3 percent of global grain use on a gross basis (Figure 2). Because roughly one-third of the volume of grain processed for ethanol actually was used to produce animal feed, it is appropriate to suggest that the equivalent of 95 million tonne of grain were used to produce fuel and the remaining equivalent 47.5 million tonne entered the feed market as co-products. Thus, ethanol production represented 4.2 percent of total global grain use in 2010/11 on a net basis. The United States was the global leader in grain ethanol production, accounting for 88 percent of total grain use for ethanol. The European Union accounted for 6 percent of grain use for ethanol, followed by China (3.4 percent) and Canada (2.3 percent). The vast majority of grain processed for ethanol by the United States was maize, though grain sorghum represented a small share (approximately 2 percent). Canada's industry primarily used wheat and maize for ethanol, while European producers principally used wheat, but also processed some maize and other coarse grains. Maize also accounted for the majority of grain use for ethanol in China.

Sugar cane

Aside from grains, sugar cane is the other major ethanol feedstock in wide use today, particularly in tropical or subtropical regions. Sugar cane is typically processed by mills that are capable of producing both raw sugar and ethanol.



In the sugar cane ethanol process, mills normally wash incoming sugar cane stalks to remove soil and other debris. Washing is followed by a process known as "breaking," in which cane stalks are crushed to expose sugar-rich fibres. These fibres are then mechanically pressed to extract sugars and form sugar "juice". At most facilities, the juice typically is then divided into two streams: one stream for raw sugar production and the other stream for ethanol fermentation. For the stream dedicated to ethanol production, suspended materials are strained out of the juice, followed by another refining step known as the "clarification" process. The clarified sugar juice typically is then concentrated via evaporation. Next, clarified and concentrated sugar juice is fermented and distilled into alcohol.

The fibrous residue remaining after sugars are extracted is known as "bagasse". Whereas the co-products of grain ethanol are used primarily as animal feed, bagasse is used predominantly as a fuel source to generate steam and electricity to operate the sugar mill. Some research has been conducted on using bagasse as a feed ingredient for cattle, but this is a rare application with limited commercial acceptance.

In 2010, more than 98 percent of the world's sugar cane ethanol output came from Brazil, while Colombia provided 1 percent. A total of 292.3 million tonne of sugar cane was processed for ethanol in 2010 (F.O. Licht, 2011).

Sugar beet

Though far less common than grains or sugar cane, sugar beet is occasionally used as an ethanol feedstock. The

process and technology used to convert sugar beet into ethanol is quite similar to the sugar cane ethanol process. However, the fibrous component of the sugar beet that remains after sugars are extracted (known as "beet pulp") is most often dried and marketed as an animal feed ingredient. Currently, the use of sugar beet for ethanol occurs mainly in the European Union. An estimated 6.9 million tonne of sugar beet was used for ethanol in 2010 (F.O. Licht, 2011).

Sugar cane and beet molasses

Molasses is a by-product of raw sugar production from sugar cane and beets. It contains minerals regarded as impurities in the raw sugar, but also retains some fermentable sugars. Molasses has generally been used as an animal feed ingredient, but is also used as a feedstock for ethanol production in facilities that have integrated sugar and ethanol production capabilities. Fermentation of the sugars found in molasses is conducted in a manner similar to fermenting sugars from other feedstocks. An estimated 18.4 million tonne of molasses was processed into fuel ethanol in 2010, with Brazil representing 74 percent of total use, followed by Thailand (7 percent) and India (5 percent) (F.O. Licht, 2011).

Cassava

Cassava, also known as tapioca, is an annual crop that is cultivated in tropical regions. The cassava root has relatively high starch content, making it a suitable feedstock for ethanol fermentation. It is typically available in two forms for ethanol production: fresh root (high moisture, available seasonally) and dried chips (low moisture content, available throughout the year). When processing fresh root, the feedstock is washed to remove soil and debris, followed by peeling. The peeled root is then subjected to a process known as rasping, which breaks down cell walls to release starch granules. The starch is then steeped and separated from the fibrous residue and concentrated. Next, the starch is fed into the fermentation process, followed by distillation and dehydration, similar to the process for grain-based ethanol. The co-product of the cassava-to-ethanol process is root fibre, which is used as a boiler fuel source, similar to bagasse in the sugar cane ethanol process. Root fibre is not currently used as animal feed.

In 2010, the equivalent of nearly 1.3 million dry tonne of fresh cassava root was processed into ethanol. Thailand was the leading producer (50 percent), followed by China (44 percent) (F.O. Licht, 2011).

Small amounts of other feedstocks, such as cheese whey, potato and beverage waste, were probably used in 2010, but they are not discussed here because of their insignificant volumes and hence impact on global feed markets.

Biodiesel feedstocks and processes

Biodiesel is a petroleum diesel fuel replacement produced from renewable fats and oils sources such as vegetable oils, animal fats and recycled cooking oils. Chemically, biodiesel is a mono-alkyl ester of long chain fatty acids. It is produced from a diverse set of feedstocks, reflecting the natural fats or oils indigenous to specific geographical regions. Thus, European biodiesel producers rely upon rapeseed as a primary feedstock for biodiesel production. In Southeast Asia, crude palm oil or its derivatives are the primary feedstocks utilized. Meanwhile, in the United States, soybean oil is the predominant feedstock, although a host of other feedstocks, such as animal fats, yellow grease, and vegetable oil recovered from dry mill ethanol plants, contribute supplies as well.

It is estimated that global production of biodiesel in 2010 was 17.9 million tonnes (5.34 billion gallons) (Oil World, 2011). Production is expected to increase 17 percent in 2011 to 21 million tonne (6.3 billion gallons). The European Union was the global leader in biodiesel production in 2010, accounting for an estimated 52 percent of production. Almost 80 percent of the anticipated production in 2011 will be generated by the EU, United States, Argentina and Brazil.

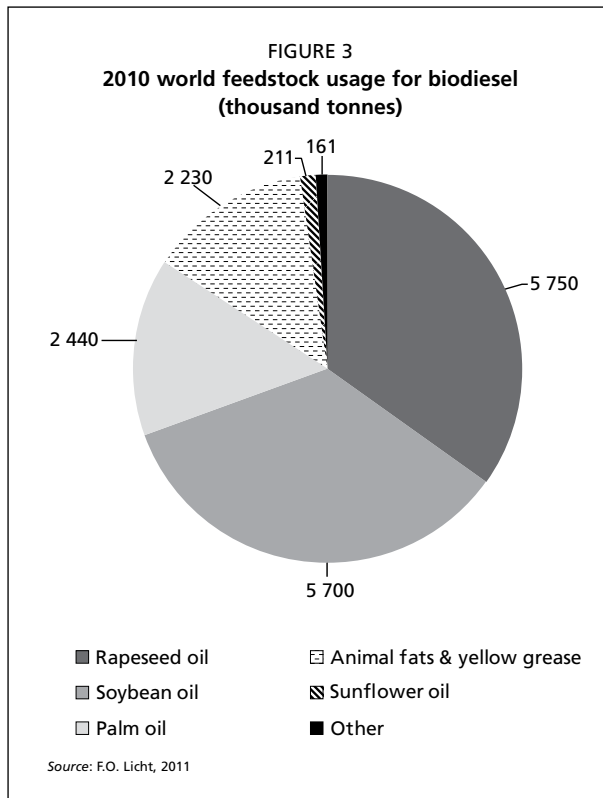
Oilseeds

Oilseeds such as rapeseed or canola and soybeans represent the most common source of vegetable oil feedstocks for biodiesel production. The biodiesel production process utilized for these feedstocks is similar. In 2010, an estimated 5.8 million tonne of rapeseed or canola oil and 5.7 million tonne of soybean oil were used globally in the production of biodiesel, representing 69 percent of the total feedstocks used in global biodiesel production (Figure 3).

Palm

Globally, palm oil is an important vegetable oil source. A unique feature of the palm tree is that it produces two types of oil; crude palm oil from the flesh (mesocarp) of the fruit, and palm kernel oil from the seed or kernel. The crude palm oil may be further refined to get a wide range of palm products of specified quality. For example, palm oil may be fractionated to obtain solid (stearin) and liquid (olein) fractions with various melting characteristics. The different properties of the fractions make them suitable for a variety of food and non-food uses.

In 2010, an estimated 2.4 million tonne of palm oil were used globally in the production of biodiesel (F.O. Licht, 2011), representing 15 percent of the total feedstocks used in global biodiesel production. Indonesia, Thailand, the EU and Colombia were the top users of palm oil for biodiesel production in 2010. Together, they represented 78 percent of global use of palm oil for biodiesel.



Animal fats and yellow grease

Animal fats are derived from the rendering process using animal tissues as the raw material. The raw material is a by-product of the processing of meat animals and poultry. The amount of fat produced is directly related to the species of animal processed and the degree of further processing that is associated with the marketing and distribution of the meat product. Current markets for rendered animal fats include use as feed ingredients for livestock, poultry, companion animals and aquaculture. In addition, products such as edible tallow are used for soap and fatty acid production. Industry analysts anticipate that roughly 25 to 30 percent of the rendered animal fat supplies could be diverted to biodiesel production given current uses (Weber, 2009).

In 2010, an estimated 2.2 million tonne of animal fats and yellow grease was used globally in the production of biodiesel (F.O. Licht, 2011), representing 14 percent of the total feedstocks used in global biodiesel production. EU producers used 54 percent of animal fats and yellow grease processed as biodiesel feedstock in 2010, followed by Brazil (16 percent) and the United States (12 percent).

Maize oil from ethanol production processes

Grain ethanol production may offer the biodiesel industry its nearest-term opportunity for a significant additive supply of plant oils for biodiesel production. Historically, maize oil has not been a viable biodiesel feedstock due to its relative high cost and high value as edible oil. However, as discussed earlier, some dry-mill ethanol plants in the United

States are now removing crude maize oil from the stillage at the back end of the process. The maize oil is typically marketed as an individual feed ingredient or sold as a feedstock for further processing (e.g. for biodiesel production). Maize oil could help to meet feedstock market demand in two ways. First, edible maize oil could displace other edible oils that could then be diverted to biodiesel production. Second, non-edible maize oil could be used directly for biodiesel production.

Biodiesel production process

Regardless of the feedstock, most biodiesel globally is produced using one of three common manufacturing methods: reaction of the triglycerides with an alcohol, using a base catalyst; reaction of the triglycerides with an alcohol, using a strong acid catalyst; or conversion of the triglycerides to fatty acids, and a subsequent reaction of the fatty acids with an alcohol using a strong acid catalyst.

In the United States and elsewhere, biodiesel is commonly produced using the base-catalyzed reaction of the triglycerides with alcohol. Methanol is currently the main alcohol used commercially for the production of biodiesel due to its cost relative to other alcohols, shorter reaction times compared with other alcohols, and the difficulty and cost of recycling other alcohols.

Use of acid catalysis is typically limited to the conversion of the fatty acid fraction in high free fatty acid feedstocks, or to treat intermediate high fatty acid/ester streams that can form in the acidification of the crude glycerin bottoms produced as a co-product of the transesterification reaction.

Stoichiometrically, 100 kg of triglycerides are reacted with 10 kg of alcohol in the presence of a base catalyst to produce 10 kg of glycerin and 100 kg of mono-alkyl esters or biodiesel. In practice, an excess amount of alcohol is used in the reaction to assist in quick and complete conversion of the triglycerides to the esters, and the excess alcohol is later recovered for re-use. All reactants must be essentially free from water. The catalyst is usually sodium methoxide, sodium hydroxide or potassium hydroxide that has already been mixed with the alcohol.

In some cases, the free fatty acid levels of the feedstock utilized are elevated to the point that an esterification step, using an acid catalyst, is incorporated into the biodiesel processing sequence. This stage involves mixing the high fatty acid material with a solution of methanol that contains an acid catalyst, typically sulphuric acid. The contained fatty acids are then converted to methyl ester. An excess of methanol and H_2SO_4 is employed to ensure conversion, and after reaction completion this excess is separated from the ester phase. The conversion of the fatty acid to ester results in the formation of water, thus after the reaction there is water in the methanol+ sulphuric acid mixture. Since this is an equilibrium reaction, the presence

of excessive amounts of water will adversely affect the conversion of the fatty acid to ester. Thus, a portion (or all) of the methanol+sulphuric acid mix is purged from the system and treated to recover the methanol and reject the water. A typical approach involves using this purge material as the acidifying agent for treating the glycerin material, followed by recovery of the methanol. In this case, the water fraction will end up in the glycerin phase.

Biodiesel co-products

The main direct co-product of biodiesel production is glycerine, which is a commonly used commercial name for products whose principal component is glycerol. More precisely, however, glycerine applies to purified commercial products containing 95% or more of glycerol. Glycerine is a versatile and valuable chemical substance with many applications. A clear, odourless, viscous liquid with a sweet taste, glycerine is derived from both natural and petrochemical feedstocks. It occurs in combined form (triglycerides) in all animal fats and vegetable oils and constitutes about 10 percent of these materials on average. Importantly, glycerine can also be utilized as a feed ingredient for livestock rations. Increased production of biodiesel has led to renewed evaluation of glycerine from biodiesel operations as a liquid feed ingredient for livestock.

In the conventional glycerine refining processes, the crude glycerine solution is initially treated with additional chemicals to remove any dissolved fatty acids or soaps, and to prepare the solution for the next stage of processing. The concentrated glycerine is then processed in a higher temperature, high vacuum distillation unit. The condensed glycerine solution is further treated to remove traces of residual fatty acids, esters or other organics that may impart colour, odour or taste to the glycerine. Typical methods for this "post-treatment" step may include activated clay addition and filtration, similar to that used in the treatment of vegetable oils for edible uses; powdered activated carbon addition, followed by filtration; and/or treatment in activated carbon columns, commonly used for trace organics removal from a range of industrial and food chemicals.

In the processing of biodiesel crude glycerine, issues typically associated with conventional crude processes, e.g. char materials, crystallized salts, etc., can be magnified, due to the higher starting impurity content. Thus, for a refinery that would process biodiesel crude only, or as a high percentage of its input, a more sophisticated processing approach may be required.

Another co-product of the biodiesel production process is fatty acids, which are derived from a variety of fats and oils, and are used directly (unreacted) or for the manufacture of derivatives. Fatty acids are used directly in a number of products such as candles, cosmetics and toiletries, animal feeds, lubricants and asphalt.

Vegetable oil meal represents a very important *indirect* co-product of biodiesel production. Oilseed crops that are crushed, either in a mechanical expelling or solvent extraction operation, will generate both crude vegetable oil and oilseed meal. Oilseed meals are an integral component of livestock rations as a source of protein and key amino acids. Although soybean oil is the most valuable part of the seed on a per weight basis, only 20 percent of the seed by weight is vegetable oil. The remaining 80 percent of the seed (the portion left after extracting the oil) is referred to as "meal". The value of oilseed meal in the animal feed market has historically been the primary economic driver of oilseed crushing, rather than the value of the oil. In other words, oilseed meal for livestock feed is the primary co-product of oilseed crushing, while vegetable oil is the secondary co-product. Thus, oilseed meal would be produced for feed regardless of the uses and demand for the oil. Accordingly, oilseed meal is not considered a direct co-product of biodiesel production.

GENERALLY ACCEPTED USES OF FEED CO-PRODUCTS IN ANIMAL DIETS

Biofuel co-products are used broadly today as feed ingredients in the diets for livestock, poultry and fish. These co-products often substitute for higher priced feeds in animal rations. For example, in recent years, DDGS has sold at a significant discount to maize and soybean meal, which are the ingredients it primarily substitutes for in animal diets (Hoffman and Baker, 2010). Ruminant animals, such as beef cattle and dairy cows, have been the main consumers of ethanol and biodiesel co-product feeds historically. However, the use of feed co-products in rations for non-ruminant animals, such as hogs and broilers, has been growing in recent years.

Numerous studies have examined the use of biofuel co-products in animal feed rations and identified key considerations for different animal species (Shurson and Spiehs, 2002; Anderson *et al.*, 2006; Whitney *et al.*, 2006; Daley, 2007; Klopfenstein, Erickson and Bremer, 2008; Schingoethe, 2008; Stein, 2008; Bregendahl, 2008; Walker, Jenkins and Klopfenstein, 2011). The amount of co-products that can be introduced into animal feed rations depends on the nutritional characteristics of the individual ingredient and unique limiting factors for the various species being fed.

Other papers have examined the mass of traditional feedstuffs displaced from typical animal feed rations by a given mass of biofuel co-products, such as distillers grain. Some of these papers show that due to the concentration of certain nutritional components, a given mass of distillers grains can displace more than the equivalent mass of maize and soybean meal in some animal rations. Arora, Wu and Wang (2008), for example, found that 1kg of

distillers grain can displace 1.2 kg of maize in a typical beef ration. Hoffman and Baker (2011) found that "...in aggregate (including major types of livestock/poultry), a metric ton of DDGS can replace, on average, 1.22 metric tons of feed consisting of maize and soybean meal in the United States."

In general, studies show that distillers grains can account for approximately 30 to 40 percent in beef cattle rations, although higher rates can be used (Vander Pol *et al.*, 2006). Animal feeding studies generally indicate effective distillers grain inclusion rates of 20 to 25 percent for dairy cows, 20 percent for farrow-to-finish hogs, and 10 to 15 percent for the grow-finish stages of poultry feeding. Gluten feed from wet mills is typically fed to beef cattle at an inclusion rate of 30 to 50 percent of the ration, while gluten meal is fed at much lower levels to both ruminant and non-ruminant animals. Gluten meal is also a common ingredient in pet food products. Pressed or shredded beet pulp is typically fed to ruminant animals at no more than 15 to 20 percent of the diet. Glycerine from the biodiesel process can be added to beef and dairy diets at low levels, typically representing no more than 10 percent of the ration. Research is also under way to determine appropriate levels of glycerine inclusion in swine and poultry rations (Flores and Perry, 2009).

HISTORICAL VOLUMES OF FEED FROM BIOFUEL CO-PRODUCTS

Currently, there are no regular or comprehensive efforts to collect and report data on biofuel feed co-product produc-

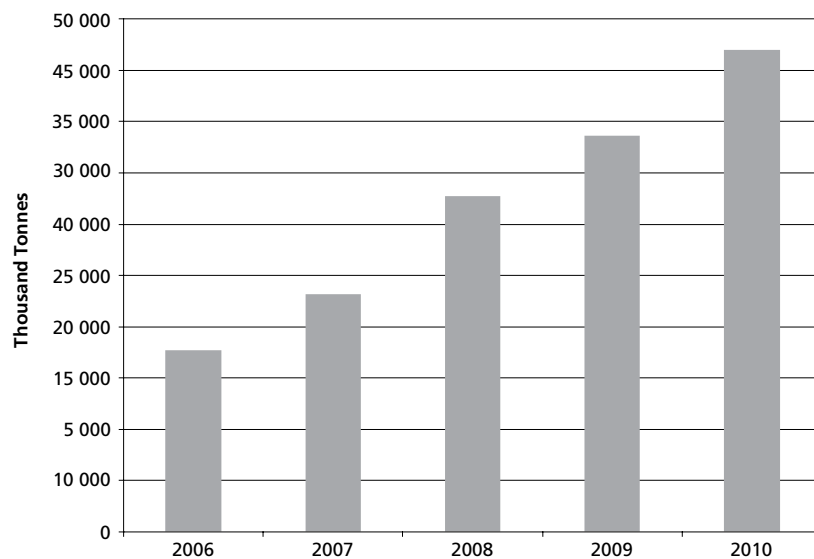
tion volumes. However, several studies have approximated co-product output volumes, based on generally accepted conversion factors per tonne of feedstock and government estimates of feedstock use for biofuel production (Hoffman and Baker, 2010). As a general rule of thumb, a tonne of grain processed by an ethanol biorefinery will generate approximately one-third of a tonne of feed co-products. Thus, global grain ethanol co-product production can be estimated (Figure 4) by applying this simple conversion to estimates of total feedstock use, as provided by F.O. Licht (2011).

As most of the world's grain ethanol output comes from the United States, most of the world's DDGS and other feed co-products also originate in the United States. In recent years, as much as 25 percent of U.S. feed co-product output has been exported.

The amount of crude glycerine generated by the biodiesel industry is directly proportional to overall biodiesel production. Generally about 10 percent, by weight, of the lipid source will be glycerine. In reality, approximately 0.4 kg of glycerine are produced per litre of biodiesel production. An economic analysis prepared by IHS Global Insight suggests expected biodiesel feedstock supplies in the United States could support 9.5 billion litres of biodiesel by 2015 (IHS Global Insight, 2011).

With increased production of biodiesel and a resultant increase in crude glycerine supplies, it is likely that expanded feed applications will continue to be pursued. A 2010 survey of National Biodiesel Board (NBB) member companies reported that 48 percent of NBB members sold

FIGURE 4
Global production of grain ethanol animal feed co-products



Source: RFA calculation based on F.O. Licht, 2011

their glycerine output to refiners to be processed for high-value uses, 33 percent marketed glycerine to be used for livestock feed, 4 percent sold the co-product as fuel, and the remaining survey respondents either did not specify a use or listed a minor use.

Impacts on global livestock and poultry markets

Numerous studies have examined the potential impacts of increased biofuels production on animal feed supplies and prices, as well as the production levels and prices of meat, milk, eggs and other agricultural products (Taheripour, Hertel and Tyner, 2010a, b; Elobeid *et al.*, 2006; Banse *et al.*, 2007; Birur, Hertel and Tyner, 2007; Westcott, 2007; USDA, 2007). Many of these studies have employed computable general equilibrium (CGE) or partial equilibrium economic models to estimate the potential long-term impacts of biofuel policies. While most of these studies suggest that large-scale biofuel production results in higher long-term prices for certain agricultural commodities (thus increasing input costs for the livestock and poultry industries), the magnitude of the impacts is generally modest. For example, in its analysis of the impacts of the United States' Renewable Fuel Standard (RFS), the U.S. Environmental Protection Agency (EPA, 2010) found that full implementation of the programme's biofuel consumption mandates might result in price increases of just 0.8% for soybeans, 1.5% for soybean oil and 3.1% for maize by 2022 over a baseline scenario with no biofuels mandate. Similarly, one recent study indicated that, from 2005 to 2009, prices for rice, wheat, soybean and maize would have been only marginally lower (-0.2, -1.3, -1.7 and -3.3 percent on average, respectively) if U.S. ethanol policies had not existed (Babcock, 2011).

Most of these studies indicate that the production and consumption of meat, milk, eggs and other agricultural goods may be slightly reduced due to higher feed input costs induced by biofuels expansion, but again, the impacts are found to be small. For example, the U.S. Environmental Protection Agency found that full implementation of the RFS biofuel consumption mandates could be expected to result in just a 0.05% reduction in consumption of livestock products and 0.03% reduction in consumption of dairy products by 2022 (EPA, 2010). In an analysis of the agriculture market impacts of achieving the 2015 RFS mandate for conventional (maize starch) biofuels, the U.S. Department of Agriculture (USDA) found no change in U.S. chicken output, an average -0.2% reduction in milk output and an average -0.3% reduction in pork output over baseline values between 2007 and 2016 (USDA, 2007). Beef output actually increased an average of 0.1% in the USDA analysis, as beef cattle production was assumed to benefit from increased production of distillers grain.

While the results of these economic analyses are instructive, many of the studies have failed to properly incorporate

the recent economic impacts of increased consumption of biofuels co-products by the livestock and poultry sector (Taheripour, Hertel and Tyner, 2010b). In recent years, prices for biofuel feed co-products have generally declined relative to competing feedstuffs, which is not accurately accounted for in most economic modelling studies examining adjustments by the livestock and poultry sectors in response to increased biofuel production. Recent pricing patterns indicate that biofuel co-products can help the livestock and poultry industry offset minor cost increases for traditional feedstuffs that might result from expanded biofuel demand. Many of the economic modelling studies discussed here were conducted prior to the establishment of sustained price discounts for key biofuel feed co-products relative to traditional feedstuffs.

Recognizing this shortcoming in previous modelling efforts, Taheripour, Hertel and Tyner (2010a) introduced an improved co-product substitution methodology to the Global Trade Analysis Project (GTAP) model, a popular CGE model used by government agencies and other entities in the U.S., EU, and Brazil. Based on the improved methodology and updated modelling results, Taheripour, Hertel and Tyner (2010b) concluded that "In general, the livestock industries of the US and EU do not suffer significantly from biofuel mandates, because they make use of the biofuel byproducts to eliminate the cost consequences of higher crop prices". The study further found that "...while biofuel mandates have important consequences for the livestock industry, they do not harshly curtail these industries. This is largely due to the important role of by-products in substituting for higher priced feedstuffs".

While Taheripour, Hertel and Tyner (2010a) represented an advancement in the analysis of the impact of expanded biofuels production on livestock, it did not take into account the ability of DDGS to displace more than an equivalent mass of maize and soybean meal, as documented by Arora, Wu and Wang (2008) and Hoffman and Baker (2011). Nor did the Taheripour study account for likely continued improvements in the feed conversion efficiency of livestock and poultry.

Specifically pertaining to biodiesel production, research has been conducted to evaluate the impact of increased biodiesel production from oilseeds on the livestock sector (Centrec, 2011). Utilizing a partial equilibrium model called the Value Chain Analysis (VCA) developed for the United Soybean Board, the impacts of single soybean oil supply or demand factors were examined in isolation from other factors. A decrease in soybean oil demand for biodiesel was isolated and analysed. The analysis found that reduced demand for soybean oil for United States biodiesel production would result in lower soybean oil prices, reduced soybean production and significantly higher soybean meal prices. Thus, the analysis showed that increased demand

for vegetable oil for biodiesel results in larger supplies of oilseed meal for livestock feed and, in turn, lower prices.

The results of the Centrec work were confirmed in 2011 in an economic analysis conducted by IHS Global Insight (2011) that analysed United States and international feedstock supplies, projected petroleum pricing, edible oil demand, and energy policy to estimate potential biodiesel industry growth in the United States. Potential acreage shifts, commodity price impacts, and global trade effects were also examined. The analysis demonstrated a significant decrease in soybean meal values due to increased oilseed production.

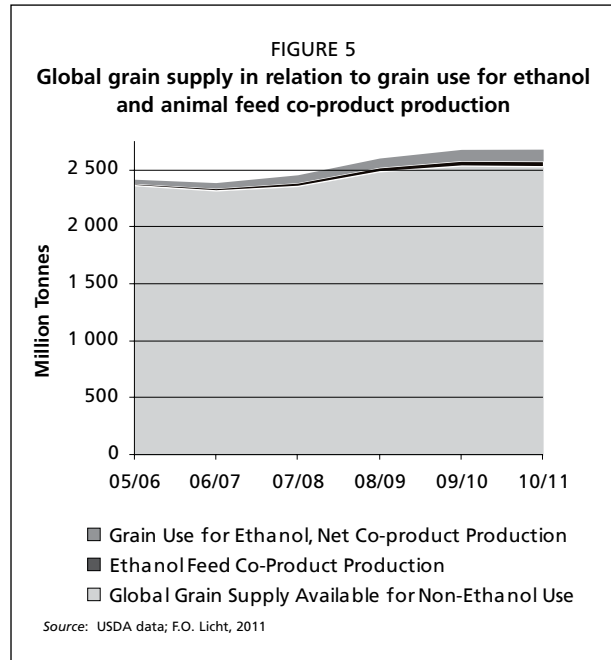
Aside from the effect of substituting relatively lower-cost feed co-products from biofuels production for traditional feedstuffs, the modest impacts of expanded biofuels production on the livestock sector can be partially explained by steadily increasing supplies of food and feed crops. That is, the global grain and oilseed supply has grown substantially in recent years, such that increased use of these commodities for biofuels production has not led to reduced availability for feed or feed use.

As an example, the global grain supply (wheat, rice, maize, sorghum, barley, oats, rye, millet and mixed grains) totalled 2 423 million tonne in 2005/06. Grain use for ethanol and co-product production was 54 million tonne on a gross basis in 2005/06 (F.O. Licht, 2011), meaning 2 369 million tonne of grain remained available for uses other than ethanol and feed co-products. By comparison, the global grain supply was a record 2 686 million tonne in 2009/10. Grain use for ethanol and co-products totalled 143 million tonne in 2009/10, meaning 2 543 million tonne of grain were available for non-ethanol uses. Thus, the supply of grain available for non-ethanol uses (i.e. grain remaining after accounting for grain use for ethanol) grew 7 percent between 2005/06 and 2009/10. Further, the supply of grain ethanol feed co-products grew 268 percent during this period. The combined supply of grain for non-ethanol use and ethanol feed co-products totalled 2 586 million tonne in 2009/10, compared with 2 386 million tonne in 2005/06. Figure 5 shows recent growth in the global grain supply relative to grain use for ethanol and feed co-product production.

The amount of grain available for uses other than ethanol production is expected to grow more significantly in the long term, as grain use for ethanol moderates in accordance with slowing national mandates.

BIOFUELS AND CO-PRODUCT OUTLOOK TO 2020

Market factors and government policies are expected to continue to support expanded biofuels production and use in the long term. Growth in grain and oilseed use for biofuels is expected to be maintained or accelerated



in some nations or blocs throughout the decade. In the EU, for instance, USDA (2011) projects biodiesel production will increase 22 percent and ethanol production will increase more than 40 percent by 2020 in response to biofuels blending mandates. Further, USDA projects Brazilian ethanol production will increase 45 percent by 2020, largely because of stronger expected export demand. Ethanol and biodiesel production increases from traditional feedstocks are also projected in Canada and Argentina.

However, growth in the use of certain agricultural commodities as biofuels feedstocks is expected to moderate in the next 10 years in some other nations. For example, USDA projects maize use for ethanol in the United States will be 128 million tonne in 2011/12, but will grow only gradually (1 percent per year) to 140 million tonne by 2020/21 (USDA, 2011). There are two major reasons for the expected slower rate of growth in the use of agricultural feedstocks for biofuels in the United States and some other nations. First, government policies in several nations place restrictions on the amount of agricultural commodities that may be used for biofuels. For example, the United States' RFS caps the amount of maize starch ethanol that can qualify for the mandate at a maximum of 57 billion litres (15 billion gallons) per year beginning in 2015. Similarly, China recently imposed regulations to limit grain ethanol production to current levels, effectively restricting any further growth in grain use for ethanol (USDA, 2011). The second reason for moderation in the growth in the use of agricultural commodities for biofuels is the expectation that future growth in biofuels production will primarily come from new feedstocks that currently have no or limited application in the animal feed market, such as perennial grasses (switch grass, miscanthus), agricultural residues (maize

stalks, wheat straw), algae, jatropha, pennycress, municipal solid waste, forestry residues and other materials.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

While animal feed co-products from biofuels production have played an important role in the global livestock and poultry industries for many years, several critical knowledge and information gaps remain. First, as highlighted by Taheripour, Hertel and Tyner (2010b), many studies examining the impact of biofuels demand on commodity prices and livestock and poultry markets do not properly account for the sustained price discount of co-product feeds versus traditional feedstuffs. There appears to be a general lack of understanding of how pricing trends and fluctuations affect co-product feeding decisions and dietary inclusion levels. The dynamic pricing relationship among animal feed co-products from biofuels processes and traditional feedstuffs, and the impacts of pricing relationships on substitution rates, is an area for further future research.

Additionally, understanding of the impact of biofuel feed co-products on livestock and poultry markets has been greatly hindered by a lack of public data and information on co-product production volumes by type and geography. Government agencies that track and publish public market data for traditional feedstuffs and commodities generally do not provide adequate coverage of co-product feed production volumes, types, etc. This is a significant information gap that, if filled, would enhance the collective understanding of co-product animal feed markets.

Finally, little is known about the effect of maize oil extraction on feeding and pricing of DDGS. This again is an area for future research.

CONCLUSIONS

Recent years have seen a tremendous increase in the production of biofuels from agricultural commodities. Growth in biofuel production has been accompanied by increased output of animal feed co-products from common biofuel processes. Globally, these feed co-products are growing in volume and importance. While the increased use of agricultural commodities for biofuels is generally expected to contribute to slightly higher input costs for certain livestock and poultry feeds, the impacts are expected to be modest and can be mitigated in part by increased substitution of co-products for traditional feedstuffs. Increased agricultural productivity has allowed the global supply of crops available for non-biofuel uses to continue to grow over the long term. Growth in the use of agricultural commodities for biofuels is expected to continue through to 2020, but growth rates will slow in key producing countries as government-imposed limits on grain use for biofuels are reached and new non-agricultural feedstocks are commercialized.

ACKNOWLEDGEMENTS

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Chapter 2

An outlook on EU biofuel production and its implications for the animal feed industry

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ABSTRACT

While GHG emissions of most carbon sources in the EU have been falling, GHG emissions from road transport have been rising. The EU has therefore enacted mandates to reduce the GHG emissions from road transport by 2020, and this will primarily be achieved by biofuel blending in the EU. This chapter describes the road transport mandates, which must be met in 2020; the alternative processes and crops that can be used for biofuel production; the EU animal feed balance; and sustainability of EU biofuels. Based on this background information, expected scenarios are developed for 2020, to show the estimated biofuel production and changes in animal feed balance in the EU, and the associated carbon benefits.

The EU animal feed co-product production will depend on the split between biodiesel and bio-ethanol to meet the 2020 target. The estimated animal feed from dried distillers grain with solubles (DDGS) and oil meal co-product output varies from 23 million tonne per year for a low ethanol scenario to 35 million tonne per year for a high ethanol scenario. The use of animal feed co-products in the EU compound feed market will directly or indirectly displace a mixture of EU cereals and imported soybean meal, mainly from South America. Most of the additional EU crop output will be achieved by increased crop yields, and the remainder from continuing to use arable land that would otherwise have been released from use. Since the yields of biofuel crops grown in the EU are substantially higher than those of soybean in South America, the overall result of these changes is an increase in biofuel production of about 500 PJ/year (12 million toe/year), with a net reduction in required global land area of about 3 million hectare.

INTRODUCTION

The primary purpose of European Union (EU) biofuel mandates is to reduce greenhouse gas (GHG) emissions associated with road transport fuels. However, for EU biofuel production, there are also implications for the animal feed industry by making available biofuel co-products, such as protein-rich dried distillers grain with solubles (DDGS) and oilseed meals. Previous work has generally focused on the GHG emissions of biofuel production, without consideration of either the land use changes or GHG implications of the animal feed industry. This chapter aims to take a broader view across the biofuel and animal feed industries to show that biofuel co-products used as animal feed give a major carbon benefit in addition to the immediate carbon saving benefit of the biofuel. In order to develop this broader view, background information is presented under a few major headings:

- The need for biofuels to help tackle climate change.
- Biofuel mandates and target to be met by 2020.
- Biofuel processes and crops.
- EU animal feeds.
- Biorefining of crops for biofuel and animal feed.
- Sustainability of biofuels and animal feed.

Based on this background information, expected scenarios are developed for 2020, to show the biofuel production and changes in animal feed balance in the EU and the associated carbon saving benefits.

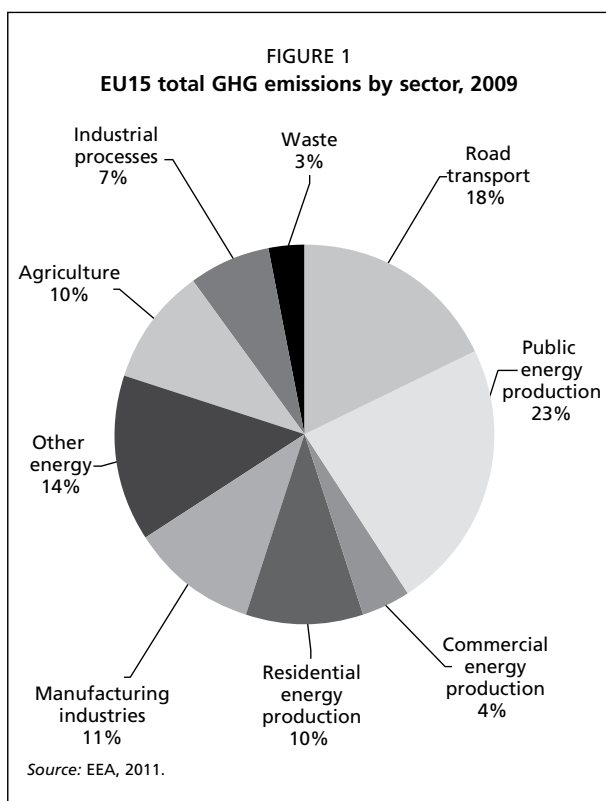
THE NEED FOR BIOFUELS TO TACKLE CLIMATE CHANGE

The main cause of global warming is GHG emissions. The breakdown of EU GHG emissions by sector is shown in Figure 1, which shows that 18 percent of GHG emissions are related to transport fuels. The growth of EU GHG emissions by sector is shown in Figure 2.

Since 1990, while nearly all other sectors have seen a significant reduction in GHG emissions, the GHG emissions from transport have increased by more than 25 percent. This highlights the vital need to reduce the GHG emissions from road transport, by decarbonization of road transport fuels, the development of more efficient engines and encouraging the use of more efficient means of transport. Commercially viable options for decarbonization of transport fuels are much more limited than for power generation, and the only cost-effective technology in the foresee-

MAIN MESSAGES

- Since 1990, while all other sectors have seen a significant reduction in GHG emissions, the GHG emissions from transport have increased by more than 25 percent. This shows the vital need to reduce the GHG emissions from road transport.
- There is a loss of only 1 to 2.5 percent in the overall energy efficiency from biorefining food crops for biofuel and animal feed. The non-extracted oil and fermentation by-products are all conserved to provide metabolizable energy in the animal feed co-products, while all the other plant components, such as protein and minerals are concentrated in the animal feed.
- The blending of animal feed co-products in the EU compound feed market will directly or indirectly displace a mixture of EU cereals and imported soybean meal, mainly from South America.
- The use of cereal crops and oilseed rape for additional biofuel production will reduce the rate of abandonment of arable land in temperate regions, while the use of other oil seeds and sugar cane for biofuels will increase the demand for arable land in South America and SE Asia.
- Biofuel production from EU crops will give substantial GHG savings and animal feed co-products will provide substantial additional GHG savings from ILUC, due to soybean meal displacement.
- The yields of biofuel crops grown in the EU are substantially higher than those of soybean in South America, so the overall balance of biofuel production from EU crops is a net biofuel output of 12 million tonne oil equivalent annually, with a net reduction in global land area of about 3 million hectare.



able future is the use of transport biofuels to replace fossil fuels. While there are other options for non-carbon transport, based on plug-in electricity or hydrogen fuel cells, these vehicles would use marginal base load power, which for many EU countries will be provided by coal-fuelled power stations. Plug-in electric vehicles also incur substantial additional GHG emissions in the production of the battery pack (Patterson, Alexander and Gurr, 2011). These

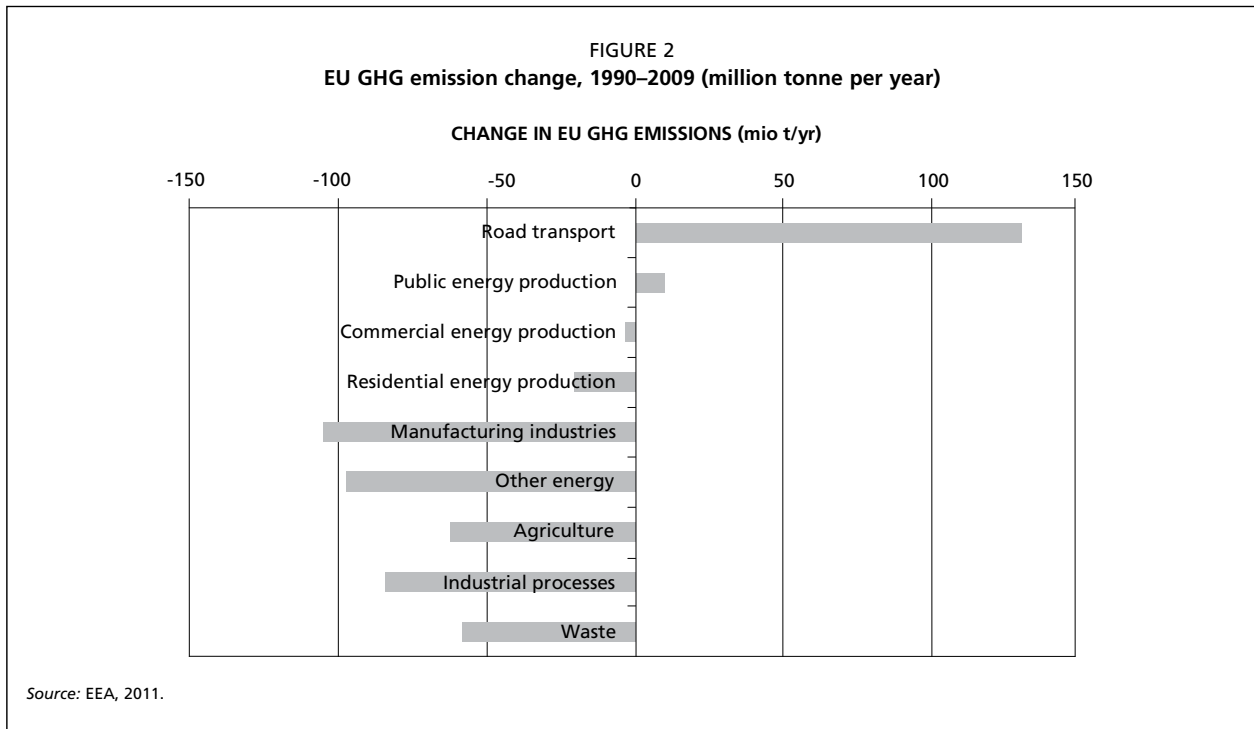
vehicles will therefore not provide worthwhile GHG savings until coal power stations are shut down and the base load power generation sector is substantially decarbonized.

EU BIOFUEL PRODUCTION

Biofuel legislation and outlook

Biofuels are required to meet two pieces of legally binding EU legislation:

- The EU Renewable Energy Directive (RED) (EC, 2009), which mandates Member States to meet 10 percent of surface transport energy from renewable sources by 2020. Rail electrification and electric vehicles may make a contribution to the RED, but biofuels are expected to make up the majority of the effort. Biofuels will only count towards the RED targets if they meet specified sustainability criteria. These include meeting a minimum threshold in GHG savings compared with fossil fuels of 35 percent by 2013, and 50 or 60 percent by 2017.
- The EU Fuel Quality Directive (FQD), which mandates transport fuel suppliers to meet a 6 percent reduction in the carbon intensity of road transport fuels by 2020. The minimum obligation is expected to be met through improved industrial practices in the extraction and refining of fossil fuels (reductions in flaring and venting) and the use of lower GHG-emitting biofuels and alternative fuels. Analysis by fuel suppliers has suggested that little if any of the target can be met by improvements in the production of fossil fuels. If it is assumed that there is no improvement in the refining GHG intensity, then biofuels will have to provide the full 6 percent of the GHG emission reduction. While some biofuels, such as those from lignocellulosic feedstocks and from wastes and residues,

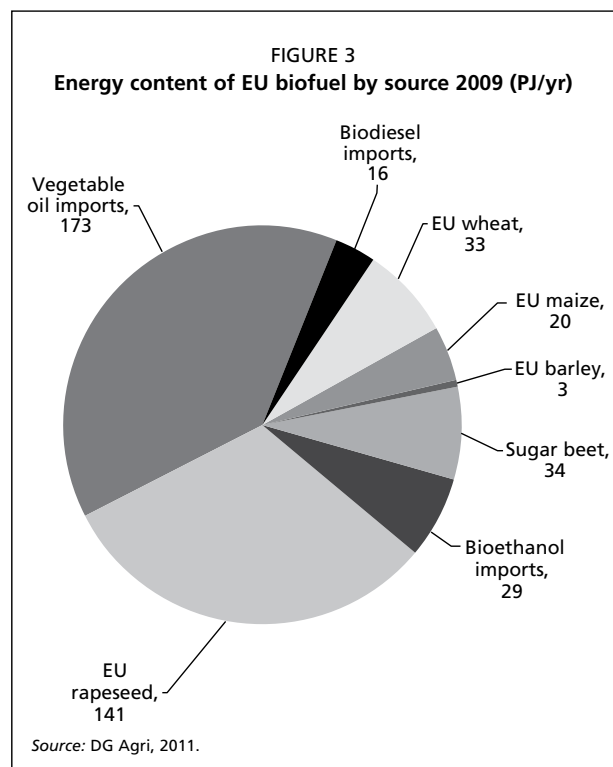


count double towards the RED target, this does not apply to the FQD target.

Member States have developed National Renewable Energy Action Plans (NREAPs) with estimates of how they intend to meet the RED targets, but not how they intend that fuel suppliers will meet the FQD targets. In most cases, the quantities of biodiesel and bio-ethanol are extrapolations of existing biofuel supply positions, or are based on extrapolations of the trend in fossil fuel diesel/petrol split with similar biofuel contents in each fossil fuel. While it has been assumed for many estimates that fossil fuels will have a similar energy blend of biodiesel in diesel as for bio-ethanol in petrol, there is no reason why this should be the case. High blends, such as E85 (85 percent ethanol + 15 percent petrol) and B100 (100 percent biodiesel) enable any bio-ethanol/biodiesel ratio to be accommodated. However, current vehicles cannot tolerate ethanol blends above 10 percent v/v and biodiesel blends above 7 percent v/v, so the timescale for introduction of vehicles with higher biofuel blend capability is a key factor in meeting the RED target. Also it is not clear that any Member States have taken into account the higher GHG thresholds in 2017, nor the need for fuel suppliers to meet the FQD target. These two issues will drive a substantially higher bio-ethanol to biodiesel ratio than so far suggested by NREAPs. This point is developed further below in considering likely biofuel scenarios for 2020.

EU biofuel sources

Estimates of the amount of biofuel required in 2020 to meet the RED target depend on estimates of surface transport



energy needs and of double-counting biofuels, and have ranged between 1000 and 1200 petajoules (PJ). The latest estimate is 1100 PJ (DG Agri, 2011). This compares with sales of biofuels in the EU in 2009 of about 450 PJ. The breakdown of biofuel by source in 2009 is shown in Figure 3.

The total biodiesel supply was 330 PJ, mainly from EU-grown rapeseed and from vegetable oil imported as oil or as oilseeds, from soybean and palm. The total bio-

ethanol supply was 120 PJ from EU cereals and sugar beet, with imports of sugar cane bio-ethanol.

BIOFUEL PROCESSES

Different crop products are used to make biofuels, using alternative technologies:

- Sugar and starch fermentation to bio-ethanol.
- Vegetable oil transesterification or hydrogenation to biodiesel.
- Anaerobic digestion to biomethane.
- Hydrolysis of lignocellulosic feeds followed by fermentation to bio-ethanol.
- Gasification of lignocellulosic feeds followed by biodiesel synthesis.

Starch in cereal crops and sugar in crops such as sugar cane and sugar beet are converted to bio-ethanol using fermentation, leaving the remaining DDGS from cereals and pulp from sugar beet for use as animal feed. Vegetable oils are extracted from oilseed crops, such as rapeseed and soybean, and converted to biodiesel using transesterification or hydrogenation processes, leaving the remaining oilseed cakes or meals for use as animal feed. Transesterification of vegetable oils uses methanol and gives aglycerine co-product. While various work has been done to show that glycerine can be used as an animal feed, it is unlikely that it will be used to any significant extent. This is because purification of crude glycerine would probably be needed to eliminate the risk from associated methanol and because there are alternative higher value markets for glycerol: upgrading for pharmaceutical use, manufacture of chemicals and in the EU there are incentives to use crude glycerol for renewable power generation.

Anaerobic digestion is able to utilize a large range of feedstocks to produce biogas, which can be used to generate heat and power, or purified to make biomethane. The biomethane can be fed into the gas grid or used as a biofuel. Anaerobic digestion decomposes the starch, sugar, oil and protein in the feedstock to produce methane, while the

remaining components, including phosphate and potash and the nitrogen from the protein fraction, are returned to land as digestate, or decomposed in an aerobic oxidation unit.

Lignocellulosic feedstocks such as wheat straw, maize stover and wood need more aggressive processing to access components for biofuels. One option is to use hydrolysis of the feedstocks to extract the sugar, for fermentation to ethanol. The other option is gasification of the feedstock to hydrogen and carbon monoxide, followed by synthesis processes to produce methanol, ethanol, dimethyl ether or middle distillate using Fischer-Tropsch synthesis and hydrocracking. The remaining components are used as fuel to drive the process. These processes all require a large capital investment. Only the sugar-, starch- and oilseed-based processes (so called “First-generation” processes) normally provide animal feed as a co-product.

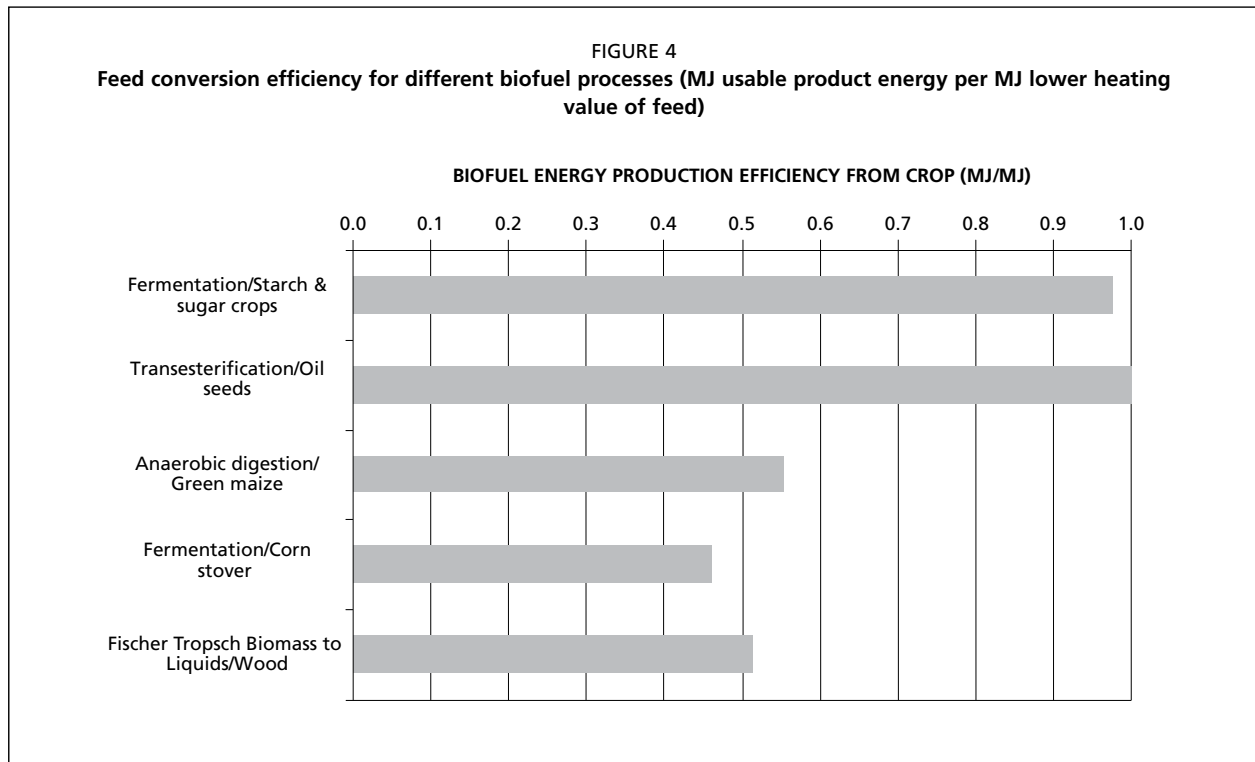
Biofuel production process efficiencies

A comparison of biofuel recovery and energy efficiency losses for different biofuel processes (Ingledew 1993; Aden *et al.*, 2002; FNR, 2009; Nexant, 2007) is shown in Table 1. The biofuel component is the component in the feedstock that is used to make the biofuel, and different technologies are used. The extraction efficiency is the proportion of the available feedstock component that is extracted or utilized for the biofuel process. For example, in anaerobic digestion, lignin and cellulose can not all be utilized in the process. The biofuel selectivity is the proportion of the biofuel component that is converted to biofuel, while the rest is converted to other by-products. The crop energy efficiency is the proportion of energy in the crop or feedstock that is converted to useful energy products such as biofuel or animal feed.

Although some potential biofuel is lost in fermentation and transesterification technologies due to inefficiencies in vegetable oil extraction and in fermentation, there is a loss of only 1 percent to 2.5 percent in the overall energy

TABLE 1
Biofuel recovery and energy efficiency losses for different biofuel processes

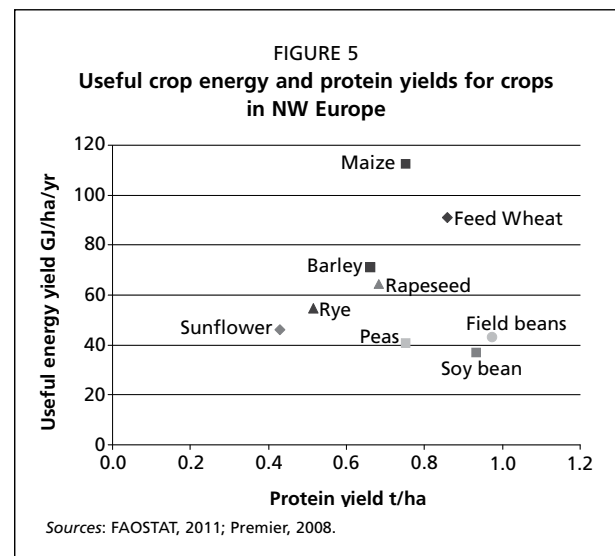
Parameter	Biofuel feedstock				
	Cereals and sugar crops	Oil seeds	Green maize	Maize stover and straw	Wood
Component	Starch and sugar	Vegetable oil	Carbon	Carbohydrate and sugar	Carbon
Component extraction technology	Hydrolysis	Extraction	Anaerobic digestion	Hydrolysis	Gasification
Component extraction efficiency	98.5%	80%–96%	81%	58%	100%
Biofuel production technology	Fermentation	Transesterification	Anaerobic digestion	Fermentation	Fischer-Tropsch process
Product	Ethanol	Fatty acid methyl ester	Methane	Ethanol	Middle distillate
Component selectivity	93%	95%	75%	82%	74%
Stoichiometric energy conversion efficiency	95.9%	99.7%	90%	96%	69%
Crop energy efficiency	97.5%	99.9%	55%	46%	51%



efficiency of food crops for biofuel, due to the heat of reaction in the biofuel conversion process. The non-extracted oil and fermentation by-products are all conserved to provide metabolizable energy in the animal feed co-products, while all the other plant components, such as protein and minerals, become concentrated and conserved in the animal feed. Processes that are used for non-food feeds have a lower feed efficiency, with a large proportion of the feedstock energy lost as unprocessed feed, by-product losses, or heat release during reaction stages. Any protein in the feedstock is decomposed and lost.

The overall feed conversion efficiencies of producing alternative biofuels by the different processes are shown in Figure 4. Low feed efficiencies are often associated with lower inputs of fossil energy to the conversion process, or potential export energy. These vary depending on the detailed process design and will determine the plant's GHG emissions, but are not included in Figure 4.

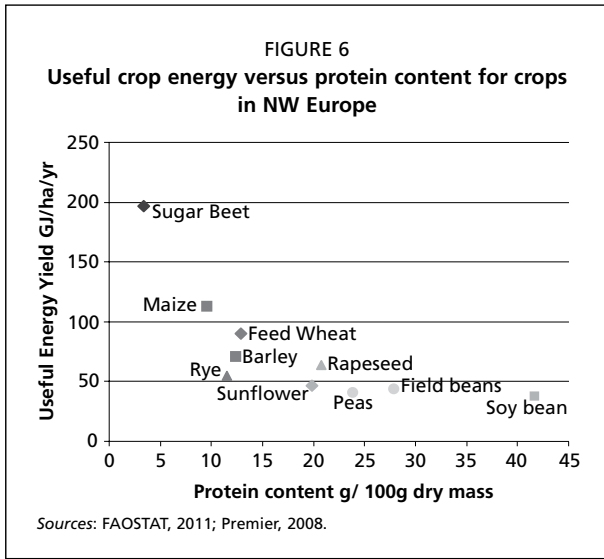
This demonstrates that due to the low energy efficiency of anaerobic digestion and gasification processes, they should only be used for feedstocks that are not suitable for starch, sugar or vegetable oil extraction. The average harvested yields of lignocellulosic crops in the EU, such as miscanthus and short rotation coppice, are about 11 dry t/ha and are similar to the yields of food crops, such as cereals (including straw and stover). However, there are large yield variations due to weather, land quality and crop management, and substantially higher than average yields can be obtained both for lignocellulosic crops and food crops. Thus, despite comparable yields of lignocellulosic and food



crops, the low energy efficiency of processes using lignocellulosic feedstocks results in there being no carbon benefit compared with processing food crops.

BIOFUEL CROPS

Food crops produce several commercially useful plant products, primarily protein, carbohydrate and lipid (oil or fat) and minerals. It is convenient for understanding the implications of biofuels for the animal feed industry to focus on the protein and energy levels and yields in different crops. The protein and energy yields for a range of medium- and high-protein crops grown in NW Europe are shown in Figure 5.



The useful crop energy is the average metabolizable energy (for ruminants, pigs and poultry) of the crop, or for oil seeds it is the metabolizable energy of the meal (Premier, 2008) plus the lower heating value of the extracted vegetable oil. The crop yields for NW Europe are the average for United Kingdom, Ireland, France, Germany, Denmark, Belgium and the Netherlands for the period 2005 to 2009 (FAOSTAT, 2011). Figure 5 shows that the protein yields of many crops, including wheat, beans and soybean are fairly similar, with yields around 0.8 to 1.0 t/ha. Although soybeans and field beans are recognized as being protein crops, due to their high protein content, the protein yield

is little higher than for wheat and maize. However, the energy yields of cereal crops such as wheat and maize are substantially higher than protein crops such as soybean and field beans.

An important advantage of oil seeds and legumes is that they have a higher protein concentration, compared with cereals, which is important for animal feed compounding.

The energy yields of biofuel crops grown in NW Europe and the metabolizable energy of associated co-products are compared in Figure 7.

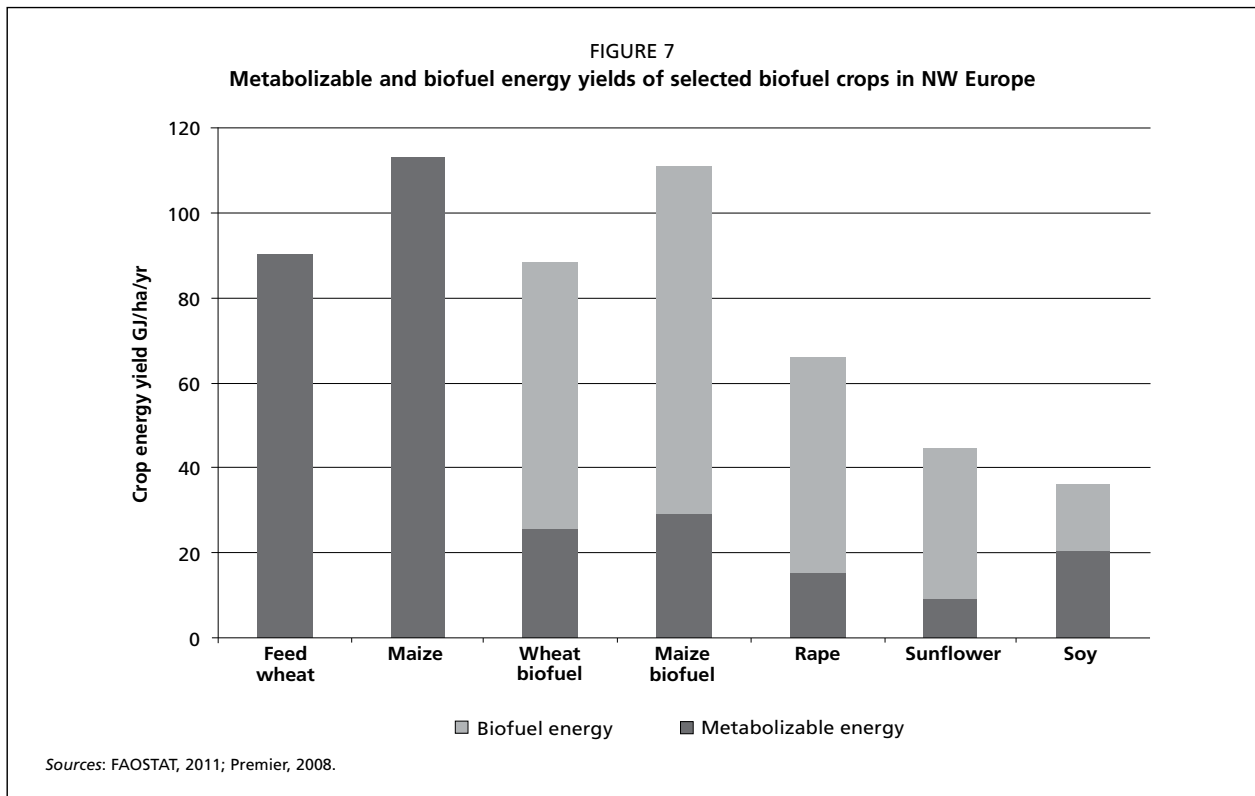
There is a clear relationship: the higher the concentration of protein per unit dry mass of recovered crop, the lower the useful energy yield of the crop. Crops with high protein content are grown to provide high-protein animal feeds, despite their similar protein and lower energy yields.

The energy yields of biofuel crops grown in NW Europe are compared in Figure 7.

It is assumed from the data in Figure 4 that all the energy in oilseed crops is conserved when they are used for biofuel production. Figure 7 demonstrates that nearly all the energy in cereal crops is also conserved when they are used for biofuel production. While both the biofuel energy and metabolizable energy yields are higher for cereal crops than oilseed crops, cereal crops need a break crop, typically oilseed rape, every three or four years as part of the crop rotation.

Biofuel crop capacity and growth rates

The proportion of EU crops used to produce EU biofuels from cereals, oilseeds and sugar beet in 2009 is shown in



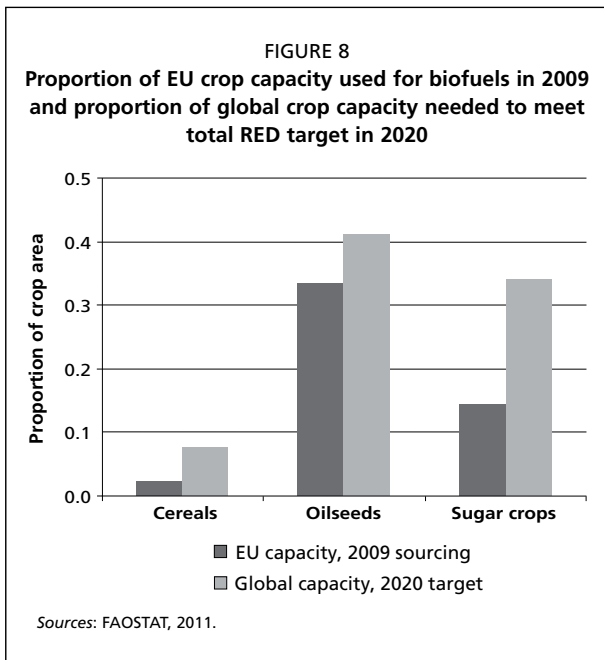


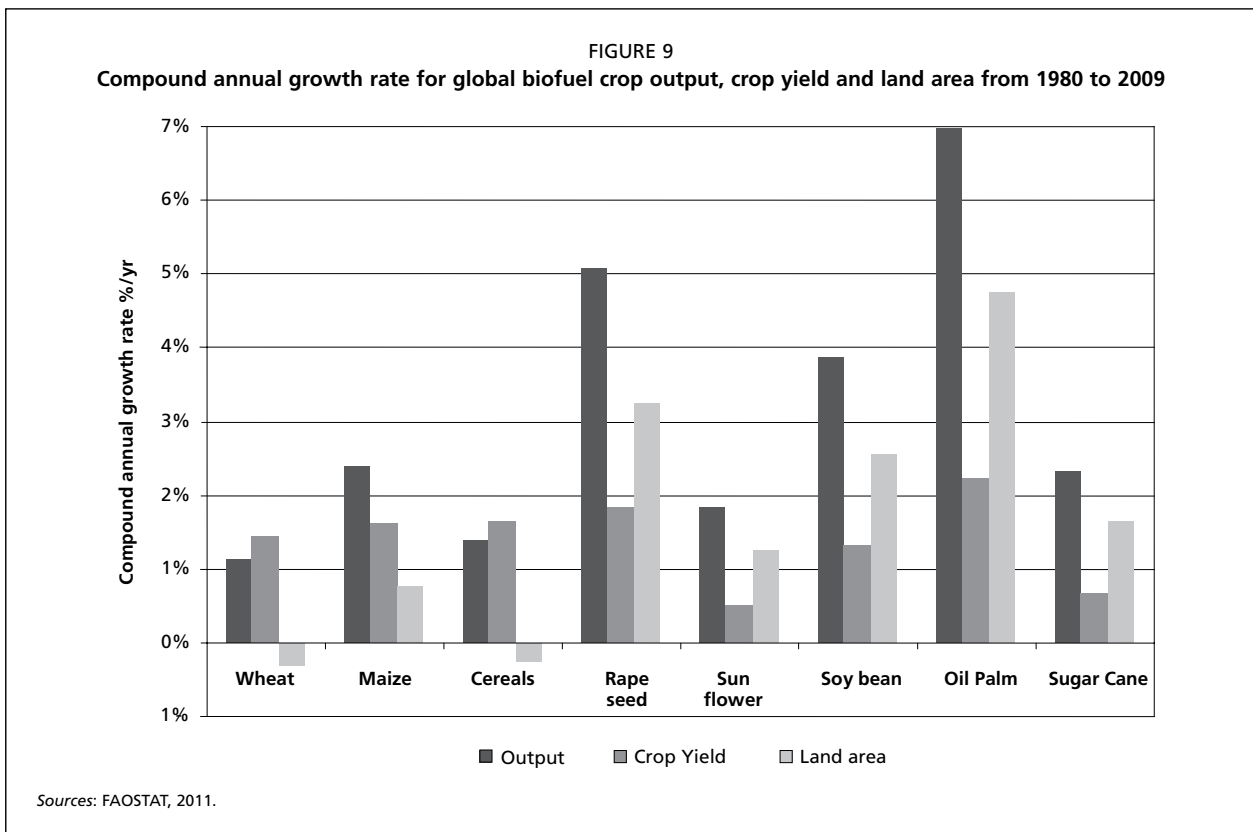
Figure 8. It can be seen that while only 2.3 percent of EU cereals was being used for biofuels in 2009, an order of magnitude higher – 33 percent – of EU oilseed capacity was being used for biodiesel.

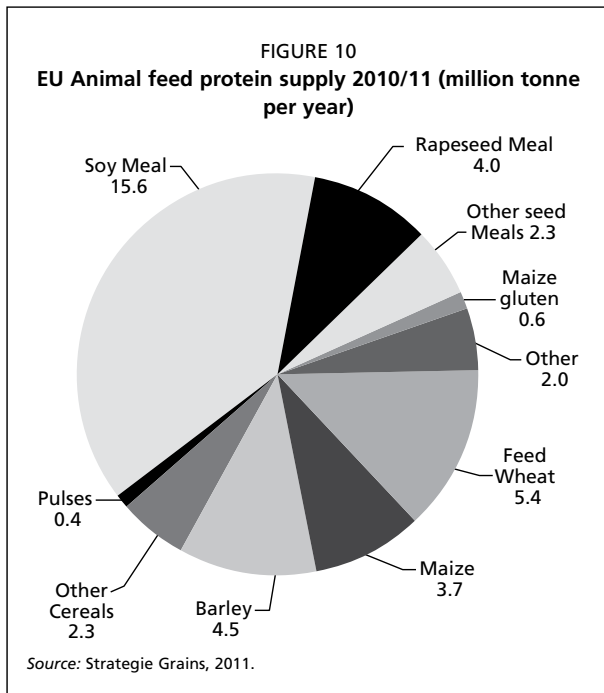
The proportion of current global crop needed to meet the full EU 2020 biofuel target for each crop type is also shown in Figure 8. Sugar crops include sugar beet and sugar cane. A much higher proportion of global oilseed

or sugar crops would be needed to meet the 2020 target, compared with cereals. That is mainly because cereal crops take a much higher share of the global crop area than oilseeds and sugar crops. While the majority of EU biofuel has so far been from biodiesel, it is unlikely that oilseed crops will be able to expand fast enough to meet the 2020 target, and a higher proportion of the biofuel growth will therefore have to be met from bio-ethanol crops.

The historic global growth rates of biofuel crops (FAOSTAT, 2011) are shown in Figure 9.

This shows that the yield increases of cereal crops (wheat, maize, barley and rye) have been greater than the increase in demand, such that the global land area needed to grow these crops has actually fallen since 1980. This has led to a steady release of arable land in temperate regions such as the EU (Lywood, 2011), Eastern Europe and the United States. While the rates of yield increase for oil seed crops and sugar cane are comparable to those of cereals, the higher increases in demand for these crops has required substantial expansion of crop areas. The growth of oilseed rape has been in the EU, Ukraine and Canada, while for other oil crops and sugar cane the expansion has primarily been in South America and SE Asia. The use of cereal crops and oilseed rape for additional biofuel production will therefore reduce the rate of abandonment of arable land in temperate regions, while the use of other oil crops and sugar cane for biofuels will continue to increase the demand for arable land in South America and SE Asia.





EU ANIMAL FEED SUPPLY

In order to produce meat efficiently, animals are fed with a range of feeds to provide energy and protein. These feeds are mainly from seed crops, and include wheat, maize, rapeseed meal and soybean meal, and are blended to provide an optimum feed for different animals. The blending is operated primarily to meet the optimum energy levels and protein levels or amino acid levels for animal feed, but also to meet many other factors, such as mineral requirements.

The energy content of different animal feeds is fairly similar, but the protein content varies widely. The source of supply of protein in animal feed in the EU is shown Figure 10. Soybean meal accounts for 38 percent of all the protein used in animal feed in the EU. Rapeseed is supplied from within the EU, but most of the other oilseed meals are imported to the EU.

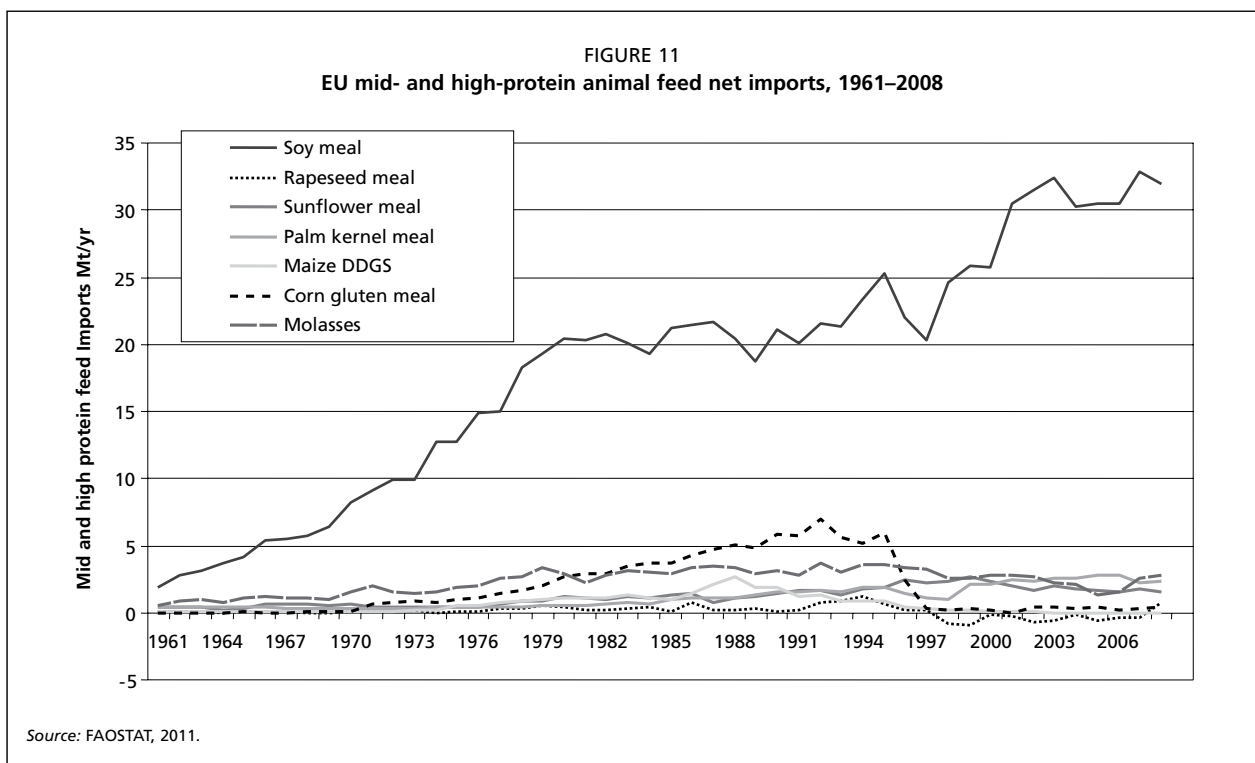
Historic mid-protein and high-protein animal feed imports to the EU are shown in Figure 11.

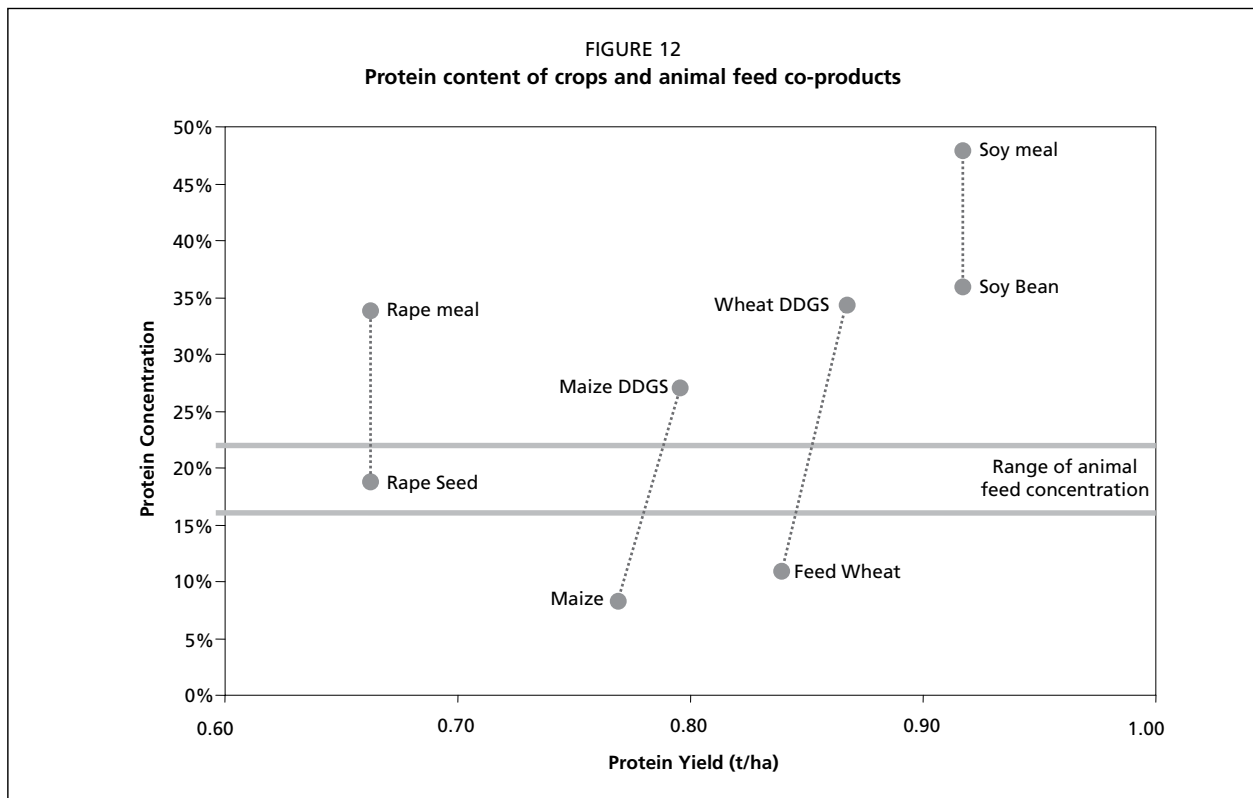
Soybean meal is by far the major import protein source, and rates have increased steadily in the EU and now account for about 90 percent of the total imported animal feed protein requirements. However, there are serious concerns with this large expansion of soybean, because of its high rate of land expansion and as a major cause of deforestation in South America (FOE, 2008).

BIOREFINING OF CROPS FOR BIOFUEL AND ANIMAL FEED

Animal feed requirements

In order to maximize the rate of animal growth, the optimum level of protein (as fed) in animal feed is typically in the range of 16 to 22 percent. Cereal grains have (as fed) protein levels of 8 to 13 percent, while oilseeds, such as soybean and oilseed rape, have protein contents of 18 to 36 percent. Vegetable oil is typically extracted from oil seeds and used either in the food sector or to produce biodiesel fuel, while the co-product oilseed meals are used as animal feeds. These oilseed meals, with protein contents ranging from 33 to 48 percent, are blended with cereals to give





the optimum animal feed concentration. In an analogous process, starch is extracted from cereals by fermentation to produce bio-ethanol, while the dried distillers grain with solubles (DDGS) co-product, with typical protein contents of 27 to 35 percent, is used in animal feeds. The effect of these oil and starch extraction processes is to increase protein levels of biofuel co-products, and is shown in Figure 12, using average NW European protein yields for rape, wheat and maize, and South American protein yield for soybean.

In all cases, the extraction raises the co-product protein concentration above that of typical animal feed, so that it can then be blended with cereals to provide optimum animal feed protein concentrations. Some additional protein is produced during the fermentation process from yeast biomass growth (supported by added mineral nitrogen) and this increases both the protein concentration and the protein yield.

Animal feed is formulated from up to 20 components to meet an optimum specification for each animal group. The specification includes required levels of about a dozen nutritive components, including: metabolizable energy, digestible protein or amino acids, minerals, vitamins, fats and maximum levels of various anti-nutritive factors. For ruminants, the protein from all animal feeds is digested to fairly similar extents, so digestible protein is based on crude protein levels. For mono-gastric animals, the diet must include required digestible levels of essential amino acids (EAAs). Also higher levels of dietary fibre in DDGS and rapeseed meal give lower protein digestibility than lower-

fibre sources such as wheat and soybean meal. Soybean meal has a better amino-acid profile and a higher protein digestibility than other animal feed co-products, but in the EU, deficiencies in EAAs are largely made up by the addition of synthetic or crystalline EAAs during feed compounding. For normal inclusion levels of DDGS in animal diets, the limiting EAAs are lysine and tryptophan for maize DDGS, and lysine and threonine for wheat DDGS. These amino acids are cost effective for use in compound animal feed to overcome most EEA limitations. Average levels of protein digestibility of different animal feeds for pigs and poultry feed are calculated (Premier, 2008) from the weighted average digestibility of amino acids, assuming that synthetic EAAs are added when formulating.

Co-product displacement

Animal feed dietary formulation targets are driven by economic considerations. In the United States, a substantial quantity of maize DDGS is used as liquid feed or as dried direct feed in local feedlots. The significant logistics costs associated with moving protein components from surplus regions to deficit ones means that the low cost of local protein sources relative to other components can justify the use of local DDGS as a cheap energy source. However, the animal feed industry in the EU operates differently from in the United States. In the EU, since most protein-rich animal feed is imported, primarily as soybean meal (Figure 11), protein is a relatively expensive dietary component, so there is a strong economic incentive to use it efficiently in diets, by

TABLE 2
Animal feeds prices – United Kingdom average price, 2008–2010 inclusive

Feed	Price in GBP per tonne	Source
Feed wheat	110	Farmers Weekly
Soybean meal	302	Farmers Weekly
Rapeseed meal	182	Farmers Weekly
Wheat DDGS	179	LMC Intl. Ltd

accurately targeting optimum dietary protein or amino-acid levels. In the EU, feed wheat that is in excess of demand is exported and soybean meal imports are adjusted to meet the EU demand for animal feed protein. Feed wheat can therefore be regarded as the marginal animal energy feed and imported soybean meal as the marginal high-protein feed. Average prices of selected animal feed materials are shown in Table 2.

Using substitution ratios from Table 3, the average value of the soybean meal and wheat displaced by wheat DDGS is GBP 221/t of DDGS. Compared with the DDGS price of GBP 179/t this gives a good margin to cover costs for blending and feed supplements. The DDGS and rapeseed meal prices will vary with soybean meal and wheat prices to ensure all co-product is utilized in animal feed. Co-products, such as DDGS and rapeseed meal, from EU biofuel production will therefore displace a mixture of soybean meal imported from South America and EU feed wheat in the animal feed formulation.

The substitution ratios of biofuel co-products for cereals and soybean meal can be determined accurately by animal feed formulation models and checked by animal feed trials. Most formulation work has been done to determine the addition of DDGS and rapeseed meal for particular diets. These tend to show that DDGS displaces mainly a mixture of soybean meal and cereal in monogastric diets (Lywood, Pinkney and Cockerill, 2009a) and a mixture of soybean meal, cereal and other mid-protein components in ruminant diets (Weightman *et al.*, 2010). However, all the mid-protein animal feeds components (rapeseed meal, sunflower meal and maize

gluten) are secondary co-products from the production of vegetable oils or wet maize milling, and the imports of all these components to the EU are small (Figure 11). They will therefore continue to be produced and will be used elsewhere in animal feed formulations, for example displacing a mixture of soybean meal and wheat in pig and poultry feeds. Therefore DDGS and rapeseed meal will directly or indirectly replace cereals and imported soybean meal. In order to determine whether the DDGS goes directly into pigs and poultry, or whether the DDGS goes into ruminants and displaces other mid-protein crops in pigs and poultry, will require more advanced animal feed modelling, which maintains the total usage of mid-protein animal feed co-products.

The displacement of soybean meal and cereals by biofuel co-products has been explored in various studies and the results for DDGS and rapeseed meal for weighted average livestock groups in the EU are shown in Table 3.

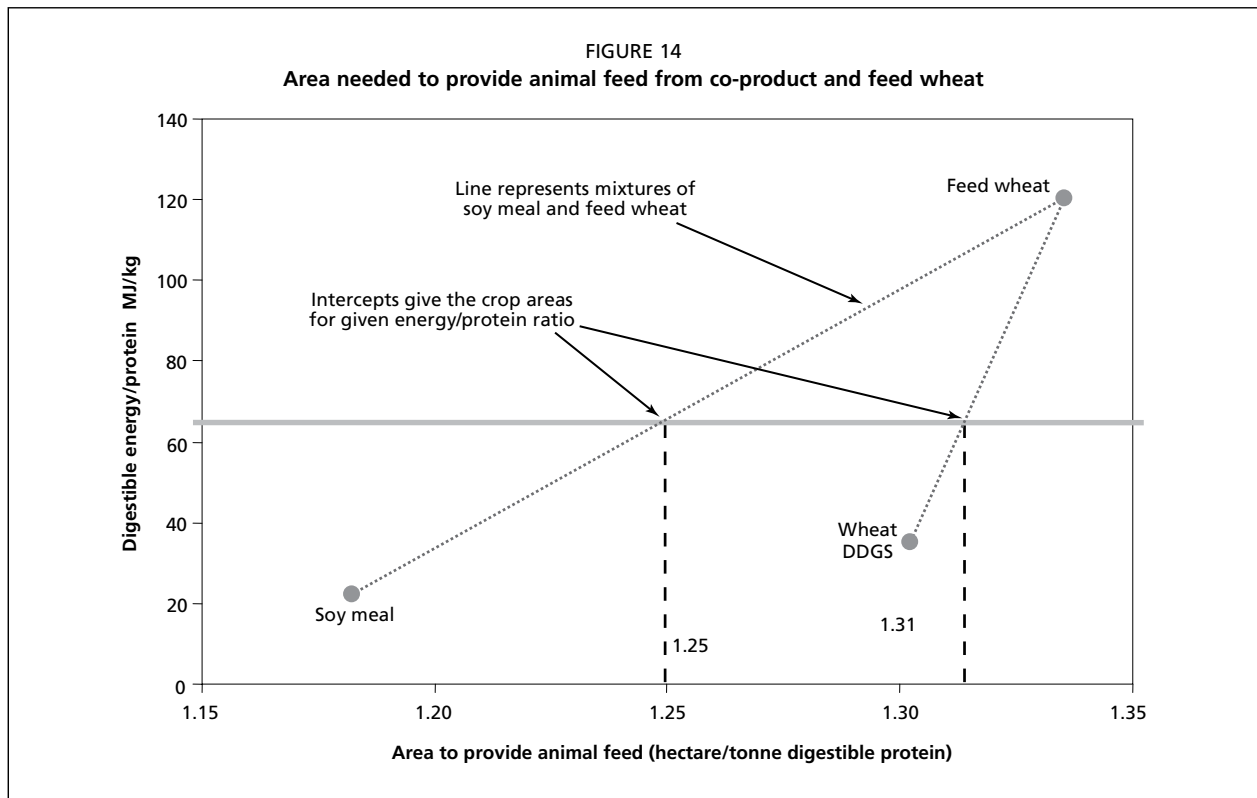
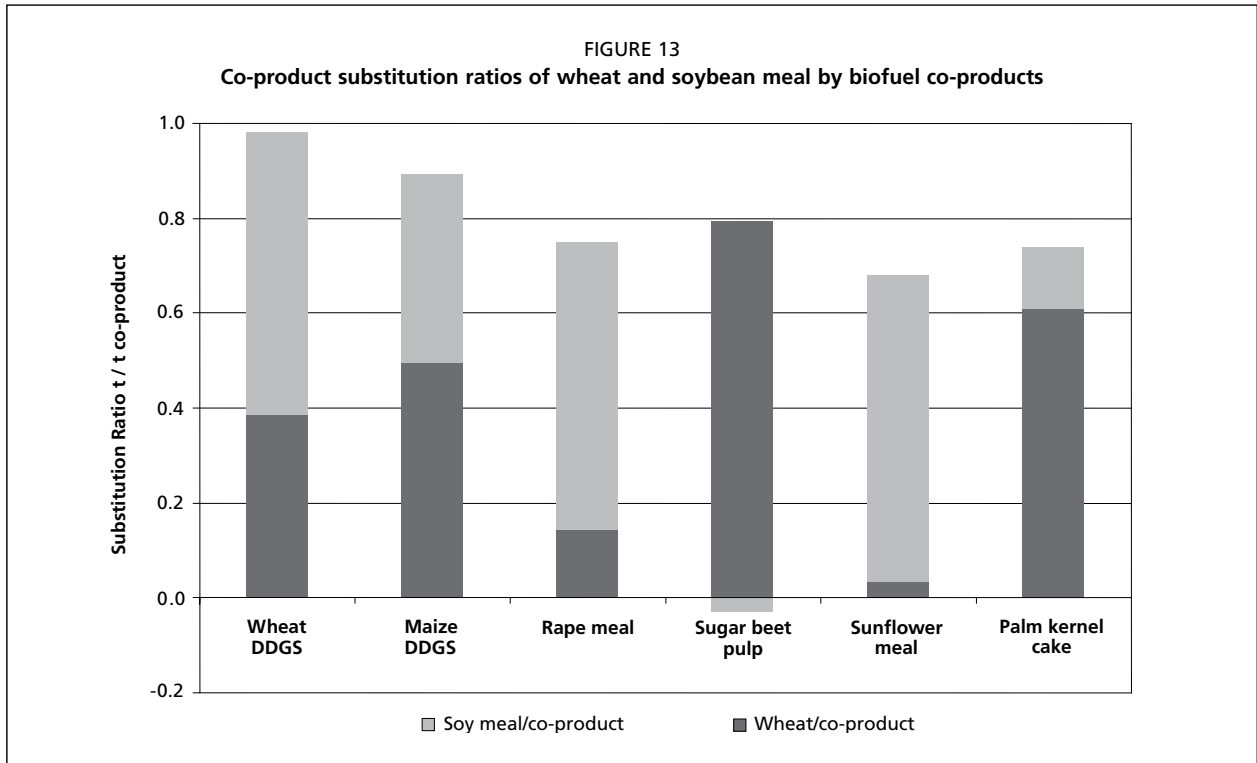
It may be seen that there is reasonably good agreement between the figures from different studies. It has been shown (Lywood, Pinkney and Cockerill, 2009a) that these results are reasonably consistent with a model whereby the co-products displace soybean meal and cereal to give the same metabolizable energy and digestible protein in animal feed. This approach provides substitution ratios for a range of biofuel co-products and is used to determine net land use for this study. These substitution ratios for different biofuel co-products for feed wheat and soybean meal are illustrated and compared in Figure 13. In practice, these ratios will vary depending on the quantity of biofuel co-product (Weightman *et al.*, 2010), variations in relative prices of soybean meal and wheat, relative abundance of alternative animal feeds, and variations in quality of animal feeds from different sources.

In all cases, the animal feed co-product will be blended with more feed wheat to give the desired animal feed composition, as shown in Figure 14 for the cases of soybean meal plus wheat, and wheat DDGS plus wheat, blended to give a typical animal feed energy:protein ratio.

TABLE 3
Substitution ratios for biofuel co-products in the EU

Co-product	Substitution (t/t co-product)		Notes	Source
	For soybean meal	For cereal		
Wheat DDGS	0.50	0.66		CE Delft, 2008
Maize DDGS	0.45	0.69		CE Delft, 2008
Rapeseed meal	0.66	0.26		CE Delft, 2008
Wheat DDGS	0.59	0.39		Lywood, Pinkney and Cockerill, 2009a
Maize DDGS	0.40	0.49		Lywood, Pinkney and Cockerill, 2009a
Rapeseed meal	0.61	0.15		Lywood, Pinkney and Cockerill, 2009a
Wheat DDGS	0.60	N.A.	High usage scenario	Weightman <i>et al.</i> , 2010
Wheat DDGS	0.60	0.68	Aglink-Cosimo model	Blanco-Fonseca <i>et al.</i> , 2010

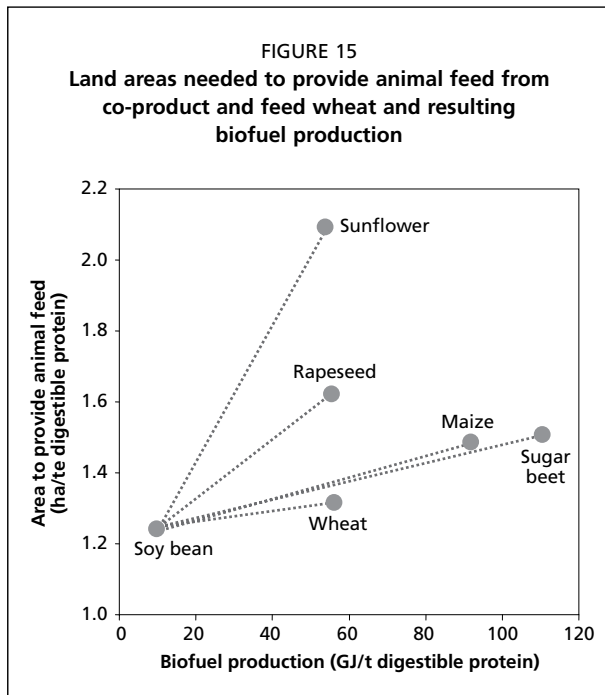
Notes: N.A. = not available.



Land use for biofuel crops with co-products

In order to reduce global warming, the competing requirements for global land, not only for food but also for transport fuels and possibly also for power generation, need to be addressed. Land use efficiency is therefore important when considering the options for alternative biofuels and

their associated animal feed co-products. In calculating the land requirement for biofuels, account should be taken of land saved by the use of biofuel co-products for animal feed. The “direct” land use for biofuels does not account for co-products, while the “net” land use does account for co-products. The net land use of biofuels production from



crops, such as cereals and rape, where co-products are used for animal feed are significantly lower than the direct land use because biofuel co-products used for animal feed displace a mixture of soybean meal and cereals. Since soybean is grown primarily for the meal, the reduced demand for soybean meal will tend to slow the rate of soybean expansion. It may be argued that just because the demand for soybean from the EU animal feed market is reduced, it does not necessarily mean that soybean production will reduce: for example, soybean producers may find other markets for their crop. However, the basic premise of land use change is that an increase in demand causes an increase in the land area needed for crops to be grown. Conversely, if there is a reduction in demand for a crop, then the corresponding reduction in land area needed to grow that crop must be recognized as a credit. This credit has either been ignored completely in modelling work on the evaluation of grain crops for biofuel production, e.g. the IFPRI-IMPACT model (Edwards, Mulligan and Marelli, 2010), or credit is only taken for the energy in the co-products and not the protein component: IFPRI-MIRAGE (IFPRI, 2010) and GTAP (Edwards, Mulligan and Marelli, 2010).

The “net” land use for biofuel can either be calculated by subtracting a land credit for the crops displaced by the biofuel co-product, or can be determined from the additional land and additional biofuel from producing a fixed amount of animal feed from different biofuel crops. Both methods give the same result. The net land use calculation by adding a land credit for the biofuel co-product is detailed in Lywood, Pinkney and Cockerill (2009a). The alternative approach of comparing additional land areas and biofuel production after producing a fixed amount of animal feed

is demonstrated in Figures 14 and 15 using protein yields from Figure 12.

In Figure 14, the mixture line between soybean meal and feed wheat gives the land area needed to provide an animal feed at any concentration by blending these components. For example to supply animal feed with a metabolizable energy level of 65 MJ per kg of digestible protein, the area required is 1.25 ha per tonne of digestible protein. The area required to give the same amount of animal feed from DDGS is 1.31 ha per tonne of digestible protein. A similar analysis to that in Figure 14 has been done to determine the land area needed to produce the same amount of animal feed for other biofuel co-products. This net land use for animal feed crops is shown as the y-axis in Figure 15. For each animal feed co-product, there is an associated biofuel production rate (as shown in Figure 7), which is plotted on the x-axis of Figure 15.

In Figure 15, the slope of the line joining the point for each biofuel crop to the point for soybean gives the additional area per additional unit biofuel energy for each biofuel crop compared with soybean. This slope is the “net” land use (ha/GJ) for each biofuel after credit for the co-product displacing soybean meal and cereal. The choice of energy:protein ratio (i.e. 65 MJ/kg digestible protein) in Figure 14 will change the position of the point for the crop in Figure 15, but does not change the slope of the blue line. The “net” land use is compared with the “direct” land use in Figure 16.

Figure 16 shows that for biofuels with significant animal feed co-products, the net land use is much lower than the direct land area. In some cases the net land use is significantly lower than for crops such as sugar cane and oil palm, which do not have significant animal feed co-products. In the case of wheat bio-ethanol the net land area is only about 10 percent of the direct land area. These data show that it is vital to take into account the biofuel co-products when comparing land area or yields of biofuel crops.

Since there is a large continuing growth of soybean in South America, any displacement of soybean meal by animal feed co-products in the EU will reduce the rate of growth of soybean area, rather than cause a reduction in area. It should also be noted that with changes in crop demand and output, there will be associated changes in crop yields (Lywood, Pinkney and Cockerill, 2009b). This has not been taken into account in Figure 16, but will act to further reduce the net land use of biofuels with animal feed co-products.

SUSTAINABILITY OF BIOFUELS AND ANIMAL FEED

As was discussed in the section on climate change at the beginning of this chapter, a primary purpose of the introduction of biofuels is to reduce the GHG emissions from

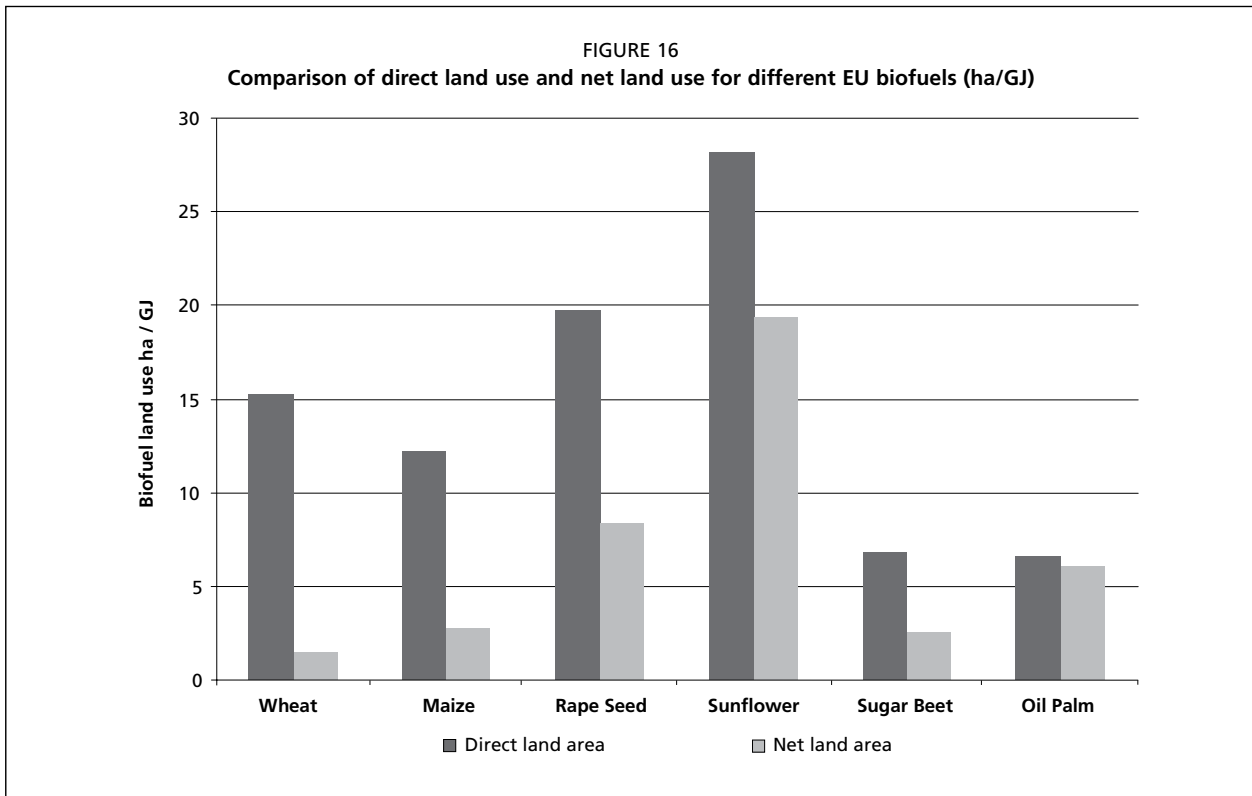


TABLE 4.
Typical GHG savings of biofuel from selected crops given in the Renewable Energy Directive (RED)

Source	Biofuel	Typical GHG saving as % of fossil fuel emissions
Sugar beet ethanol	Bio-ethanol	61
Wheat ethanol gas CHP	Bio-ethanol	53
Maize ethanol gas CHP	Bio-ethanol	56
Sugar cane ethanol	Bio-ethanol	71
Palm oil	Biodiesel	36
Soybean	Biodiesel	40
Rapeseed	Biodiesel	45
Sunflower	Biodiesel	58
Waste vegetable oil	Biodiesel	88

Notes: CHP = combined heat and power

road transport. The GHG savings are considered below, both for direct biofuel production and for the indirect land-use changes associated with biofuel production in the EU.

Biofuel GHG savings

The calculation of biofuel GHG emissions includes crop cultivation, oil extraction, the biofuel production process and transport of crops and biofuel. For crops with animal feed co-products, the upstream GHG emissions are allocated between the biofuel and co-product according to the energy content of each product. There are substantially different GHG emissions for each biofuel crop and also a range of GHG emissions for each biofuel crop, due to differences in cultivation and processing. Typical GHG emission savings

TABLE 5.
Biofuel cultivation GHG emissions (g CO₂ eq/MJ biofuel)

	Wheat	Maize	Rapeseed	Sugar beet
Typical RED	23.0	20.0	29.0	12.0
United Kingdom	21.0	—	31.0	14.0
Netherlands	24.1	16.2	25.3	8.7
Germany	21.5	14.2	23.7	11.6
France	21.0	10.5	24.0	9.5
Ireland	20.0	—	24.0	12.0
Cultivation saving	2	6	5	3

for different biofuel crops are provided in the RED (EC, 2009) and some data are shown in Table 4.

However, substantial improvements can be made to these figures. The European Commission has published data submitted by EU Member States with estimates of the GHG emissions from cultivation in different regions (EC, 2011). Some of these data are shown in Table 5.

The cultivation saving is an indicative difference between the typical RED GHG emissions and those achieved in some regions of Member States. These data show that improvements of up to 6 g CO₂ eq/MJ biofuel (equal to 7 percent of fossil fuel GHG emissions) can be achieved from lower cultivation emissions in some regions. Some biofuel process improvements can give substantial GHG savings benefits. For example: adding methane capture to palm oil processing would provide an additional GHG saving of 26 percent, while recovery of CO₂ from fermentation processes to replace fossil fuel CO₂ could provide similar gains. GHG

TABLE 6
Anticipated biofuel GHG savings for EU biofuels by 2017–2020

	GHG saving	GHG saving per RED credit
Crop-based biodiesel	52.5%	52.5%
Crop-based bio-ethanol	72.5%	72.5%
Waste oil biodiesel	88.0%	44.0%
Lignocellulosic biofuels	88.0%	44.0%

savings figures are reported in the United Kingdom for all biofuel production and can be used as a guide to the improvements in GHG emissions of some biofuels. United Kingdom sugar beet bio-ethanol has average GHG savings of 77 percent (DfT, 2011) compared with the typical RED figure of 61 percent. United Kingdom wheat bio-ethanol is now reporting GHG savings of 78 percent (DfT, 2011) compared with the RED figure of 53 percent. These examples show that, without any monetary incentive to do so, biofuel producers are already making process improvements that reduce GHG emissions and are able to report GHG savings substantially better than the typical quoted RED values. When monetary incentives are available, biofuel producers can improve GHG emissions by sourcing crops from regions that have low cultivation GHG emissions, and work with farmers to grow crops with higher yields and lower inputs to realize even lower cultivation GHG emissions. The average GHG savings achieved will therefore be significantly greater than the typical values quoted in the RED.

All biofuels will have to meet a GHG savings threshold of 50 percent in 2017, while new plants will have to meet a 60 percent GHG savings threshold. Anticipated GHG savings for different types of biofuels for 2017–2020 are shown in Table 6. The typical GHG saving for sugar cane bio-ethanol is 71 percent, and given the results for sugar beet- and wheat-based ethanol in the United Kingdom, an illustrative average saving of 72.5 percent for bio-ethanol has been assumed. An average figure for crop-based biodiesel is estimated at 52.5 percent, but since the threshold figure is significantly higher than crop-based biodiesel GHG savings reported so far, the amount of crop-based biodiesel that will be able to meet this level of savings is unclear.

To align FQD and RED targets, oil companies will need to meet high average GHG savings per RED credit. For crop-based biofuels, the GHG saving per RED credit will be the normal GHG savings figure. However, because waste oil biodiesel will count double towards the RED, but will not count double towards the FQD, the GHG saving of about 88 percent, will only give a GHG saving of 44 percent per RED credit.

Indirect land use change

In situations where farmers use previously uncultivated land to grow crops for biofuel production, there is direct land use change (LUC) and methods are included within

the RED to calculate the GHG effect of LUC. For example, oil palm grown on previously forested land will incur a substantial GHG penalty from LUC. For all other cases where part of a crop is used to produce biofuel, this will increase the demand for the crop and therefore it is assumed there will be an indirect land use change (ILUC) to meet the increased crop production. If the biofuel production gives co-products that can displace other crops, this will also lead to an ILUC credit. Reflecting the ILUC credit of co-products, the net ILUC effect may be either a penalty or a credit in GHG savings. Concerns have been expressed that as a result of consideration of ILUC, the overall GHG savings of some biofuels may be negative and this is undoubtedly an issue for some biofuels, such as palm oil biodiesel. Various macro-economic models purport to model ILUC effects, but they do not properly take into account several of the factors that determine ILUC, especially for EU biofuel crops with co-products.

Detailed work done specifically to determine the carbon stock changes for crops in the EU and for growing soybean in South America, allows a relatively simple and transparent ILUC calculation for EU biofuel crops. The calculation is based on the land areas used for Figure 16 (Lywood, Pinkney and Cockerill, 2009a). Changes in crop output as a result of biofuels demand will give changes in crop yields. While these changes were not taken into account in Figure 16, they are included here by using factors for the proportion of increase in crop output that derives from land area increase (Lywood, Pinkney and Cockerill, 2009b).

Figures for the carbon stock change for LUC in the EU range from 2.2 t CO₂/ha/yr (Lywood, 2011) to 6.2 t CO₂/ha/yr (Heiderer *et al.*, 2010). These figures are substantially different. Lywood (2011) takes account of growth of biofuel crops in the EU reducing the historic abandonment of arable land in the EU (DG Agri, 2011), so the carbon stock change is the foregone carbon sequestration that would otherwise have occurred from carbon accumulation on the abandoned land. The figure from Heiderer *et al.* (2010) does not take account of land abandonment and assumes that 38 percent of all new cropland in the EU will be obtained from deforestation. Figures for the carbon stock change for LUC associated with growing soybean in South America are 12.9 t CO₂/ha/yr (Weightman *et al.*, 2010) and 13.5 t CO₂/ha/yr (Heiderer *et al.*, 2010). These carbon stock changes for LUC are high, because most of the land for soybean expansion is from conversion of cerrado grassland and deforestation (Dros, 2004).

The ILUC calculation, using averages of the carbon stock change values above, is shown in Table 7. More research work needs to be done to obtain better agreement on the data used for ILUC modelling.

It can be seen that there are substantial ILUC credits from growing cereals and rapeseed for biofuel in the EU.

TABLE 7
GHG savings from ILUC for EU crops with animal feed co-products

Parameter			Source		
Increased cereal output from increased land area		22%	Lywood, Pinkney and Cockerill, 2009b		
Increased soybean output from increased land area		90%	Lywood, Pinkney and Cockerill, 2009b		
Carbon stock change for land in EU	t CO ₂ eq/ha/year	4.22	See text		
Carbon stock change for soybean in South America	t CO ₂ eq/ha/year	13.2	See text		
Fossil fuel GHG emissions	kg CO ₂ eq/GJ	83.5	RED, 2009		
EU biofuel crop			Wheat	Maize	Rapeseed
Crop area (EU)	ha/TJ		15.3	12.2	19.7
Wheat area credit (EU)	ha/TJ		1.9	2.3	0.7
Soybean area credit (South America)	ha/TJ		11.6	7.1	10.6
Net land use	ha/TJ		1.7	2.8	8.4
Increased crop output from increased land area			22%	22%	63%
Net EU land area after yield changes	ha/TJ		2.94	2.17	12.27
South American land area after yield changes	ha/TJ		10.48	6.36	9.54
Net carbon stock changes	kg CO ₂ eq/GJ		-126	-75	-74
GHG savings from ILUC			150%	89%	89%

These GHG savings arise because the carbon stock changes from the co-product displacement of soybean are higher than the carbon stock changes associated with EU land use. The GHG savings in Table 7 are additional to those shown in Table 4. Note that the figure for rapeseed only applies if the rape area change is proportionate to the cereal area change. If rapeseed area increases at the expense of other break crops in a crop rotation, the ILUC figure will be different.

This shows that biofuel production from EU crops will give substantial GHG savings and that co-products will enable substantial additional net GHG savings from ILUC, due to soybean meal displacement.

BIOFUEL AND ANIMAL FEED SCENARIOS FOR 2020

Biofuel split scenarios to meet FQD target

The most economic way of meeting the FQD target will be chosen by oil companies depending on their petrol:diesel supply ratio, the degree by which they can meet their FQD target by improvements in their refinery operations, and the GHG emission savings from different biofuels. While vehicles using renewable electricity and biomethane can count towards meeting the RED 10 percent target for 2020, the impact will be small. Also, while the FQD target can be met

by reducing the GHG emissions of refinery operations, oil companies claim that the scope is very limited. Therefore, at a first approximation, in order to align meeting the FQD target of 6 percent GHG savings and the RED target of 10 percent renewable energy, an average biofuel GHG saving of 60 percent (including double counting) will be required by 2020.

When the RED GHG threshold is increased to 50 percent in 2017, it is likely that much soybean and palm biodiesel will be unable able to meet this target, so the availability of sustainable biodiesel feedstocks will be reduced from 2017. There are also impending changes to GHG thresholds or GHG calculations to account for ILUC and these are most likely to restrict the use of palm oil and soybean biodiesel. However, the 50 percent threshold and ILUC issues will not limit bio-ethanol production. Table 6 shows that the average GHG savings of crop-based bio-ethanol are significantly higher than both that of biodiesel and the average savings of 60 percent to meet the FQD target. Table 8 shows a rough economic comparison for oil companies to increase GHG savings, by supplying a higher ratio of bio-ethanol to biodiesel, or by blending more biofuel than is required by the RED target, for high and low blends. Low blends are blends where the amount of biofuel addition is small and does not change the price charged per litre of blend.

TABLE 8
Comparison of options for meeting the FQD target

	Low blends	High blends		
Average oil company margin 2008–2010				
Bio-ethanol (€/GJ)	-7.1	-13.1		
Biodiesel (€/GJ)	-10.3	-11.3		
	Cost of changes (€/GJ)		GHG saving (from Table 6)	Relative cost per GHG saving
Increased bio-ethanol or biodiesel	-3.2	1.9	20%	9
Increased biodiesel above RED	7.1	13.1	52.5%	25

TABLE 9
Illustrative biofuel splits to meet RED and FQD targets in 2020

RED biofuel energy target		10.0%			
Average GHG savings for FQD		60%			
EU fuel energy from petrol blends in 2020		32%			
<i>Source: DG Agri, 2011.</i>					
Scenario		A	B	C	D
Biofuel energy fraction of RED target					
Double count biodiesel		0.0%	2.5%	4.6%	7.5%
Crop-based biofuels		100%	95%	91%	85%
Crop-based bio-ethanol		38%	45%	51%	60%
Crop-based biodiesel		63%	50%	39%	25%
Total biodiesel		63%	52%	44%	32%
Total biofuel		100%	98%	95%	92.5%
Blending ratios					
Bio-ethanol : biodiesel	energy	0.60	0.82	1.06	1.52
Bio-ethanol : petrol	energy	11.6%	14.0%	16.0%	18.7%
Biodiesel : diesel	energy	9.2%	7.7%	6.5%	4.7%
Bio-ethanol blend	vol/vol blend	16.6%	19.8%	22.3%	25.9%
Biodiesel blend	vol/vol blend	9.9%	8.3%	7.0%	5.1%

The oil company margins are the differences in quoted prices between Rotterdam biofuel prices and fossil fuel prices. The figures indicate that it is more economic for oil companies to supply a higher ratio of bio-ethanol to biodiesel, rather than blending more biofuel than is required by the RED target. Therefore the FQD target should be met by oil companies by adjusting the biofuel split to increase the amount of bio-ethanol relative to biodiesel.

Biofuels from lignocellulosic feedstock and from wastes and residues count double towards the RED. It has been shown (Nexant, 2007; Wright and Brown, 2007) that biofuel production from lignocellulosic feedstock is unlikely to be economic before 2020, and product will only be available from demonstration plants. However, significant quantities of biodiesel will be produced from waste vegetable oil. The blends required to meet the RED and FQD targets can be easily calculated and an illustrative example is shown in Table 9 using GHG savings for different biofuel types from Table 6 and different levels of biodiesel from waste oil.

The quantity of crop-based biofuels follows directly from the assumed quantity of double counting biodiesel. The split between crop-based bio-ethanol and biodiesel is determined to meet the FQD target, based on their relative GHG savings. The biofuel blending ratios are determined from the quantities of biofuel, the split between diesel and petrol in the vehicle market and the fuel energy densities.

If there were no double-counting biodiesel (e.g. that from waste oil), then the average bio-ethanol and biodiesel volume content in petrol and diesel blends would need to be 16.6 percent and 9.9 percent, respectively. Since biofuels from wastes and residues count double for RED, but not for FQD, the use of double-counting biofuels helps in meeting the RED target, but does not help to meet the FQD target. As the level of double-counting biodiesel in the fuel mix

increases, the ratio of bio-ethanol to biodiesel has to be increased substantially to align the RED and FQD targets. It may be seen that if the level of double-counting biodiesel reaches 4.6 percent of the RED energy target, there is no need for a higher biodiesel blend above the current standard 7 percent blend. Double counting biofuels will be spread around EU Member States, to average out their contribution to meeting the RED target and to avoid excessive bio-ethanol/biodiesel ratios to meet the FQD target in any particular Member State. Member States will need to take action to ensure that the vehicle park in 2020 is such that the average blends shown in Table 9 can be utilized. This can be achieved by the introduction of E85 blends.

For case B in Table 9 and a total annual road transport fuel use of 310 million tonne of oil equivalent, an energy split of 0.82 bio-ethanol:biodiesel would require a bio-ethanol volume of 23 billion litres and a biodiesel volume of 18 billion litres.

Biofuel production scenarios in EU

There are several ways in which the production of biofuel crops can be increased within the EU in order to provide feed for biofuel production. These are:

- increasing crop yields. Data is from the Gallagher review (Kindred *et al.*, 2008);
- switching from lower yielding to higher yielding crops;
- maintaining the EU arable land area by growing biofuel crops; and
- cereals displaced by co-products become available for additional biofuel production.

A likely scenario for biofuel production in the EU in 2020 is shown in Table 10.

The data for all crop areas and increases in crop use for food and other non-biofuel uses are from DG Agri

TABLE 10
Scenario projection for biofuel and co-product production in the EU in 2020

		Wheat	Maize	Barley	Rape	Sunflower	Total
		Bio-ethanol			Biodiesel		
Current data							
EU27 crop production (2009)	×10 ⁶ t/yr	139.0	58.0	62.0	21.4	7.0	
EU27 crop area	×10 ⁶ ha	25.7	8.4	13.9	6.5	3.9	58.4
EU27 crop yield 2009	t/ha	5.4	6.9	4.5	3.3	1.8	
Crop used for biofuel production 2009	×10 ⁶ t/yr	3.9	2.3	0.4	—	—	
Biofuel yield from crop	t/t	0.32	0.33	0.28	0.95	0.95	
Vegetable oil yield	t/t crop	—	—	—	0.41	0.42	
Biofuel lower heating value	MJ/kg	26.8	26.8	26.8	37.2	37.2	
EU biofuel production 2009	PJ/yr	33.4	20.6	3.1	—	—	
Projected crop data for 2020							
Increase in yield	per yr	1.6%	1.4%	1.5%	1.3%	1.4%	
Additional crop from yield	×10 ⁶ t/yr	26.5	9.3	11.1	3.2	1.1	
Extra land area	×10 ⁶ ha	1.1	0.8	-1.2	0.8	-0.5	1.0
Additional crop from extra land	×10 ⁶ t/yr	7.1	6.4	-6.3	3.0	-1.0	
Increase in non-biofuel crop consumption	×10 ⁶ t/yr	1.8	0.1	0.0	—	—	1.9
Additional crop grown	×10 ⁶ t/yr	31.8	15.6	4.8	6.2	0.1	
Additional oil available	×10 ⁶ t/yr	—	—	—	2.5	0.0	2.6
Increase in non-biofuel oil consumption	×10 ⁶ t/yr	—	—	—	0.5	0.1	0.6
Additional oil available for biodiesel	×10 ⁶ t/yr	—	—	—	2.0	-0.1	2.0
Cereal displaced by co-product	t/t crop	0.127	0.158	0.127	0.081	0.003	
Cereal displaced by co-product	×10 ⁶ t/yr	4.6	2.9	0.7	0.5	0.0	
Additional biofuel crop for biofuel	×10 ⁶ t/yr	37.0	18.5	5.5	—	—	
Projected biofuel data for 2020							
Additional biofuel production in EU	×10 ⁶ t/yr	11.8	6.2	1.6	1.9	-0.1	
Additional EU biofuel production	PJ/yr	317	166	42	72	-2	594
Projected animal feed co-product data for 2020							
Additional animal feed co-product	×10 ⁶ t/yr	12.2	5.9	2.3	2.8	-0.1	23
Soybean meal displaced by co-product	t/t crop	0.20	0.13	0.20	0.34	0.65	
Reduction in soybean meal requirement	×10 ⁶ t/yr	7.2	2.3	1.1	1.7	-0.1	12.2

TABLE 11
Potential crop yield growth

	Wheat	Maize	Barley	Rape	Sunflower	Soybean
	EU	EU	EU	EU	EU	S. America
Yield growth BAU ⁽¹⁾	0.7%	0.9%	0.7%	0.8%	0.9%	1.0%
Yield growth max. ⁽¹⁾	2.5%	1.8%	2.4%	1.7%	1.9%	1.7%
Yield growth UK ⁽²⁾	2.4%	—	—	1.6%	—	—

Notes: BAU = Business-as-usual. Sources: (1) Data from Kindred *et al.*, 2008. (2) Data from Spink *et al.*, 2009.

(2011). The increased crop area of 1 million ha for biofuel crops in 2020 comes from a reduction in the area of other cereals crops, so the total EU arable crop area remains the same.

It can be seen that crop areas are forecast to change in favour of higher yielding crops: i.e. maize and wheat will displace barley, while rapeseed will displace sunflower seed. Oilseed crops, such as rapeseed, are normally grown as a break crop in the EU, so the area will be tied to a ratio of the cereal land area and will depend on the area of cereal crops. The increase in yield will depend amongst other things on the increase in biofuel demand. Estimates of potential yield growth are shown in Table 11.

It is important to note that these are potential yield increases and technology development work will be needed to obtain these yields. The yield increases in Table 11 use rates of yield increases that are mid-way between the Kindred *et al.* (2008) “business as usual” and “maximum improvement” increases given in Table 11.

Analysis of EU data shows that nearly all the change in EU demand for cereals is provided by changes in EU production and very little is met by changes in trade. It is therefore assumed in Table 7 that there is no change in the EU balance of trade for cereal crops. The breakdown for the increase in crops available for biofuel production for this case is shown in Figure 17.

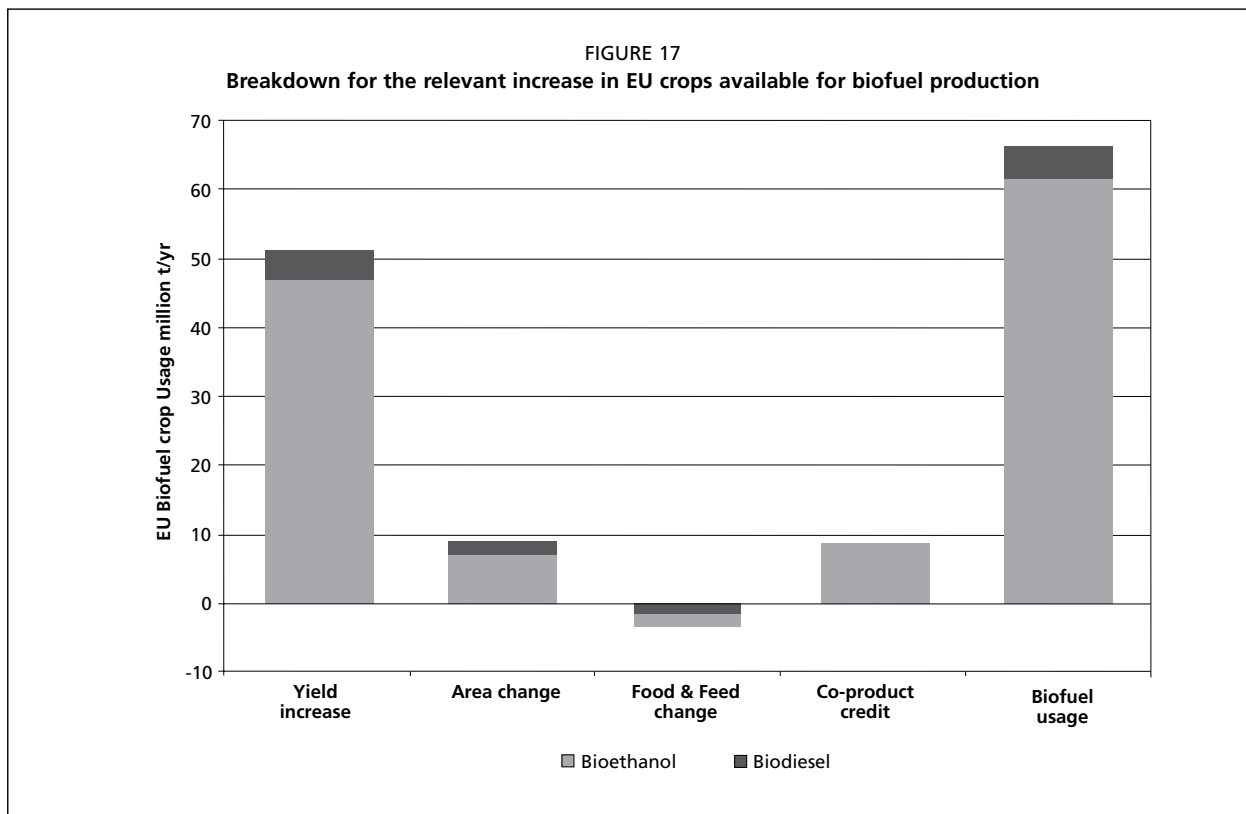


TABLE 12
Summary of projected EU biofuels demand and production in 2020

Parameter	Unit	Bio-ethanol	Biodiesel	Total
EU biofuel production 2009	PJ/yr	91	337	427
Low ethanol demand scenario				
Additional EU biofuel production	PJ/yr	525	69	594
Additional animal feed	$\times 10^6$ t/yr	20	3	23
EU biofuel production 2020	PJ/yr	615	406	1021
EU biofuel production	$\times 10^9$ L/yr	29	12	—
Proportion of 2020 road transport fuel		4.7%	3.1%	7.9%
High ethanol demand scenario				
Additional EU biofuel production	PJ/yr	786	95	881
Additional animal feed	$\times 10^6$ t/yr	31	4	35
EU biofuel production 2020	PJ/yr	876	432	1308
EU biofuel production	$\times 10^9$ L/yr	41	13	—
Proportion of 2020 road transport fuel		6.7%	3.3%	10.1%

Notes: Calculations based on a projected transport fuel demand in 2020 of 13 000 PJ/yr (DG Agri, 2011).

A summary of the EU biofuel balance is shown in Table 12. The case for “low ethanol” relates to data shown in Table 10. This shows that the bio-ethanol blending required to meet the EU 2020 FQD target of 23 billion litres in Case B of Table 9 can all be met by EU bio-ethanol production. However, there will only be a small increase in biodiesel production from EU oilseed crops and a large part of the biodiesel demand would need to be imported, or produced from imported crops.

The “high ethanol” case assumes maximum yield improvements (Table 11) and shows that all the additional biofuel in the 2020 biofuel target could be met from EU

crop production, with no increase in imported biofuels or biofuel production from imported crops.

Overall biofuel and land balance

Table 10 shows the biofuel and land balance for the EU, but does not account for the changes in soybean area and soybean oil production in South America. The overall balances for biofuel, high protein animal feed and land are shown in Table 13.

The high-protein co-product from the production of ethanol from cereals within the EU, if used as animal feed, will reduce the increase in imports of soybean meal

TABLE 13
Overall balance for land and biofuel

Parameter		
Reduction in soybean meal requirement in EU	×10 ⁶ t/yr	12.2
Soybean diesel yield	t/t meal	0.22
Biofuel lower heating value	MJ/kg	37.2
Reduced soybean biodiesel production	PJ/yr	101
Additional EU biofuel production	PJ/yr	594
Net extra biofuel available	PJ/yr	493
South American soybean average yield 2005–2009	t/ha	2.55
South American soybean yield annual growth		1.4%
Reduced soybean land use	×10 ⁶ ha	4.0
Extra EU biofuel crop area	×10 ⁶ ha	1.0
Net decrease in land use	×10⁶ ha	3.0

(mainly from South America) by 12 million tonne per year by 2020. This will reduce the areal growth of soybean production in South America and provide a substantial improvement in the security of supply of animal feed in the EU. The loss of vegetable oil production as a result of reduced soybean bean output is accounted for as a reduction in biodiesel from the soybean oil of 102 PJ/yr. The net increase in biofuel production is thus about 500 PJ/yr. The reduced EU demand for soybean will avoid the use of 4 million ha of new land in South America, which would come from destruction of forest or cerrado grassland. The net reduction in global land area requirement is therefore 3 million ha.

These results show that the growth of the production of biofuel from cereals and oilseeds in the EU enables re-optimization of crops for food and fuel, in order to better utilize existing agricultural land. This will give an overall increase in biofuel production in the EU of about 500 GJ/yr, with a reduction in net land area need of 3 million hectare.

Soybean meal balance

Some concerns have been expressed that the increased use of biofuel co-products could be constrained due to a lack of markets for animal feed. The comparison of soybean meal displacement by co-products with EU soybean imports is shown in Table 14.

Table 14 shows that for the low-ethanol case (shown in detail in Table 10) only 25 percent of the estimated

soybean meal imports in 2020 would be displaced by EU biofuel co-products. This rises to 38 percent for the high-ethanol EU biofuels case (maximum yield improvements). This means that EU biofuel use can continue to grow substantially after 2020 without reaching a limit on soybean meal displacement.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

There are many uncertainties in the data and assumptions used in the analysis presented, but the major uncertainty in the supply of biofuel co-product for animal feed up to 2020 is the extent to which the EC 2020 targets for road transport fuel will be met by bio-ethanol or by biodiesel. If the target is met by more bio-ethanol, this can be supplied by EU crops and provide DDGS co-product. If the target is met by more biodiesel, some will be supplied by rape seed in the EU and provide rapeseed meal co-product. However, this will be limited by the extent to which rapeseed can be grown in the crop rotation, so most biodiesel will be supplied by imports of biodiesel or vegetable oils, providing little extra animal feed co-product to the EU animal feed industry. The extent to which the EC 2020 targets for road transport fuel will be met by bio-ethanol or biodiesel depend on several factors. These are the knowledge gaps to 2020:

- Fossil fuel diesel vs petrol split in 2020.
- GHG savings vs fossil fuel of typical bio-ethanol and biodiesel fuels.
- Level of double counting biofuel from wastes residues and lignocellulosic feedstocks.
- Ability of EU Member States to avoid hitting blend wall limits.
- Impact of any changes in legislation regarding indirect land use change.

Most of these knowledge gaps are unlikely to be resolved by research, as they depend on legislation (diesel to petrol split; blend walls; indirect land use change) or investment choices by biofuel producers and oil companies (GHG savings; double counting biofuels).

Beyond 2020, the supply of biofuel co-product for animal feed will depend on:

TABLE 14
Projected EU protein animal feed balance

Parameter	Unit		
EU soybean meal imports in 2008 ⁽¹⁾	×10 ⁶ t	32	
EU import growth annually since 1990 ⁽¹⁾		2.9%	
Projected EU soybean meal imports in 2020	×10 ⁶ t	49	
Scenario projection		Low ethanol	High ethanol
Soybean meal displaced by co-products in 2020 ⁽²⁾	×10 ⁶ t	12	19
Soybean meal displaced by co-products in 2020		25%	38%

Notes: (1) Data from FAOSTAT. (2) Data from Table 10.

- Setting of further transport fuel GHG savings targets.
- Extent to which targets are met by biofuels from lignocellulosic feedstocks and wastes.
- Extent of introduction of renewable electricity-based road transport solutions.

CONCLUSIONS

The outlook for biorefining of EU crops for biofuel production depends on the likely split between bio-ethanol and biodiesel usage in the EU in 2020. If the target is met by more bio-ethanol, this will be supplied to a large extent by EU crops and EU biofuel production, and will provide DDGS co-product. If the target is met by more biodiesel, some will be supplied by rape seed in the EU and provide rapeseed meal co-product, but most will be supplied by increased imports into the EU of biodiesel or vegetable oils, providing little additional animal feed co-product to the EU animal feed industry.

The estimated animal feed co-product for a low-ethanol scenario is 23 million tonne per year, while the high ethanol scenario gives 35 million tonne per year.

The blending of animal feed co-products in the EU compound feed market will directly or indirectly displace a mixture of EU cereals and imported soybean meal, which mainly comes from South America. This will give a reduction of between 25% and 38% in imported soybean meal in 2020.

With continued agricultural development, most of the additional EU crop output can be achieved by increased crop yields and the remainder from using arable land that would otherwise be abandoned, so there would be no increase in EU arable land.

Because the yields of biofuel crops grown in the EU are substantially higher than those of soybean in South America, the overall biofuel and land balance of these changes is an increase in biofuel production of about 500 PJ/yr (12 million tonne oil equivalent per annum), with a net reduction in global land area of about 3 million ha.

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Chapter 3

Impact of United States biofuels co-products on the feed industry

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ABSTRACT

Although 140 biodiesel plants produced 1.2 billion litres of biodiesel in 2010, very little crude glycerin has been used in animal feeds in the United States due to the relatively low volume produced compared with ethanol industry co-products, and its higher value for consumer products and industrial manufacturing. Distillers grain (DG) co-products have been fed to livestock for more than a century and the feed industry acceptance over time coincided with the evolution of our nutritional knowledge and growing supply of these ingredients. DG serves primarily as an energy source in animal feeds, but also contributes a significant amount of amino acids, and is high in digestible phosphorus compared with other grains and grain by-products used in animal feeds. Because of the abundant supply, excellent feeding value, and low cost relative to maize and soybean meal, DG has become the most popular alternative ingredient used in beef, dairy, swine and poultry diets in the United States and in over 50 countries worldwide. Dietary inclusion rates have been increasing in recent years because of the increasing price of maize and the high energy value DDGS provides to animal feeds at a lower cost. The relative value of DG varies by species, the price differential between maize and soybean meal and geographical region. The widespread acceptance of DG by the international feed industry is a result of several key factors, including: (1) extensive research defining the benefits and limitations of using DG at various levels in ruminant and non-ruminant diets; (2) media attention and use of a variety of information dissemination technologies and programmes; (3) economic value relative to record high prices of competing ingredients; and (4) extensive promotion and export market development efforts.

The most significant barrier for domestic and international feed industry acceptance has been the variability of nutrient content and digestibility among DG sources. Because colour has been historically used as a qualitative indicator of nutrient digestibility, particularly for amino acids, dried DG with solubles (DDGS) with a light, golden colour has become the preferred physical quality characteristic in the market. Currently there are no grading systems or quality standards to differentiate quality, and efforts to develop such systems have not been successful. As a result, various feed industry companies have recently developed and commercialized "nutritional tools" to provide more accurate nutrient loading values for diet formulation and approaches to compare value among DG sources.

Other concerns impeding the acceptance of DG in the feed industry include: mycotoxins, antibiotic residues, sulphur content and risk of introducing bacterial pathogens. An emerging concern about the extent of lipid oxidation in DDGS and its effects on health and performance of monogastrics requires further research.

As ethanol production technology continues to evolve, so does the composition and diversity of co-products resulting from these processes. Significant research has been conducted to develop, evaluate and implement front-end fractionation technologies, but because of a number of challenges, there are only a few ethanol plants using these technologies and producing fractionated maize co-products for use in the feed industry. Therefore, front-end fractionation and its associated co-products have had minimal impact on the United States feed industry. In contrast, back-end oil extraction technologies are being widely implemented in several dry-grind ethanol plants, resulting in reduced-oil DG co-products becoming available in significant quantities. However, the impact of oil extraction on energy value of these co-products for livestock and poultry has not been determined, but it will probably result in lower value and reduced dietary inclusion rates in animal feeds. Use of alternative ethanol feedstocks, such as other grains, sources of cellulose and algae, together with the possibility of phytate phosphorus extraction and other technologies, will dramatically change the availability and nutritional composition of co-products. Emerging new markets for DG include aquaculture, horse and companion animal feeds, and human foods, but research to support these market applications is needed.

MAIN MESSAGES

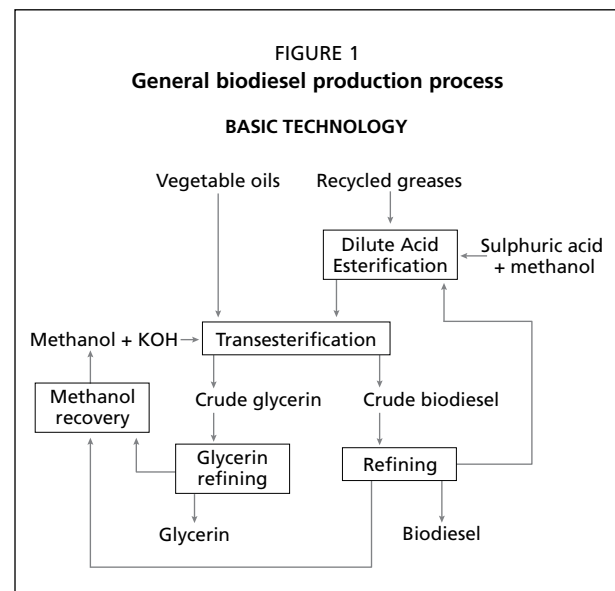
- Although 140 biodiesel plants produced 1.2 billion litres of biodiesel in 2010, very little crude glycerin has been used in animal feeds in the United States due to relatively low volume produced compared with ethanol industry co-products, and competition with higher value consumer product and industrial uses.
- DG serves primarily as an energy source in animal feeds, but also contributes a significant amount of protein and amino acids, and is high in digestible phosphorus compared with other grains and grain by-products used in animal feeds.
- Dietary inclusion rates have been increasing in recent years because of the increasing price of maize and the high energy value DDGS provides to animal feeds at a lower cost.
- Relative value of DG varies by animal species, the price differential between maize and soybean meal, and geographical region.
- DG has become the most popular alternative ingredient used in beef, dairy, swine and poultry diets in the United States and in over 50 countries worldwide because of abundant supply, excellent feeding value and low cost relative to maize and soybean meal.
- The most significant barrier for domestic and international feed industry acceptance has been the variability of nutrient content and digestibility among DG sources.
- As ethanol production technology continues to evolve, so does the composition and diversity of co-products resulting from these processes.
- Use of alternative feedstocks, such as other grains, sources of cellulose, and algae, along with the possibility of phytate phosphorus extraction and other technologies, will dramatically change the availability and nutritional composition of co-products.
- Emerging new markets for DG include aquaculture, horses and companion animal feeds, and human foods, but further research to support these market applications is needed.

INTRODUCTION

As a result of the exponential growth of the United States ethanol industry during the past decade, distillers grain (DG) co-products from dry-grind ethanol production have been produced in great quantities, and have affected the feed industry to a much greater extent than other biofuel co-products combined. Lesser amounts of maize co-products (maize gluten feed, maize gluten meal and maize germ meal) are produced by the wet-milling segment of the fuel ethanol industry, but have also been extensively used in the United States feed industry for more than 30 years.

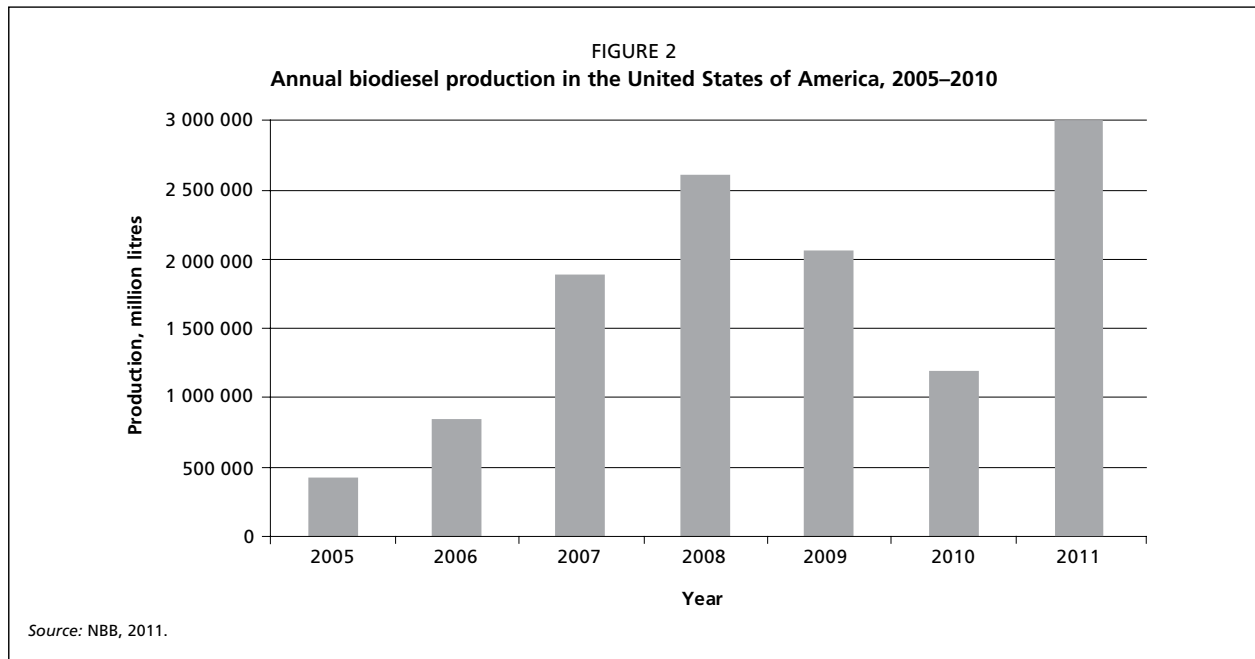
Biodiesel is produced using a variety of esterification technologies. New or used vegetable oils and animal fats are used as the initial feedstock. These feedstocks are filtered and pre-processed to remove water and contaminants, followed by mixing with an alcohol (usually methanol) and a catalyst (sodium or potassium methylate). This causes the oil molecules (triglycerides) to be broken apart into methylesters and glycerin, which are then separated from each other and purified (Figure 1).

Biodiesel production in the United States rapidly expanded from 2005 (424 million litres) to 2008 (2.6 billion litres), but declined from 2009 (2.1 billion litres) to 2010, with 1.2 billion litres being produced from 140 plants within the United States (Figure 2). The principal co-product of the biodiesel production is crude glycerin (Ma and Hanna, 1999; van Gerpen, 2005), with 0.08 kg of crude glycerin



generated for every litre of biodiesel produced. As a result of the recent decline in biodiesel production and consequent reduced availability of crude glycerin, along with competing uses in consumer and industrial products, very little crude glycerin has been used in the United States feed industry. Examples of non-feed uses of glycerin include:

- moistening, sweetening and preserving foods and drinks (soft drinks, candies, cakes, casings for meats and cheese, dry pet foods, etc.);



- use in drugs and pharmaceuticals (capsules, anaesthetics, cough remedies, lozenges, emollient for skin medications, etc.);
- serving as a moisturizing agent or emollient for cosmetics and toiletries (toothpaste, skin creams, deodorants, make-up, lipstick, mascara, etc.);
- keeping tobacco moist and soft to prevent breaking and crumbling during processing (also adding flavour to chewing and pipe tobaccos, and used to manufacture cigarette filter tips);
- softens and reduces shrinkage during paper manufacturing (grease-proof paper, food wrappers, printing ink manufacturing, etc.);
- sizing and softening yarn and fabric, and producing a renewable propylene glycol (humectants, antifreeze and de-icing solutions, etc.);
- combining glycerin and citric acid to produce biodegradable polymers; biodegradable films, sheets, plastics and gel-like coatings; propylene glycol; and
- using *Escherichia coli* to convert glycerin into ethanol.

Unless economic incentives promote the United States biodiesel production industry to expand to a larger volume in the future, very little, if any crude glycerin will be available for use in animal feeds. In addition, the methanol remaining in crude glycerin can be toxic to animals at moderately low concentrations. In the United States, feed companies tend to be very conservative regarding the use of crude glycerin in proprietary products and in animal feed formulations. In fact, some feed companies have elected to not use crude glycerin in any feed products because the quality of crude glycerin can vary considerably among sources, with concerns regarding methanol content and due to its handling characteristics.

Unlike crude glycerin, DG has revolutionized the global feed industry during the past decade. In fact, many nutritionists often refer to these novel feed ingredients as “The biggest change in feeding animals since soybean meal.” (Comment made to Dr Harold Tilstra by Pete Kitzman at Land O’Lakes Purina Feed LLC in 2002). These sentiments were also expressed by Dr Terry Klopfenstein, Professor and beef cattle nutritionist at the University of Nebraska, who has been a leader in DG research for more than 40 years, who said “By-products from ethanol are having a bigger impact on the cattle industry than anything I’ve experienced during my 41 years with the university” (Quoted in *Farm Industry News*, 1 Sept. 2006). Although the exponential growth of the United States fuel ethanol industry has been controversial relative to its impacts on using maize for fuel vs feed, environmental costs and benefits, government policy and subsidies, as well as the dependence on imported crude oil, it continues to be the largest emerging segment of United States agriculture. Currently, worldwide there are over 200 ethanol plants producing more than 35 million tonnes of maize-based distillers co-products that are being fed to livestock, poultry and fish in over 50 countries.

DG is a co-product of the beverage and fuel ethanol industry. In the USA, maize is the predominant feedstock used to produce ethanol, but other grains and carbohydrate containing feedstocks can be used. Maize DG with solubles has high feeding value in animal feeds and is considered to be a high energy, mid-protein ingredient. In fact, DG contains equal or more energy than maize for all animal species except poultry. However, despite its moderate protein content (27 percent), DG has poor protein quality for monogastric animals because of the low lysine content relative to the crude protein (CP) concentration. The type of

feedstock used to produce ethanol determines the nutrient content and values of DG co-products. The two main types of ethanol production processes are wet-mill and dry-grind ethanol plants. Both process maize and mix it with yeast to convert starch into ethanol and carbon dioxide. After distillation of ethanol, the residual co-products are centrifuged to remove water, and are often dried to produce co-products for the feed industry. The type of milling and further processing determines the nutritional value and composition of co-products. Wet mills use maize to produce ethanol, maize gluten feed, maize gluten meal, steep water, maize germ meal and crude maize oil. Dry-grind ethanol plants are the predominant ethanol production process used in the United States today, and produce an even more diversified group of maize co-products, which includes: wet DG, condensed distillers solubles, modified wet DG, dried distillers grain, dried distillers grain with solubles (DDGS), high-protein DDGS (from fractionation), de-oiled or de-fatted DDGS (following oil extraction), maize germ meal, maize bran and crude maize oil. Of all of these co-products, DDGS is the predominant form produced and available to the global feed industry.

Although widespread use of DG by the feed industry has only occurred within the past decade, these co-products have been produced and fed to livestock for more than a century. Beverage alcohol production has occurred in the United States throughout history. In the 1800s through to the early 1900s, farmers used on-farm or small “backwoods” stills to convert grain to alcohol for personal consumption. Some of these facilities grew into distilleries that produced whiskey, and the resulting by-product was referred to as “mash” or “slop”. Local farmers fed this mash to cattle and hogs and began seeking information on how to utilize this by-product more effectively in animal feeds. With the exception of the alcohol prohibition years from 1919 to 1933, there are records dating back to 1900 indicating that feeding mash to dairy cows resulted in an increase in milk production (Lindsey, 1900–1903). Research reports increased in frequency in the late 1940s and 1950s and provided information on optimizing the use of distiller’s co-products in dairy, beef, swine and poultry diets.

EVOLUTION OF DG PRODUCTION AND USE IN THE UNITED STATES FEED INDUSTRY

1950s and 1960s

Up until the 1950s, DG was treated primarily as a protein ingredient and used to partially replace other protein ingredients in cattle feeds. During this period, little was known about essential nutrients required by animals, and diets were formulated more on the basis of ingredient substitution than to meet specific nutrient requirements. During the 1950s and 1960s, most of the essential nutrients were

identified and nutrient requirements were established for most animal species. Diets in the 1960s and 1970s, including those containing DG, were typically formulated using CP as the primary criterion. This diet formulation approach was appropriate for cattle feeds, but was not precise enough for formulating swine and poultry feeds in order to achieve optimal animal performance because the lysine (an essential amino acid and most likely to be deficient relative to the animal’s requirement) level relative to crude protein content is low in DDGS and all other maize co-products. As a result, if DDGS was included in swine and poultry diets, it was added at low levels (<10 percent) to avoid reductions in animal performance, and was generally considered an inferior ingredient for these animals. This stigma was not overcome until the late 1990s, and DG was used almost exclusively in beef and dairy feeds.

1970s and 1980s

Large wet-milling maize plants were built in the 1970s and 1980s and began producing enough ethanol to consider using it as an additive to gasoline. Wet gluten feed produced by these wet mills became a popular ingredient used in beef cattle feed lots. During this same period, there were several dry-grind ethanol facilities built in the western corn-belt states. These facilities were typically much smaller than the existing wet-mill operations, were usually owned by a group of local maize producers and investors, and often incorporated equipment and technology that proved difficult to operate. Many struggled to stay in operation due to production and economic challenges. However, some of these early projects succeeded, and as time went on, more dry-grind ethanol plants were built.

Beef feedlots were the earliest significant users of DG co-products in the United States. Many feedlots made use of ‘commodity sheds’ with multiple storage bays easily accessible by front-end loaders. This provided convenient handling of a variety of ingredients that varied widely in texture, moisture and other physical attributes. One of the most important considerations in deciding ethanol plant locations during this period was proximity to significant numbers of beef cattle. In fact, many maize producers who joined ethanol plant cooperatives were also beef cattle feedlot owners.

One of the key lessons learned from the first attempts at building a dry-grind ethanol industry in rural America was the importance of political involvement to help get a new industry started. It took creative thinking to come up with ideas and develop plans that enabled farmers to pool their capital to construct and operate an ethanol production facility. Farmers had to be politically active to ensure that states and communities were open to construction of new facilities and did not set unreasonable barriers and undermine the sustainability of these plants.

1990s to early 2000s

The State of Minnesota was an early example demonstrating the importance of political involvement in further developing the United States ethanol industry. Key state officials in the legislative and executive branches of state government recognized that supporting the growth of the fuel ethanol industry could stimulate rural development. Minnesota maize producers worked with the state legislature to pass legislation in the 1980s and 1990s which became known as the "Minnesota Model" in the United States ethanol industry (See <http://www.mda.state.mn.us/en/renewable/ethanol/about.aspx>). There were two key concepts included in this legislation:

- requiring the use of 10 percent of ethanol in gasoline sold in the state for use in automobiles; and
- providing ethanol plants with a payment from the State of Minnesota of US\$ 0.05 per litre for the first 57 million litres of ethanol produced per year for ten years.

This legislation stabilized the intra-state development of new ethanol producing companies by assuring a market for ethanol and by providing a supporting revenue stream for these ethanol facilities. Groups of farmers in several communities formed cooperatives that owned and operated dry-grind ethanol plants. These farmers bought shares in the cooperatives and committed to delivering a pre-determined amount of maize each year to their ethanol plant. The maize price received by farmer members was dependent upon the profitability of the ethanol production cooperative.

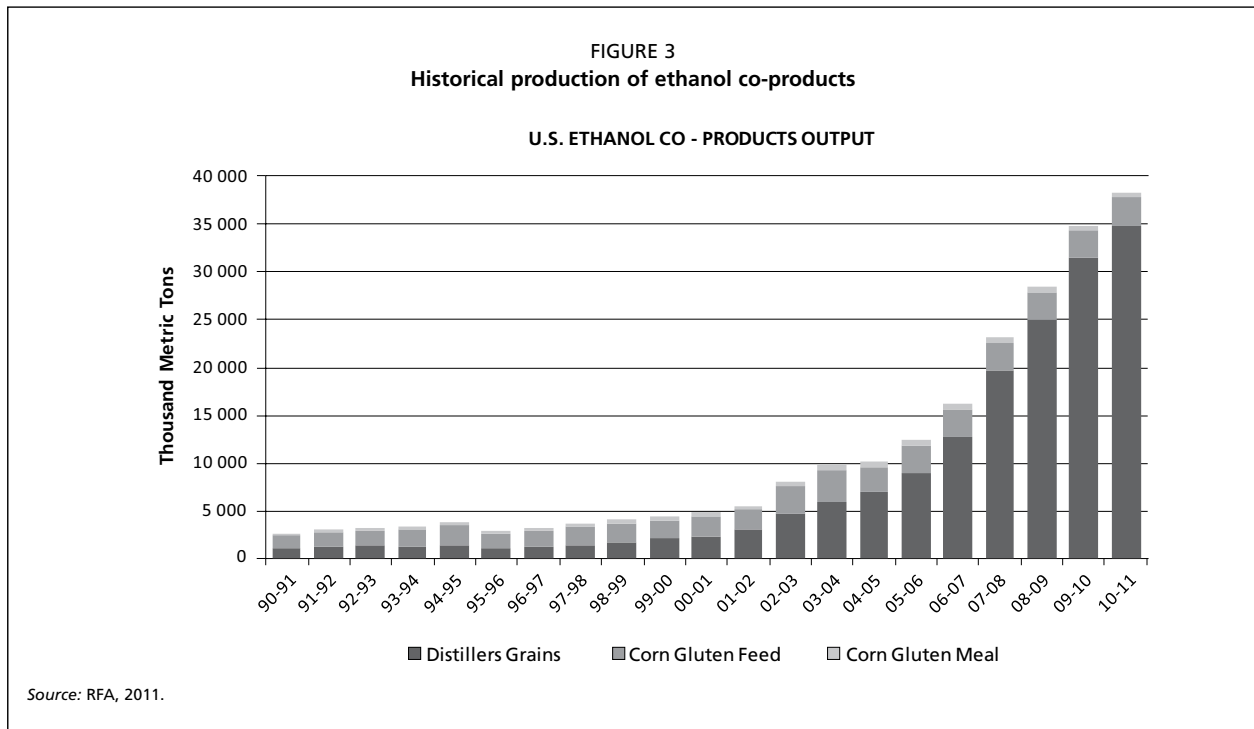
These new businesses also introduced a steady supply of new feed ingredients into their local areas which included DDGS, wet DG with (WDGS) or without (WDG) solubles, and condensed distillers solubles (CDS). These co-products became economical partial replacements for key ingredients in animal feeds. Addition of WDGS replaced some of the maize silage, maize grain and protein supplement in beef and dairy rations, and including DDGS in swine diets replaced part of the maize grain, soybean meal and inorganic phosphorus supplements. However, the bulk of these co-products continued to be used in beef cattle rations, primarily in finishing feedlots. These co-products were fed at relatively low levels (usually less than 15 percent of the diet on a dry matter (DM) basis) to avoid potential challenges in handling wet co-products and possible reductions in animal performance.

Co-product pricing during this period was often based more on the need for ethanol facilities to get rid of the co-products due to limited storage capacity (less than ten days of production) than on its nutritional value. Ethanol plants that sold WDGS or WDG needed to have these co-products sold and moved out of their storage facilities every day, whereas CDS needed to be sold and moved out within two to four days after it was produced. DG co-product market-

ers were challenged to maximize the price received while at the same time keeping the co-products moving out of the plants in a timely fashion.

Beginning in the autumn of 1998, a group of Minnesota ethanol plant managers became concerned about the potential future growth of the fuel ethanol industry, and whether there would be a market for the distiller co-products being produced. Engineering design and production technologies used in constructing and operating new ethanol plants in Minnesota and South Dakota, led by Fagen, ICM and Broin (now known as POET), resulted in the production of a "golden coloured" DDGS which was perceived to have higher feeding value than traditionally produced dark-coloured DDGS, because of less heat damage during drying. Approximately 98 percent of the DDGS produced prior to 1998 was fed to dairy and beef cattle. However, because of the perceived higher quality and feeding value of DDGS being produced by these new ethanol plants, plant managers began questioning why more DDGS could not be used to feed to swine and poultry. This led to the formation of a consortium of ethanol plants in Minnesota and South Dakota that implemented a voluntary "check-off" programme that collected US\$ 0.10/ton of DDGS produced, which was used to support the initial research studies at the University of Minnesota to determine the feeding value and optimal diet inclusion rates of this "golden coloured" DDGS for swine and poultry. Commodity Specialists Company (now CHS) was the marketing group that coordinated the funding and worked directly with researchers at the University of Minnesota to prioritize relevant research projects needed to increase demand for DDGS in swine and poultry feeds. Results from the first research project from these efforts were published by Spiehs, Whitney and Shurson in 2002. This hallmark study showed that the nutrient content of DDGS produced by these relatively new, relatively small, farmer-owned ethanol plants was higher than DDGS produced by older ethanol plants in the industry. Subsequent studies conducted at the University of Minnesota demonstrated that the energy content, amino acid and phosphorus digestibility were also higher in DDGS sources produced by these relatively "new" ethanol plants, compared with DDGS produced by older ethanol plants, and these research results showed that this "new" DDGS was an excellent alternative feed ingredient suitable for use in swine and poultry diets.

These research findings led to a need to differentiate quality among DDGS sources in the ethanol and feed industry in order to ensure that the DDGS source being fed would result in optimal performance of swine and poultry. As a result, the terms "new generation" vs "old generation" and "golden" DDGS were created to informally use lightness and yellowness of DDGS colour as an indicator of superior quality, feeding value and suitability for use in



swine and poultry diets. These terms were used and adopted widely throughout the ethanol as well as the domestic and international feed industry because there were no official feed industry or government grading systems to differentiate quality and feeding value among DDGS sources. It was common for “golden” DDGS to trade at a premium price compared with “old generation” DDGS. Surprisingly, these terms are still used today among DDGS users in countries around the world. This was the beginning of the ethanol industry referring to DDGS as a co-product rather than a by-product.

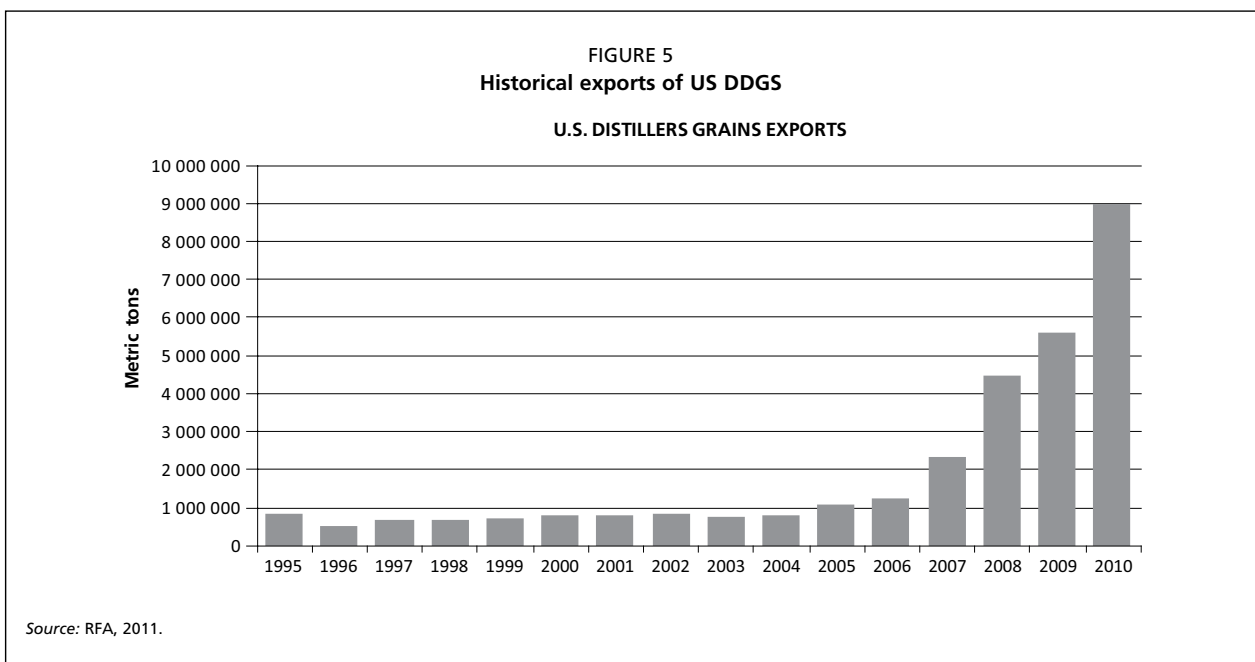
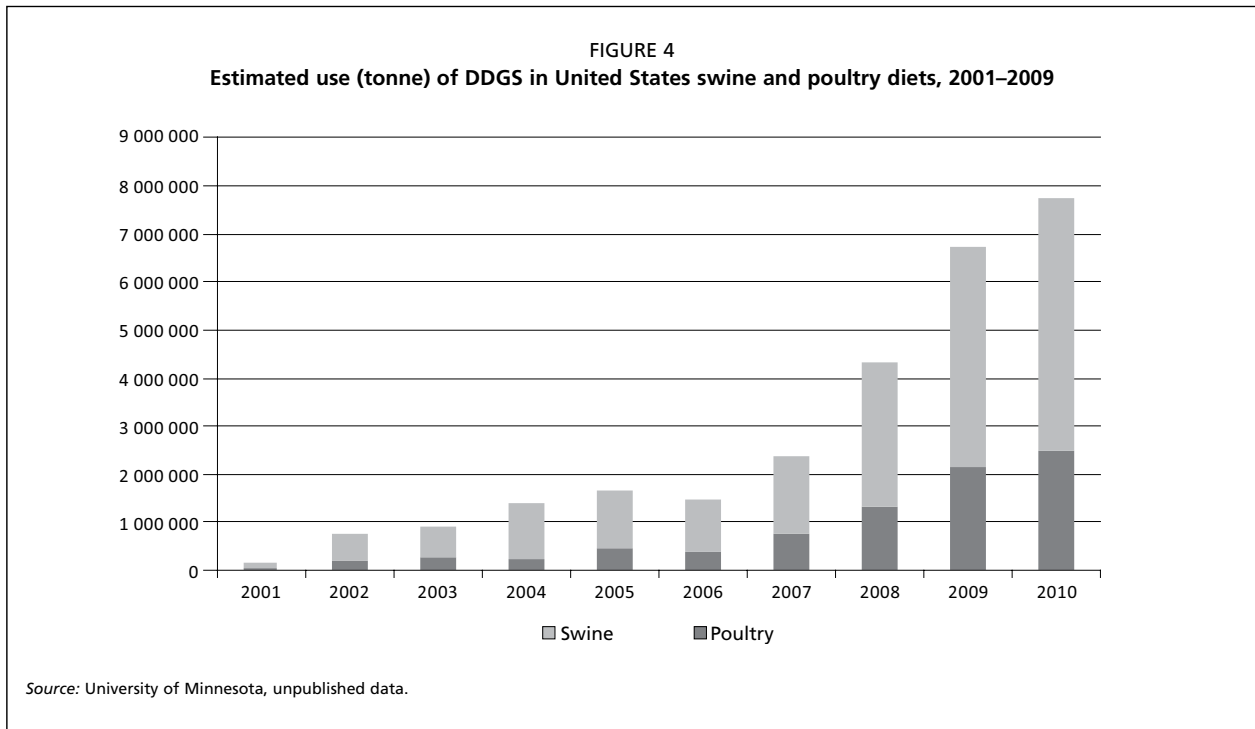
2000 to present

The period from 2000 to 2008 was an exciting time in the ethanol and feed industries, and DG production increased dramatically (Figure 3). This era was defined by the question of “What are we going to do with the ‘mountains’ of DDGS produced by the exponential growth of the ethanol industry?”

This concern, accompanied by the increased volume of DG being produced, prompted several state maize grower associations, as well as livestock and feed industry organizations to provide a dramatic increase to several universities of funding for animal nutrition research on feeding applications of DG co-products. Like the exponential growth of the ethanol industry, there was a dramatic increase in research conducted at universities and feed industry companies to more completely define the benefits and limitations of using DG in livestock, poultry and aquaculture diets. Leading DG researchers in dairy (Schingoethe *et al.*, 2009), beef (Klopfenstein, Erickson and Bremer,

2008) and swine (Stein and Shurson, 2009) published summaries of results from numerous research studies conducted during this period. Their research, and the publication of these summaries, made a tremendous impact, with widespread feed and livestock industry acceptance of these maize co-products. Several key research studies were also conducted and results were published for poultry (Lumpkins, Batal and Dale, 2004, 2005; Lumpkins and Batal, 2005; Batal and Dale, 2003, 2006). Likewise, these initial studies were instrumental in expanding the use of DDGS in poultry feeds around the world. Because of the extensive amount of research conducted during this decade, maximum recommended dietary inclusion rates were established for using DG with solubles in animal feeds. Maximum inclusion rates established are: lactating dairy cows – 30 percent; beef feedlot cattle – 40 percent; swine – 30 percent all phases, and up to 50 percent in gestation; and poultry – 5 percent.

In addition to new knowledge generated from extensive research, widespread media attention on the growing ethanol industry and the development of university and industry Web sites (e.g. www.ddgs.umn.edu) devoted to communicating research results and feeding recommendations, were instrumental in communicating knowledge to DDGS end users around the globe. Interest in using DDGS in new market segments grew dramatically because there was a positive story to tell. Research results showed the excellent feeding value of DDGS for all species, including swine and poultry, and for the first time in history, a significant market for DDGS in the United States swine and poultry industry was created (Figure 4).



Furthermore, the U.S. Grains Council began focusing much of its export market development efforts on promoting the use of DDGS in countries around the world. These efforts led to a dramatic increase in DDGS exports from 2004 to 2010 (Figure 5). In 2010, the major United States export markets for DDGS were Canada, China, Mexico, Republic of Korea [South Korea] and Viet Nam, with most of the DDGS being used in swine and poultry feeds.

Perhaps the biggest challenge in DDGS acceptance in the feed and livestock industry during this time was the

realization that nutrient content and digestibility varied considerably among sources. Nutritionists and livestock producers began demanding more consistency and predictability in the quality and nutrient content of the DDGS sources they were using. Unlike maize grain, there is no grading system to differentiate quality or nutritional value among DDGS sources. With DDGS being a relatively “new” feed ingredient to many nutritionists and livestock producers, there was much skepticism and a lack of knowledge of how to value its use in animal feeds and manage quality and nutrient variability.

In the early 2000s, some ethanol plants and DDGS marketing groups attempted to respond to the demands from their DDGS customers to implement more extensive quality assurance programmes focused on DDGS in order to provide a more consistent quality. These attempts failed for several reasons. First, the primary economic focus of ethanol plants was on ethanol production and not the co-products when ethanol profits were high. Second, as long as ethanol plants were able to “get rid of” their DG in a timely fashion there was little economic incentive to invest time and money in developing a programme to improve DDGS quality and consistency because there was no guarantee of a price premium or financial return for this investment.

There were also formal and informal attempts to form coalitions among segments of the ethanol and maize industry to develop strategies to differentiate the DDGS they were producing from other DDGS sources on the market. With the exception of a few ethanol companies that developed branded co-products, these attempts also failed. Many ethanol companies did not want more transparency and methods to differentiate quality in the market, perhaps out of fear that the co-products they were producing would be discounted in price relative to competitor sources. Furthermore, there were legal concerns related to the risk of being accused of market collusion that prevented these early efforts from materializing. However, in autumn 2005, the American Feed Industry Association (AFIA), the Renewable Fuels Association (RFA), and the National Corn Growers Association formed an industry-wide initiative to focus on two aspects related to DDGS quality issues: first, to cooperatively design a study that would lead to recommendations on the most applicable analytical testing methods for DDGS; and, second, to review the applicability of current American Association of Feed Control Officials (AAFCO) and AFIA definitions of distillers co-products. The outcome of this effort was published in a final report by AFIA in February 2007. The committee provided recommended analytical testing methods for moisture, CP, crude fat and crude fibre. These recommendations were neither mandatory nor regulated by the government, but were voluntary to encourage all DDGS suppliers to use common analytical methods to minimize discrepancies in description of nutrient content of DDGS that occur when different analytical procedures are used. The committee also decided at that time that the current AAFCO definitions were adequate to define the distillers co-products being produced, and any changes would tend to limit trade rather than provide further clarity between buyers and sellers. However, this same committee agreed that the current AFIA Ingredient Guidelines be updated for definitions of DDGS and maize condensed solubles. Although these industry initiatives failed to create an industry-wide quality assur-

ance programme and standards, they did result in greater awareness among many ethanol plants in the industry, which motivated ethanol plants to implement improved practices and quality assurance programmes to produce more consistent and higher quality co-products. In 2009, the Chicago Board of Trade (CBOT) initiated a DDGS futures contract with specified minimum product standards. While this futures contract has been only lightly traded since its inception, its launch was indicative of the growing importance of DDGS in the feed ingredient market, and offered some level of price discovery and standardization that some market participants thought was missing. Following is commentary received from the CME Group, owner of CBOT, concerning the current status of the DDGS futures contract:

“Since 1877, when the Chicago Board of Trade (CBOT) began trading Corn, Wheat, and Oat futures contracts, price discovery and price risk management for many agricultural products have occurred on organized futures exchanges. New products for trade have been added over time including Soybean futures (1936), Soybean Oil and Meal futures (1950 and 1951), Agricultural Options (1985), and Ethanol futures (2005). DDG futures were launched in 2010 to provide a platform for open and transparent price discovery and a tool for managing price risk for livestock feeders, feed manufacturers, importers and exporters, producers, and marketers. While the CBOT DDG futures contract has yet to gain industry traction, it is not uncommon for new futures contracts to take several years to build regular activity. Lack of a quality standard and a rapidly changing industry make DDG futures a more difficult futures product compared with more standardized commodities like maize or soybeans. It is clear, however, that the growing DDG industry is in need of the price discovery and price risk management tools offered through exchange-traded futures contracts. The CBOT continues to work with the DDG industry to build a futures contract that will allow producers the ability to hedge their production margins and users to hedge their feed input needs.”

(CME Group as pers. comm. to Dr Harold Tilstra, 4 August 2011)

Despite, these efforts, the challenge of selecting and managing nutrient variability and digestibility among DDGS sources continues. Out of necessity, some independent feed industry companies have commercially developed and implemented the use of various methods and services – including near-infrared spectroscopy, energy prediction equations, and *in vitro* laboratory methods to estimate amino acid digestibility – to help end users of DDGS more accurately determine specific nutrient loading values for specific DDGS sources as well as differentiate feeding value among DDGS sources for specific animal species. Use of some of these “nutritional tools” and services show that, depending on the DDGS source, nutritional value can be as much as US\$ 45 per tonne more than the actual price paid for the ingredient, as demonstrated by one of the present author’s (Dr Gerald Shurson) experiences with the use of

TABLE 1
Ingredient composition changes on a percentage as-fed basis in typical growing swine diets in the decades before and after 2000

Ingredient	Before 2000	At current maize, soybean meal and DDGS prices	At current maize, soybean meal and DDGS prices, with competitively priced canola meal
Maize	70	53	44
Soybean meal	25	11	5
Canola meal	0	0	15
DDGS	0	30	30
Choice white grease	2	3	3
Other ingredients, vitamins, minerals, amino acids	3	3	3
Total	100	100	100

TABLE 2
Ingredient composition changes (on a percentage of DM basis) in typical beef feedlot diets in the decades before and after 2000

Ingredient	Before 2000	Current with moderate maize price	Current with high maize price
Cracked and/or high moisture maize	75.0	52.0	44
Maize silage	15.0	15.0	
DG		25.0	45.0
Alfalfa hay	5.0		
Grass hay		5.0	5.0
Maize stalks			3.0
Soybean meal	3.0		
Urea	0.5		
Vitamin-mineral mix	1.5	3.0	3.0
Total	100	100	100

Illuminate® from Value Added Science and Technology (VAST), Mason City, Iowa, to estimate relative economic value and nutrient loading values among DDGS sources for swine. As these tools become more refined and widely adopted, they could become the ultimate “grading system” used by DDGS users in the feed as well as livestock and poultry industries to manage and differentiate quality.

During the past decade, there have been dramatic changes in the composition of commercial animal diets due to acceptance and widespread use of DG in the feed industry. Table 1 shows examples of differences in diet composition as a result of variation in availability and value of commonly used feed ingredients for 60-kg growing pigs located in west-central Minnesota. Prior to 2000, very little, if any DDGS was used in swine diets. Currently, because of lower price relative to maize, soybean meal and canola meal, DDGS is added at levels of 30 percent or more to partially replace portions of maize and soybean meal, resulting in significant differences in diet costs.

A similar comparison can be made by looking at possible diets for a 340-kg feedlot steer in a west central Minnesota feedlot. DG would most likely be fed as “wet” (~30 percent DM) or as “modified” (~50 percent DM) co-product, but the displacement of other ingredients is readily apparent in either case. As in swine diets, the price of DDGS relative to maize affects usage rates in beef feedlot rations.

Prior to 2000, rations consisted of 75 percent cracked or high moisture maize, with soybean meal and urea providing the necessary protein (nitrogen) (Table 2). Today, depending on maize price, 25 to 45 percent DG is being fed to reduce the amount of maize in the ration and, because DG is moderately high in protein, no soybean meal or urea is needed. The readily available supply of DG products in the western corn belt has resulted in much less maize being harvested as whole-plant maize silage. As a result, grass hay and maize stalks have replaced alfalfa hay and maize silage as forage sources.

In both the swine and beef examples, the proportions of ingredients in the diet changes as distiller co-products become more available and more price competitive. DG is primarily an energy source in animal diets, and with recent market prices (as of June 2011), DDGS sells at a substantial discount to maize grain (the predominant energy source in traditional diets). Thus, formulation systems tend to include DG at levels up to pre-set maximums.

The relative value of DG in diets for various animal species has also changed during the past decade (Table 3). In commercial diets fed in the early 2000s, DDGS had the highest value in lactating dairy cow diets, followed by beef cattle feedlot diets, laying hen diets and growing-finishing swine diets, when maize, as the primary energy source in the diet, was 40 percent the price of soybean meal (a

TABLE 3
Historical break-even price (US\$ per tonne) comparison for using DDGS in diets for different animal species

	Early 2000s	Mid-July 2011
Assumptions:		
U.S. #2 Corn	79	306
Soybean meal (48% protein)	193	376
DDGS break-even values:		
Dairy – lactation	126	290
Beef – feedlot	119	297
Swine – grower-finisher	106	315
Poultry – layers	114	262

Notes: Data based on a 10% dietary inclusion rate and central Iowa prices. Source: Land O' Lakes Purina Feed LLC, unpublished data.

TABLE 4
Comparison of break-even price (US\$/tonne) of DDGS in diets for different animal species in three geographical areas of the United States based on maize and soybean meal prices in mid-July 2011

	Central Iowa	South Carolina	Pennsylvania
Assumptions:			
U.S. #2 Corn	306	356	351
Soybean meal (48% protein)	376	437	441
DDGS break-even values:			
Dairy – lactation	290	357	379
Beef – feedlot	297	346	338
Swine – grower-finisher	315	332	335
Poultry – layers	262	322	362

Notes: Calculations are based on a 10% dietary inclusion rate. SOURCE: Land O' Lakes Purina Feed LLC, unpublished data.

primary protein source). During this time, DDGS was used more as a protein supplement rather than as an energy source in animal feeds. However, as the price of maize (primarily an energy source) increased to 80 percent of the price of soybean meal, and because DDGS is primarily an energy source in animal feeds, it changed the relative value of DDGS among these animal species. Currently, DDGS has the highest value in swine diets, followed by beef, dairy and poultry.

There are also geographical differences in relative value of DDGS for various animal species (Table 4). These break-even values are based on the nutrient value of the DDGS at the point of animal consumption using mid-July 2011 maize and soybean meal market prices, 10 percent dietary inclusion rate and typical assumptions on animal performance, diet digestibility, etc. The values do not reflect marketing, processing and transportation cost, nor the competitive effect of other ingredients in the market place, all of which help determine the market value of DDGS. Currently, in central Iowa, DDGS has the highest value in swine and beef cattle diets; in South Carolina it has the highest value in dairy and beef cattle diets; and in Pennsylvania it has the highest value in dairy and poultry diets. The regional differ-

ences in break-even price of DDGS observed in this comparison were largely determined by availability and market price of other competing ingredients at each location, and by restricting the dietary inclusion level to 10 percent of the complete feed for all species. Higher inclusion levels of DDGS in diets results in different break-even values to those used in this comparison.

On a volume basis, DG products have become the third most used feedstuff in United States livestock and poultry diets, following maize and soybean meal. This increased use occurred in conjunction with the rapidly increasing supply of DG produced by the dry-grind ethanol production industry. Record high feed grain and ingredient prices have caused livestock producers to adopt any and all opportunities to lower feed cost. As of early May 2011, the DDGS price was less than 80 percent of the market value of maize grain. Relatively low price, coupled with greater nutrient concentration, provides a tremendous economic incentive for livestock and poultry producers to maximize dietary inclusion rates of DG. Based on diet cost economics, the questions United States livestock producers have asked during the evolution of the ethanol industry and DG production have transitioned from "Can we use distillers co-products in animal feeds?" to "How much distillers co-products can we use?" to "Can we use even more co-products than that?"

Marketing of co-products

Over the past ten years, the United States ethanol industry has increased DG production from 2.7 million tonne in 2000, to 32.5 million tonne in 2010. Beef, dairy, swine and poultry have been the primary consumers of these co-products. Research is being conducted that is expanding the use of DG co-products into small ruminant diets and aquaculture feeds, as well as for companion animal feed and human food products.

The dissemination of knowledge about the use, value and application of co-products is essential for creating new markets. The U.S. Grains Council has been in the forefront of new market development for ethanol co-products worldwide. Much of the information used to develop the international market for ethanol co-products has also been used to develop the domestic market. The marketplace continues to value the many positive attributes of distillers co-products and is becoming more knowledgeable at managing the challenges that feeding some of co-products have created for some animal species. DG marketers are delivering better information about product characteristics and nutrient profiles so that the end user can better evaluate and realize the value from these co-products.

A number of approaches are used to market ethanol co-products. Many ethanol plants have on-site, staff merchandisers that market their co-products to local livestock

producers, to re-sellers that transport large quantities of dried co-products to other destinations and to firms that specialize in exporting co-products. There are also marketing firms that have contractual relationships with ethanol plants to market on a commission-fee basis all of the co-products produced. In addition, there are a few large ownership-based networks of ethanol plants with centralized marketing groups handling all of the network's co-product production. Some of the marketers of DG co-products are listed at <http://www.ddgs.umn.edu/links-marketing.htm>.

Distillers co-products are also available to end users as an ingredient in many branded feed products purchased by livestock, poultry and aquaculture producers and feed industry groups. Examples include complete feeds in meal, pelleted, extruded or liquid forms. Supplements and mineral products are also available in meal, liquid, pelleted, block or tub forms for a variety of species.

DDGS exports

From 1995 to 2004, less than 1 million tonne of DDGS were exported (Figure 3). During this decade, most of the DDGS was exported to Europe. However, beginning in 2005, DDGS exports to more areas began to increase, reaching approximately 9 million tonne in 2010. Today, due primarily to DDGS market development efforts by the U.S. Grains Council, DDGS is exported to more than 50 countries around the world. The major DDGS importing countries in 2010 were Canada, China, Mexico, Republic of Korea [South Korea] and Viet Nam. The majority of DDGS exports are being used in swine and poultry diets, but use in aquaculture feeds is increasing dramatically. Due to declining carryover stocks of grain in many regions around the globe, increased meat demand in China causing increased feed demand, and adverse weather conditions limiting acreage planted and yields harvested, export demand is expected to continue to increase for distillers co-products.

FUTURE IMPACT OF UNITED STATES ETHANOL PRODUCTION ON THE FEED INDUSTRY

Maize co-products from front-end fractionation and back-end oil extraction technologies

Current United States wet-mill capacity is 4.6 billion litre/year and that capacity is expected to remain constant between now and 2022. Depending on government policy, dry-grind ethanol production is expected to increase by 2022, but at a significantly reduced rate compared with the 2004 to 2008 period. Although the majority of maize co-products being produced by the dry-grind ethanol industry include wet and dried DGS, limited quantities of new maize co-products are also becoming available to the feed industry. These new maize co-products are a result of some ethanol plants implementing front-end fractionation of the maize kernel or back-end oil extraction, or both.

Fractionating maize kernels into different chemical and structural components has been utilized to produce various industrial and food-grade maize products for many years. More recently, maize fractionation technologies have been developed, evaluated and implemented by a few ethanol plants in an attempt to remove non-fermentable components of the maize kernel and improve ethanol yield. The main incentives for dry-grind ethanol plants to consider using fractionation technologies include: increased ethanol yield, less enzyme use during fermentation, lower production of co-product mass that requires drying, reduced drying costs and heat damage to proteins in co-products, less energy and water use, reduced need for frequent cleaning of the system to remove oil, ability to market or use the maize oil for other, higher value applications, and increased diversity of co-products to potentially add value and create new markets. It is important to recognize that although front-end fractionation was a popular concept a few years ago, very few ethanol plants have implemented these technologies in the United States ethanol industry because of:

- the high capital investment required during recent periods of low or negative profits in ethanol production;
- the difficulty of starting up and of keeping the technology functional in ethanol plants;
- a greater emphasis on back-end oil extraction due to more favourable economic return on investment; and
- the undeveloped and uncertain market for new co-products with different nutritional characteristics, resulting in minimal demand for these fractionated maize co-products, with the exception of high-protein DDGS in some export markets.

As a result, only small amounts of some fractionated maize co-products are currently being produced, with minimal effects on the feed industry. Furthermore, even though university researchers began evaluating various fractionated co-products for use in livestock and poultry feeds in anticipation of them becoming more readily available to the feed industry, it is unlikely that front-end fractionation and the resulting co-products will increase in the future.

In contrast, back-end oil extraction is becoming widely implemented in the United States ethanol industry, as expressed by Randy Ives, Gaviol, LLC, Omaha, Nebraska, who is quoted as saying that

“as of mid-2011 approximately 25 percent of the United States dry-grind ethanol plants are removing some maize oil by centrifuging the solubles. I expect that number to probably double by the end of 2011 to 50 percent. We will probably see over 80 percent of the ethanol plants removing at least some of the maize oil by the end of 2012.”

(Personal correspondence with Dr Tilstra, 3 August 2011, and used with permission)

Currently, the range in crude fat content of DDGS sources is increasing (6 to 14 percent on a DM basis) compared with the typical range in crude fat content in DDGS only a few years ago (9 to 13 percent on a DM basis). However, depending upon the extraction equipment and methodology, crude fat levels in DDGS can be as low as 5 percent on a DM basis. Unfortunately, the effects of this oil extraction on digestible, metabolizable and net energy content for livestock and poultry are not known, but research is being conducted to obtain this information, which will be essential for establishing price and value differentials among DDGS sources relative to crude fat content, as well as for accurate diet formulations using reduced-oil co-products.

Front-end fractionation

Fractionation involves separating the maize kernel into three components: the endosperm, the germ and the bran (tip and pericarp). The endosperm represents about 83 percent of the maize kernel and is primarily composed of starch, whereas the germ (about 12 percent of the kernel) is high in oil, protein, ash and non-fermentable carbohydrates. The remaining bran portion is almost exclusively composed of fibre (non-fermentable carbohydrates).

Front-end fractionation involves separating the endosperm, germ and bran fractions before fermentation. The endosperm fraction (rich in starch) is fermented to produce ethanol and a maize co-product. Maize oil is extracted from the germ fraction and marketed, or utilized for various industrial and feeding applications, leaving a maize germ meal as a feed co-product. The bran fraction is also separated and used as a high-fibre feed, primarily for ruminants.

Back-end oil extraction

Back-end oil extraction often involves a two-step process to extract maize oil after the entire maize kernel is fermented to produce ethanol. Crude maize oil is extracted from thin stillage, resulting in low-fat syrup, which undergoes a second extraction along with whole stillage to separate more maize oil. The remaining residue is used to produce a reduced-fat DG co-product.

There are a number of additional fractionation and oil extraction technologies being developed and evaluated, but they have not been widely implemented for ethanol and co-product production. The following are examples of the types of technologies being researched and developed to improve ethanol yield, which if implemented would result in a wider variety of co-products with differing nutrient compositions for use in animal feeds.

1. Efforts to improve the efficiency of fermentation and ethanol production of maize.
 - a) Adding proteases in addition to carbohydrases (Wang *et al.*, 2009b).
 - b) Comparison of raw starch hydrolyzing enzyme

with conventional liquefaction and saccharification enzymes (Wang *et al.*, 2007b).

- c) Use of endogenous liquefaction enzymes (Singh *et al.*, 2006).
 - d) Comparison of enzymatic (E-Mill) and conventional dry-grind maize processes using a granular starch hydrolyzing enzyme (Wang *et al.*, 2005).
2. Pre-treatments and fermentation of DDGS components to increase ethanol yield.
 - a) Pre-treatment protein separation (Brehmer *et al.*, 2008).
 - b) Pre-treatment and enzymatic hydrolysis (Perkis, Tyner and Dale, 2008).
 - c) Fermentation of DDGS hydrolysates to solvents and value-added products by solventogenic clostridia (Ezeji and Blaschek, 2008).
 - d) Use of hot water and ammonia to expand fibre components in DDGS (Dien *et al.*, 2008; Kim *et al.*, 2008b; Kim, Mosier and Ladisch, 2008; Lau, Dale and Balan, 2008).
 - e) Water solubilization of DDGS using phosphate esters (Oshel *et al.*, 2008).
 - f) Use of solid-state fermentation products grown on DDGS (Hoskins and Lyons, 2009).
 3. Fibre separation to enhance ethanol yield.
 - a) from DDG and DDGS (Srinivasan *et al.*, 2005, 2007a, b, 2008a, b; Srinivasan and Singh, 2006; Srinivasan, To and Columbus, 2009).
 - b) Decortication (Corredor *et al.*, 2006).
 - c) Quick germ, quick fibre and enzymatic milling comparisons with the conventional dry-grind maize process (Singh *et al.*, 2005).
 - d) Dry de-germ and de-fibre to separate germ and pericarp fibre of maize prior fermentation of the endosperm fraction fermentation and lipid removal (Murthy *et al.*, 2006).
 4. Oil extraction efficiencies.
 - a) Maize processing methods (Wang *et al.*, 2009a).
 - b) Supercritical CO₂ and hexane extraction of lipids from DDGS (Wang *et al.*, 2008, 2007a).
 - c) *In situ* transesterification for the production of fatty acid esters from DDGS (Haas *et al.*, 2007).
 5. Integrated production of ethanol and biodiesel from DDGS (Balan *et al.*, 2009).
 6. Zein extraction from DDGS (Xu, Reddy and Yang, 2007).

NUTRIENT COMPOSITION, DIGESTIBILITY AND FEEDING VALUE OF NEW MAIZE CO-PRODUCTS FOR LIVESTOCK AND POULTRY

Because fractionation and oil extraction are relatively new and emerging technologies in fuel ethanol production,

TABLE 5
Nutrient composition of new, fractionated maize distiller's co-products (DM basis)

Company co-product	Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)
Typical maize DDGS	89.3	30.9	10.7	7.2	6.0
POET Dakota Gold HP	91.6	44.8	3.9	7.3	2.1
POET Dakota Bran	ND	14.6	9.8	3.8	4.6
POET Dehydrated Maize Germ	93.2	16.9	18.9	5.5	5.8
Maize Processing Innovators Quick Germ/Quick Fibre DDGS	ND	49.3	3.9	6.8	3.2
Maize Processing Innovators E-Mill DDGS	ND	58.5	4.5	2.0	3.2
Cereal Process Technologies Hi-Protein DDGS	ND	35.0–37.0	4.0–6.0	4.0–6.0	ND
Renessen Enhanced DDGS	ND	40.0–50.0	2.5–4.0	7.0–11.0	ND
Solaris NeutraGerm	97.0	17.5	45.0	6.0	1.9
Solaris Probran	90.0	9.5	2.0	16.6	1.0
Solaris Glutenol	90.0	45.0	3.3	3.8	4.0
Solaris Energia	90.0	30.0	2.5	8.2	2.5
FWS Technologies Enhanced DDGS	ND	35.0–37.0	6.5	ND	3.8
De-Oiled DDGS	89.9	31.3	2.3	ND	6.2
J. Jireh Products Dried Condensed Solubles	93.4	21.6	4.7	3.1	8.3

Notes: ND = not determined. Source: Shurson and Alghadi, 2008.

there are limited data on nutrient composition, digestibility and feeding value of these new co-products. Dry matter, crude protein, crude fat, crude fibre and ash concentrations for most of the known fractionated co-products are shown in Table 5 (Shurson and Alghadi, 2008). In general, most fractionated maize co-products are higher in crude protein and crude fibre and lower in crude fat than DDGS. Although amino acid concentration may slightly increase in many of the high-protein fractionated co-products, the protein quality (amino acid balance) is still poor relative to the requirements of monogastric animals. The reduced fat and increased fibre content of these fractions will probably result in lower energy value for swine and poultry. Therefore, the feeding and economic value of these fractions may be reduced compared with that of "typical"

DDGS for monogastric animals. However, based on the nutrient composition of these co-products, they generally appear to have greater value in ruminant diets because the amino acid balance of maize protein is not as critical in ruminant diets as it is in swine, poultry and aquaculture diets. Furthermore, the increased amount of readily fermentable fibre can provide a good source of energy for ruminants, and the lower fat content may allow higher dietary inclusion rates for lactating dairy cows and reduce concerns regarding milk fat depression at high feeding levels.

A summary of published studies evaluating the nutritional value of some of the new, fractionated maize co-products to various livestock and poultry species are shown in Table 6. The majority of these studies have evaluated

TABLE 6
Summary of published studies involving feeding new fractionated maize co-products to livestock and poultry

Species	HP-DDG	De-oiled DDG	Maize germ	Maize bran	Other
Beef feedlot cattle				Bremer <i>et al.</i> , 2006; Berger and Singh, 2009	Partial DDGS fractionation – Depenbusch <i>et al.</i> , 2008
Lactating dairy cows	Kelzer <i>et al.</i> , 2007; Mjoun <i>et al.</i> , 2009b	Mjoun <i>et al.</i> , 2009a, b	Kelzer <i>et al.</i> , 2007; Abdelqader <i>et al.</i> , 2006	Kelzer <i>et al.</i> , 2007; Janicek <i>et al.</i> , 2007	
Growing-finishing swine	Widmer, McGinnis and Stein, 2007; Widmer <i>et al.</i> , 2008; Gutierrez, Kil and Stein, 2009; Anderson, Shurson and Kerr, 2009	Anderson, Shurson and Kerr, 2009	Widmer, McGinnis and Stein, 2007; Widmer <i>et al.</i> , 2008; Anderson, Shurson and Kerr, 2009	Anderson, Shurson and Kerr, 2009	Yeast product – Stein <i>et al.</i> , 2005; Dried condensed soluble and dehydrated, de-germed maize co-products – Anderson, Shurson and Kerr, 2009; Helembai, Hausenblas and Mezes, 2006
Broilers	Batal, 2007; Kim <i>et al.</i> , 2008a		Batal, 2007; Kim <i>et al.</i> , 2008a	Batal, 2007	
Layers	Batal, 2007; Kim <i>et al.</i> , 2008a		Batal, 2007; Kim <i>et al.</i> , 2008a	Batal, 2007	
Turkeys	Batal, 2007; Kim <i>et al.</i> , 2008a		Batal, 2007; Kim <i>et al.</i> , 2008a	Batal, 2007	High protein hydrolyzed maize co-product – Abe <i>et al.</i> , 2004

nutrient content and digestibility, but not maximum dietary inclusion rates or their effects on animal performance. Until significant quantities of new maize co-products are produced commercially—and more research conducted—it is difficult to determine their comparative feeding value for various animal species.

OTHER EMERGING OR POTENTIAL PROCESSING AND MAIZE CO-PRODUCT PRODUCTION TECHNOLOGIES

Phosphorus extraction

The phosphorus content of maize is concentrated three times during the ethanol and DDGS production process, and also becomes more bio-available (50 to 60 percent) in DDGS for non-ruminant animals compared with maize (approximately 15 percent), because a portion of phytate P is converted to inorganic P (Noureddini *et al.*, 2009). This makes DDGS an excellent phosphorus source for animal feed. However, the total phosphorus in DDGS is significantly higher than maize and 40 to 50 percent of it remains stored in phytate-mineral complexes. At current dietary DDGS inclusion rates, animals are consuming more phosphorus than they require. Under normal physiological conditions, the dietary phytate phosphorus that cannot be utilized by the animal will pass through the gastrointestinal tract and eventually result in high phosphorus content in manure that could pollute surface waters and create environmental concerns.

Phytic acid and phytate are valuable chemicals that are widely used in many commercial applications in the food, industrial and medical fields. Currently, there are no major phytic acid manufacturers in the United States and all of the phytic acid and its salts sold domestically are either imported or toll-manufactured. The major commercial production companies are primarily located in Japan and China. Phytic acid can be produced from many types of feed commodities, such as bran from maize, rice and wheat, and from cottonseed meal. However, maize co-products can be a practical source for manufacturing phytic acid in the United States, considering the volume of co-products currently being produced. The extraction is a relatively simple and mature process, and easy to implement commercially. Furthermore, after phytate extraction, the residual material can still be used as a valuable feed ingredient in animal feeds. Applying this process to DDGS would create extra profits and job opportunities for ethanol plants by selling a commercially valuable product currently imported. Phytate extraction has many positive benefits, including removal of undigestible phytate phosphorus from DDGS, decreasing phosphorus excretion in manure, and increasing nutritional value of DDGS by improving the digestibility of other nutrients previously immobilized by phytate.

Fibre extraction

Several methods have been developed to extract the fibre from maize before fermentation, or extract fibre from the DG after fermentation, which have created substantial opportunities to add value to the ethanol and maize co-product production process (Brekke, no date). Maize grain contains about 2 percent fibre, and can be more conveniently and economically utilized as a feedstock for further ethanol production (with the appropriate technology) compared with most other sources of fibre because alternatives imply substantial cost for material handling and transport. Once fibre is removed by fractionation, it has two potentially valuable uses in an ethanol plant: it can serve as fuel source in a biomass boiler for operating the plant; or, if cellulosic ethanol production technologies are added to an existing maize ethanol plant, fibre can serve as an additional feedstock for ethanol production. In either case, the DG resulting from a process that removes some, or most, of the fibre (and only the fibre) would be expected to be higher in protein, fat and other nutrients than DG co-products produced using the entire maize kernel.

Maize fibre also has potential food and feed uses outside of the ethanol plant. For example, maize fibre can be used as a fibre supplement in human nutrition. For animal feeds, POET, one of the major ethanol producers in the United States, is producing and marketing a product called Dakota Bran™ that includes maize bran as one of its principle components (DakotaGold, 2007).

Drying systems

Nutritional quality and digestibility of DDGS is highly dependent on the drying process used in ethanol production facilities. In general, the dry-grind ethanol industry utilizes rotary drum driers that are fueled with natural gas. Internal temperatures range from 500 °F [250 °C] to as much as 1100 °F [600 °C]. As ethanol fermentation efficiency improves, drier system capacity can be exceeded, resulting in shorter drying times and the use of higher temperatures to increase throughput, which can negatively affect the nutritional value of DDGS. High temperatures and prolonged drying time can result in damage to amino acids and destruction of some other nutrients. As a result, some ethanol plants use multiple rotary drum driers in their systems to reduce heat damage to DDGS.

Use of microwaves is a relatively new drying technology, and is only being used by a few ethanol plants in the industry. Microwaves are radio waves that cause water molecules to vibrate very fast, and the friction between the vibrating water molecules produces the heat that allows drying. According to literature from Cellencor™, one of the companies providing microwave systems to the ethanol industry in the United States, the temperature of the DDGS does not exceed 200 °F [93 °C] in the drying process, “so the

full nutritional goodness of the DDG/DDGS is preserved.” (Cellencor, 2011). However, in a recent study by Anderson, Shurson and Kerr (2009), there was not a large difference in digestible or metabolizable energy for swine between DDGS produced by a rotary drum-drier or a microwave-drying system in the same ethanol plant.

Industrial microwave systems appear to be more energy efficient than natural gas powered systems, requiring less than half as much energy per kg of water removed from the DG during the drying process. In addition, microwave drying systems can be used to dehydrate a variety of co-products produced by the various fractionation systems being developed and implemented in the ethanol industry.

Feedstock source: maize vs other grains

Ethanol production is a result of the fermentation of readily fermentable carbohydrates (sugars and starch) into alcohol. Therefore, when feedstocks with high sugar or starch content are used, greater ethanol yield occurs (Table 7). However, the availability of grains, agronomic growing conditions (e.g. soils and weather) and local markets also determine the type of feedstock used to produce ethanol. For example, Brazil, the world's second-largest fuel ethanol producer and a leading exporter of ethanol, utilizes sugar cane as a feedstock because it has higher sugar content than cereal grains, and there are favorable soil and weather conditions that allow economic production of large quantities of sugar cane (Conti, 2006).

The agronomic conditions in Canada and Europe are best suited for wheat and barley production; therefore, those grains are used as feedstock for ethanol production. In the United States Midwest, maize is the feedstock of choice because of its high yield, low cost and abundant supply. A few ethanol plants in Great Plains states of the United States blend milo [grain sorghum] with maize to produce ethanol, in order to take advantage of availability and low local prices. Depending upon the feedstock used, ethanol yield varies and the nutrient content and digestibility of the co-products varies as well. The extent of future ethanol growth in the United States and the world will be determined by the availability and price for various feedstocks, and this will determine the amount and nutritional

characteristics of the co-products available to the feed industry in the future.

Feedstock source: cellulosic

Many politicians, agriculturalists and environmentalists have questioned the long-term sustainability of using maize as the feedstock for ethanol production in the United States, particularly now with very low carryover stocks in the maize inventory, as well as record high maize, feed, livestock and food prices. In order to reduce demand for maize used in ethanol production and use more sustainable feedstocks, millions of research dollars have been invested over the past decade—and continue to be invested—in intensive research and development of cellulosic ethanol technology. Cellulosic ethanol is often referred to as “second-generation bio-ethanol”. Although ethanol from cellulosic feedstocks is not currently being produced commercially, several ethanol production plants are under development that will use various biochemical and thermochemical conversion processes.

Feedstocks high in cellulose are more difficult to convert into fermentable sugars than starch (from grain) and sugars (sugar cane). Therefore, additional biochemical treatments are required beyond current ethanol production processes. These include pre-treatment of the feedstock to convert the hemicellulose fraction into simple sugars and separate them for fermentation, along with cellulose hydrolysis to produce glucose, which can then be used as the substrate for yeast to produce ethanol. Alternatively, thermochemical processes can be used to convert cellulosic biomass into ethanol. This process involves heat and chemicals to convert the biomass into syngas, which is a mixture of carbon monoxide and hydrogen, and these molecules are converted into ethanol. Regardless of the type of process used, the resulting by-products will probably consist of lignin, minerals and perhaps other residual fibrous material, and will have very low, if any, feeding value for animals.

Feedstock source: algae

There is increasing interest in using algae as a feedstock for second-generation biofuels production, and research is being conducted to develop technologies. Algae contain relatively high amounts of starch inside their cells, and cellulose in their thin cell walls, both of which, with the appropriate processes, can be more easily converted into ethanol than those used in cellulosic ethanol production. The lipids in algae oil can be used to produce biodiesel. However, the actual number and commercial value of algal biofuel co-products is unknown because these co-products do not exist today. Algal biofuel processes may theoretically result in more co-products with higher value relative to maize ethanol, but these technologies and co-products have not been evaluated on a commercial scale.

TABLE 7
Starch content and ethanol yield of various feedstocks

Feedstock	Moisture (%)	Starch (%)	Ethanol yield (litres per tonne)
Starch	–	100.0	720
Sugar cane	–	–	654
Barley	9.7	67.1	399
Maize	13.8	71.8	408
Oats	10.9	44.7	262
Wheat	10.9	63.8	375

Source: Saskatchewan Agriculture and Food, 1993.

The process of producing ethanol from algae begins with growing starch-accumulating, filament-forming or colony-forming algae in an aquaculture environment. After the algae have been grown, they are harvested to provide biomass, and decomposition of the biomass initiated. The decomposition process can be done mechanically or non-mechanically to rupture the cells. Yeast is added to the biomass to begin fermentation and convert carbohydrates to ethanol. Ethanol is then harvested after fermentation is complete. It is not known if the residual biomass from algae bio-ethanol production will have significant feeding value for animals. Additionally, there is research underway to determine the feasibility of producing ethanol from seaweed, as well as combining cellulosic ethanol and biodiesel technology in an attempt to gain greater efficiencies in biofuels production than from those processes currently being used.

FEED AND FOOD SAFETY QUESTIONS

We are at a critical point in history, where intelligent decisions need to be made not only about the need to continue to develop and use new food production technology to feed the world, but also to provide realistic risk assessment of any potential short- and long-term consequences of using these technologies. For example, some countries have embraced the use of genetically modified grains in animal feeds and recognize them as safe. In contrast, other countries have been reluctant to accept the use of this technology, which consequently limits their choices and increases the cost of food.

Several characteristics of DG have been identified as potential animal or human health risk factors. However, knowledge, tools and product options exist to mitigate or eliminate many or all of the potential risk factors discussed in this section.

Genetically modified grains

Approximately 98 percent of the maize produced in the United States is from genetically modified varieties. Farmers prefer to grow these varieties because of their better yields, whether economic or agronomic. As a result, maize seed companies are continually developing new genetically modified maize varieties that possess economically important agronomic traits. For example, a new genetically modified maize variety (Event 3272, released as cv. Enogen) has been developed by Syngenta Seeds, Inc., with the goals of improving ethanol yields while reducing energy costs and greenhouse gas emissions. The use of Enogen grain by United States ethanol producers could provide a 380 million litre ethanol plant with annual efficiency improvements that save 1.7 million litres of water, 1.3 GWh of electricity and 244 billion BTUs of natural gas, which is equivalent to the amount of power needed to heat several thousand

homes, while reducing carbon dioxide emissions by 48 000 tonne. These are all very positive. Syngenta requested that the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) grant non-regulated status to its alpha-amylase maize ('Event 3272') in 2005. It was approved by the US Food and Drug Administration (FDA) for human food consumption in 2007, and in February 2011 APHIS announced its decision to deregulate this new variety of maize, which has now been cleared by USDA for production. However, it is important to recognize that only a few thousand acres have been planted with this new maize cultivar, and only a few ethanol plants are involved in evaluating its potential benefits. What will be the acceptance of the maize by-products for feed use in countries outside of the United States?

Sulphur

Sulphur is an essential mineral for animals and serves many important biological functions in the animal's body. However, when excess sulphur is present in ruminant diets, neurological problems can occur. When feed and water containing high levels of sulphur (>0.40 percent of diet DM) are fed to ruminants, a condition called polioencephalomalacia (PEM) can occur. PEM is caused by necrosis of the cerebrocortical region of the brain of cattle, sheep and goats, and if not treated with thiamin within 48 hours after the onset of this condition, animals will die. Ruminants are more vulnerable to PEM when their diets are abruptly changed from being primarily forage to primarily grain based, causing a shift in rumen microbial populations to produce thiaminase, resulting in a thiamin deficiency. Sulphur appears to have a significant role and interaction with thiaminase production to cause this condition, but the mechanism is not well understood (Boyles, 2007). This condition does not occur in non-ruminant animals (pigs, poultry, fish).

Sulphur levels can be highly variable among DDGS sources and can range from 0.31 to 1.93 percent (average 0.69 percent) on a DM basis. Sulphuric acid is commonly added during the dry-grind ethanol production process to keep pH at desirable levels for optimal yeast propagation and fermentation to convert starch to ethanol, and is used because of its lower cost relative to other acids. According to AAFCO Official Publication 2004, p. 386, sulphuric acid is generally recognized as safe according to US Code of Federal Regulation (21 CFR 582) and is listed as an approved food additive (21 CFR 573). In addition, maize naturally contains about 0.12 percent sulphur, and this is concentrated three times like all other nutrients when maize is used to produce ethanol and DDGS. Yeast also contains about 3.9 g/kg sulphur, and naturally creates sulphites during fermentation.

Table 8 shows examples of the impact on final diet sulphur levels of adding different dietary levels of DDGS,

TABLE 8
Effect of sulphur content of DDGS and dietary inclusion rate (DM basis) on total dietary sulphur content in maize+maize silage-based diets for beef cattle

DDGS inclusion rate (% DM)	0.60% S in DDGS	0.80% S in DDGS	1.0% S in DDGS
20	0.21	0.25	0.29
30	0.27	0.33	0.37
40	0.33	0.41	0.49

Source: from Boyles, 2007.

containing different levels of sulphur, to beef cattle diets comprising maize and maize silage, assuming low sulphate levels in drinking water. These data show that at high dietary inclusion rates (40 percent of DM intake) and sulphur levels in DDGS greater than 0.80 percent, total dietary sulphur levels would exceed the 0.40 percent considered to be the threshold level for causing PEM. If DDGS is fed to cattle, the sulphur content should be determined and considered along with the feeding level and sulphur contributions from other dietary ingredients to ensure that total dietary sulphur content does not exceed 0.40 percent.

The sulphur content of DDGS may also contribute to an increased animal risk of Mulberry Heart Disease, which is a vitamin E or selenium deficiency, or a combination. High dietary sulphate (Halvorson, Guss, and Olson, 1962) or cysteine (Lowry and Baker, 1989) concentrations can be antagonistic to the utilization and bioavailability of high levels of selenium, in the form of selenate or selenite (Halvorson and Monty, 1960; Ardüser, Wolfram and Scharrer, 1985). However, little research has been conducted to examine the sulphur-selenium relationship in animal diets containing supplemental selenium. In addition to selenium, vitamin E (dl- α -tocopheryl acetate) bio-availability may be impaired as a result of excess dietary sulphur (Boyazoglu, Jordan and Meade, 1967). These results indicate that feeding excessive dietary sulphur may decrease the bio-availability of both selenium and vitamin E, and should be considered when formulating diets containing DDGS with high sulphur levels.

Mycotoxins

If mycotoxins are present in the grain used to produce ethanol and DDGS, they are not detoxified during the production process but instead, are concentrated by a factor of approximately three. In a recent review, Zhang *et al.* (2009) showed that unless there is a high prevalence of mycotoxin contamination during a given crop year, there is minimal concern regarding mycotoxins in the resulting DDGS. Their results indicated that: (1) none of the samples contained aflatoxin or deoxynivalenol levels higher than the FDA guidelines for use in animal feed; (2) no more than 10 percent of the samples contained fumonisin levels higher than the recommendation for feeding equids and rabbits, and the rest of the samples contained fumonisin lower than

FDA guidelines for use in animal feed; (3) none of the samples contained T-2 higher than detection limit, and no FDA guidance levels are available for T-2 toxin; (4) most samples contained no detectable zearalenone levels, and no FDA guidance levels are available for zearalenone; (5) the containers used for export shipping of DDGS did not contribute to mycotoxin production.

Fat oxidation

DDGS contains approximately 10 percent maize oil. Maize oil contains high levels of polyunsaturated fatty acids (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation. During lipid peroxidation, a wide variety of toxic aldehydes are produced that have been shown to be related to cell death, gene mutations and a series of diseases, including cancer in both animals and humans (Kritchevsky, 1991; Owen *et al.*, 1997; Hussain, Hofseth and Harris, 2003). Drying temperatures used by ethanol plants can vary substantially (185 °F to 1100 °F [85 °C to 600 °C]) and increased drying time and temperature used during the drying process accelerates lipid peroxidation. To better understand the levels of lipid peroxidation, as well as the toxic aldehydes among DDGS sources, Song, Csallany and Shurson (2010) measured thiobarbituric acid reactive substances (TBARS) and peroxide value (PV), which are common analytical methods, to measure lipid peroxidation, in DDGS samples obtained from 31 ethanol plants in the USA. The range in TBARS among DDGS samples was from 1.0 to 5.2 ng malondialdehyde equivalents/mg oil, and PV ranged from 4.2 to 84.1 meq/kg oil. The DDGS sample with the highest TBARS and PV values was 25 and 27 times greater, respectively, than the level found in maize. These results suggest that lipid peroxidation level varies among DDGS sources. It appears that feeding high levels of highly oxidized sources of DDGS to pigs may require supplementation with higher levels of antioxidants (e.g. vitamin E) than are currently being fed, in order to minimize the potential negative effects on pig health, growth performance and quality of meat products. Harrell, Zhao and Reznik (2011) and Harrell *et al.* (2010) showed that adding an antioxidant (AGRADOPLUS, Novus International Inc.) to the diet improved growth performance for pigs fed DDGS and the oxidized maize oil diets. The beneficial effects of dietary vitamin E on improving oxidative stability of pork chops and ground pork during storage were also demonstrated by Asghar *et al.* (1991).

Antibiotic residues

Antibiotics have been used for many years to control bacterial infections during fermentation in ethanol production, and virginiamycin and penicillin have been the most commonly used. When antibiotics are used, they are added to fermenters in very small quantities relative to usage rates in

animal feeds. For example, when virginiamycin (Lactrol) is added to fermenters, it is typically added at levels of 0.25 to 2.0 ppm, whereas when virginiamycin (Stafac) is added to swine feeds it is at levels 5.5 to 110 ppm. In November 1993 the FDA's Center for Veterinary Medicine issued a "letter of no objection" for the use of virginiamycin in ethanol and DDGS production. No other antibiotics were included in this letter of no objection. Currently, there are minimal guidelines and no FDA regulatory enforcement and monitoring of antimicrobial residues in distillers co-products produced by fuel ethanol plants. Recently, the FDA has expressed three primary concerns related to antibiotic residues in DG: (1) the potential for transfer of antibiotic residues from DG to animal tissues; (2) the potential harm to humans who eat animal tissues containing antibiotic residues; and (3) the potential harm to animal health if antibiotic residues are present in DG. The prevalence of antibiotic use in the ethanol industry, the level of residue detection and the presence of biological activity in residues in DG is unknown. Because of these concerns and limited data on the extent and levels of antibiotic use in ethanol and DG production, the FDA initiated a nationwide survey in December 2007. The FDA has not published these results, nor commented on their health and safety implications, nor implemented regulatory action to date.

Bacteria in distillers co-products

The possibility of bacterial presence in co-products does exist. The Center for Veterinary Medicine at the FDA conducted a survey of plant-derived protein animal feed ingredients in 2003, of which 79 samples were collected from a variety of oil-seed meals and cereal grain-based products. Some of the samples showed presence of *Salmonella*, *E. coli* or *Enterococcus* bacteria, either singly or in combination (Headrick *et al.*, 2004). Enteric strains of bacteria are associated with food-borne illness in animals and people. When serious human infections occur, they are newsworthy, as shown by the 2011 *E. coli* outbreak in Europe that was associated with consumption of alfalfa sprouts from a single source.

In 2007, there was a dramatic increase in interest in identifying and understanding the possible reasons for the increases in *E. coli* O157:H7 in ground beef contamination in the United States. Because of the exponential increase in ethanol and DG production during this same period, there were some suspicions that feeding DG were contributing to this problem. As a result, researchers began conducting studies to determine if there was a relationship between feeding DG with solubles and the increased incidence of *E. coli* O157:H7 in beef. A series of controversial studies conducted by researchers at Kansas State University (Jacob *et al.*, 2008a, b, c) showed low prevalence and inconsistent responses to *E. coli* O157:H7 shedding in feedlot cattle fed

DG diets. Despite these inconsistent results, these researchers concluded that feeding DG increased faecal *E. coli* O157:H7 shedding in beef feedlot cattle.

Subsequent to the Kansas State University reports, researchers at the University of Nebraska (Peterson *et al.*, 2007) fed up to 50 percent (DM basis) wet DG diets and showed that *E. coli* O157:H7 shedding occurred, but the level of shedding was no different from cattle fed diets containing no DG. These results were not in agreement with those reported by Jacob *et al.* (2008a, b, c). Furthermore, Nagaraja *et al.* (2008) collected manure samples from 700 cattle fed either control or DDGS diets for 150 days and showed that the overall prevalence of *E. coli* O157:H7 shedding was low (5.1 percent) and feeding DDGS had no effect. The most recent study conducted by Jacob *et al.* (2009) showed no differences in faecal prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in cattle fed dry-rolled maize or DDGS. Currently, there is no scientific evidence suggesting that the levels of DDGS being fed is a cause for *E. coli* O157:H7 contamination in ground beef. It is important to recognize that bacterial contamination (including *E. coli* O157:H7) in the meat supply can occur during many segments of the food chain, and is not restricted to feed or feed ingredients. In contrast, studies in swine have actually shown that feeding diets containing DDGS have positive effects in improving gut health of pigs (Whitney, Shurson and Guedes, 2006; Perez and Pettigrew, 2010).

EXPANDED USES OF CO-PRODUCTS

Aquaculture

Aquaculture is one of the fastest growing food production industries in the world. Fishmeal has traditionally been used in commercial fish feed as a major source of dietary protein for many years. However, when global fishmeal production declines and fishmeal prices increase, fish nutritionists begin considering less expensive plant protein sources. Plant protein sources have traditionally been considered to be inferior to fishmeal in fish diets. However, when two or more complementary plant protein sources (DDGS and soybean meal) are added to the diet, the potential exists to replace all of the fishmeal in the diet. Therefore, to reduce diet cost, fish nutritionists are continually evaluating alternative plant protein sources as a means to reduce or replace expensive fishmeal sources. As a result, there is increasing interest in using DDGS in aquaculture diets around the world, due to its moderately high protein content, relatively low phosphorus content and low cost compared with fishmeal. Furthermore, DDGS does not contain antinutritional factors found in other protein sources such as soybean meal (trypsin inhibitors) or cottonseed meal (gossypol).

Aquaculture, like livestock and poultry production, is also subject to increasing environmental regulation. The

two nutrients of greatest concern in fish farm effluent water are nitrogen and phosphorus. Soybean meal and DDGS are relatively high in protein, but much lower in phosphorus than fishmeal. As a result, substituting DDGS and soybean meal for fishmeal in aquaculture diets reduces the total phosphorus level in the diet and lowers the level of phosphorus in fish farm discharge water. There have been a considerable number of research studies conducted on the effects of feeding various levels of DDGS to different species of fish, but, unfortunately, DDGS use in aquaculture feeds has been limited. Based upon the following research studies, maximum dietary inclusion of DDGS in aquaculture diets are shown in Table 9.

- Channel catfish (*Ictalurus punctatus*) – Wilson and Poe, 1985; Shiao, Chuang and Sun, 1987; Jauncey and Ross, 1982; Robinson, 1991; Tidwell, Webster and Yancey, 1990; Webster, Tidwell and Yancey, 1991; Webster *et al.*, 1993; Robinson and Li, 2008; Lim, Yildirim-Aksoy and Klesius, 2009.
- Rainbow trout (*Oncorhynchus mykiss*) – Cheng and Hardy, 2004a, b; Cheng, Hardy and Blair, 2003; Stone *et al.*, 2005.
- Freshwater prawns (*Macrobrachium rosenbergii*) – Tidwell *et al.*, 1993a, b; Coyle, Najeeullah and Tidwell, 1996.
- Pacific white shrimp (*Litopenaeus vannamei*) – Lim, Yildirim-Aksoy and Klesius, 2009.
- Tilapia (*Oreochromis niloticus*) – Wu *et al.*, 1994; Wu, Rosati and Brown, 1996, 1997; Tidwell *et al.*, 2000; Lim *et al.*, 2007.
- Sunshine bass (*Morone chrysops* × *M. saxatilis*) – Thompson *et al.*, 2008.

TABLE 9
Current, revised recommendations for maximum dietary inclusion rates of DDGS for various species of fish

Species	% DDGS	Comments
Catfish	Up to 30%	
Trout	Up to 15%	Without synthetic lysine and methionine supplementation
Trout	Up to 22.5%	With synthetic lysine and methionine supplementation
Salmon	Up to 10%	
Freshwater prawns	Up to 40%	Can replace some or all of the fishmeal in the diet
Shrimp	Up to 10%	Can replace an equivalent amount of fishmeal
Tilapia	Up to 20%	Without synthetic lysine and supplementation in high protein diets (40% CP)
Tilapia	Up to 82%	With synthetic lysine and tryptophan supplementation in low protein diets (28% CP)

Horses, rabbits and companion animals

There are significant opportunities to increase the use of DG co-products in non-traditional markets for use in horse, rabbit and companion animal feeds. Based upon the limited research information available, it appears DDGS is a very suitable ingredient for use in horse (Bonoma *et al.*, 2008; Hill, 2002; Leonard, Baker and Willard, 1975; Orme *et al.*, 1997; Pagan, 1991), rabbit (Villamide, de Blas and Carabano, 1989) and dog (Allen *et al.*, 1981; Corbin, 1984) diets. However, very little DDGS has been used in diets for companion animals, primarily due to current perceptions about the risk of mycotoxin contamination.

Human foods

One potentially large, undeveloped market for distillers co-products is the human food and nutraceutical market. DG is suitable for human consumption provided that the maize used to produce ethanol and DG is food grade and the production facility is approved for food production. Components in DG that are a potential concern for use in human foods include: amount of yeast cells (nucleic acids), bacteria, low lysine relative to protein levels, metal contamination and antibiotic residues. However, considerable research must be conducted to determine appropriate human food applications for DG.

DG has many nutritional components that give it potential for use as a functional food in human nutrition, and it also has nutraceutical properties (Plate and Gallaher, 2005). Unfortunately, there is limited information on the effects of distillers co-product consumption on human health. Some of the important nutritional components found in maize distillers co-products include unsaturated fatty acids; antioxidants and phenolic acids; beta glucans; fibre; and xanthophylls.

Several components of maize (i.e. arabinoxylans, phytosterols and xanthophylls) have been shown to be effective in lowering cholesterol, which may have benefits for reducing cardiovascular disease in humans. Maize and its co-products are higher in natural antioxidants (e.g. ferulic acid) than other grains, and these antioxidants have been shown to be effective in reducing colon cancer and controlling type 1 diabetes. Maize distillers co-products are high in carotenoid pigments called xanthophylls. Two of these pigments, lutein and zeaxanthin, are uniquely concentrated in the macular region of the retina and are associated with preventing macular degeneration, several types of cancer, and coronary artery disease.

Research is being conducted to evaluate therapeutic uses for maize-based distiller's co-products, thereby increasing the value of these co-products. The value of distiller's co-products will be increased if it can be demonstrated that they have functional food or nutraceutical properties beneficial for human health, rather than being strictly used as

an animal feed ingredient. This could potentially change the marketing strategy and profit margins of an ethanol plant, where higher value and increased margins from distillers co-products would allow an ethanol plant to sell ethanol at a lower price and still remain profitable. Demonstrating that maize-based distiller's co-products have functional food or nutraceutical properties will also result in job creation in the food sector, because companies will explore new and innovative uses for these co-products in human food and nutraceutical products. Using sound, scientific research to show human health benefits from maize-based distillers co-products will increase demand and establish a potentially large, new market for these co-products.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Although much is known about the nutritional value, feeding applications, and benefits and limitations of maize distillers co-products, research is needed to obtain new knowledge in several important areas to further increase demand and expand the market for these ingredients.

- Continue to develop and refine prediction equations and various “nutritional tools” to provide practical, inexpensive and rapid estimates of nutrient content and digestibility, as well as relative value among identity-preserved DDGS sources.
- Explore nutritional strategies to overcome limitations affecting maximum dietary inclusion rates for livestock and poultry.
- Determine nutrient content, digestibility and feeding applications of new and emerging maize co-products.
- Determine the effects of feeding maize co-products on animal health and feed safety.
- Determine the need for antioxidants to preserve shelf life of DDGS under hot, humid conditions and in long-term storage, as well as in animal feeds.
- Evaluate feeding applications in aquaculture, pet foods, horses and rabbits.
- Determine nutraceutical properties of distillers co-products and their potential benefits to human health and use in human foods.

CONCLUSIONS

Although 140 biodiesel plants produced 1.19 billion litres of biodiesel in 2010, very little crude glycerin has been used in animal feeds in the United States due to the relatively low volume produced compared with ethanol industry co-products, and its higher value for consumer products and industrial manufacturing. DG co-products have a long history of being fed to food producing animals, but only recently, have they revolutionized animal diets around the world. Most of these changes have been a result of an abundant and growing supply of co-products, increased

nutritional knowledge of how to optimize their use and value, and competitive prices relative to competing ingredients. Further opportunities exist to expand their use in current and other, as yet undeveloped, markets, but production processing and other nutritional technologies must be developed through additional research and education to overcome their real or perceived limitations. As the United States ethanol industry continues to evolve, there will be new niches and feeding applications developed for the new co-products produced.

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Chapter 4

Utilization of wet distillers grains in high-energy beef cattle diets based on processed grain

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ABSTRACT

Distillers grains (DG) are used extensively by beef cattle feeding operations in the United States, including the Southern Great Plains. Our regional research consortium has conducted research focused on utilization of wet DG in feedlot diets based on steam flaked maize (SFC). Effects of DG on feedlot cattle performance are influenced by source and concentration of DG in the diet. In SFC-based diets, DG concentrations of 15 to 60 percent in the dry matter (DM) decreased gain efficiency, with effects seemingly related to the neutral-detergent fibre (NDF) content of wet DG and associated changes in ruminal DM and NDF digestibility. Thus, the exchange of starch for fibre plays an important role in digestion and animal performance as DG is added to a SFC-based diet. Wet DG contributes a unique source of fat to the diet; however, our findings indicate that fat contained in sorghum DG and in a commonly used commercial source (yellow grease – an animal-vegetable fat blend) are utilized in a similar manner. Exchanging DG for SFC and oilseed meals typically decreases degradability of crude protein (CP) and often increases the total dietary CP. Our results indicated that between 0.52 and 0.78 percent urea was needed to optimize feedlot performance with diets containing 15 percent wet DG, but added urea was not beneficial when the diet contained 30 percent DG, presumably reflecting recycling of excess CP in 30 percent DG diets. Although interactions between DG concentration and grain processing method have been reported, our results with SFC- vs dry-rolled maize-based diets have not provided evidence of an interaction. The relative difference in net energy for gain (NE_g) concentration between DG and the basal grain it replaces seems to provide a reasonable explanation for differences in feedlot performance when DG is fed with different processed grains. High concentrations of sulphur (S) and their effects on health and performance of feedlot cattle are a practical concern when including DG in feedlot diets. In our work, feed additives like ionophores and antibiotics did not increase *in vitro* ruminal hydrogen sulphide, but H₂S production was clearly responsive to dietary S concentration. Manure production varies with the concentration of DG in the diet, basal grain processing method, and other dietary ingredients. The quantity of manure collected and the phosphorus (P) excreted in manure from feedlot pens increases with addition of DG to finishing diets. When applied to meet crop P requirements, farmland required to utilize the manure increases approximately 20 percent for each 10 percent increase in wet DG in the diet. Greenhouse gas emissions from feedlots using DG are variable and need further study. Overall, our results suggest that DG can be a useful source of energy and protein in feedlot diets, but optimal concentrations of DG are less in SFC-based diets than in diets based on minimally processed maize.

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MAIN MESSAGES

- Effects of DG on feedlot cattle performance are influenced by source and concentration of DG in the diet.
- The exchange of starch for fibre plays an important role in digestion and animal performance as DG is added to an SFC-based diet.
- The relative difference in NE_g concentration between DG and the basal grain it replaces provides a reasonable explanation for differences in feedlot performance among different grain processing methods.
- Partially replacing highly processed grain in feedlot diets with wet DG will probably increase excretion of N, P and S, and high S concentrations might have negative consequences for animal health.
- Implications for animal health and digestive disorders of feeding wet DG have not been extensively evaluated and could be an important area of research.

INTRODUCTION

Cattle feedlots in the United States have historically relied on inexpensive supplies of grain, with diets formulated to maximize grain inclusion and limit use of more costly protein and roughage sources. In the Southern Great Plains region, feedlots further refined grain utilization through extensive processing to gelatinize starch, typically by steam flaking of maize and sorghum (Vasconcelos and Galvayan, 2007). Expansion of fuel ethanol production in the United States via dry milling has greatly increased availability of grain byproducts, such that distillers grains (DG; with or without condensed distillers solubles) are now widely available in the major beef cattle feeding regions of the United States, including the Southern Great Plains. From the standpoint of overall returns, replacing grain by DG is often economically advantageous, which has markedly increased the use DG in feedlot diets (Vasconcelos and Galvayan, 2007). Based on research conducted in the Midwest United States, primarily with diets based on minimally processed grain (e.g. dry-rolled maize – DRC), adding DG to feedlot diets has positive effects on performance, even at concentrations up to 50 percent of the dietary dry matter (DM) with wet DG (Klopfenstein, Erickson and Bremer, 2008). Because steam-flaked maize (SFC) is the primary grain in feedlot diets in the Southern Great Plains, and its net energy (NE) value is considerably greater than DRC (Zinn, Owens and Ware, 2002), we embarked on a 4-year cooperative effort involving a consortium of 4 institutions and agencies to evaluate utilization of wet DG in feedlot diets based on SFC. Our objective in this review is to summarize research we have conducted with feedlot cattle in our major focus areas: source and concentration of DG; the role of specific nutrients and feed ingredients; potential interactions of grain processing and feed additives; and the environmental impact of using wet DG in feedlot diets in terms of nitrogen (N), phosphorus (P) and greenhouse gas emissions.

CONCENTRATION AND SOURCE OF DISTILLERS GRAINS

The effect of DG on performance seems to be influenced by both the source and concentration of DG in the diet. Klopfenstein, Erickson and Bremer (2008) presented results of 2 meta-analyses that suggested DG has a feeding value as much as 78 percent greater than DRC. In contrast, values reported from research in the Southern Plains with SFC-based diets were much less, with Vasconcelos *et al.* (2007) reporting 12 percent decrease in gain:feed ratio (G:F) when 15 percent wet sorghum DG was included in a SFC-based diet. In our more recent work, Luebbe *et al.* (2010a) observed decreased G:F with incorporation of 15 to 60 percent DG into SFC-based diets. Figure 1 shows the difference in G:F response to adding wet DG to SFC-based diets with supplemental fat added to achieve a minimum dietary fat concentration of 6.5 percent (Luebbe *et al.*, 2010a) compared with adding wet DG to DRC-based diets

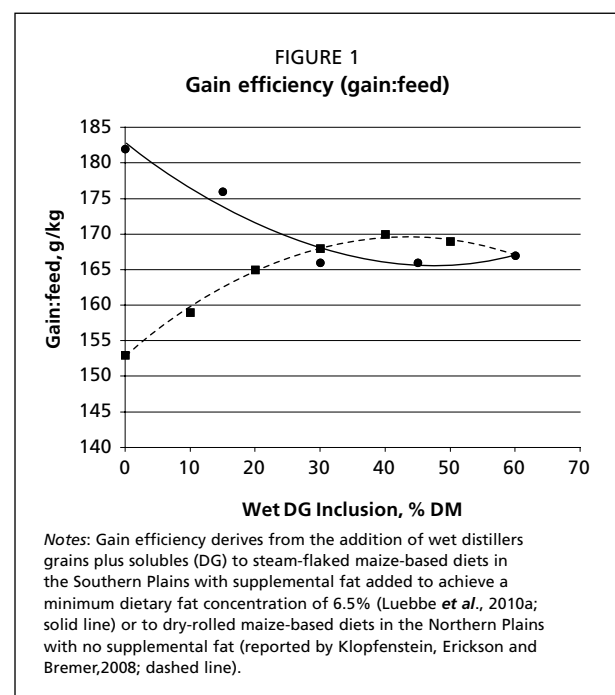


TABLE 1

Effects of wet distillers grains (DG) concentration in finishing steer diets on ruminal, post-ruminal, and total-tract digestion

Item	Dietary treatment ⁽¹⁾						SEM	Maize	P-value ⁽²⁾	
	DRC	SFC	15DG	30DG	45DG	60DG			Lin	Quad
No. of steers	6	6	7	6	6	6	--	--	--	--
Intake, kg/day										
DM	9.0	9.1	9.6	9.3	9.8	8.8	0.6	0.91	0.76	0.17
OM	8.6	8.7	9.1	8.7	9.1	8.2	0.5	0.96	0.41	0.14
NDF	1.23 c	1.34 c	1.74 b	2.08 b	2.56 a	2.63 a	0.16	0.54	<0.01	0.26
Starch	4.91 a	5.05 a	4.77 a	3.83 b	3.16 c	2.02 d	0.25	0.57	<0.01	0.05
Ruminal digestibility, %										
OM	53.5 abc	60.8 a	56.4 ab	50.5 bc	40.5 d	43.7 cd	4.8	0.14	<0.01	0.39
NDF	20.3 bc	18.9 c	30.6 abc	35.1 ab	36.8 a	43.7 a	7.8	0.87	<0.01	0.55
Starch	78.4 b	90.2 a	89.7 a	89.5 a	90.6 a	88.5 a	2.2	<0.01	0.60	0.73
Post-ruminal digestibility, % entering										
OM	61.1	57.4	62.1	61.8	63.7	59.3	4.2	0.39	0.57	0.17
NDF	21.9	10.8	14.5	17.7	18.0	21.7	7.8	0.14	0.13	0.89
Starch	78.3 bc	80.7 bc	89.9 a	86.0 ab	79.2 bc	76.2 c	4.1	0.61	0.05	0.03
Total-tract digestibility, %										
OM	82.3 ab	83.3 a	83.4 a	81.4 abc	78.6 c	79.0 bc	1.5	0.51	<0.01	0.99
NDF	34.1 c	30.5 c	39.9 bc	42.3 bc	48.5 ab	57.2 a	5.7	0.56	<0.01	0.89
Starch	96.0 d	98.1 bc	99.0 a	98.6 ab	98.2 b	97.4 c	0.3	<0.01	<0.01	<0.01

Notes: (1) DRC = dry-rolled maize control diet; SFC = steam-flaked maize control diet; 15DG, 30DG, 45DG and 60DG = diets with 15, 30, 45 and 60% wet distillers grains plus solubles; DM = dry matter; OM = organic matter; NDF = neutral-detergent fibre. (2) Maize = pre-planned contrast of DRC vs SFC; Lin = Linear effect of wet DG concentration; Quad = quadratic effect of wet DG concentration. a,b,c,d Values with different suffixes in a row differ at $P < 0.05$. Source: from Luebbe *et al.*, 2010b.

in the Northern Plains with no supplemental fat, as reported by Klopfenstein, Erickson and Bremer (2008). Although not directly comparable because of different locations, DG sources, diet formulation strategies, and so on, this comparison illustrates that replacing SFC by wet DG might negate a portion of the energetic benefit of steam flaking grain in Southern Plains feedlots and that optimal performance would be realized with lower dietary concentrations of DG than in feedlots of the Northern Plains.

Using ruminally and duodenally cannulated steers in a Latin square design, Luebbe *et al.* (2010b) measured ruminal and post-ruminal digestion characteristics of finishing diets with 0, 15, 30, 45 and 60 percent wet DG in the dietary DM of SFC-based diets. A DRC control treatment also was included to compare effects of maize processing method. Dietary fat was formulated at a minimum of 6.5 percent of dietary DM, with an animal-vegetable blended fat (yellow grease) used to balance fat. Ruminal organic matter (OM) digestibility decreased linearly with increasing wet DG inclusion ($P < 0.01$), probably reflecting decreased starch intake and increased neutral-detergent fibre (NDF) intake (Table 1). Wet DG concentration had no effect on ruminal starch digestion ($P > 0.60$). In contrast to starch, ruminal NDF digestibility increased linearly ($P < 0.01$) with increasing NDF intake. These observations were paired with a concomitant increase in the acetate-to-propionate ratio (A:P) in ruminal fluid with increasing DG inclusion (data not shown). Nonetheless, the A:P in SFC-based diets with DG was less than the values observed in the DRC

control diet. No differences in post-ruminal OM digestibility were detected ($P > 0.17$). Post-ruminal starch digestibility increased quadratically with increasing wet DG inclusion ($P = 0.03$), perhaps as a result of increased amylase production associated with a greater intestinal protein supply (Richards *et al.*, 2002) or simply less starch entering the intestine with wet DG diets. Overall, it seems likely that the exchange of starch for fibre plays an important role in digestion and animal performance as DG is added to the diet.

In contrast to the negative effects of DG inclusion on G:F noted earlier in the Luebbe *et al.* (2010a) study, we have observed animal performance similar to SFC-control diets in steers fed diets containing 20 (MacDonald, 2008) or 35 percent DG (MacDonald, 2009) in the dietary DM. Differences in composition of DG among studies might help explain differences in experimental results. MacDonald (2011) measured *in situ* digestibility to more closely evaluate two wet DG products that resulted in the best and worst animal performance observed by our research group. The greatest difference in nutrient content for these two DG sources was fibre. Variability in the nutrient composition of DG both within a plant, and across plants is well documented (Spiehs, Whitney and Shurson, 2002; Buckner *et al.*, 2011), with NDF and acid-detergent fibre (ADF) generally the most variable nutrients reported (Spiehs, Whitney and Shurson, 2002). The low- and high-NDF wet DG were derived from maize and sorghum, respectively, and contained 23.3 and 43.9 percent NDF, respectively. These data should not be interpreted as a direct comparison of maize

and sorghum wet DG because differences between the two sources were also probably confounded by the concentration of solubles in the products. Six ruminally cannulated steers were fed either SFC- or DRC-based diets containing no wet DG or 20 percent low- and high-NDF wet DG in an *in situ* digestion study. The Latin square design was such that each steer received each diet, and each DG source was incubated (0, 2, 4, 8, 16, 24, 48 and 96 hours) only in steers fed the same DG source. The wet DG samples, but not the *in situ* residues, were extracted with acetone to remove fat. An exponential model was used to estimate the rapidly soluble fraction (*A*), potentially degraded fraction (*B*), and fractional rate of degradation of the *B* fraction (*c*). Effective ruminal degradability (ERD) was calculated using the equation:

$$\text{ERD} = A + [(B \times c)/(c + k)]$$

with a passage rate (*k*) = 0.05/hour. Source of DG had the greatest effect on the parameter estimates (Table 2). For DM disappearance, the low-NDF DG source had a larger *A* fraction ($P < 0.01$) and greater ruminal ERD ($P < 0.01$) than the high-NDF source. The low-NDF DG also had a lower *B* fraction for DM when incubated in steers consuming DRC-based diets ($P < 0.05$); however, this was offset by a numerically greater rate of digestion, such that ERD did not interact with maize processing method ($P = 0.60$). The trends for parameters of NDF disappearance were the inverse of DM. The high-NDF source had a larger *A* fraction of NDF ($P < 0.01$) and greater ERD of NDF ($P < 0.01$). The low-NDF DG again had a lower *B* fraction of NDF when incubated in steers consuming DRC-based diets ($P < 0.01$); however, this also was offset by a numerically greater rate, such that ERD of NDF did not interact with maize processing method ($P = 0.77$). We believe that incubating these DG samples in steers that were fed diets containing the same DG source allowed us to detect differences because the DG sample consumed by the animal influenced ruminal charac-

teristics such as pH. Concomitant pH measurements (data not shown) supported the NDF digestion estimates. When steers consumed high-NDF wet DG, the ruminal pH was greater than when they consumed the low-NDF source, whereas the pH for low-NDF DG was not different from the controls, which did not contain DG. Maize processing also affected ruminal pH, but it did not interact with inclusion of wet DG. Overall, these data are interpreted to suggest that NDF content of wet DG can markedly affect animal performance because of differences in ruminal DM digestibility. The NDF digestibility seems to be more influenced by NDF content than by maize processing method.

EFFECTS OF SPECIFIC NUTRIENTS AND FEED INGREDIENTS

Nitrogen and fat considerations

Exchanging wet DG for SFC inherently increases dietary CP, changes the proportion of ruminally degradable crude protein, and contributes a unique source of fat to the diet. Ensuring an adequate ruminal supply of degradable N is critical to optimize ruminal organic matter (OM) fermentation and feedlot performance (Brown, 2009). Our research has focused on determining the extent to which the greater CP associated with feeding wet DG provides adequate ruminal N via recycling to the gut and whether the fat supplied by DG is comparable to common fat sources fed in the Southern Plains.

Silva *et al.* (2007) fed 400 yearling crossbred heifers a control SFC-based diet with 0.9 percent urea and cottonseed meal (13.5 percent dietary CP) with 0 or 3 percent added yellow grease as a fat source, and 3 diets with 15 percent of the dietary DM as wet sorghum DG, with 0, 1.5 or 3.0 percent added yellow grease. The DG averaged 11.0 percent crude fat; thus, the fat concentration of the diet containing 0 percent DG and 3 percent yellow grease was

TABLE 2
Effects of corn processing method and wet distillers grains (DG) source on kinetics of *in situ* digestion of DG with a low and high neutral detergent fiber (NDF) content⁽¹⁾

Item	Low NDF DG		High-NDF DG		SEM	Maize	P-value	
	DRC	SFC	DRC	SFC			DG	Interaction
Dry matter								
A, % ⁽²⁾	60.3	59.9	38.1	41.4	1.9	0.31	<0.01	0.21
B, % ⁽²⁾	16.1 c	38.1 bc	56.3 ab	33.1 bc	12.8	0.95	0.10	0.05
c, %/h ⁽²⁾	5.10	2.82	2.21	3.15	1.10	0.57	0.29	0.19
ERD, % ⁽³⁾	67.6	66.3	50.1	50.3	2.1	0.73	<0.01	0.60
NDF								
A, % ⁽²⁾	13.7	13.9	22.8	27.8	2.8	0.22	<0.01	0.23
B, % ⁽²⁾	19.9 c	24.1 bc	38.4 a	27.7 b	2.9	0.13	<0.01	<0.01
c, %/h ⁽²⁾	12.7	6.3	5.1	4.8	4.3	0.29	0.16	0.33
ERD, % ⁽³⁾	24.2	24.7	40.1	39.8	2.3	0.95	<0.01	0.77

Notes: (1) Low- and high-NDF wet DG were derived from corn and sorghum, respectively, and contained 23.3, and 43.9% NDF, respectively. The wet DG was incubated in the rumen of steers consuming diets containing 20% of the wet DG being incubated (low or high NDF) and 60% dry-rolled corn (DRC) or steam-flaked corn (SFC) in a Latin square design. (2) A = rapidly soluble fraction; B = potentially degradable fraction; c = fractional degradation rate of fraction B. (3) Effective ruminal degradability (ERD) = $A + [(B \times c)/(c + k)]$ with $k = 0.05/\text{hour}$. Source: from MacDonald, 2011.

similar to the diet containing 15 percent DG and 1.5 percent added yellow grease. All diets with DG also contained 0.9 percent urea. For the 106-day period, heifers fed 15 percent sorghum DG ate 5 percent more feed ($P < 0.05$), gained body weight (BW) 5 percent more rapidly ($P < 0.05$), and had a G:F ratio similar to that of heifers fed 0 percent sorghum DG (Table 3). The greater DM intake (DMI) with sorghum DG presumably reflected changes in ruminal acidity, digesta passage or other unknown factors that stimulated intake, with a corresponding increase in ADG, such that gain efficiency was not changed. Added yellow grease did not alter performance by heifers fed 0 percent DG, but carcasses of heifers fed yellow grease were fatter, with an increased yield grade and greater quality grade ($P < 0.10$; data not shown). Increasing yellow grease in diets with 15 percent DG linearly increased ($P < 0.05$) G:F, but carcass quality and yield grades were not altered by adding yellow grease in DG diets. Overall, results suggest that yellow grease and the fat contained in sorghum DG are utilized in a similar manner.

As noted previously, exchanging DG for SFC and oilseed meal typically decreases the relative degradability of CP supplied by basal ingredients and often increases the total dietary CP content. Thus, whether more or less ruminally degraded CP is needed in diets with wet DG depends on the extent to which N recycling from the excess CP can offset a potential ruminal N deficiency. To study this question, Ponce *et al.* (2009) evaluated the effects of wet DG concentration and supplemental urea in SFC-based diets on performance and carcass characteristics of yearling steers (9 or 10 steers/pen; 8 pens/treatment). Treatments were arranged in a $2 \times 3 + 1$ factorial of DG (15 or 30 percent of DM) and urea concentration (0, 0.53 and 1.06 percent of DM) with a positive control diet containing 1.06 percent urea and cottonseed meal (13.5 percent CP). Diets had a similar fat concentration, and the source of wet DG was a blend of 90 percent maize and 10 percent sorghum grains

that had an analysed composition of 33.4 percent CP, 36.3 percent NDF and 12.2 percent crude fat (DM basis).

Trends for interactions between DG and urea concentration were evident for average daily gain (ADG; $P = 0.12$) and feed efficiency ($P = 0.06$). Increasing dietary urea linearly increased DMI (Table 4; $P < 0.05$) to a maximum of 0.30 to 0.35 kg/day at 1.06 percent urea. Averaged across urea concentrations, ADG was decreased with 30 vs 15 percent DG ($P < 0.02$), and G:F was less ($P < 0.02$) when steers were fed 30 vs 15 percent DG. No interactions were evident for carcass characteristics. Hot carcass weight was greater ($P < 0.01$) for steers fed the control diet than for those fed 30 percent DG, and greater ($P < 0.01$) for those fed 15 vs 30 percent DG ($P < 0.01$). Dressing percent was less with both 15 ($P < 0.001$) and 30 percent DG ($P < 0.01$) compared with the control diet. Plasma urea N (Figure 2) increased with increasing WDGS and urea concentrations ($P \leq 0.04$). Similar results with blood urea N were reported recently by Jenkins *et al.* (2011).

In a subsequent study, Ponce *et al.* (2010) fed steer calves a control diet similar to the previous study or diets with 15 percent DG and 0, 0.52, 0.78, or 1.04 percent urea to more closely titrate the non-protein nitrogen (NPN) needs of diets with a lower inclusion of wet DG. The DG was derived from a blend of 78 percent maize and 22 percent sorghum grain (averaged over the study period). Daily DMI and ADG were greater for cattle fed wet DG than for control (Table 5; $P < 0.01$), whereas G:F did not differ among treatments.

Overall, results of our studies suggest that between 0.52 and 0.78 percent urea was needed to optimize growth performance with diets that contained 15 percent wet DG, but added urea was not beneficial when the diet contained 30 percent DG. This finding suggests that increased N recycling with the 30 percent wet DG diet was sufficient to offset the needs for additional dietary urea, which is consistent with the report by Jenkins *et al.* (2011).

TABLE 3
Growth performance for a 106-day feeding period by heifers fed wet sorghum distillers grains plus solubles (DG) and yellow grease

Item	DG, % of DM					SE ⁽¹⁾	Contrasts ⁽²⁾			
	0%	0%	15%	15%	15%		1	2	3	4
	Yellow grease, % of DM									
	0%	3.0%	0%	1.5%	3.0%					
Initial shrunk BW, kg	373	373	373	374	373	11.2	-	-	-	-
Final shrunk BW, kg	521	521	524	535	530	21	0.88	0.05	0.30	0.14
Adjusted final BW, kg ⁽³⁾	520	523	522	535	531	21	0.64	0.05	0.13	0.09
DMI, kg/day	8.29	8.33	8.69	8.99	8.73	0.74	0.81	0.001	0.77	0.04
Adjusted ADG, kg/day	1.39	1.42	1.41	1.52	1.49	0.09	0.59	0.04	0.12	0.11
Adjusted G:F g/kg ⁽³⁾	167.3	170.7	161.9	169.8	171.6	5.4	0.47	0.64	0.05	0.39

Notes: (1) Standard error of least squares means; n = 8 pens/treatment with 9 to 10 animals/pen; BW = body weight; DMI = dry matter intake; ADG = average daily gain; G:F = gain:feed ratio. (2) Significance level of each contrast is reported. Contrasts include: 1 = 0% DG, 0% yellow grease vs 0% DG, 3% yellow grease; 2 = 0% vs 15% DG; 3 and 4 = linear and quadratic effects, respectively, of yellow grease within 15% DG. (3) Calculated as hot carcass weight divided by (overall dressing percent divided by 100). Source: from Silva *et al.*, 2007.

TABLE 4
Effects of wet distillers grains with solubles (DG) and non-protein nitrogen (NPN) on performance by finishing steers

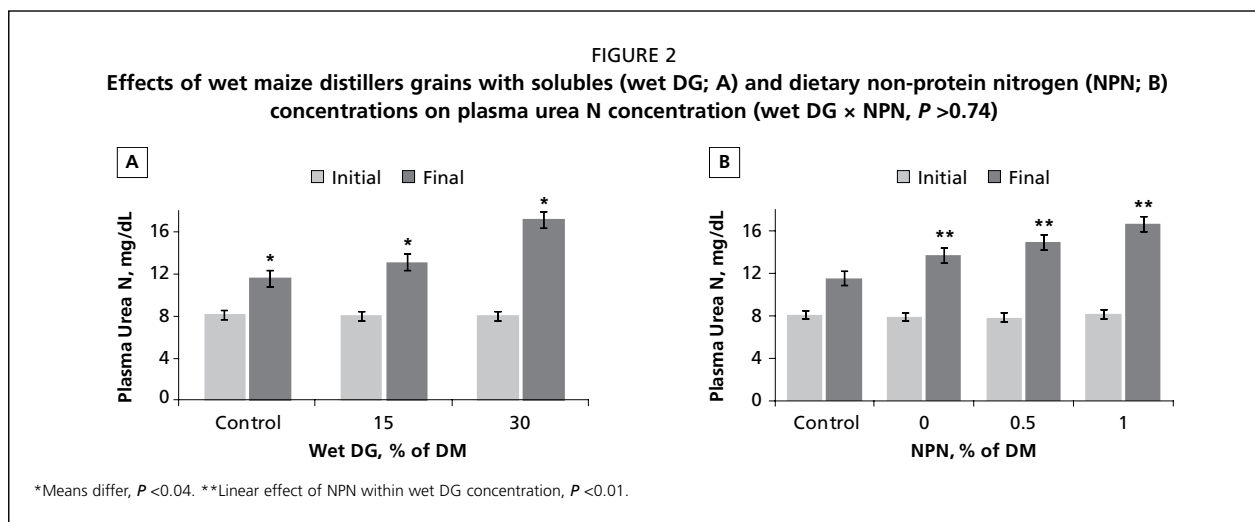
Item	Control	DG, % of DM						SE ⁽¹⁾
		15%			30%			
		NPN, % of DM			NPN, % of DM			
	0	1.5	3.0	0	1.5	3.0		
Initial BW ⁽²⁾ , kg	373	374	372	372	372	373	373	13
Final BW ⁽²⁾ , kg ^{b,c}	600	597	611	598	588	587	588	12
Adjusted final BW ⁽³⁾ , kg ^{b,c}	600	591	600	598	586	585	586	9
DMI, kg/d ^a	9.75	9.47	9.78	9.82	9.45	9.45	9.76	0.26
Adjusted ADG, kg/d ^{b,c}	1.76	1.68	1.75	1.74	1.66	1.65	1.65	0.04
Adjusted G:F, g/kg ^{b,c}	180.9	177.3	179.5	178.2	175.6	174.2	168.9	3.73
Hot carcass weight, kg ^{b,c}	385	379	385	384	376	376	376	6
Dressing percent ^{b,d}	65.1	64.3	64.0	64.3	64.0	64.0	63.9	0.35
12 th rib fat, cm	1.24	1.21	1.24	1.29	1.23	1.20	1.25	0.08
Longissimus muscle area, cm ²	92.3	91.1	90.0	92.0	93.2	91.7	92.7	1.53
Yield grade	2.77	2.75	2.86	2.83	2.63	2.67	3.06	0.20
Marbling score ⁽⁴⁾	400	387	405	402	395	400	393	11

Notes: (1) Standard error of treatment means, n = 8 pens/treatment. DMI = dry matter intake; ADG = average daily gain; G:F = gain:feed ratio. (2) A pencil shrink of 4% was applied. (3) Adjusted body weight (BW) was calculated as hot carcass weight divided by the overall average observed dressing percent (64.22%). (4) 300 = Slight; 400 = Small. (a) Linear effect of NPN, $P < 0.05$. (b) Control vs. 30% DG; $P < 0.09$ for Adjusted ADG and G:F; $P < 0.01$ for hot carcass weight and dressing percent. (c) 15 vs 30% DG, $P < 0.02$ for Adjusted ADG and G:F; $P < 0.01$ for hot carcass weight. (d) Control vs 15% DG, $P < 0.001$. Source: from Ponce *et al.*, 2009.

TABLE 5
Effects of non-protein nitrogen (NPN) on performance by finishing steers fed 15% wet distillers grains with solubles

Item	Control	15% DG			SE ⁽¹⁾	Contrasts ⁽²⁾		
		NPN, % of DM				1	2	3
		1.5	2.25	3.0				
Initial BW ⁽³⁾ , kg	344	345	344	344	12	0.51	0.23	0.56
Final BW ⁽³⁾ , kg	576	596	592	593	9.2	0.002	0.70	0.68
Adjusted final BW ⁽⁴⁾ , kg	576	595	590	596	10.5	<0.01	0.92	0.39
DMI, kg/day	7.86	8.46	8.29	8.26	0.15	0.001	0.22	0.65
Adjusted ADG ⁽⁴⁾ , kg/day	1.39	1.52	1.49	1.52	0.04	0.005	0.99	0.49
Adjusted G:F ⁽⁴⁾ , g/kg	176.7	179.5	179.8	184.1	3.77	0.15	0.25	0.58

Notes: (1) Standard error of treatment means, n = 9 pens/treatment. DMI = dry matter intake; ADG = average daily gain; G:F = gain:feed ratio. (2) Contrasts included: 1) control vs. average of 15% DG; 2) the linear effect of NPN among 15% DG; and 3) the quadratic effect of NPN among 15% DG. (3) A pencil shrink of 4% was applied. (4) Adjusted body weight (BW) was calculated as hot carcass weight divided by the overall average observed dressing percent (65.2%). Source: from Ponce *et al.*, 2010.



Roughage concentration and source

The proportion of traditional roughage sources added to feedlot diets is typically low because this optimizes G:F and decreases problems with handling and conveying bulky material. Indeed, the small amount of fibre supplied by the roughage component of feedlot diets is thought to primarily help performance by maintaining a healthy rumen and minimizing digestive disorders like acidosis and bloat. Despite low inclusion rates, roughage concentration and source can significantly affect feedlot performance, primarily through changes in DMI. Arelovich *et al.* (2008) reported positive linear relationships between dietary NDF and DMI ($r^2 = 0.96$) by feedlot cattle. In addition, dietary NDF concentration and NE for gain (NEg) intake were closely associated ($r^2 = 0.86$) in feedlot beef cattle diets that ranged from 7.5 to 35.3 percent total NDF. Given that NEg intake is key in determining performance, when the price of roughage is low, increasing dietary NDF concentration through changes in roughage concentration can increase DMI and thereby ADG by cattle. Moreover, with differences in physical or chemical characteristics of NDF among roughage sources, changing the roughage source at a fixed dietary roughage concentration can result in similar effects to changes in roughage concentration (Galyean and Defoor, 2003). Based on their meta-analysis, Arelovich *et al.* (2008) suggested that roughage sources could be exchanged on an equal NDF basis to achieve equal DM and NEg intakes, a recommendation that is generally supported by the literature (Marshall *et al.*, 1992; Theurer *et al.*, 1999). Nonetheless, the possibility of unique physical characteristics related to particle size, density and fibre composition (i.e. physically effective NDF) probably need to be considered.

With observed relationships between DMI and NDF (Arelovich *et al.*, 2008), one might expect DMI to increase when DG is added to feedlot diets; however, this expectation has not been observed consistently in practice. For example, adding 15 percent wet sorghum DG to the DM of feedlot diets did not affect DMI in either DRC- or SFC-based diets (Leibovich, Vasconcelos and Galyean, 2009), despite decreased ADG. Similarly, Vander Pol *et al.* (2009) observed no effects on DMI with inclusion of up to 40 percent (DM basis) maize DG in DRC-based feedlot diets, and Depenbusch *et al.* (2008) reported no effects on DMI when maize DG (0 or 25 percent of dietary DM) was added to SFC-based diets. Indeed, Drouillard *et al.* (2005) reported a linear decrease in DMI as sorghum DG concentration increased from 0 to 40 percent of the dietary DM.

Recent results of our collaborative studies in feedlot steers suggest that both roughage concentration (May *et al.*, 2011) and source (Quinn *et al.*, 2011) need to be considered in diets containing wet DG. May *et al.* (2011) evaluated two dietary concentrations of wet DG (15 or 30 percent of the DM; DG was approximately 90 percent

maize and 10 percent sorghum) and alfalfa hay (7.5, 10 or 12.5 percent; DM basis) plus a non-DG control diet that contained 10 percent alfalfa hay. No DG \times alfalfa hay interactions ($P > 0.12$) were detected, and final shrunk BW, ADG ($P > 0.15$), and DMI ($P = 0.38$) did not differ between the two DG concentrations over the feeding period. Increasing alfalfa hay concentration tended ($P < 0.08$) to increase DMI linearly, and it resulted in a linear decrease ($P < 0.05$) in G:F and calculated dietary NE concentrations. Hot carcass weight did not differ ($P > 0.15$) among treatments nor did dressing percent or loin muscle area; however, there was a tendency ($P < 0.06$) for 12th rib fat and marbling score to be greater with 15 vs 30 percent DG in the diet. When the same treatments were used as substrates for *in vitro* fermentation measurements, increasing DG concentration from 15 to 30 percent increased ($P < 0.05$) H₂S production because of increased S concentration, but effects of treatments on DM disappearance and molar proportions or total concentration of volatile fatty acids (VFA) were not large.

As a follow-up to the work of May *et al.* (2011), Quinn *et al.* (2011) evaluated a SFC-based control diet with no DG and 10 percent alfalfa hay, and diets with either 15 or 30 percent wet DG (DM basis; DG was a blend of approximately 70 percent maize and 30 percent sorghum). Roughage sources in the DG diets were alfalfa hay, coastal bermudagrass hay or sorghum silage, with all sources provided at an equivalent percentage of NDF to 7.5 percent alfalfa hay. In contrast to the results of May *et al.* (2011), cattle fed 15 percent DG diets had greater ($P < 0.04$) final BW, ADG and G:F than those fed 30 percent DG. Moreover, using alfalfa hay as the roughage source resulted in decreased final shrunk BW and ADG ($P < 0.02$) compared with bermudagrass and sorghum silage, but sorghum silage decreased ($P = 0.01$) G:F relative to bermudagrass hay. Hot carcass weight was greater ($P < 0.01$) for steers fed 15 vs 30 percent DG, and it tended ($P = 0.06$) to be less for diets with alfalfa hay as the roughage source. As in the May *et al.* (2011) study, the effects of roughage source and DG concentration on *in vitro* DM fermentation measurements were not large. Overall, results suggested that substituting roughages on an equivalent NDF basis might not result in similar DMI with feedlot diets that contain up to 30 percent DG, and further work seems necessary to characterize the physically effective NDF component of various roughage sources.

POTENTIAL INTERACTIONS WITH GRAIN PROCESSING AND FEED ADDITIVES

Grain processing

The increased digestibility of both starch and non-starch OM associated with steam flaking greatly increases the NE concentration of SFC compared with DRC. In a summary of the results of several experiments, SFC increased the NEg concentration by 18.8 percent compared with dry-

processed maize (Zinn, Owens and Ware, 2002). A practical concern for feedlot nutritionists is whether replacing SFC with DG affects the NE value of the diet and ultimately animal performance. As noted previously, with diets based on minimally processed grain, positive effects of adding DG have been reported for feedlot ADG and G:F. For example, in a summary of 9 experiments with DRC or high-moisture maize (HMC) as the grain source, G:F responded quadratically as wet DG increased in the diet, with a maximal response at 30 to 50 percent DG in the DM (Klopfenstein, Erickson and Bremer, 2008).

As noted previously, our data with SFC diets (e.g. Figure 1) and other reports in the literature lend credence to the idea that the feeding value of DG differs depending on the basal grain processing method. May *et al.* (2007) detected an interaction between maize processing method and inclusion level of sorghum DG ($P < 0.01$), with decreased G:F when up to 30 percent sorghum DG was included in SFC-based diets, but a positive G:F response with DRC-based diets. Similarly, Corrigan *et al.* (2009) reported an interaction ($P < 0.01$) between maize processing method and DG concentration in feedlot diets, such that G:F increased linearly ($P < 0.01$) with increasing wet maize DG concentration up to 40 percent of the dietary DM with diets based on DRC and HMC, but with no change in G:F when DG replaced SFC. Providing further evidence of a DG \times grain processing interaction, Cole *et al.* (2009) summarized data from 37 experiments and concluded that the NEg concentration of DG was greater in DRC vs SFC-based diets.

In contrast to experiments suggesting an interaction with grain processing method, Leibovich, Vasconcelos and Galyean (2009) reported that including 15 percent sorghum DG in the dietary DM decreased G:F to a similar degree with both SFC- and DRC-based diets. Likewise, *in vitro* DM digestion and gas production data with the same treatments used in the feedlot study did not provide evidence of an interaction between DG concentration and grain processing method.

Given the importance of SFC in the Southern Great Plains, our research group set out to further investigate the

purported DG \times grain processing interaction. MacDonald (2008, 2009) conducted two experiments with DG added to DRC- or SFC-based diets. In the first experiment, 264 crossbred yearling heifers were used in a randomized complete block design with a 2 \times 2 factorial arrangement of treatments: SFC and DRC control diets vs SFC and DRC diets with 20 percent (DM basis) wet maize DG with solubles. The DG was purchased in Hastings, NE, United States, transported to the experimental feedlot near Amarillo, TX, United States, and stored in plastic silo bags after being mixed with chopped alfalfa hay (75 percent DG:25 percent alfalfa hay). All diets contained 10 percent alfalfa hay, 1.2 percent urea and 2.0 percent yellow grease; thus, diets with 20 percent DG had greater CP and ether extract concentrations than control diets. The heifers were adapted to the final 90 percent concentrate diet over a 21-day period and were fed for an average of 154 days. In the second experiment, 54 steer calves were used in a randomized incomplete block design. The steers were trained in a Calan gate feeding system, thereby allowing individual animals to be the experimental units. Treatments and the source of DG were the same as in the first experiment, except that DG was fed at 35 percent of the dietary DM, and diets were formulated to equalize ether extract concentration across treatments.

Neither of the two experiments reported by MacDonald (2008, 2009; Tables 6 and 7) provided evidence of a grain processing \times DG inclusion interaction ($P \geq 0.22$ for performance variables). In both studies, the response to SFC was as expected, with lower DMI and increased G:F ($P < 0.01$) than with DRC. In contrast to the results of Corrigan *et al.* (2009), adding DG improved ($P \leq 0.05$) G:F with both SFC- and DRC-based diets. Although composition of DG varies among plants, the fact that MacDonald (2008, 2009) used maize DG from an ethanol plant in Nebraska would seem likely to minimize the effect of DG source on comparisons with the work of Corrigan *et al.* (2009). Nonetheless, differences in diet formulation, cattle type, environmental factors, etc., could be involved. Based on these results, however, the significance of a DG \times processing method interaction in cattle feedlots in the Southern Great Plains

TABLE 6
Effects of maize processing method and dietary wet distillers grains plus solubles (DG) inclusion on animal performance of yearling heifers

Item	0% DG		20% DG		SE ⁽³⁾	Proc	P-value ⁽¹⁾	
	SFC ⁽²⁾	DRC ⁽²⁾	SFC	DRC			DG	Proc \times DG
Initial BW, kg	354.2	353.7	354.2	354.2	15.87	0.91	0.75	0.34
Final BW ⁽⁴⁾ , kg	537.4	532.4	542.0	542.4	8.16	0.62	0.10	0.55
ADG, kg ⁽⁵⁾	1.19	1.17	1.23	1.23	0.032	0.77	0.14	0.69
DMI, kg	9.02	9.80	9.16	9.80	0.245	<0.01	0.74	0.76
G:F	0.133	0.120	0.135	0.126	0.003	<0.01	0.05	0.38

Notes: (1) P-values of the F-test for: Proc = main effect of maize processing method; DG = main effect of dietary DG inclusion; Proc \times DG = interaction of maize processing method and dietary DG inclusion. (2) Maize processing method; SFC = steam-flaked maize; DRC = dry-rolled maize. (3) Standard error of treatment means, n = 6 pens/treatment. DMI = dry matter intake; ADG = average daily gain; G:F = gain:feed ratio. (4) Final individual body weight (BW) measured on a live basis and shrunk by 4%. Cattle were fed for an average of 154 days. Source: from MacDonald, 2008.

TABLE 7
Effects of maize processing method and dietary wet distillers grains plus solubles (DG) inclusion on animal performance of steer calves

Item	0% DG		35% DG		SE ⁽³⁾	Proc	P-value ⁽¹⁾	
	SFC ⁽²⁾	DRC ⁽²⁾	SFC	DRC			DG	Proc × DG
Initial BW, kg	309.8	306.1	307.5	307.0	8.16	0.76	0.93	0.81
Final BW ⁽⁴⁾ , kg	561.9	544.2	556.0	560.5	11.34	0.49	0.60	0.25
ADG, kg ⁽⁵⁾	1.45	1.37	1.43	1.45	0.045	0.51	0.57	0.29
DMI, kg	8.53	9.16	8.21	8.98	0.227	<0.01	0.26	0.75
G:F	0.170	0.149	0.175	0.163	0.004	<0.01	0.03	0.22

Notes: (1) P-values of the F-test for: Proc = main effect of maize processing method; DG = main effect of dietary DG inclusion; Proc × DG = interaction of maize processing method and dietary DG inclusion. (2) Maize processing method: SFC = steam-flaked maize; DRC = dry-rolled maize. (3) Standard error of treatment means, n = 12 to 14 steers/treatment. Because of unbalanced numbers among treatments, the largest SE value is reported. ADG = average daily gain; DMI = dry matter intake; G:F = gain:feed ratio. (4) Final individual body weight (BW) measured on a live basis and shrunk by 4%. (5) Cattle were fed for 174 days. Source: from MacDonald, 2009.

might not be a major concern, at least when diets are balanced for fat content.

Reasons for the possible interaction between DG concentration and grain processing method are not clear. Cole *et al.* (2009) suggested several possibilities, including effects of dietary fat or energy concentration, contamination of DG with ethanol, effects of yeast cells that remain in DG, differences in methane production and measurement errors in DM content of DG. With respect to nutrient digestibility, Corrigan *et al.* (2009) reported that there was no interaction ($P > 0.10$) between maize processing method and wet maize DG concentration (0 or 40 percent) for total tract digestion measurements, with greater digestibility of DM and OM in 0 percent DG diets. In our laboratory, SFC-based diets with either 15 percent (DM basis) maize or sorghum DG did not differ in total tract digestibility from a non-DG control diet (May *et al.*, 2010).

Changes in ruminal fermentation patterns might be related to different responses to DG with various processing methods. Corrigan *et al.* (2009) reported less change in A:P with SFC-based diets that contained wet maize DG than for diets based on DRC and HMC (interaction P -value ≤ 0.09). Vander Pol *et al.* (2009) also reported that A:P was less ($P < 0.10$) in steers fed 40 percent wet maize DG vs a control diet based on DRC. DiLorenzo and Galyean (2010) suggested that the relatively high hemicellulose concentration of DG might contribute to changes in A:P, as fermentation of hemicellulose by ruminal microorganisms results in a lower A:P than fermentation of cellulose (Murphy, Baldwin and Koong, 1982). In addition, DiLorenzo and Galyean (2010) suggested that fermentation of the solubles fraction added to DG also might help explain decreased A:P when feeding DG. Thus, differences among studies in the proportion of solubles added to the DG might explain variable responses noted with different processing methods. Besides ruminal effects, factors such as total and unsaturated fat intake (Zinn, 1989) and changes in digesta passage and ruminal degradation rates of various nutrients could also result in DG × grain processing interactions.

As noted previously, the NEg concentration of SFC is of the order of 19 percent greater than DRC (Zinn, Owens and Ware, 2002). May *et al.* (2011) reported that the NEg concentration of wet DG (a 90:10 blend of maize and sorghum) in a SFC-based diet ranged from 88.2 to 105.1 percent of the value of SFC. Thus, if DG, which is of equal energy value to SFC, is substituted into SFC-based diets, little change in G:F would be expected. In contrast, substituting DG into a DRC-based diet should increase the NEg concentration of the diet and improve performance. This scenario fits well with the DG × grain processing interaction for G:F reported by Corrigan *et al.* (2009). Responses for individual experiments would depend on the NEg concentration of DG relative to SFC, which might be affected by digestion or fermentation changes noted previously. Thus, for a given source of DG, the relative difference in NEg concentration between the DG and the processed grain to which it is compared seems to provide a reasonable explanation for observed effects on feedlot performance.

Ionophores

Ionophores, antibiotics and probiotics are used extensively in the United States beef feedlot industry. Among the ionophores, monensin is included in virtually all conventional feeding programmes in United States feedlots. Lasalocid and laidlomycin propionate also are approved for use with confined beef cattle. Ionophores alter transport of ions across bacterial membranes, shifting ruminal microbial populations in a way that leads to decreased acetate and increased propionate (Russell and Strobel, 1989), with potential effects on methane production and ammonia concentrations. Although early data with monensin suggested improvements in feed efficiency of 7.5 percent (Goodrich *et al.*, 1984), results with processed grain diets indicate responses approximately half as large (Laudert, 1992).

Effects of ionophores in diets containing DG are not as well defined as in non-DG diets. Meyer *et al.* (2009) reported increased G:F with the addition of monensin and monensin plus tylosin to diets with 25 percent wet DG

(DM basis), regardless of basal grain source (DRC or SFC). Depenbusch *et al.* (2008) evaluated effects of monensin and monensin plus tylosin in 371 heifers fed SFC-based diets that contained 0 or 25 percent wet maize DG. In contrast to the results of Meyer *et al.* (2009), feed additives did not affect performance ($P \geq 0.20$), and feed additive \times diet interactions ($P \geq 0.77$) were not observed. In our laboratory, classical *in vitro* shifts in VFA (e.g. decreased A:P) were observed when monensin was added at concentrations of 0, 2, 4 and 6 mg/L with a substrate containing 15 percent wet DG (Smith *et al.*, 2010). Thus, it does not seem likely that DG has unique substrate effects that would alter the response to ionophores.

Sulphur and feed additives

Use of sulphuric acid to control pH during the fermentation in grain ethanol production and as a cleaning agent for equipment results in potentially increased and variable concentrations of S in DG. Sulphur concentrations vary among ethanol production facilities, and solubles typically contain more S than the residual grain fraction (wet cake). Concentrations ranging from 0.40 to 1.30 percent S in the DM have been reported in wet and dry DG (Crawford, 2007; Klopfenstein, Erickson and Bremer, 2008). The recommended maximum tolerable dietary concentration of S is 0.4 percent of the DM for beef cattle (NRC, 2000); however, the NRC (2005) recently suggested a maximum tolerable concentration of 0.3 percent for high-concentrate diets and 0.6 percent for high-forage diets. High S intakes from feed and water can result in polioencephalomalacia (Gould, 1998), which seems to occur when ruminal bacterial reduce S to hydrogen sulphide (H_2S) that is absorbed through the ruminal wall or via the lungs when animals eructate ruminal gases. Thus, high concentrations of S and their effects on health and performance of feedlot cattle have been an issue of considerable research focus with DG.

Work in our group initially evaluated the potential interaction between S concentration and ionophores. Based on *in vitro* experiments, Kung, Bracht and Tavares (2000) suggested that adding monensin to batch culture fermentations increased H_2S production, resulting in practical concerns as to whether use of monensin in feedlot diets with a high concentration of S might increase ruminal H_2S . The Kung, Bracht and Tavares (2000) research was conducted using ruminal fluid from cattle that were not adapted to monensin, and the concentration of S in the incubation substrate was 1.09 percent of the DM, a value that is considerably greater than the S concentration of typical feedlot diets, even with a high inclusion of DG. We used a batch culture fermentation and gas collection system to evaluate effects of the ionophores monensin, lasalocid and laidlomycin propionate, as well as the antibiotics chlortetracycline and tylosin (Quinn *et al.*, 2009) on H_2S production. In contrast to the work by Kung, Bracht and Tavares (2000),

in vitro H_2S production was not affected by the ionophore and antibiotic treatments with a substrate that contained 0.42 percent S (DM basis). Subsequently, Smith *et al.* (2010) evaluated monensin concentrations of 0, 2, 4 and 6 mg/L in combination with S concentrations ranging from 0.2 to 0.8 percent S (DM basis) in a high-grain substrate. Increasing substrate S concentration linearly increased *in vitro* H_2S production; however, no effect of monensin concentration was detected. Moreover, responses did not depend on adaptation of ruminal fluid donor cattle to monensin. Shifts in the A:P typically associated with ionophores were observed in both the Quinn *et al.* (2009) and Smith *et al.* (2010) studies.

Although commonly used feed additives did not increase *in vitro* ruminal H_2S , our work and *in vivo* observations clearly demonstrate that H_2S production is responsive to dietary S concentration. Moreover, an inverse relationship between ruminal H_2S and pH has been reported (Gould, 1998), so whether well-buffered *in vitro* systems adequately mimic the potential effects of DG *in vivo* is open to question. Thus, practical concerns remain regarding high S concentrations in DG, particularly S that is added to DG during production.

Probiotics

Probiotics are used frequently in United States feedlot diets, reflecting, in part, growing public concern over the use of growth-promoters and antibiotics, as well as beneficial effects of some probiotics on faecal shedding of foodborne pathogens (Stephens, Loneragan and Brashears, 2007; Vasconcelos *et al.*, 2008). The mode of action of probiotics is not fully defined, and responses depend on the type of product fed and, for viable cultures of microorganisms, on the dose. Performance responses to probiotics have been equivocal and generally not large. Krehbiel *et al.* (2003) summarized research findings and reported a 2.5 percent increase in G:F with feedlot cattle supplemented with lactate-utilizing and lactate-producing bacteria.

To our knowledge, the effects of adding probiotics to feedlot diets containing DG have not been reported. Given the greater NDF and fat content associated with addition of DG to feedlot diets, further research is needed to determine whether possible interactions exist between DG concentration and probiotic use. With limited evidence suggesting the possibility that adding DG to the diet might increase faecal shedding of *E. coli* O157 (Jacob *et al.*, 2008), research to evaluate the efficacy of probiotics that have been shown to decrease faecal shedding of *E. coli* O157 needs to be conducted for diets with varying concentrations of DG.

ENVIRONMENTAL EFFECTS OF FEEDING WET DISTILLERS GRAINS IN HIGH-ENERGY, PROCESSED GRAIN DIETS

Feeding livestock in confinement concentrates feed nutrients such as N, P and other minerals and salts in a small

geographical area. Extraneous losses of these nutrients to groundwater, surface water and the atmosphere, and removal of accumulated manure, are significant environmental concerns to the livestock industry.

Manure quantity and quality

In general, feeding more digestible diets decreases the quantity of faeces excreted and thereby the quantity of manure that must be managed. Effects of feeding DG on diet digestibility and manure production vary depending on the quantity of DG in the diet, the grain processing method and other dietary ingredients. In diets based on SFC, replacing a portion of the maize with DG decreased diet digestibility by 3 to 5 percentage units (Cole, 2008, 2010; Cole, Brown and MacDonald, 2008; Cole *et al.*, 2011). In contrast, replacing DRC with DG seems to have little effect on digestibility of finishing diets (Cole, 2008, 2010; Cole, Brown and MacDonald, 2008; Pritchard *et al.*, 2010). Because of effects on both digestibility and intake, total manure production and the quantity of manure collected from feedlot pens will increase by 10 to 20 percent with addition of DG to the finishing diet (Cole, Brown and MacDonald, 2008; May *et al.*, 2009; Uwituze *et al.*, 2010).

Typically, replacing maize with DG in high-concentrate diets will increase the N, P and S content of the diet, and increase the quantity of these nutrients excreted onto the pen surface (Benson *et al.*, 2006; Spiehs and Varel, 2009; Gilley *et al.*, 2010b). Based on a summary of 6 feeding trials, we noted that although the concentration of P in manure was not altered by feeding DG, the total quantity of P in manure was increased by 3.4 g/animal daily for each 10 percent wet DG substituted in the diet (Cole, Brown and MacDonald, 2008).

Effects of DG on manure chemical composition vary with the quantity and source of DG fed in the diet. In several feeding studies, we noted no effect of DG on manure N, P or N:P ratio, whereas in other studies, manure N and N:P increased (Cole, 2008; 2010; Cole, Brown and MacDonald, 2008). Gilley *et al.* (2010b) reported that manure from cattle fed 40 percent wet DG diets had more ammonium-N and total P than manure from steers fed control diets containing no DG; however, diet did not affect runoff of soluble P, particulate P, total P or total N from feedlot pens.

Effects on air quality

Air pollutants in feedlots originate from many sources, including pens, manure stockpiles, alleys, the feed milling and storage areas, lagoons, settling basins and retention ponds. In general, emissions of greatest concern are dust, odours, ammonia, greenhouse gases (CO₂, methane, nitrous oxide) and H₂S (NRC, 2002).

Ammonia emissions are affected greatly by environmental factors and by dietary factors such as protein quantity,

protein degradability and carbohydrate degradability (Cole *et al.*, 2005, 2006; Todd, Cole and Clark, 2006; Todd *et al.*, 2009, 2011). When fed at low concentrations in the diet (15 percent of DM or less) DG generally do not significantly increase dietary N concentration, urinary N excretion or N volatilization losses (Cole, 2008, 2010; Cole, Brown and MacDonald, 2008). In addition, feeding 15 percent DG in iso-nitrogenous diets increased OM on the pen surface and decreased the pH of the surface manure (Cole, Brown and MacDonald, 2008). Feeding wet DG at greater concentrations, however, increased the N content of the diet, resulting in increased urinary N excretion (Cole and Todd, 2009) and greater ammonia losses (Todd *et al.*, 2009; 2011). Todd *et al.* (2009) reported that ammonia emissions from a commercial feedlot increased by about 50 percent when the finishing diet contained approximately 25 percent wet DG on a DM basis.

Effects of feeding DG on greenhouse gas emissions from feedlots have not been studied extensively, and results of currently available studies are somewhat inconsistent, presumably reflecting differences in diet composition. In a review of data from several laboratories, Wainman, Dewey and Brewer (1984) reported that feeding brewers grains decreased enteric methane production in ruminants. Similarly, McGinn *et al.* (2009) reported that replacing barley grain with maize dried DG in finishing diets decreased enteric methane losses by almost 20 percent, which was most likely the result of a 3 percent increase in dietary fat concentration. Nonetheless, increased N excretion with DG feeding could potentially increase N₂O and ammonia emissions.

Using lab-scale *in vitro* tests, Behlke *et al.* (2008) reported that replacing maize with dried DG did not affect total methane production but increased methane production per unit of digested OM. An increase in enteric methane production as determined by the sulphur hexafluoride technique was noted when dried DG replaced DRC and maize oil in the diet. In contrast, with a SFC-based diet, Hales, Cole and MacDonald (2011) reported that feeding 30 percent wet DG in the diet did not affect enteric methane emissions when the diets were balanced to have equal fat concentration.

Composting beef cattle manure has a number of agronomic benefits; however, the composting process also has environmental effects. Composting decreases mass by 30 to 50 percent as a result of losses of C (46 to 62 percent loss) and N (19 to 42 percent loss) (DeLuca and DeLuca, 1997; Eghball, *et al.*, 1997). Hao *et al.* (2011) measured greenhouse gas emissions from compost comprising manure from cattle fed control (85 percent barley) or 65 percent wheat-dried DG diets. Manure from the cattle fed the dried DG-based diet tended to have greater total N and ammonium-N concentrations initially and after 99 days of

active composting (windrows turned at 7- to 21-day intervals). Carbon dioxide and methane emissions were similar for both composts; however, as might be expected from the increase in nitrate concentrations, N₂O emissions were significantly greater for dried DG than for control manure (0.053 vs 0.115 kg/tonne of compost).

Production of H₂S in feedlots is highly episodic, normally occurring after a rainfall. Because DG can contain high concentration of S, they are a potential source for production of H₂S or other S-containing odorous compounds. Studies in South Dakota (Benson *et al.*, 2005) indicated that feeding of dried DG (0 percent vs 35 percent of diet DM) increased the atmospheric concentration of H₂S (0.66 vs 2.22 ppb), but feeding DG did not affect odour emissions measured by an olfactometry panel. In contrast, Varel *et al.* (2008) and Spiehs and Varel (2009) reported increasing concentrations of H₂S and several odorous volatile organic compounds in manure slurries as the concentration of wet DG in the diet increased. Using a lab-scale wind tunnel, Miller *et al.* (2010) also noted greater H₂S emissions from manure of cattle fed 20 to 60 percent wet DG than from manure of cattle fed a control diet with no DG. As previously noted, feeding wet DG might also affect enteric H₂S production.

Most pathogens in feedlot air are easily killed by radiation and desiccation; therefore few living pathogenic bacteria can be cultured in air collected at feedlots (Wilson *et al.*, 2002; Purdy *et al.*, 2004). With respect to the effects of DG on pathogen excretion and survival, Varel *et al.* (2008) noted that *E. coli* were more persistent in slurries of manure from cattle fed 20 or 40 percent wet DG than in manure of cattle fed a control diet with no DG. Yang *et al.* (2010) also reported longer survival of *E. coli* O157:H7 in faeces from cattle fed 40 percent wheat or 40 percent maize dried DG compared with a control diet with no DG.

Manure as a fertilizer

In general, the most economically feasible use of manure is as a fertilizer for crops or pastures. Nonetheless, there is potential for loss of nutrients to surface and groundwater when manures or inorganic fertilizers are improperly applied (Kellogg *et al.*, 2000; Cole, Schwartz and Todd, 2005). As previously noted, feeding DG typically increases P excretion in manure. When manure is applied to farmland to meet the P requirements of the crop, the quantity of farmland required to utilize the manure increases approximately 20 percent for each 10 percent wet DG in the diet (DM basis; Benson *et al.*, 2005; Cole, 2010; Cole, Brown and MacDonald, 2008). Gilley *et al.* (2010a) noted that runoff from field plots treated with wet DG manure contained more particulate P and tended to contain more nitrates and ammonium than manure from cattle fed control diets. In a greenhouse study, Benke *et al.* (2010) reported that soil total P and available P were greater in plots treated with

manure from cattle fed 60 percent dried DG diets in comparison with cattle fed a control diet.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Economics, nutritional characteristics and availability of wet DG are the primary factors that have influenced its use in feedlot diets. Based on knowledge gained from our cooperative research efforts, we suggest the following areas of research to fill gaps in knowledge related to efficient use of DG.

Characteristics of wet DG

Data on ruminally degraded protein and physically effective NDF contents of DG are needed. Effects of effective fibre and physical characteristics of wet DG on feedlot intake management programmes should also be evaluated. The role of tannin concentrations, particularly in sorghum-based DG, and mycotoxins on the feeding value of DG needs to be assessed. Approaches (e.g. mathematical expressions related to chemical or physical characteristics) to define the energy value of wet DG deserve further study.

Wet DG supplementation, animal performance, and environmental implications

Effects of grain processing (e.g. SFC vs DRC) relative to concentration of wet DG on fibre digestion, NE value and needs for ruminally degraded CP would benefit from further study. In addition, effects of probiotics (e.g. direct-fed microbials) and various feed additives (e.g. ionophores) on feedlot performance when wet DG is fed are largely not known. Additional data on ruminal and post-ruminal digestion patterns across a range of DG concentrations should be explored further, as should effects of wet DG inclusion on adaptation of feedlot cattle to finishing diets. Implications of feeding wet DG on animal health and digestive disorders have not been extensively evaluated and could be an important area of research. Methods to decrease potential negative environmental effects of wet DG use in feedlot diets need to be further evaluated.

CONCLUSIONS

Wet DG is an effective source of protein and energy in feedlot cattle diets. Feedlot performance can be positively affected by supplementation of wet DG, but effects depend on concentration of DG and potentially interact with basal grain processing, supply of ruminally degraded CP, and NDF and fat concentrations in the DG. Despite added fibre from DG, dietary roughage should not be eliminated, and for optimal cattle performance the concentration and source of roughage need to be considered in wet DG diets. Partially replacing highly processed grain in feedlot diets with wet DG will probably increase N, P and S excretion, and high S concentrations might have negative consequences for animal

health. Feeding wet DG at concentrations greater than 10 to 15 percent of the dietary DM might increase urinary N excretion and ammonia and nitrous oxide emissions.

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Chapter 5

Utilization of feed co-products from wet or dry milling for beef cattle

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ABSTRACT

Recent expansion of the ethanol industry has led to an increase in production of co-products that are used extensively in the cattle industry. A variety of different co-products are being produced, all with slightly different nutrient compositions. Maize [corn] gluten feed (CGF) is the main co-product of the wet milling industry, while distillers grains with solubles (DGS) is the main feed produced by dry milling plants. These co-products have little to no starch remaining, which reduces acidosis challenges in feedlot cattle and reduces negative associative effects of starch digestion on fibre digestion for cattle on high forage diets. The extent to which an ethanol plant dries these co-products affects their nutritional value. For feedlot cattle, wet DGS (WDGS) have a feeding value 30–40 percent greater than maize when included at 10–40 percent of diet DM. Modified and dried DGS have feeding values 15–30 percent and 13 percent greater than maize, respectively. Because feeding DGS results in improved cattle performance, cattle can be fed for fewer days resulting in decreased costs. Feeding high levels of DGS increases the sulphur content of diets and may decrease performance or result in polioencephalomalacia (PEM), particularly if sulphur levels exceed 0.47 percent of diet DM. Increasing roughage levels in the diet appears to be an effective way of minimizing sulphur impacts and maintaining cattle performance. In addition, lower quality roughages could be fed in feedlot diets containing WDGS without diminishing performance. Intense maize processing increases the value of diets containing CGF. However, greater performance responses have been seen with less intensely processed maize in diets containing DGS. There appear to be many complex interactions that cause these differences in performance, and warrant further study.

The environmental impacts of these co-products are quite important. The ideal scenario for reducing greenhouse gas (GHG) emissions of ethanol involves feeding WDGS to feedlot cattle within 100 km of the ethanol plant. In this scenario, GHG emissions can be reduced by 56–62 percent compared with gasoline due to improved cattle performance and decreased energy costs at the ethanol plant when DGS is not dried. Feeding high levels of co-products increases nitrogen (N) and phosphorous (P) in the diet, which increases the N and P content of the manure. Capturing these nutrients in the manure and applying to crop land as fertilizer increases the value of the manure above the costs to apply it. These co-product feeds are an excellent source of energy, protein and P for cattle on high forage diets, and quadratic increases in average daily gain (ADG) and final bodyweight (BW) have been observed with increasing levels of DGS supplementation.

INTRODUCTION

Two primary types of grain milling processes currently exist, resulting in quite different feed products. These processing plants produce and market a variety of feed products, but in general, the dry milling process produces distillers grains with solubles (DGS), and the wet milling process produces maize gluten feed (CGF). These feeds can be marketed as wet feeds, or they can be dried and marketed as either dry maize gluten feed (DCGF) or dried distillers grains (DDG) with or without solubles. The majority of ethanol plant expansions are dry milling plants that produce DGS; however, an increase in supply of wet maize gluten feed (WCGF) is

also expected. Therefore, these feeds may be very attractive for beef producers to use as feed sources. This report will focus on the production, composition, feeding values and environmental issues of using these co-products in both growing and finishing beef cattle diets. Management strategies will be discussed as well, including type of grain, grain processing, roughage levels and the effects of fat, protein, phosphorus (P) and sulphur (S) with these products.

Wet milling

Wet milling is a process that requires the use of high quality (U.S. No. 2 or better) maize, and fractionates the maize

MAIN MESSAGES

- **WDGS has a feeding value 30–40 percent greater than maize when included at 10–40 percent of diet DM.**
- **MDGS has a feeding value 15–30 percent greater than maize when included at 10–40 percent of diet DM.**
- **DDGS has a feeding value 13 percent greater than maize when included at 20–40 percent of diet DM.**
- **High inclusions of DGS increase the sulphur content of diets, which results in reduced DMI and ADG, but has little effect on efficiency.**
- **Feeding WDGS to feedlot cattle located close to an ethanol plant reduces GHG emissions 56–62 percent compared with gasoline.**
- **Distillers grains are an excellent supplement for cattle on high-forage diets because of the high energy, protein and P contents, and lack of starch.**

kernel to produce numerous products, some of which are intended for human use. Fresh water enters the milling system in the final stage of starch washing. Subsequently, it runs countercurrent with respect to the flow of maize, passing through numerous screens and separating implements, acquiring soluble nutrients at each step. Ultimately, this solution will serve as the resource to steep the maize as the initial step in the process. Lactic acid-producing bacteria in the steeping process ferment the soluble carbohydrates collected by the water to further kernel softening. Following the steeping process (Figure 1), maize kernels are separated into kernel components of maize bran, starch, maize gluten meal (high in protein), germ and soluble components.

If the wet milling plant is fermenting starch into ethanol, a portion of the steep water (now called steep liquor) is added to the fermentation vats to supply nutrients for the ethanol-producing yeast cells to grow. The ethanol is distilled off after the fermentation process. The solution exiting the still is called distillers solubles, not to be confused with dry milling distillers solubles. This product contains very lit-

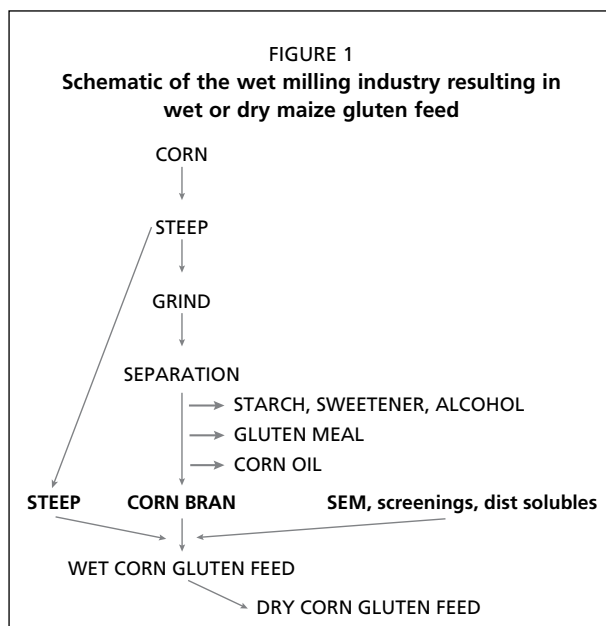
tle maize residue, almost no fat, and is high in protein from the remnants of yeast cells from the fermentation process. The distillers solubles and a portion of the steep liquor are added to the bran fraction of the maize resulting in WCGF. The WCGF can have a portion of the germ meal added if the plant has those capabilities. For a more complete review of the wet milling process, please refer to Blanchard (1992). The actual composition of WCGF can vary depending on the plant capabilities. Steep, a combination of steep liquor and distillers solubles, contains more energy (136 percent the feeding value of maize) and protein than maize bran or germ meal (Scott *et al.*, 1997). Therefore, plants that apply more steep to maize bran or germ meal will produce WCGF that is higher in crude protein (CP) and energy. For instance, Sweet Bran™ is a trademarked WCGF product that Cargill produces. This product contains more steep and germ meal than other WCGF, causing it to have a higher energy value (112 percent the feeding value of maize).

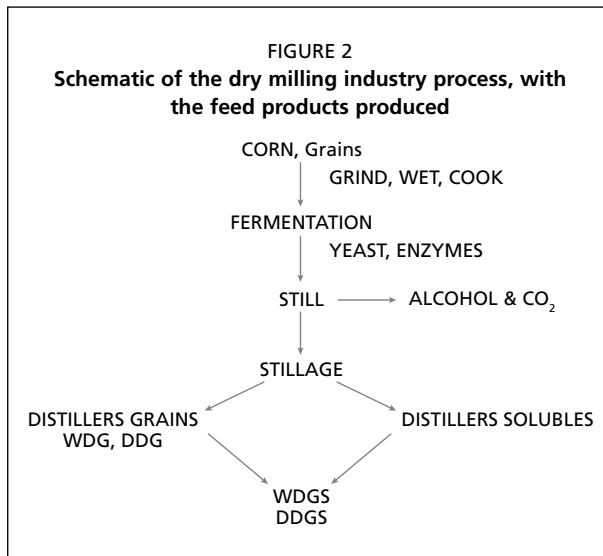
Wet CGF contains 16 to 23 percent CP, of which about 70 percent is degraded in the rumen (degradable intake protein, DIP) and used by rumen microbes. During wet milling, maize gluten meal is removed and marketed in higher value markets. Maize gluten meal should not be confused with WCGF because they are different products. Maize gluten meal contains approximately 60 percent CP of which 40 percent is DIP and 60 percent is bypass protein (also known as undegradable intake protein, UIP).

Dry milling

The dry milling ethanol process (Figure 2) is relatively simple.

Maize (or another starch source such as sorghum [milo]) is ground and then the starch source is converted to ethanol and CO₂ (fermentation). Approximately one-third of the dry matter (DM) remains as a feed product following starch fermentation, assuming the starch source is approximately two-thirds starch. As a result, all the nutrients are concentrated three-fold, because most grains contain approximately two-thirds starch. For example, if maize is 4 percent fat, the DGS will contain approximately 12 percent fat.





After the ethanol distillation step, the resulting product, referred to as stillage, is centrifuged. The purpose of the centrifuging step is to separate the distillers grains from the distillers solubles. These distillers solubles are evaporated and are partially dried. Typically, the distillers solubles are added back to the distillers grains, although individual plants vary in the amount of solubles that are returned to the grains. The nutrient composition may vary depending on the relative ratios of distillers grains to distillers solubles and if the distillers grains are dried partially before the solubles are added. If all of the solubles are added back to the grains, DGS is approximately 80 percent distillers grains and 20 percent distillers solubles on a dry matter (DM) basis (Corrigan *et al.*, 2007). Most distillers grains contain some solubles, but the amount varies from plant to plant. Solubles are a good source of protein, are high in fat, P and S, and low in fibre (Corrigan *et al.*, 2007). Solubles contain 20 to 25 percent CP, 15 to 20 percent fat, >1.0 percent P, 0.92 percent S and 2.3 percent neutral-detergent fibre (NDF). Distillers solubles have become a popular base for

liquid feed supplements. As molasses prices have increased, liquid supplement companies are using steep from the wet milling industry and distillers solubles from the dry milling industry as partial replacement of molasses for liquid supplements. All dry milling plants produce wet DGS (WDGS; 30 to 35 percent DM), but some remove moisture to manufacture modified DGS (MDGS; 42 to 50 percent DM), or dried DGS (DDGS; 88 to 92 percent DM).

Composition

As noted previously, due to production process differences, maize milling co-products can vary in nutrient composition from plant to plant. An overview of this variability in composition of co-products is presented in Table 1. Variation exists from plant to plant, and even day to day within a given plant. These table values are indicative only, and should not replace sampling and analysis of feed from individual plants. The DDGS, WDGS and maize condensed distillers solubles (CCDS) represented in the table are all from one plant in Nebraska and represent average values for 2003.

Examples of plants with an excellent database on variability are the Cargill facilities in Blair, Eddyville and Dalhart in the United States. The standard deviations are low for DM change from load to load. This is a result of two things: process development to minimize variation, and a quality control culture of personnel operating the plants to minimize variation in feed products.

The DDGS composition data in Table 2 are based on the relative ratios of dried distillers grains to solubles ratio in DDGS (Corrigan *et al.*, 2007). The ethanol plant's normal DDGS averaged 19 percent solubles. However, in this study, distillers grains products were produced with 0 to 22 percent solubles added back to the grains portion. Increasing the amount of solubles decreased the DM, CP and NDF content of the DDGS. However, the fat level increased in the DDGS as more solubles were added. As more solubles

TABLE 1
Nutrient composition of selected maize milling co-products

Feedstuff ⁽¹⁾	DRC	WCGF	Sweet Bran	DDGS ⁽²⁾	WDGS ⁽²⁾	CCDS ⁽²⁾	Steep ⁽³⁾ (4)
DM	90.0	44.7	60.0	90.4	34.9	35.5	49.4 (49.0)
SD	0.88	0.89	0.05	1.70	3.60	1.40	1.00 (5.8)
CP (% DM)	9.8	19.5	24.0	33.9	31.0	23.8	35.1
SD	1.10	0.63	0.51	1.30	0.90	1.50	1.10
UIP (% DM)	60.0	20.0	20.0	65.0	65.0	65.0	20.0
P (% DM)	0.32	0.66	0.99	0.51	0.84	1.72	1.92
SD	0.04	0.03	0.04	0.08	0.06	0.27	0.11
NEg (Mcal/kg)	1.54	1.56	1.76	1.72	1.91	1.91	1.94

Notes: DM = dry matter; SD = standard deviation; CP = crude protein as % of DM; UIP = undegradable intake protein, as % of DM; P = phosphorus, as % of DM; NEg = Net energy for gain; DRC = dry-rolled maize [corn]; WCGF = wet maize [corn] gluten feed; DDGS = dried distillers grains with solubles; WDGS = wet distillers grains with solubles; CCDS = maize [corn] condensed distillers solubles. (1) DRC values based on NRC (1996) values with approximately 3500 samples. (2) DDGS, WDGS and CCDS values are from spring, 2003, from only one plant in Nebraska that produces DDGS, WDGS and CCDS, with standard deviations based on weekly composites. (3) DM values represent variation from daily composites for a 60-day period. Other nutrients are based on monthly composites for 2002 and half of 2003. (4) Values in parentheses are monthly composites for 2003 from one plant in Nebraska with the assumption that it is a mixture of steep and distillers solubles.

TABLE 2
Composition of dried distillers grains with solubles (DDGS)

Parameter	Solubles level (% DM)				
	0	5.4	14.5	19.1	22.1
DM (%)	95.5	92.1	90.8	89.3	89.6
CP (%)	32.1	31.9	31.5	30.7	30.9
Fat (%)	6.9	8.9	10.4	12.7	13.3
NDF (%)	36.8	34.9	31.9	30.3	29.3

Notes: NDF = neutral-detergent fibre. CP = crude protein; DM = dry matter. Solubles level calculated using % NDF of solubles (2.3%) and 0% solubles DDG. Source: Adapted from Corrigan *et al.*, 2007.

were added to the grains, from 0 to 22 percent, the resulting DDGS changed from a golden yellow colour to a brown colour. However, the change in colour was not related to total digestive tract protein digestibility because the protein was 97 to 98 percent digestible in all samples.

Samples (n=1200) of WDGS and MDGS were collected for five consecutive days, across four different months and within six dry-milling plants, and analysed for DM, CP, fat, P and S (Buckner *et al.*, 2011). Variation in DM content within each plant was minimal (coefficient of variation (CV) less than 3 percent), but DM was different across plants. Producers should therefore be aware of the DM for each DGS product produced, particularly when buying DGS from more than one plant. On average, DGS contained 31.0 percent CP, 11.9 percent fat, 0.84 percent P and 0.77 percent S. Variation within days, across days, and within the same plant remained small for CP and P (CV less than 4 percent), but P varied slightly more across plants. Fat content variation was slightly more but remained relatively small (CV less than 5 percent) within plants and within days, but larger variation was observed among ethanol plants. Fat content varied from 10.9 to 13.0 percent by plant, probably due to varying amounts of distillers solubles that the plants return to the grains. Therefore, producers should know the fat content from each plant and be less concerned with fat variation within a plant. Variation in S content was the largest for all nutrients tested, as CV within days and across days (within the same ethanol plants) ranged from 3 to 13 percent. These data suggest S values should be routinely monitored because high S levels can lead to nutritional challenges.

A review of several published articles summarized nutrient variability for DGS (Benton, 2010). Average nutrient composition for DGS was 31.5 percent CP, 10.5 percent fat, 6 percent starch, 37.9 percent NDF, 0.51 percent P and 0.57 percent S. Relatively low variation was observed for CP, NDF, P and S, with CVs of 10.7, 10.5, 8.4 and 6.3 percent, respectively. Greater variation was observed for fat and starch, with CVs of 31.4 and 36.3 percent, respectively. This large variation in fat and starch makes some logical sense as this is a summary of many samples over many ethanol plants. Not every ethanol plant combines the same proportion of distillers solubles with distillers grains, nor do they use the same procedure for analysing fat content. Ethanol

plants are also not likely to ferment the same amount of starch from maize for ethanol production.

Although DM variation is probably of greatest importance with wet co-products, both fat and S levels can vary in DGS. Fat variation can lead to changes in feeding value, and S has potential for toxicity (polioencephalomalacia – PEM). It is therefore critical to have accurate analyses of feed ingredients and S analysis of the water that cattle drink. Previously, NRC suggested that diets should not exceed 0.4 percent S (NRC, 1996), or even 0.3 percent S in high-grain feedlot diets (NRC, 2000). However, research has been conducted and will be presented that evaluates performance for cattle fed DGS diets with greater than 0.4 percent S. In addition, thiamine is commonly added at 150 to 200 mg/steer daily to offset challenges related to sulphur-induced PEM. This is an important issue to be aware of and to treat cattle as quickly as possible if any PEM symptoms are observed.

BEEF FINISHING

In terms of philosophy used by nutritionists, the first units of co-products added to a ration are primarily used to replace protein from urea or natural protein sources in the ration. Subsequent additions of co-products to the ration replace maize and other grains, so are considered an energy source. Clearly, the fat and fibre in DGS is used for energy by the animal and associated microbes when DGS is fed. In feedlot diets with DGS at levels less than 15 to 20 percent of diet DM, the DGS serves to meet the protein requirements of the animal. Conversely, when DGS is above 20 percent inclusion, the beef animal utilizes the DGS as both a protein source and an energy source, due to replacement of traditional energy sources. When protein is supplied above the animal's requirements, UIP that is digested is used primarily as an energy source. Therefore, excess protein fed when DGS inclusion is greater than 15 to 20 percent of diet DM is used as energy as well.

PROTEIN SUPPLEMENTATION

In certain production situations, light (less than 341 kg) finishing cattle may need to be supplemented with UIP (bypass) protein to meet metabolizable protein (MP) requirements. Wet or dry DGS is an excellent source of UIP.

TABLE 3
Wet and dry distillers grains for calves

Supplement	ADG	Protein efficiency ⁽¹⁾	ADIN
Urea	0.45	—	—
WG	0.66	2.6	—
DDGS	0.65	2.0	9.7
DDGS	0.67	1.8	17.5
DDGS	0.70	2.5	28.8

Notes: ADIN = acid-detergent-insoluble N; WG = wet grains; DDGS = dried distillers grains with solubles. (1) kg gain/kg supplemental protein.

Wet grains were compared with dry grains and the value of the protein was similar (Table 3). This suggests that the high escape protein value of DGS is due to the innate characteristics of the protein and not to drying or moisture content, and does not appear to be influenced by acid-detergent-insoluble protein, which is a common measure of heat damaged protein.

Distillers grains contain approximately 65 percent UIP (as percentage of CP), consequently diets that include DGS fed as an energy source (generally greater than 15 percent diet DM) are commonly deficient in DIP but contain excess MP. Cattle convert excess MP to urea, which can be excreted in the urine or recycled to the rumen to serve as a source of DIP. Jenkins *et al.* (2011) fed DDGS to finishing cattle at either 10 or 20 percent of diet DM, with or without added urea. No advantage was observed for cattle supplemented with urea (DIP) or not, suggesting recycling was occurring in finishing diets that included 10 or 20 percent DDGS. However, some numerical differences suggested a conservative approach to balancing diets based on protein needs would be to follow NRC (1996) guidelines for DIP supplementation if DGS are provided at less than 20 percent of diet DM. Jenkins *et al.* (2011) also fed 0, 0.5 and 1.0 percent urea (DIP) to dry-rolled maize (DRC)-based diets containing 25 percent WDGS. The diet containing 1.0 percent urea was the only diet that was calculated to meet DIP requirements. In the first 61 days on feed of the 142-day feeding period, dry matter intake (DMI) was similar across urea levels, but average daily gain (ADG) increased with added urea, resulting in an increased gain:feed (G:F) ratio. However, there were no cattle performance differences over the entire feeding period. These data suggest that when DGS are fed with DRC at inclusions greater than

20 percent of diet DM, then recycling occurs and is sufficient to meet the DIP requirements.

ENERGY REPLACEMENT

The feeding value of DGS and CGF is dependent on whether the co-products are fed wet or dry, and the level of dietary inclusion. Although the feeding value of WCGF is better than maize (100 to 112 percent of the feeding value of maize), the feeding value of DCGF is 88 percent of DRC when fed at 25 to 30 percent of diet DM (Green, Stock and Klopfenstein, 1987; Ham *et al.*, 1995).

There have been several research experiments conducted to evaluate inclusion levels of WDGS, MDGS and DDGS on cattle performance. To summarize these experiments, statistical meta-analyses were conducted to evaluate each of these types of DGS and account for differences observed across experiments conducted at the University of Nebraska (Bremer *et al.*, 2011). The inclusion of DGS replaced equal DM portions of DRC and/or high-moisture maize (HMC). In the meta-analysis that summarized 20 trials for feeding up to 40 percent WDGS (of diet DM), quadratic effects were observed for DMI, ADG and G:F (Table 4).

Optimum inclusion of WDGS was observed at 15.8 percent for DMI, 28.4 percent for ADG, and 40 percent for G:F, calculated from the first derivative of the quadratic equation. These improvements in G:F resulted in 30 to 40 percent greater feeding value for WDGS compared with maize at inclusions of 10 to 40 percent. Although these were quadratic relationships, feeding 40 percent WDGS resulted in greater ADG and G:F compared with a traditional maize-based diet. Greater 12th rib fat thickness and marbling scores result from feeding WDGS, and were also quadratic relationships. The meta-analysis that summarized MDGS in four feeding trials up to 40 percent diet DM also indicated quadratic relationships for DMI, ADG and G:F (Table 5). Optimum inclusion of MDGS for DMI was at 22.5 percent and 29.4 percent for ADG, and 40 percent for G:F.

These improvements in cattle performance resulted in 15 to 30 percent greater feeding value for MDGS compared with maize, in which cattle had greater ADG and G:F for all inclusions up to 40 percent. A quadratic relationship was observed for 12th rib fat thickness and a linear relationship for marbling score for feeding MDGS. These

TABLE 4
Performance measurements for cattle fed increasing levels of wet distillers grains plus solubles (WDGS)

	Control diet	10% WDGS	20% WDGS	30% WDGS	40% WDGS
DMI (kg/day) ⁽¹⁾	10.5	10.6	10.6	10.5	10.2
ADG (kg) ⁽¹⁾	1.60	1.71	1.77	1.79	1.76
G:F ⁽¹⁾	0.155	0.162	0.168	0.171	0.173
12th rib fat (cm)	1.22	1.32	1.37	1.40	1.40
Marbling score ⁽²⁾	528	535	537	534	525

Notes: Levels are as a % of diet DM. DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. (1) Quadratic response to level of WDGS in the diet ($P < 0.01$). (2) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. Source: Adapted from Bremer *et al.*, 2011.

TABLE 5
Performance measurements for cattle fed increasing levels of modified distillers grains with solubles (MDGS) as percentage of diet DM

	Control diet	10% MDGS	20% MDGS	30% MDGS	40% MDGS
DMI (kg/day) ⁽¹⁾	11.0	11.4	11.6	11.5	11.3
ADG (kg) ⁽¹⁾	1.68	1.79	1.85	1.85	1.81
G:F ⁽²⁾	0.152	0.156	0.160	0.162	0.162
12th rib fat (cm)	1.30	1.45	1.52	1.52	1.47
Marbling score ⁽³⁾	559	554	550	545	540

Notes: (1) Quadratic response to level of MDGS in the diet ($P < 0.01$). (2) Quadratic response to level of MDGS in the diet ($P = 0.07$). (3) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. ADG = average daily gain; G:F = gain-to-feed ratio. Source: Adapted from Bremer *et al.*, 2011.

TABLE 6
Performance measurements for cattle fed increasing levels of dried distillers grains with solubles (DDGS), as a percentage of diet DM

	Control diet	10% DDGS	20% DDGS	30% DDGS	40% DDGS
DMI (kg/day) ⁽¹⁾	11.0	11.5	11.8	11.9	11.9
ADG (kg) ⁽²⁾	1.57	1.63	1.69	1.75	1.80
G:F ⁽²⁾	0.141	0.143	0.145	0.147	0.148
12th Rib fat, cm	1.12	1.24	1.30	1.30	1.22
Marbling score ⁽³⁾	569	569	569	569	569

Notes: (1) Quadratic response to level of DDGS in the diet ($P = 0.03$). (2) Linear response to level of DDGS in the diet ($P < 0.01$). (3) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. Source: Adapted from Bremer *et al.*, 2011.

cattle performance changes for MDGS were not as great as with WDGS.

Another meta-analysis that summarized DDGS in four trials also resulted in a quadratic effect for DMI, as optimum inclusion was between 20 and 40 percent of diet DM (Table 6). Linear relationships were observed for ADG and G:F, as optimum inclusion was 40 percent DDGS. This resulted in a 13 percent improvement in feeding value when feeding DDGS compared with maize. A quadratic relationship resulted for 12th rib fat thickness, while no effect was observed for marbling score due to feeding DDGS compared with maize. This improvement in cattle performance was not as great as MDGS, suggesting that drying DGS decreases its feeding value.

Although all of these meta-analysis summaries have a large amount of data to support the results and are representative over many experiments, the three types were never fed in the same experiment, until recently. Nuttelman *et al.* (2010b) fed WDGS, MDGS, and DDGS in the same trial at 0, 20, 30 and 40 percent dietary DM inclusions. No interactions between co-product level (20, 30 or 40 percent) and type (WDGS, MDGS and DDGS) were observed. Therefore, only the main effects of co-product level (Table 7) and co-product type (Table 8) were summarized. Optimum inclusion of DGS was 40 percent for ADG and G:F. A linear increase was observed for fat depth, with marbling score unchanged, as DGS inclusion increased. Therefore, these data suggest that cattle performance is enhanced the most with increasing levels of DGS up to 40 percent, similar to the conclusions drawn from the meta-analyses.

Within co-product type, no differences were observed for ADG, but DMI was greatest for DDGS, least for WDGS, and

TABLE 7
Performance measurements for cattle fed increasing levels of distillers grains with solubles (DGS) as percentage of diet DM⁽¹⁾

	0% DGS	20% DGS	30% DGS	40% DGS
DMI (kg/day)	11.2	12.0	11.8	12.0
ADG (kg)	1.63	1.85	1.84	1.90
G:F ⁽²⁾	0.146	0.156	0.157	0.161
Carcass characteristics				
HCW (kg)	378	400	398	405
Marbling score ⁽³⁾	607	609	599	603
12th rib fat (cm)	1.27	1.57	1.57	1.65

Notes: DMI = dry matter intake; ADG = average daily gain; HCW = hot carcass weight; G:F = gain-to-feed ratio. (1) Overall main effect for level of DGS, including WDGS, MDGS and DDGS. (2) Linear response to level of DGS in the diet ($P < 0.01$). (3) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. Source: Adapted from Nuttelman *et al.*, 2010b.

TABLE 8
Performance measurements for cattle fed wet (WDGS), modified (MDGS) or dried distillers grains with solubles (DDGS)⁽¹⁾

Parameter	WDGS	MDGS	DDGS
DMI (kg/day)	11.3 a	12.0 b	12.3 b
ADG (kg)	1.87	1.90	1.84
G:F	0.165 a	0.158 b	0.150 c
Carcass characteristics			
HCW (kg)	401	403	399
Marbling score ⁽²⁾	610	599	602
12th rib fat (cm)	1.60	1.63	1.52

Notes: DMI = dry matter intake; ADG = average daily gain; HCW = hot carcass weight; G:F = gain-to-feed ratio. (1) Overall main effect of feeding DGS at 20, 30 and 40% DM inclusion. (2) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. a,b,c = Means within the same row without a common suffix differ ($P < 0.05$). Source: Adapted from Nuttelman *et al.*, 2010b.

TABLE 9
Performance measurements for cattle fed increasing levels of 'Sweet Bran' (SB) WCGF as a percentage of diet DM

	Control diet	10% SB	20% SB	30% SB	40% SB
DMI (kg/day) ⁽¹⁾	9.9	10.1	10.4	10.6	11.1
ADG (kg) ⁽¹⁾	1.67	1.73	1.78	1.84	1.90
G:F ⁽¹⁾	0.168	0.169	0.171	0.172	0.174
12th rib fat (cm)	1.17	1.19	1.24	1.27	1.32
Marbling score ⁽²⁾	492	497	501	506	511

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. (1) Linear response to level of SB in the diet ($P \leq 0.03$). (2) Marbling score: 400 = Slight, 500 = Small, 600 = Modest. Source: Adapted from Bremer, Erickson and Klopfenstein, 2008.

intermediate for MDGS. This suggests that cattle consume more feed to support the same gain for dried (DDGS) or partially dried (MDGS) distillers compared with no drying (WDGS).

Distinct differences exist for WCGF, even within companies, due to plant-to-plant variation. Stock *et al.* (1999) divided WCGF into two main categories, depending on the ratio of steep to bran. Based on differences in the amount of steep added, WCGF has 100 to 109 percent the feeding value of DRC when fed at levels of 20 to 60 percent of diet DM (Stock *et al.*, 1999). Higher feeding value (and protein) is associated with increases in steep added in WCGF. 'Sweet Bran' (Cargill, Blair) has more steep relative to maize bran and is of higher feeding value than traditional WCGF. However, feeding WCGF results in better performance than DCGF (Ham *et al.*, 1995). A meta-analysis was conducted by Bremer, Erickson and Klopfenstein (2008) to evaluate increasing levels of 'Sweet Bran' in feedlot diets. Cattle consumed more DM and had greater ADG and G:F when fed 'Sweet Bran' compared with maize (Table 9). Each of these parameters resulted in a linear relationship, thus indicating that performance theoretically continues to increase up to 40 percent 'Sweet Bran', the maximum included in this dataset. Cattle fed 'Sweet Bran' had greater 12th rib fat thickness and marbling scores.

The improved animal feeding performance from co-product feeds translates into increased 12th rib fat thickness and either equal or greater marbling scores compared with maize. Cattle gain weight quicker when fed co-products compared with feedlot cattle fed maize. Therefore, cattle either require fewer days on feed to reach the same end weight, backfat and marbling score, or they will be slaughtered heavier and fatter with co-products in the diet. The increased fat thickness and marbling is presumably due to improved daily gains and energy content of the diets when co-products are fed.

HIGH INCLUSIONS

Co-product feeds can be priced cheaply due to supply and demand fluctuations, and may be a very attractive feed when grains are priced high. Therefore, some research has been conducted to evaluate feeding greater amounts (>50 percent diet DM) of WDGS in finishing diets to

determine impact on performance. Providing other low-fat co-products or greater roughage inclusions might offset the risk related to high S and PEM, or high fat resulting in decreased cattle performance.

Loza *et al.* (2010) conducted three experiments evaluating combinations of WCGF and WDGS up to 75 percent of diet DM, with varying levels of forage. Cattle fed a 1:1 ratio of WCGF and WDGS had similar or improved performance compared with cattle fed a maize-based diet. Some PEM symptoms were observed in cattle fed diets with >60 percent co-products and 0 percent roughage.

Wilken *et al.* (2009) evaluated four diets containing higher (>50 percent diet DM) amounts of co-products compared with a DRC-based control diet and a DRC diet with 44 percent WDGS. All diets contained 7.5 percent alfalfa hay. The four experimental diets were: (1) 33 percent WDGS plus 33 percent 'Sweet Bran' with 22 percent DRC; (2) 33 percent WDGS, 33 percent 'Sweet Bran' and 22 percent soyhulls, with no DRC; (3) 44 percent WDGS plus 44 percent 'Sweet Bran' with no DRC or soyhulls; and (4) 66 percent WDGS with 22 percent brome grass hay. Cattle diet (3) had the lowest DMI, probably due to high dietary energy (Table 10). Cattle fed diet (4) had the greatest DMI. Cattle fed 44 percent WDGS with maize had the greatest ADG and G:F. However, when cattle were fed diets containing a co-product combination with no soyhulls or 66 percent WDGS with 22 percent grass hay, cattle performance was considered acceptable and similar to the maize control diet.

Because the previous trial indicated that feeding a higher inclusion of WDGS with a larger amount of roughage yielded acceptable performance with no incidences of PEM, a second trial was conducted by Rich *et al.* (2010) that evaluated high inclusions of WDGS with varying levels of wheat straw. Two dietary treatments were similar to Wilken *et al.* (2009) in this trial: a DRC-based control diet and a DRC diet with 40 percent WDGS. Five other dietary treatments were: (1) 70 percent WDGS plus 8 percent straw, with 17 percent DRC; (2) 77.5 percent WDGS plus 9 percent straw, with 8.5 percent DRC; (3) 85 percent WDGS plus 10 percent straw replacing all maize; (4) 70 percent WDGS plus 25 percent straw replacing all maize; and

TABLE 10
Effect of feeding high levels of co-products on cattle performance

	Diet (see notes for details)					
	83maize	44DG:maize	33DG:33SB:maize	33DG:33SB:hulls	44DG:44SB	66DG:hay
DMI (kg/day)	11.9 bc	11.5 ab	11.9 bc	11.7 abc	11.3 a	12.1c
ADG (kg)	1.83 b	2.03c	1.89 b	1.70 a	1.80 b	1.83 b
G:F	0.154 bc	0.177 a	0.159 b	0.144 d	0.160 b	0.151c

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio.

Key to diets: 83maize = 83% maize-based control; 44DG:maize = 44% WDGS in maize based diet; 33DG:33SB:maize = 33% WDGS with 33% 'Sweet Bran' and 22% maize; 33DG:33SB:hulls = 33% WDGS with 33% 'Sweet Bran' and 22% soyhulls; 44DG:44SB = 44% WDGS with 44% 'Sweet Bran'; 66DG:hay = 66% WDGS with 22% grass hay. Represented as a % of diet DM. Percentage S in diets on DM basis: 83maize = 0.153%; 44DG:maize = 0.403%; 33DG:33SB:maize = 0.475%; 33DG:33SB:hulls = 0.476%; 44DG:44SB = 0.587%; 66DG:hay 0.549%. a,b,c,d = Means within the same row without a common suffix differ ($P \leq 0.06$). Source: Adapted from Wilken *et al.*, 2009.

TABLE 11
Effect of feeding high levels of WDGS in combination with straw on cattle performance

	Diet (see notes for details)						
	83maize	40DG:maize	70DG:8straw	77DG:9straw	85DG:10straw	70DG:25straw	77DG:17straw
DMI (kg/day)	10.3	10.4	9.2	8.6	8.1	8.3	8.9
ADG (kg)	1.64 b	1.97 a	1.66 b	1.62 b	1.31 d	1.13 e	1.40 c
G:F	0.159 c	0.189 a	0.181 b	0.186 ab	0.162 c	0.137 d	0.157 c
DOF (n)	183	183	183	183	225	225	225
12th rib fat (cm)	1.07	1.55	1.22	1.09	1.09	0.69	1.27

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio; DOF = degrees of freedom.

Key to diets: 83maize = 83% maize-based control; 40DG:maize = 40%WDGS in a maize-based diet; 70DG:8straw = 70% WDGS with 8% straw; 77DG:9straw = 77% WDGS with 9% straw; 85DG:10straw = 85% WDGS with 10% straw; 70DG:25straw = 70% WDGS with 25% straw; 77DG:17straw = 77% WDGS with 17% straw. Represented as a % of diet DM. Percentage S in diets on a DM basis: 83maize 0.05%; 40DG:maize = 0.38%; 70DG:8straw = 0.57%; 77DG:9straw = 0.61%; 85DG:10straw = 0.66%; 70DG:25straw = 0.55%; 77DG:17straw = 0.60%. a,b,c,d,e = Means within the same row without a common suffix differ ($P < 0.05$). Source: Adapted from Rich *et al.*, 2010.

(5) 77.5 percent WDGS plus 17.5 percent straw replacing all maize. Feeding more than 70 percent WDGS and no maize (elevated straw diets) resulted in the poorest cattle performance, with the lowest DMI, ADG and G:F (Table 11). In fact, daily gains were considerably less, so that cattle had to remain on these diets for an additional 42 days in an attempt to get those cattle to reach equal market weight. This suggests that to maintain adequate cattle performance, low quality roughages should not be used to replace all maize inclusion in high WDGS diets. As expected, cattle fed 40 percent WDGS in a DRC-based diet had the best cattle performance. Feeding 70 percent WDGS with 8 percent straw and 77.5 percent WDGS with 9 percent straw resulted in similar ADG compared with the maize-control diet, but DMI was less, and G:F improved compared with the maize control. This study suggests that cattle fed 70–77 percent WDGS with less than 10 percent straw and some inclusion of DRC results in adequate performance. When WDGS is priced below 70 percent of expensive maize, these diets may become feasible up to 77 percent of diet DM. However, inclusion of poor quality roughage should be less than 10 percent with high inclusions of WDGS. No sulphur-induced PEM was observed in this study.

Including roughages above normal levels appears to be an appropriate avenue of maintaining cattle performance compared with an all-maize diet. The feasibility of these high-WDGS diets largely depends on the price of WDGS

and forages and the hauling cost for WDGS. Both of these experiments proved to be appropriate means to feed high inclusions of WDGS in combination with 'Sweet Bran' or roughage, as long as some maize remained in the diet.

ROUGHAGES

Forages ("roughages") are often included at low levels (<12 percent of diet DM) to control acidosis and maintain intake in feedlot cattle (Stock and Britton, 1993). Since co-products reduce the occurrence of acidosis in feedlot cattle, then perhaps roughage levels could be reduced from conventional levels in diets containing co-products. Farran *et al.* (2004) fed either 0 or 35 percent WCGF with 0, 3.75 or 7.5 percent alfalfa hay at each level (i.e., treatments were factorialized with WCGF level and hay level). There was a significant interaction between WCGF and alfalfa level on G:F. Therefore, only simple effects were discussed (Table 12). Increasing alfalfa hay level with 0 percent WCGF increased ADG and DMI with no effect on G:F. With 35 percent WCGF, increasing alfalfa hay increased ADG and DMI, but hindered (decreased) G:F linearly. Roughages can perhaps be reduced in DRC-based diets containing 35 percent or more WCGF. However, ADG was reduced for the 0 percent hay and 35 percent WCGF treatment, so a small amount of roughage is recommended even when WCGF is included. Similar results have been observed with steam-flaked maize (SFC) based diets where alfalfa can be

TABLE 12

Effect of increasing alfalfa hay level in diets with and without wet maize gluten feed (WCGF) for finishing yearlings fed dry-rolled maize (DRC)-based diets

	0% WCGF			35% WCGF		
	0	3.75	7.50	0	3.75	7.50
Alfalfa level						
DMI (kg/day) ⁽¹⁾	10.3	10.8	11.0	10.6	11.3	11.6
ADG (kg) ⁽¹⁾	1.67	1.82	1.82	1.79	1.85	1.85
G:F ⁽²⁾	0.161	0.168	0.166	0.168	0.164	0.160

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. (1) Non-significant interaction between WCGF and alfalfa level; significant ($P < 0.10$) increase due to WCGF; significant ($P < 0.03$) linear increase for alfalfa level. (2) WCGF \times alfalfa level interaction ($P < 0.09$); Linear effect ($P < 0.06$) of alfalfa level within 35% WCGF; no effect of alfalfa hay with 0% WCGF. Source: Adapted from Farran *et al.*, 2004.

TABLE 13

Effects of increasing alfalfa hay in steam-flaked maize diets containing 25% dried distillers grains with solubles on cattle performance

	Alfalfa hay (as percentage of diet DM)				
	3	6	9	12	15
DMI (kg/day) ⁽¹⁾	10.7	11.1	11.3	11.9	11.5
ADG (kg) ⁽¹⁾	1.54	1.59	1.62	1.66	1.61
G:F	0.144	0.143	0.143	0.140	0.140

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. (1) Quadratic response to level of alfalfa hay in the diet ($P < 0.01$). Source: Adapted from Miller *et al.*, 2009.

TABLE 14

Effect on cattle performance of alfalfa hay level in steam-flaked maize diets containing 15 or 30% wet distillers grains with solubles (WDGS)

	Control	Treatment (see notes)					
		15% DG-L	15% DG-M	15% DG-H	30% DG-L	30% DG-M	30% DG-H
DMI (kg/day)	8.9	9.0	9.0	9.4	8.9	9.0	9.2
ADG (kg)	1.48	1.54	1.52	1.59	1.54	1.46	1.51
G:F	0.168	0.172	0.169	0.167	0.173	0.163	0.166

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio.

Key to diets: Control = 0% WDGS with 10% alfalfa hay; 15DG-L = 15% WDGS with 7.5% alfalfa hay; 15DG-M = 15% WDGS with 10% alfalfa hay; 15DG-H = 15% WDGS with 12.5% alfalfa hay; 30DG-L = 30% WDGS with 7.5% alfalfa hay; 30DG-M = 30% WDGS with 10% alfalfa hay; 30DG-H = 30% WDGS with 12.5% alfalfa hay. Source: Adapted from May *et al.*, 2011.

reduced to 2 percent with at least 25 percent WCGF (Sindt *et al.*, 2003).

Parsons and Stanton (2000) observed no change in G:F when alfalfa hay was decreased from 9 to 0 percent in SFC diets containing 40 percent 'Sweet Bran', but DMI and ADG decreased linearly. Just as with results in conventional maize-based diets, the optimum amount of roughage appears to be dependent on grain processing and level of WCGF.

Alfalfa hay levels have also been fed to feedlot cattle at increasing levels of 3, 6, 9, 12 and 15 percent (of diet DM) in SFC-based diets containing 25 percent DDGS (Miller *et al.*, 2009). A quadratic response was observed for DMI and ADG with increasing level of alfalfa hay in diets, but with no response in G:F (Table 13).

The optimum inclusion level of alfalfa hay in this trial was 9 to 12 percent. A second trial evaluated alfalfa hay levels of 7.5, 10 and 12.5 percent (of diet DM) in SFC-based diets containing 15 or 30 percent WDGS in a 3 \times 2 factorial arrangement of treatments (May *et al.*, 2011). These treatments were also compared with a control diet containing 10 percent alfalfa hay with no WDGS. Regardless of 15 or

30 percent WDGS, greater inclusions of alfalfa hay promoted greater DMI and poorer G:F, with no effect on ADG (Table 14). The control diet resulted in the lowest DMI and ADG, suggesting that WDGS promotes greater cattle performance. These data agree with Miller *et al.* (2009) in that including increasing amounts of alfalfa hay up to 10 percent promotes greater DMI. Although DDGS and WDGS may offset some acidosis challenges, these trials suggest some roughage should remain in the diets to promote DMI and sometimes aid ADG and G:F.

Benton *et al.* (2007) fed alfalfa hay, maize silage or maize stalks as the roughage source in 30 percent WDGS (DM basis) diets. Each of the sources were included at a conventional level, one-half that level, and compared with a diet with no roughage (Table 15).

The normal level was equal to 8 percent alfalfa hay and the low level was equal to 4 percent alfalfa hay. Maize silage and maize stalks diets were formulated to provide NDF (from roughages only) equal to the alfalfa hay diets. In general, conventional roughage levels increased DMI and ADG. When roughage was eliminated from the 30 percent

TABLE 15
Effects of roughage source and level compared with no roughage inclusion on performance of steers fed diets containing 30% wet distillers grains with solubles

	Treatment (see notes)						
	Control	LALF	LCSIL	LCSTK	NALF	NCSIL	NCSTK
Roughage (%) ⁽¹⁾	0.0	4.0	6.1	3.0	8.0	12.3	6.1
DMI (kg/day)	10.1 a	11.1 b	11.0 b	11.4 bc	11.7 c	11.5 c	11.6 c
ADG (kg)	1.97 a	2.05 ab	2.05 a	2.18 c	2.16 bc	2.16 bc	2.18 c
G:F	0.195	0.186	0.187	0.192	0.185	0.188	0.188

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio; LALF = low alfalfa hay; LCSIL = low maize silage; LCSTK = low maize stalks; NALF = normal alfalfa hay; NCSIL = normal maize silage; NCSTK = normal maize stalks. (1) Inclusion level of each roughage source in the finishing diet (DM basis). a,b,c = Means in a row with unlike suffixes differ ($P < 0.05$). Source: Adapted from Benton *et al.*, 2007.

WDGS diets, G:F was improved, but DMI and ADG were decreased compared with diets containing normal levels of alfalfa (8 percent), maize stalks (6 percent) or maize silage (12 percent). Therefore, it is not beneficial to completely eliminate roughage sources from finishing diets containing 30 percent WDGS (DM basis). Interestingly, feeding maize stalks was either similar or better in terms of performance to other roughages. Feeding wet co-products allows for lower quality roughages to be used because protein is not needed with higher protein in co-products compared with maize, and mixing and palatability are aided with WDGS. The moisture in diets containing WDGS should allow for decreased sorting of low quality forage (Benton *et al.*, 2007). As roughages contain different amounts of fibre content, roughages can be exchanged on the basis of NDF in the roughage (Galyean and Defoor, 2003).

GRAIN PROCESSING

Feeding maize milling co-products in feedlot diets reduces acidosis-related challenges. Both WCGF and WDGS have little or no starch remaining following the milling process. Therefore, feeding these co-products will dilute dietary starch that is fed and influence rumen metabolism.

Feeding WCGF helps prevent the risk of acidosis with high-grain diets, as observed by greater rumen pH in metabolism steers (Krehbiel *et al.*, 1995). In many studies, feeding WCGF resulted in increased DMI, which would be a common response to decreased subacute acidosis. However, processing maize increases the rate of digestion by rumen microbes. As a result, more rumen acid is produced, which increases the risk of acidosis (Stock and Britton, 1993). Feeding co-products may affect the feeding value or acidosis challenge, or both, with different maize processing types.

Numerous studies have been conducted at the University of Nebraska to determine if feeding values are improved in diets containing WCGF when maize is more intensely processed. Scott *et al.* (2003) evaluated various maize processing techniques and observed improved G:F as processing intensity of the maize increased when fed to calves or yearlings (Table 16). Ranking of processing based on G:F (lowest to highest) was whole maize, DRC, HMC and steam-flaked maize (SFC) when fed to finishing calves. Relative improvements in G:F for DRC, HMC and SFC compared with whole maize were 6.8, 11.1 and 12.5 percent, respectively. When fed to yearlings, response to processing was not as

TABLE 16
Effect of maize processing on cattle performance when fed with wet maize gluten feed (WCGF)

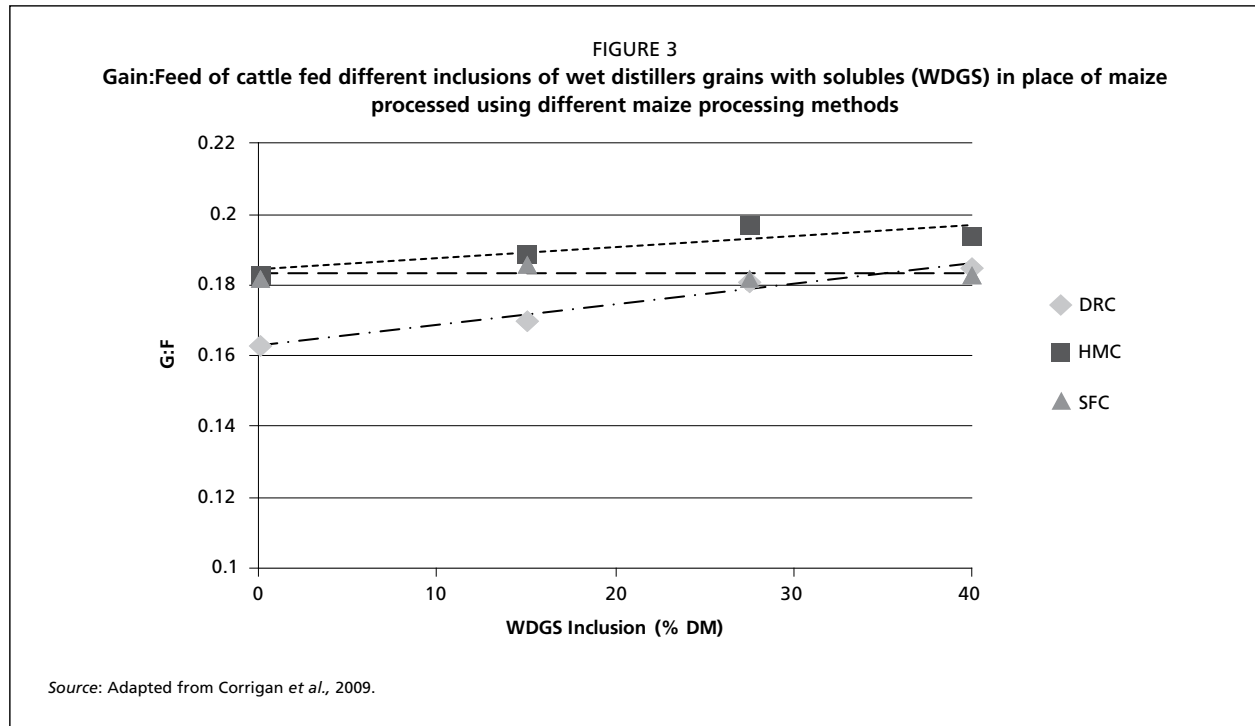
	Processing method (see notes)			
	25% WCGF			
	GHMC	DRC	RHMC	SFC
ADG (kg)	1.93	1.92	1.91	1.97
G:F	0.198 b	0.182 c	0.195 b	0.204 a
NEg (maize) (Mcal/kg)	1.71	1.54	1.68	1.77
Faecal starch (%)	8.4 b	19.2 c	10.6 ab	4.1 a
	32% WCGF with calves			
	Whole	DRC	RHMC	SFC
ADG (kg)	1.9	1.93	1.89	1.93
G:F	0.169 d	0.181 c	0.190 b	0.193 a
	22% WCGF with yearlings			
		DRC	RHMC	SFC
ADG (kg)		1.81 a	1.83 a	1.92 b
G:F		0.164 b	0.168 b	0.181 a

Notes: ADG = average daily gain; G:F = gain-to-feed ratio. Key to processing methods: DRC = dry rolled maize; RHMC = rolled high moisture maize; GHMC = ground high moisture maize; SFC = steam-flaked maize; whole = whole maize. NEg = Net energy for gain; a,b,c,d = Means with different suffixes differ ($P < 0.05$). Sources: Adapted from Scott *et al.*, 2003, and Macken *et al.*, 2006.

TABLE 17
Effect of maize processing on cattle performance when fed diets containing 30 percent WDGS

	Processing method (see notes)				
	Whole	DRC	DRC/HMC	HMC	SFC
DMI (kg/day)	10.5 a	10.3 a	9.8 b	9.5 bc	9.3 c
ADG (kg)	1.75 a	1.84 b	1.78 ab	1.77 ab	1.63 c
G:F	0.165 a	0.176 bc	0.178 bc	0.183 c	0.174 b

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. Key to processing methods: Whole = whole maize; DRC = dry rolled maize; DRC/HMC = 50:50 blend of dry rolled maize and high moisture maize; HMC = high moisture maize; SFC = steam-flaked maize. a,b,c,d = Means within a row with different suffixes differ ($P < 0.05$). Source: Adapted from Vander Pol *et al.*, 2008.



favourable as with calves. Feeding HMC did not significantly improve G:F compared with DRC. Macken *et al.* (2006) fed DRC, SFC and HMC processed as either rolled (roller mill, RHMC) or ground (tub grinder, GHMC) to calves, with all diets containing 25 percent WCGF. Whole maize was not fed in this study, but performance was improved as the maize was more intensely processed (Table 16). Net energy calculated from performance (NRC, 1996; Owens, Hinds and Rice, 2002.) was increased by 9.1, 11.0 and 14.9 percent for RHMC, GHMC and SFC, respectively, compared with DRC.

HMC appears to have greater feeding value when diets contain WCGF than previously observed in diets without WCGF. Because HMC has greater ruminal starch digestibility than DRC or SFC (Cooper *et al.*, 2002), cattle fed HMC have a greater potential for acidosis when HMC is fed alone. However, feeding HMC in combination with WCGF appears to increase efficiency of HMC utilization, perhaps by reducing acidosis. For example, the feeding value of HMC in diets containing HMC as the only grain source is lower than that observed when fed in combination with other grains (Stock *et al.*, 1991) or maize co-products.

Previous reviews reported that HMC feeding resulted in 2 percent greater efficiency than DRC (Owens *et al.*, 1997). However, based on research with HMC-based diets containing 20 to 35 percent WCGF, cattle are 5 to 10 percent more efficient than those fed WCGF and DRC. Our conclusion is that intense maize processing (HMC or SFC) has tremendous value in diets containing WCGF.

However, optimal maize processing in diets containing WDGS appears to be somewhat different than in diets containing WCGF. Vander Pol *et al.* (2008) fed diets containing 30 percent WDGS with either whole maize, DRC, HMC, a 50:50 blend of HMC and DRC (DM basis), or SFC to finishing steers for 168 days. Cattle fed DRC, HMC or a combination of HMC and DRC gained more and were more efficient than cattle fed whole maize alone (Table 17). Interestingly, cattle fed SFC did not gain as efficiently.

Corrigan *et al.* (2009) investigated feeding DRC, HMC or SFC in diets containing 0, 15, 27.5 or 40 percent WDGS. They found greater performance responses for greater WDGS inclusion in diets based on DRC and HMC (Figure 3). Optimal ADG and G:F resulted from 40 percent WDGS in

TABLE 18
Feeding value of wet distillers grains with solubles (WDGS) in dry-rolled maize (DRC) or combinations of high-moisture maize (HMC) and DRC diets at 0 to 40 percent DM inclusion for calves and yearlings

	Diet (see notes)				
	0WDGS	10WDGS	20WDGS	30WDGS	40WDGS
Calves					
DRC, feeding value	—	136	136	136	136
DRC:HMC, feeding value	—	124	124	124	124
Yearlings					
DRC, feeding value	—	167	159	151	143
DRC:HMC, feeding value	—	154	146	138	132

Notes: Feeding value = difference in G:F between WDGS treatment level and 0% WDGS inclusion, and divided by % of WDGS inclusion. Diets are 0WDGS = 0% WDGS; 10WDGS = 10% WDGS; 20WDGS = 20% WDGS; 30WDGS = 30% WDGS; 40WDGS = 40% WDGS. Diets expressed as a percentage of diet DM. Source: Adapted from Bremer *et al.*, 2010a.

DRC-based diets, 27.5 percent WDGS in HMC-based diets, and 15 percent WDGS in SFC-based diets. In addition, when 40 percent WDGS was included in DRC diets, cattle performed just as efficiently as cattle fed any of the SFC diets. A greater performance response to WDGS inclusion in diets based on less intensely processed grain may render them an economically attractive alternative compared to diets based on more intensely processed grains. Cattle performance is improved by steam flaking corn when diets contain WCGF. It is unclear why steam flaking did not improve performance when diets contained WDGS.

In the meta-analysis of 20 experiments for feeding increasing dietary levels of WDGS conducted by Bremer *et al.* (2010a), they evaluated feeding value differences of WDGS when fed in either DRC or DRC plus HMC blended diets, and when fed to calves or yearlings. Feeding value was calculated based on the G:F difference between a diet including WDGS and the predominately maize based diet, then divided by the percent inclusion of WDGS. For both calves and yearlings, greater feeding values resulted from including WDGS in DRC-based diets compared with the DRC plus HMC blended diets (Table 18). This further agrees with previous research that greater performance responses are observed when WDGS is included in diets with less intensely processed maize. Greater feeding values were also observed when WDGS was included in DRC or DRC plus HMC based diets for yearlings compared with calves. This suggests that cattle producers can feed WDGS to yearlings and get a greater performance response to WDGS compared with a predominately maize-based diet than with calves. It is unclear why the energy response to feeding WDGS is greater with yearlings than calves.

SULPHUR

Sulphur concentration in maize is 0.10 to 0.15 percent of DM, but S content in DGS is commonly 0.7 to 0.8 percent. Normally, nutrients are concentrated in DGS by a factor of three from that in maize, but ethanol plants typically use sulphuric acid to control pH, thereby increasing proportionately the S content in the DGS. Therefore, diets can be

high in S if a large quantity of DGS is included in diets or if the S content in the DGS is abnormally high. The common concern with feeding high dietary S is that S can be converted to hydrogen sulphide (H₂S) in the rumen and result in polioencephalomalacia ("polio" or PEM). This condition is commonly referred to as 'brainers', in which cattle experience lack of coordination. Brainers is a general term covering central nervous system problems that can be due to numerous causes, including PEM. Cattle that are chronic brainers do not recover from this condition and, if they survive, they probably will not recover in terms of performance. The key to treating cattle with PEM is early diagnosis and intravenous infusion of thiamine (Gould, 1998; Brent and Bartley, 1984). The occurrence of PEM appears to be fairly random, but is still highly correlated with dietary S concentration (and probably even more so to ruminally degradable S intake). It should be noted that while PEM is a concern, producers using less than 40 percent inclusion of any co-products (DM basis) should expect few if any cases of PEM. It should also be noted that a small incidence of PEM has been common in the feedlot industry, even before the use of DGS. However, increasing S intake exacerbates the challenge and can result in very high incidences of PEM if not monitored. Water should be tested for sulphates and accounted for in total S intake.

NRC (1996) states that 0.4 percent dietary S is considered to be a concentration that can result in PEM conditions. However, many research experiments have been conducted with co-product-containing diets where dietary S concentrations exceeded 0.4 percent, but with low PEM incidences. Thus Vanness *et al.* (2009) summarized several research experiments involving 4143 cattle in which co-products were fed to evaluate S content in the diet and incidence of PEM. Polio was defined as either identification and treatment of PEM by the health crew in the feedlot, or death due to PEM confirmed by necropsy. Very low levels of sulphates were present in the drinking water in this research feedlot (less than 100 ppm sulphate). A small incidence of PEM (0.14 percent) was observed when diets contained 0.46 percent S or less. Incidences of PEM

increased with increasing dietary S. When dietary S was 0.47 to 0.58 percent, occurrence of PEM was 0.38 percent. This incidence increased to 6.06 percent when dietary S was above 0.58 percent. A level of 0.47 percent S is typical when WDGS is included at 50 percent of diet DM. For producers it is important to be aware of the S content in their co-products and their drinking water, and perhaps monitor cattle closely for clinical signs of PEM if dietary S is above 0.47 percent.

There is evidence that high dietary S concentration may also negatively affect cattle intake and gain. Uwituze *et al.* (2009) evaluated feeding cattle two types of DDGS at 30 percent DM inclusion in either DRC or SFC finishing diets. These two types of DDGS included normal DDGS and DDGS that was spiked with sulphuric acid. The diets contained either 0.42 or 0.65 percent S. No interaction resulted from S level and grain processing. Cattle fed diets with high S had 8.9 percent lower DMI and 12.9 percent poorer ADG, resulting in 4.3 percent lighter carcass weights. These cattle also had higher concentrations of ruminal hydrogen sulphide gas. These data suggest that although cattle may not exhibit clinical signs of PEM, cattle consume less feed to offset high S intakes, and weight gain is hindered, but efficiency is not affected.

Sulphur level in DGS diets was evaluated for both DDGS and WDGS when fed at increasing levels in the diet (Sarturi *et al.*, 2010). WDGS and DDGS were fed at 20, 30 and 40 percent of DM and compared with a maize control. Each DGS contained either 0.82 percent or 1.16 percent S and were from two different ethanol plants. Cattle were individually fed (120 steers) with treatments arranged as a 2x2x3+1, factorial with factors of moisture (DDGS or WDGS), S concentration (0.82 or 1.16 percent) and three inclusions (20, 30 or 40 percent). A linear increase in DMI was observed for co-product level when feeding the low-S DDGS, but DMI was not affected for low-S WDGS. Feeding high S decreased DMI quadratically for DDGS and linearly for WDGS. These intake differences are probably due to differences in energy content between DDGS and WDGS, as DDGS has a lower energy value. Feeding the high-S DGS decreased ADG at inclusions of 30 to 40 percent DM for WDGS and 40 percent for DDGS. However, feeding DGS with low S content resulted in ADG equal to or above cattle fed the maize control diet. Feeding DDGS at either low or high S resulted in similar G:F compared with the maize control diet. However, feeding WDGS resulted in improved G:F at 20 and 30 percent DM inclusion, but was no different from maize at 40 percent inclusion. These results indicate that high S content in WDGS and DDGS decreases feed intake to offset the high dietary S intake, which probably leads to decreased ADG and no impact on G:F. In this study, feeding WDGS improved G:F compared with DDGS, similar to previous studies.

These data suggest that although no clinical signs of PEM were observed, high S content in DGS can negatively affect intake and gain, with little effect on feed conversions. The elevated S may be more challenging in WDGS than DDGS since cattle ate less and gained less at lower inclusions of high-sulphur WDGS compared with high-sulphur DDGS. Metabolism results support these findings in terms of H₂S produced in the rumen.

FORAGE-FED CATTLE

Beef calves from weaning until they enter feedlots, developing heifers and beef cows are fed primarily forage diets. Especially in the winter, forages are low in protein and P and need to be supplemented. Maize milling co-products are excellent sources of both protein and P and fit nicely into winter supplementation programmes. Maize milling co-products are also an excellent source of energy and are particularly well suited for adding to forage based diets. Co-product feeds can also be used to supply the energy needs of cattle in pasture and range situations. It is advantageous that the same commodity can be used for supplemental energy as well as protein. Because the starch is removed during the milling process, co-products cause minimal negative associative effects on fibre digestion. Sometimes the addition of starch to forage diets can cause a decrease in fibre digestion because of competition between starch- and fibre-fermenting bacteria. Increasing starch in the diet allows starch-digesting bacteria to out-compete fibre-digesting bacteria (Fieser and Vanzant, 2004). Instead of starch, maize co-products contain highly digestible fibre, which is less disruptive to digestion of the fibre in the forage.

Clearly, CGF is an excellent source of nutrients for forage-based diets. There is little to no starch in gluten feed, which results in no negative effect on fibre digestion. Maize gluten feed contains highly digestible fibre and degradable protein, which are good sources of energy and protein for rumen microbes, especially in forage-based diets (DeHaan, Klopfenstein and Stock, 1983). Wet and dry CGF were compared with DRC for growing calves fed grass hay, wheat straw and maize stalklage. The CGF or maize replaced 40 percent of the forage (Oliveros *et al.*, 1987). The supplements nearly doubled gains and improved feed conversion (Table 19). Wet and dry CGF had better feed conversions than maize, and WCGF had better feed conversion than DCGF.

The apparent feeding value of DCGF was 10 percent greater than maize, while WCGF was 31 percent higher than DCGF and 42 percent greater than maize in these forage-based diets.

In the case of DGS, a major source of the energy supplied to the animal is in the form of maize oil. Lipids contain 2.25 times more energy per unit weight than

TABLE 19
Wet (WCGF) or dry maize gluten feed (DCGF) or maize in forage-based diets (balanced for 11.5% CP) for growing calves

	Forage	Maize	DCGF	WCGF
DMI (kg/day)	5.3	8.2	7.5	7.4
ADG (kg)	0.53	1.02	0.98	1.07
G:F	0.095	0.125	0.131	0.146

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. Source: Adapted from Oliveros *et al.*, 1987.

TABLE 20
Growing calf performance over 84 days when fed native grass hay (CP = 8.7%) supplemented with either maize or dried distillers grains for two levels of gain. Net energy was 27% greater for DDG compared with maize

		Low	High
ADG (kg)	Maize	0.37	0.71
	DDGS	0.45	0.86
G:F	Maize	0.139	0.222
	DDGS	0.172	0.278

Notes: ADG = average daily gain; G:F = gain-to-feed ratio. Gain levels were: Low = supplement fed at 0.21% BW; High = supplement fed at 0.81% BW. Source: Adapted from Loy *et al.*, 2008.

other nutrients. Because DGS is about 12 percent fat, it is a concentrated source of energy. The nutrient content of DDGS can account for approximately 18 percent greater energy value than maize. However, the nutrient content alone cannot account for associative effects, positive or negative, that may exist and the actual observed energy value is much greater. A study by Loy *et al.* (2008) measured the TDN concentration of DDGS to be about 130 percent when fed at low levels, but when fed at high levels it was only about 118 percent (Table 20). This decline may be due to the fat content of the DDGS and the subsequent inhibition of fibre fermentation. Fat levels in the rumen greater than 5 percent have been shown to decrease fibre digestion through a variety of proposed – but as of yet unconfirmed – mechanisms. In the Loy *et al.* (2008) study, the fat of the high level DDGS diet was about 5.2 percent.

ENERGY SUPPLEMENTATION

Further studies have investigated the energy value of DGS. In a study by Nuttelman *et al.* (2010a) sixty crossbred steers were used to compare the energy value of WDGS vs DRC in high-forage diets at three levels. DRC was included at 22.0, 41.0 and 60.0 percent of the diet (DM), and WDGS was included at 15.0, 25.0 and 35.0 percent of the diet (DM). Diets were formulated to meet DIP and MP requirements. Cattle were limit fed for 5 days prior to and following the feeding period, and then weighed on three consecutive days to reduce variation due to gut fill. Cattle consuming WDGS gained more than DRC cattle (Table 21).

Average daily gain increased with increasing levels of DRC and WDGS. The energy value of WDGS was calcu-

TABLE 21
Effects of replacing dry-rolled maize (DRC) with wet distillers grains with solubles (WDGS) fed at 0.81% of BW in a forage-based diet

	DRC	WDGS	SEM	P-value
Initial BW (kg)	232	231	3	0.82
Ending BW (kg)	316	323	3	0.13
DMI (kg/day)	7.2	7.2	0.11	1
ADG (kg)	1.00	1.10	0.02	<0.01
G:F	0.140	0.153	0.003	<0.01

Notes: BW = body weight; DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio; SEM = standard error of the mean. Source: Adapted from Nuttelman *et al.*, 2010a.

lated using the NRC (1996) model. In this study, the net energy value of WDGS was calculated to be 146, 149 and 142 percent of the energy value of DRC.

PROTEIN SUPPLEMENTATION

Protein in forages is extensively degraded in the rumen. In certain forage situations, light-weight growing cattle may need to be supplemented with UIP to meet their MP requirements. Distillers grains (wet or dry) are an excellent source of UIP. DDGS contains approximately 65 percent UIP (as percentage of CP), consequently forage-based diets that include DDGS fed as an energy source are commonly deficient in DIP but contain excess MP. Cattle convert excess MP to urea, which is potentially recycled to the rumen and can serve as a source of DIP. Many factors influence urea recycling, and the amount of urea that is recycled when DDGS is included in a forage-based diet is not known.

Two experiments evaluated supplemental DIP requirements when DDGS was fed as an energy source in forage-based diets (Stalker *et al.*, 2004). Diets were formulated to be more than 100 g/day deficient in DIP, but with excess MP. In both experiments, no response in performance was observed when urea was added to the diet (Table 22). Sufficient urea was presumably recycled to correct the DIP deficiency. These studies indicate that adding urea to meet the DIP requirement is not necessary when DDGS is fed as an energy source in forage-based diets.

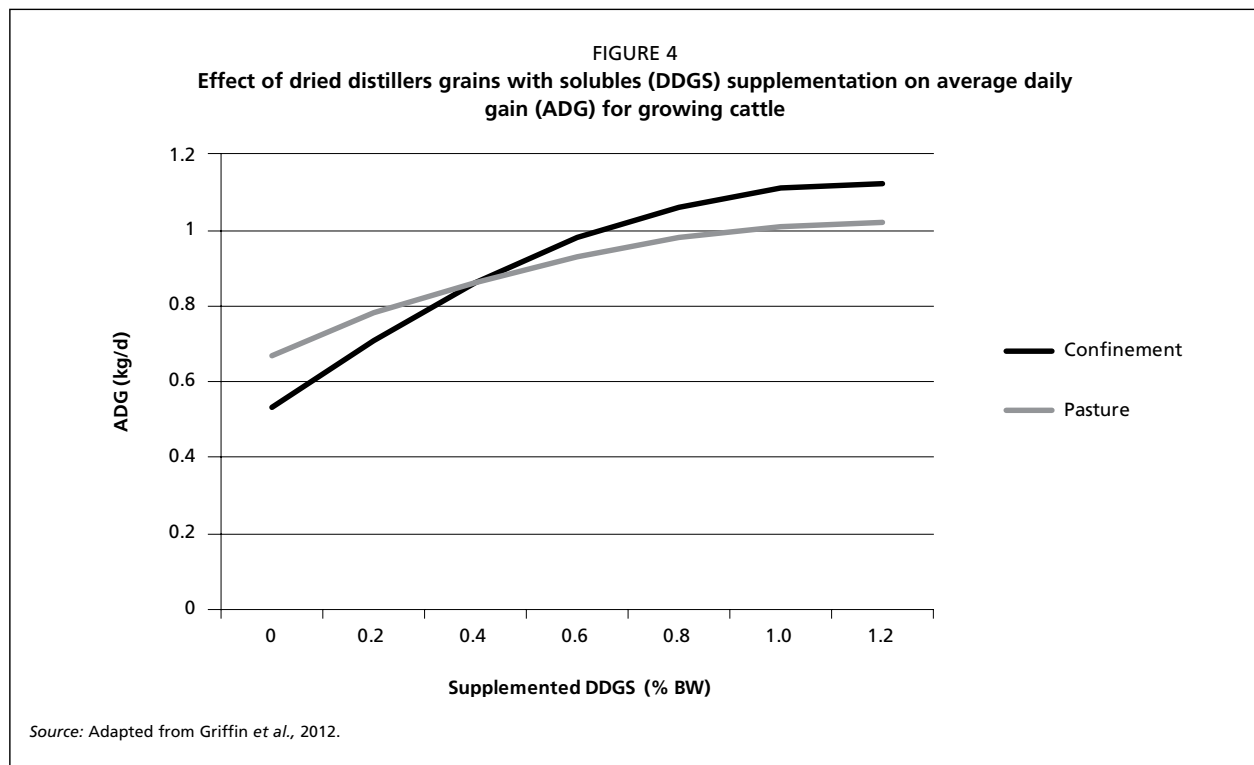
An analysis of 14 separate grazing trials in which cattle were supplemented with DDGS was conducted by Griffin *et al.* (2012) to determine effects of supplementation on ADG and final BW in pasture grazing situations. Additionally, pen studies were evaluated to determine the effects of DDGS supplementation on cattle intake, forage replacement, ADG and final BW. In both the pasture and the pen studies, ADG and final BW increased quadratically with increased level of DDGS supplementation (Figure 4). Feeding DDGS decreased forage intake quadratically; however, total intake for cattle supplemented DDGS increased quadratically with increased level of supplementation (Figure 5).

TABLE 22

Performance of animals fed diets where 0, 33, 67, 100 or 133% of the NRC-predicted degradable intake protein requirement was met with supplemental urea

	Diet					F-Test	
	0	33	67	100	133	SEM	P-value
Individually fed							
Initial BW (kg)	278	278	280	280	279	5	0.99
Final BW (kg)	315	317	309	319	319	7	0.85
ADG (kg)	0.48	0.47	0.42	0.46	0.47	0.03	0.77
DMI (kg/day)	5.1	5.2	5.2	5.2	5.2	0.09	0.95
G:F	0.200	0.185	0.167	0.185	0.189	0.004	0.54
Pen-fed							
Initial BW (kg)	205	—	—	204	—	0.5	0.10
Final BW (kg)	263	—	—	266	—	2	0.38
ADG (kg)	0.70	—	—	0.74	—	0.02	0.17
DMI (kg/day)	5.4	—	—	5.3	—	0.2	0.76
G:F	0.102	—	—	0.110	—	0.005	0.33

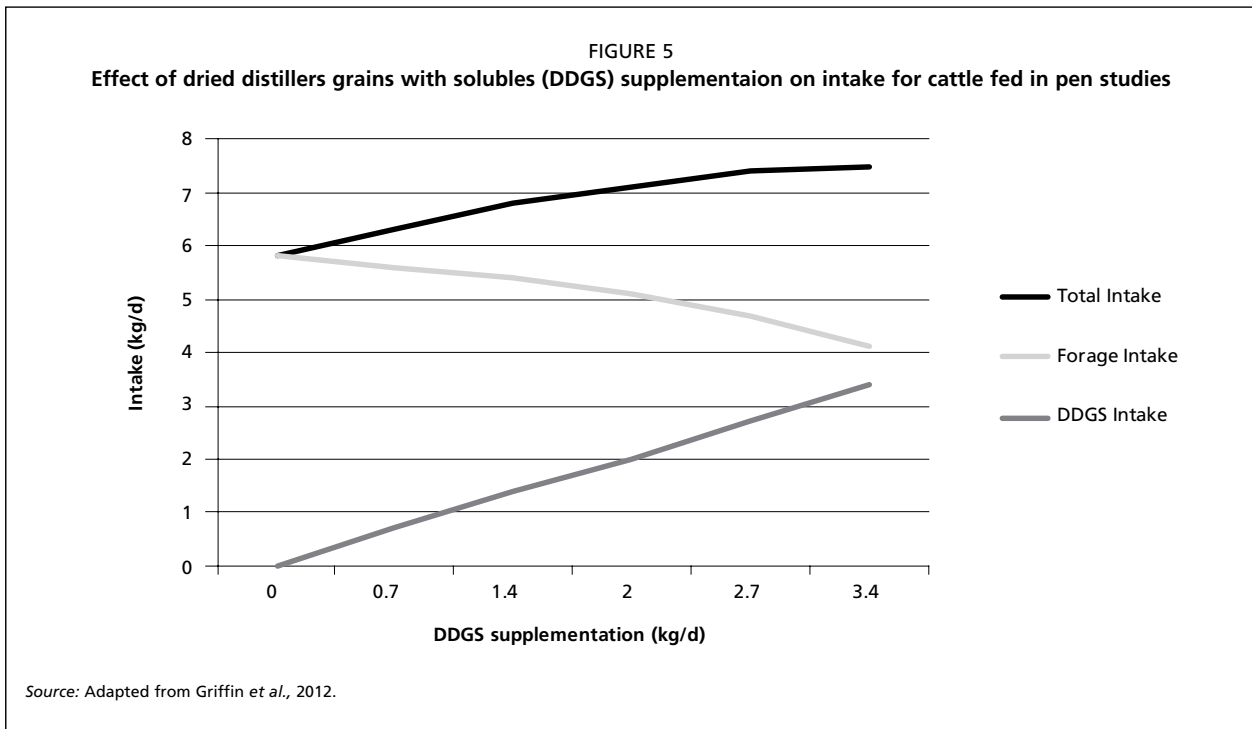
Notes: BW = body weight; DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio; SEM = standard error of the mean. Source: Adapted from Stalker *et al.*, 2004.



REPLACEMENT HEIFERS

Loy *et al.* (2004) concluded that DCGF decreases feed costs compared with conventional hay feeding when fed over the winter for developing heifers on a commercial Nebraska ranch in the Sandhills. In their study, a treatment system (TRT) was compared with their conventional management using more than 550 heifers in each group across two years. The TRT utilized only grazed winter forage and DCGF supplementation, and was compared with some winter grazing, with hay and protein supplementation. No performance differences were observed in developing heifer performance in the two treatments. The major implication

was reduced costs through the winter while maintaining excellent performance and reproduction. A similar experiment was conducted using DDGS (Stalker, Adams and Klopfenstein, 2006). Because of the higher energy content of DDGS, a smaller amount was needed to meet protein and energy requirements of these bred heifers (1353 heifers were used). Feeding DDGS and grazing winter range with heifers led to slightly better winter gains and positive changes in body condition score compared with the hay-fed, control heifers. Pregnancy rates were 97 percent for both treatments. Most important were the savings in feed costs from using DDGS and winter range versus a conven-



tional system of hay, supplement and range. Feeding DDGS as a supplement to calves grazing winter range results in similar performance and is less expensive than feeding maize and soybean meal supplement.

A two-year study (Martin *et al.*, 2007) evaluated DDGS compared with a control supplement that provided similar CP, energy, lipid and fatty acids. The protein degradability of the supplements differed such that UIP exceeded requirements for heifers consuming the DDGS supplement. The heifers were programme fed to gain 0.68 kg/day and reach 60 percent of mature weight at the time of breeding. Heifer pubertal development and overall pregnancy rate were not affected by supplement type, and averaged 89 percent for each treatment. However, artificial insemination (AI) conception rate and AI pregnancy rate were improved by feeding DDGS in the heifer development diet. The proportion of heifers detected in oestrus that conceived to AI service was higher for the DDGS treatment than for the control treatment. These data indicate that utilizing DDGS as a protein and energy source in heifer developing diets to promote moderate gains gives highly acceptable pregnancy rates and may enhance AI conception and pregnancy rates.

An experiment was conducted using maize stalk residue and supplementation as part of the development programme for replacement heifers (Larson, Cupp and Funston, 2010). While grazing maize residue, heifers were supplemented with 0.45–0.90 kg/head/day DM basis of a 28 percent CP cube. Yearling pregnancy rate varied between 84 and 92 percent and subsequent pregnancy rate as 2-year-olds of these same females ranged between 77 percent and 100 percent. These data suggest that when

heifers were supplemented at the higher rate, reproductive performance was numerically greater. In a replacement heifer development programme, DGS is an excellent source of protein, energy and P.

ENVIRONMENTAL ISSUES

N and P management

When DGS is fed as an energy source, dietary N and P exceed nutritional requirements. Excess N and P are excreted on the pen surface. Since P is not volatilized, the majority of P excreted remains in the manure. The excess N fed when DGS is included as an energy source has the potential to be volatilized from the pen surface. Luebbe *et al.* (2011) conducted a study with calf-feds in the winter and yearlings in the summer to evaluate the inclusion of WDGS at 15 and 30 percent of diet DM and its effects on nutrient mass balance. Table 23 shows nutrient intake, retention and excretion represented as kg/steer. Nutrient excretion is calculated by subtracting nutrient retention from nutrient intake.

As DGS levels in the diet increase, N and P levels increase. Because retention does not increase, excretion increases with inclusion of WDGS. Also, P is not volatilized as WDGS inclusion increases in the diet, thus manure P also increases. This amount is a direct reflection of the amount of co-products in the diet. Unlike P, a portion of N is volatilized and not available for crops. The amount of N volatilized increases with increasing levels of WDGS. However, N:P ratios remain similar. Nitrogen volatilization is greater in the summer than in the winter. About 55 percent of N is lost via volatilization in the winter, and about 70 percent is lost in the summer due to effects of temperature.

TABLE 23
Effect of dietary treatment on nitrogen (N) and phosphorus (P) mass balance

Dietary Treatment ⁽³⁾	Winter ⁽¹⁾			Summer ⁽²⁾		
	Control	15	30	Control	15	30
N intake ⁽⁷⁾	31.5	36.3	44.7	29.0	35.6	43.0
N retention ^{(4) (8)}	5.5	5.8	5.9	4.6	5.0	4.9
N excretion ^{(5) (7)}	26.0	30.5	38.8	24.4	30.6	38.1
N run-off	0.47	0.54	0.78	9.0	9.7	10.0
Manure N ⁽⁸⁾	11.5	10.9	17.3	1.2	0.86	1.5
N lost ^{(6) (7)}	14.0	19.1	20.7	14.2	20.0	26.5
N loss (%)	55.1	63.8	55.0	58.1	65.6	69.6
P intake ⁽⁸⁾	5.2	6.5	7.8	5.2	6.1	7.3
P retention ^{(4) (7)}	1.4	1.4	1.5	1.4	1.5	1.5
P excretion ^{(5) (7)}	3.9	5.1	6.4	3.8	4.6	5.8
Manure P ⁽⁷⁾	3.8	4.1	6.5	3.3	2.9	3.3
Run-off P	0.23	0.14	0.18	0.45	0.32	0.32
N:P ratio	3.06	2.81	2.65	3.06	4.03	3.95

Notes: (1) Winter values are expressed as kg/steer over a 167-day feeding period. (2) Summer values are expressed as kg/steer over a 133-day feeding period. (3) Diets are: Control = maize-based diet with no distillers grain with solubles (DGS); 15 = 15% DGS (DM basis); 30 = 30% DGS (DM basis). (4) Calculated using NRC (1996) net energy, protein, and phosphorus equations. (5) Excretion = Intake minus Retention. (6) Calculated as nutrient excretion minus run-off minus amount of nutrient removed in the manure. (7) Linear ($P < 0.05$) effect of DGS level for both winter and summer. (8) Linear ($P < 0.05$) effect of DGS level for winter. Source: Adapted from Luebbe *et al.*, 2011.

This manure can then be applied to crop fields to meet either N or P requirements. Applying on an annual P basis is expensive and unnecessary. Applying manure on an annual N basis can pose environmental problems if excess P is not accounted for. Manure should be applied on a 4-year P basis, which provides for multiple years of P in a single application. Applying on a 4-year P basis also meets crop requirements for N for one year. The following 3 years N should be applied to meet crop requirements (in years soybean is grown N will not be needed) followed by manure application again after 4 years. By implementing this rotation, manure nutrient potential is maximized and crop P requirements are met, without being exceeded. This is a more cost-efficient method. As co-products become more commonly used in feedlot diets, N and P intakes will increase, as will the amount of N and P excreted by the animal. However, if these nutrients are managed effectively through the feedlot, producers can diminish costs associated with supplementing P, reduce N lost via volatilization, and benefit from utilizing manure as fertilizer. In a Nebraska scenario, with an abundant supply of cropland, the fertilizer value of the manure exceeds the cost of handling the manure. Feedlots are able to sell the manure for a profit, especially when accounting for the fertilizer value of the P in the manure.

GREENHOUSE GAS AND LIFE-CYCLE ANALYSIS

Important considerations in utilizing DGS in cattle diets are greenhouse gas (GHG) emissions associated with bio-ethanol vs gasoline. The type of co-product used influences both cattle performance and GHG emissions, with WDGS

being more beneficial than MDGS or DDGS. The Biofuel Energy Systems Simulator (BESS; <http://nutechmarketplace.com/shoppingcart/products/BESS.html>) was developed to compare life-cycle GHG emissions from ethanol production relative to gasoline as a motor fuel, while accounting for the dynamic interactions of maize production, ethanol plant operation and co-product feeding to livestock (Bremer *et al.*, 2010b, 2011; Liska *et al.*, 2009). Meta-analysis methodology was used to develop biological performance equations for evaluating feedlot cattle when fed levels of 20 to 40 percent WDGS, MDGS, or DDGS. In all studies included in the meta-analysis, cattle were fed a high concentrate finishing diet with DGS replacing maize and urea N. Cattle performance was measured using DMI, ADG and G:F.

The most widely used and accurate method for allocating co-product GHG and energy credits to the maize-ethanol life cycle is through the displacement method in the context of 'system expansion' (Kodera, 2007). This method assumes that co-products from maize-ethanol production substitute for other feed components and offset fossil fuel use and associated GHG emissions required to produce the replaced feed components (Kodera, 2007; Liska *et al.*, 2009). Estimating the displacement credit for an individual maize-ethanol biorefinery requires quantification of the different types of co-products produced by the ethanol plant, identification of the products to be displaced in livestock diets (and displacement ratios), and calculation of the fossil fuel energy and GHG emissions attributable to the life cycle production of the displaced products (Wang, 1999; Graboski, 2002). Nutritionists' surveys indicate the current average co-product inclusion rate is 20 percent

(DM basis) with a range of 5 to 50 percent of the diet (Vasconcelos and Galylean, 2007). In the United States Corn Belt, survey data suggest that beef producers feeding DGS have an average dietary inclusion of 22 to 31 percent on a wet basis (approximately 15 to 20 percent of DM) (NASS, 2007). Respondents to both a feedlot nutritionist survey (Vasconcelos and Galylean, 2007) and a Nebraska feedlot industry survey (Waterbury *et al.*, 2009) reported that DGS are the most common ethanol co-product used by cattle feeders. The Nebraska survey indicates 53 and 29 percent of Nebraska feedlots feed WDGS and MDGS, respectively. The nutritionist survey indicated 69 percent of the 29 nutritionists were feeding DGS as the primary co-product in the diet, and these beef nutritionists were responsible for formulating diets for nearly 70 percent of cattle on feed in the United States. Feeding values of the DGS co-products relative to maize were calculated for each feedlot inclusion level of WDGS, MDGS and DDGS from measured biological feed efficiency values. These feeding values decrease as the level of co-product increases in the diets. Thus, as more DGS is included in the diet, it replaces less maize per unit increase in the substitution rate. In addition, the relative feeding value of DDGS declines at a faster rate than WDGS as inclusion levels increase, indicating that WDGS has a higher feeding value than DDGS.

For cattle, DGS inclusion in diets improves growth rates and thus reduces time in the feedlot for finishing cattle by several days, depending on the inclusion level and whether the DGS are fed dry or wet. Less time in the feedlot for finished cattle reduces fuel use for transportation of feed as well as methane emissions from enteric fermentation. Enteric methane production is calculated from cattle size, projected DMI, and energy content of the diet. Feed inputs are used to calculate gross energy intake by the cattle with standard animal energy equations (NRC, 1996). An average 2.9 percent of gross energy is lost as enteric fermentation methane by feedlot cattle (see BESS 2009.4.0 User's Guide, <http://nutechmarketplace.com/shoppingcart/products/BESS.html>). Due to a lack of data on comparison of enteric methane production between DGS and maize-based diets, the two feedstuffs were assigned the same methane production potential on a DM basis.

The feeding values of WDGS, MDGS and DDGS, when fed at 20 to 40 percent of diet DM, were 143 to 130 percent, 124 to 117 percent, and a constant 113 percent of maize (DM basis), respectively. The feeding value of DGS decreased as moisture level decreased. The feeding value of WDGS and MDGS decreased as inclusion level increased. The feeding value of DDGS was a constant 113 percent of maize DM. All scenarios evaluated had ethanol life-cycle emissions less than gasoline (Table 24). Low inclusion levels of DGS had greater reduction of GHG emissions than high-inclusion levels. This is influenced by regional variability

TABLE 24
Percentage reduction in greenhouse gas (GHG) emissions for an equivalent quantity of energy from ethanol relative to gasoline when accounting for wet (WDGS), modified (MDGS) and dried distillers grains (DDGS) moisture content and dietary inclusion level

	Beef Cattle			
	10	20	30	40
DGS, % of diet DM	10	20	30	40
WDGS, GHG % reduction to gasoline ⁽¹⁾	62.4	60.6	58.4	56.7
MDGS, GHG % reduction to gasoline ⁽¹⁾	53.9	52.6	50.9	49.7
DDGS, GHG % reduction to gasoline ⁽¹⁾	46.1	45.4	44.4	43.9

Notes: (1) Gasoline reference point is 97.7 g CO₂eq/MJ (Liska and Perrin, 2009). Source: Adapted from Bremer *et al.*, 2011.

in GHG emissions from both crop and livestock production (Bremer *et al.*, 2010b).

Feeding DGS to livestock contributes to the environmental benefit of fuel ethanol relative to gasoline. The GHG emissions benefits of ethanol are determined by how DGS moisture is managed at the ethanol production facility and what animal classes are fed. Ethanol production facilities producing DDGS require 167 percent of the energy and produce 145 percent of the GHG emissions of ethanol production facilities producing WDGS (Liska *et al.*, 2009). Feeding WDGS to feedlot cattle within 100 km of an ethanol plant resulted in the greatest reduction of GHG emissions. Cattle performance is improved with WDGS, and locating the ethanol plant close to feedlots minimizes transportation of feed co-products, which reduces costs and emissions. Not drying the DGS also reduces costs and emissions for the ethanol plant, as well as improving feedlot cattle performance compared with DDGS or a maize based diet.

NEW DEVELOPMENTS

Impact of grain feedstock use for ethanol

In the United States, maize is the primary grain used for ethanol production. Grain sorghum [milo], wheat and triticale have also been used in some locations, such as Western Canada, where maize is less readily available. Maize and sorghum have similar amounts of starch and therefore have similar ethanol yields. Al-Suwaiegh *et al.* (2002) compared sorghum and maize DGS produced at the same ethanol plant and found the maize DGS to have 10 percent greater feeding value. Galylean and Vasconcelos (2007) reported statistically similar responses in G:F for sorghum and maize DGS (0.169 and 0.176, respectively), but the feeding value of maize DGS was 25 percent greater than sorghum DGS. Mustafa *et al.* (2000) found that wheat DGS has more NDF and less fat, but more degradable protein, than maize DGS. Walter *et al.* (2010) compared wheat and maize DGS at 20 percent and 40 percent of diet DM in a barley-based

finishing diet. Inclusion level of wheat DGS had no effect on G:F, but increasing levels of maize DGS resulted in a quadratic increase in G:F. Wierenga *et al.* (2010) measured cattle performance on finishing diets with 20, 25 or and 30 percent triticale DDGS replacing barley silage in the diet. The triticale DDGS was similar in fat and NDF content to wheat DDGS, but lower in CP. Increasing inclusion levels of triticale DDGS tended to linearly increase G:F ($P = 0.06$) with no effect on ADG ($P = 0.56$).

Impact of fat and fat removal

Research has shown that feeding DGS improves cattle performance. One likely reason for DGS resulting in better performance than maize is due to the high fat content in DGS. The fat content of DGS can be affected by the process and by how much solubles are added back to the wet grains. Another factor that can affect the fat content of DGS is whether some of this maize oil is isolated in the process (similar in concept to complete removal in the wet milling industry). Numerous processes are currently being explored by ethanol plants to remove a portion of the maize oil for other purposes. It is therefore important to know the impact of the fat content in DGS on performance.

Gigax *et al.* (2011) evaluated feeding 35 percent WDGS (DM basis) with normal fat content (13.0 percent of DM) or low fat (6.7 percent of DM), and compared this with a DRC-and HMC-based control diet. Cattle consumed equal DMI, but feeding the high fat WDGS improved ADG and G:F (Table 25). Cattle fed the low fat WDGS had equivalent ADG and G:F to cattle fed the maize control diet. These data suggest that the improved performance due to

feeding WDGS is at least partially due to higher fat content in the WDGS.

In this study, the primary difference in these two products was the amount of distillers solubles added back to the wet grain. Although WDGS typically has 11 to 13 percent fat, this amount can vary due to the amount of distillers solubles (18–26 percent fat) that is added back to the wet distillers grains (~8 percent fat).

Godsey *et al.* (2009) conducted a feeding trial evaluating the proportion of solubles added to WDG at WDG:solubles ratios of 100:0, 85:15 and 70:30.

They fed these ratios in DRC-based diets at 0, 20 and 40 percent of diet DM. No interactions resulted for ratio of grains to solubles or for level of WDG±DS fed. Although there was no effect for DMI, linear improvements were observed for ADG and G:F as the level of WDG±DS was increased (Table 26). Optimum inclusion was observed at 40 percent DM inclusion. No effects of WDG to solubles ratio were detected in this experiment, suggesting that, for improving cattle performance, the level of WDGS is more important than the grain to solubles ratio.

The fat in DGS is maize oil originating from the maize grain. Maize oil is high in unsaturated fatty acids (double bonds within the fatty acids). Feeding unsaturated fat sources to cattle generally has a negative impact on the rumen microbes (particularly forage-digesting microbes). During rumen fermentation, rumen microbes will saturate the fatty acids by biohydrogenation and produce saturated fatty acids that leave the rumen and are available for absorption in the small intestine. Therefore, unless the fat is “protected” against biohydrogenation by the microbes, the majority of the fat will be saturated fatty acids at the small intestine. It is important to note that fatty acids are not absorbed in the rumen or metabolized by the rumen microbes, except for biohydrogenation. The primary site of maize oil is in the maize germ, which may be “protected” from rumen microbes.

Vander Pol *et al.* (2009) evaluated different fat sources, including wet distillers grains plus solubles, in both feeding and metabolism studies. The ratio of unsaturated fatty acids relative to saturated fatty acids increased at the small intestine in steers fed WDGS compared with maize-based

TABLE 25
Effect on cattle performance of feeding a low- or high-fat wet distillers grains with solubles (WDGS) at 35% DM inclusion compared with a maize-based control diet

	Control	Low-fat WDGS	Normal-fat WDGS
DMI (kg/day)	11.2	11.2	11.2
ADG (kg)	1.55 a	1.55 a	1.69 b
G:F	0.139 a	0.139 a	0.152 b

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. a,b = Means within the same row without a common suffix differ ($P < 0.05$). Source: Adapted from Gigax *et al.*, 2011.

TABLE 26
Effect on cattle performance of feeding increasing levels of WDG with or without distillers solubles and the ratio of WDG to distillers solubles

	Level of WDG ±DS ⁽¹⁾			Ratio of WDG:DS ⁽²⁾		
	0	20	40	100:0	85:15	70:30
DMI (kg/day)	11.6	11.6	11.4	11.5	11.4	11.6
ADG (kg) ⁽³⁾	1.68	1.76	1.77	1.76	1.75	1.80
G:F ⁽³⁾	0.144	0.152	0.156	0.153	0.154	0.156

Notes: DMI = dry matter intake; ADG = average daily gain; G:F = gain-to-feed ratio. (1) Level of wet distillers grains with or without distillers solubles (DS). Represented as a % of diet DM. (2) Ratio of wet distillers grains (WDG) to distillers solubles (DS). Represented as a proportion of the total WDGS product. (3) Linear effect for level of WDG±S fed ($P < 0.02$). Source: Adapted from Godsey *et al.*, 2009.

diets or maize-based diets with added tallow (saturated fat) or added maize oil (unsaturated fatty acids). These data suggest that a portion of the fatty acids are “protected” in the rumen in WDGS and remain intact at the small intestine. Similar results were observed by Bremer *et al.* (2010c), where the unsaturated:saturated fatty acid ratio increased from approximately 0.40–0.50 for maize, maize oil, tallow and distillers solubles, to 0.83 for WDGS. All diets in this study were approximately 8.5 percent fat, except the maize control (3.6 percent), and all had greater than 93 percent fatty acid digestibility. The fat in WDGS appears to be protected from biohydrogenation in the rumen, whereas fats in distillers solubles are not protected. Likewise, all fat sources are quite digestible.

Fractionation co-products from dry milling

The evolving ethanol industry is continually striving to maximize ethanol production efficiency. Changes associated with this progress will provide innovative new co-product feeds for producers to utilize that may be quite different nutritionally when fed to cattle. One example of a new co-product feed is Dakota Bran Cake. Bran cake is a distillers co-product feed produced as primarily maize bran plus distillers solubles produced from a pre-fractionation dry milling process. On a DM basis, bran cake contains less protein than WDGS and WCGF, similar NDF to both feeds, and slightly less fat content than WDGS. Bremer *et al.* (2007) evaluated Dakota Bran Cake in a finishing diet by comparing inclusion levels of 0, 15, 30 and 45 percent of diet DM. Results indicated improved final BW, ADG, DMI and G:F compared with feeding a blend of high-moisture and dry-rolled maize, suggesting this specific feed has 100–108 percent of the feeding value of maize. Buckner *et al.* (2007) compared dried Dakota Bran Cake with DDGS supplementation in diets for growing calves. They fed each of the two products at 15 or 30 percent of the diet, which replaced a 70:30 blend of brome grass hay and alfalfa haylage (DM basis). Animal performance improved as the inclusion of the co-products increased. Dried DGS had improved performance compared with the dried Dakota Bran Cake at both inclusion levels. Dried Dakota Bran Cake had 84 percent the feeding value of DDGS with growing steers. Previous research has shown that DDGS has about 127 percent the feeding value of maize in forage based diets. Therefore, dried Dakota Bran Cake appears to have an energy value equal to 103 percent of maize. Dakota Bran Cake is only one example of how new ethanol industry co-products will perform relative to traditional finishing rations.

FUTURE RESEARCH AREAS

Each new co-product feed is different from the next. Therefore, each new feed needs to be analysed individually for its correct feeding value. Changes to plant production

goals and production efficiency will probably have significant impacts on the feeding value of co-products produced.

Research has shown differences in cattle performance due to the interaction between level of DGS and type of grain processing. There are probably many interacting factors, including DMI, forage type and inclusion level, and differences between calf-feds and yearlings. These interactions are complex and require further research to explain.

The meta-analysis by Bremer *et al.* (2011) shows a clear performance advantage for WDGS compared with DDGS. The underlying factors leading to this are not clear and should be further researched in order to guide the ethanol industry in producing high quality co-product feeds.

Forage replacement values of DGS have been quite variable. Identifying this value will be helpful to producers using DGS as a supplement for cattle on high forage diets, especially in times of drought when forage supplies are limited.

CONCLUSIONS

Both dry and wet milling ethanol processes produce co-product feeds that are suitable for cattle diets, both high-concentrate diets and forage-based diets. These feeds are all quite different and require individual analyses to adequately describe their nutritional content. There is also variation within feeds among plants, and even within plants.

Co-products in a beef finishing diet can be added as either a protein or energy source, or both. Inclusion rates of less than 15 to 20 percent of the diet DM serve primarily as a protein supplement. Distillers grains are an excellent source of UIP, which can be recycled to the rumen as urea. Inclusion of wet, modified or dried DGS at 40 percent of diet DM in a finishing diet maximizes G:F. Maximum ADG and DMI were observed at lower levels. Feeding WDGS is the most beneficial in finishing diets, with 30–40 percent greater feeding value than maize. Modified DGS has 15–30 percent and DDGS has 13 percent greater feeding value than maize. ‘Sweet Bran’ inclusion in finishing diets up to 40 percent of diet DM had a linear increase in G:F. Higher inclusions of DGS decrease these feeding values, but still give comparable or better performance than a maize-based control, and may be economically advantageous because of decreased input costs. When feeding high levels of DGS, increased S levels may hurt performance or result in PEM. Incidences of PEM increase with increasing levels of dietary S and cattle should be monitored closely if dietary S is above 0.47 percent. Ruminally degradable S in the diet is a better indicator of H₂S production in the rumen than total S in the diet.

Environmental considerations are an important aspect of feeding DGS to cattle. Feeding DGS increases both N and P in the manure which, if captured, increases the fertilizer value of the manure. Feeding DGS to livestock also increases the environmental benefit of fuel ethanol relative

to gasoline. The GHG emissions of ethanol are dependent on whether wet, modified or dried DGS are produced and what animal classes are fed.

Maize milling co-products are excellent supplements for cattle on high-forage diets because they contain both protein and P, which are typically lacking in forage diets. In addition, the lack of starch in these products reduces the negative associative effects of starch digestion on fibre digestion. Both ADG and final BW increase quadratically with increased levels of DDGS supplementation, while forage intake decreases quadratically.

Co-product feeds from the ethanol industry are a great asset to the cattle feeding industry. Continued research should explore interactions between different types of feeds and identify ideal feeding situations in order to maximize performance.

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Chapter 6

Hydrogen sulphide: synthesis, physiological roles and pathology associated with feeding cattle maize co-products of the ethanol industry

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ABSTRACT

The toxicity of sulphur (S) is dependent upon its chemical form, amount and route of administration. Whereas elemental S is considered one of the least toxic elements, hydrogen sulphide (H₂S) rivals cyanide in toxicity. Endogenous H₂S is derived from catabolism of sulphur-containing amino acids, of which cysteine is central. Hydrogen sulphide is also produced by sulphate-reducing bacteria that are present in both ruminant and non-ruminant digestive tracts. At low concentrations, H₂S functions as a gaseous signalling molecule in animal tissues. At high concentrations, H₂S inhibits oxidative processes in nervous tissue and may lead to the central nervous system disorder in ruminants called polioencephalomalacia (PEM). Co-products from the grain wet- or dry-milling industries may be high in sulphate (0.5–1.7 percent, DM basis). As these co-products are included in the diet, sulphate concentration generally increases and the risk of cattle experiencing sulphur toxicity rises. Many S-containing compounds, when fed to ruminants, are reduced to toxic H₂S by ruminal bacteria, eructated, and then inhaled by the animal, thus bypassing liver detoxification. In contrast, H₂S produced in the gastrointestinal tract of non-ruminants is largely excreted or absorbed and detoxified (oxidized to sulphate) in the liver. Although organic and inorganic S in gastrointestinal tissues may be linked to chronic intestinal disease in non-ruminants, ruminants comprise the principal species likely to develop S toxicosis. Practical approaches to mitigation of H₂S production in the rumen, development of diagnostic tools, and development of practical approaches to alleviation of the symptoms of H₂S toxicity are major needs in research.

INTRODUCTION

Expansion of the ethanol industry has resulted in an unprecedented increase in costs of traditional feed, leaving livestock producers searching for alternatives. Distillers grain, a co-product of the ethanol industry, is exceptionally high in energy and protein, and is an economical and practical alternative feedstuff. According to the Renewable Fuels Association (RFA, no date) over 30 million tonne of distillers grain was produced from United States ethanol plants in 2010, and approximately 80 percent of this was used for feedstuff for beef and dairy cattle. Co-products from the grain wet- or dry-milling industries may be high in sulphate (0.5–1.7 percent, DM basis) because sulphuric acid is a standard treatment in these industries (McAloon *et al.*, 2000). As these co-products are included in the diet, sulphate concentration generally increases and the risk of cattle experiencing sulphur toxicity rises. Sulphur is a component of the amino acids methionine and cysteine, as well

as B-vitamins biotin and thiamine and a number of other organic compounds. It thus serves many purposes in the ruminant animal. Elemental S, sulphates, sulphuric acid and H₂S all may be present in the ruminant animal. Elemental S, sulphates, and sulphuric acid are relatively non-toxic. However, H₂S can be highly toxic at high concentrations, particularly when the H₂S catabolizing systems of the liver and kidney are bypassed. At low concentrations, H₂S functions as a signaling molecule in animal tissues (Kabil and Banerjee, 2010). At high concentrations, H₂S inhibits oxidative processes in nervous tissue and may lead to the central nervous system disorder called polioencephalomalacia (PEM; Gould, 1998). When cattle are fed diets greater than 0.56 percent sulphur, PEM occurs in 6.06 percent of the cattle population (Vanness *et al.*, 2009). As sulphur content of the diet decreases, PEM incidence decreases. PEM occurs in only 0.35 and 0.14 percent of the cattle population when dietary sulphur content decreases below 0.56 and

MAIN MESSAGES

- Co-products of the ethanol industry are high in sulphur.
- H₂S is produced by sulphate-reducing bacteria in the rumen of cattle.
- H₂S is a signal molecule in animal tissues.
- H₂S has significant effects in several tissues.
- H₂S chemically reacts with metalloproteins and oxidized cysteine residues of proteins to exert its biological effects.
- H₂S, when produced in excess, causes polioencephalomalacia in cattle.
- Cattle seem to vary in their susceptibility to H₂S toxicity.
- H₂S mitigation strategies are currently being investigated and can decrease H₂S toxicity.

0.46 percent of the diet, respectively (Vanness *et al.*, 2009).

Rumen microbes require sulphur for their normal growth and metabolism. A large portion of the sulphur found in typical ruminant diets is a component of the natural protein and most practical diets are adequate in sulphur (NRC, 1996). However, feeding diets high in non-protein nitrogen or high in rumen-undegradable intake protein may decrease the amount of sulphur available for rumen micro-organisms thus increasing the need for supplemental sulphur. For most ruminants, dietary S must be between 0.18 and 0.24 percent of DM to allow microbes to produce sufficient S-containing compounds to support microbial growth and to provide S-containing compounds for the host animal (NRC, 2005). The maximal tolerable dietary S concentration was set at 0.40 percent (DM basis). More recent guidelines (NRC, 2005) provided two recommendations based on forage concentration in the diet. For ruminant diets containing less than 15 percent forage, the maximal tolerable dietary concentration is 0.30 percent S, and for diets containing greater than 40 percent forage, the maximal tolerable dietary concentration is 0.50 percent S. The maximum tolerable dietary concentration of S for diets containing between 15 and 40 percent remains at 0.40 percent S.

DIETARY SOURCES OF SULPHUR

Typical dietary components for livestock, including maize, soybean meal, alfalfa hay and maize silage, contain relatively low to moderate S concentrations (0.1–0.5 percent, DM basis). Typical diets containing these feeds generally pose little or no danger of S toxicity. However, co-products from the grain wet- or dry-milling industries may be high in sulphate (0.5–1.7 percent, DM basis). As these co-products are included in the diet, sulphate concentration generally increases and the risk of cattle experiencing H₂S toxicity rises. Because the ethanol industry is still developing and adapting to new technology, the quality and consistency of co-products can differ greatly both within and among production plants (Spiehs, Whitney and Shurson, 2002; Buckner *et al.*, 2011). For example, Spiehs, Whitney and

Shurson (2002) reported a range for S content of distillers grain (DG) from 12 ethanol plants of 0.33 to 0.74 percent and a within-plant coefficient of variation ranging from 6.4 to 40.8 percent. Buckner *et al.* (2011) reported a range for S content of DG from 6 ethanol plants of 0.71 to 0.84 percent and a within-plant coefficient of variation ranging from 2.2 to 12.9 percent. Thus, variability in S concentration of maize milling co-products may be of greater concern, as rapid changes in feed can significantly alter ruminal fermentation. Table 1 lists the S concentration found in several common feed ingredients and co-products along with standard deviations. (Adams, 1975; Kerr *et al.*, 2008; Wagner, 2008)

Total S intake from all feed and water sources must be considered when evaluating nutritional programmes for S adequacy or excess. The cationic trace minerals zinc, copper, manganese and iron are often added to diets as the sulphate salts – primarily because the sulphate salts are inexpensive compared with organic minerals and are soluble in water and therefore often are among the most bio-available of the inorganic forms of these trace minerals. Further, S concentrations in water can vary tremendously and can be a major contributor to overall S dietary load.

TABLE 1
Sulphur concentrations (%) in typical livestock feeds

Commodity	Sulphur	SD
Barley ⁽¹⁾	0.16	–
CDS ⁽²⁾	1.62	–
Maize ⁽¹⁾	0.11	0.01
Maize gluten feed ⁽¹⁾	0.75	0.05
Maize gluten meal ⁽¹⁾	1.06	0.11
Maize silage ⁽¹⁾	0.14	0.04
Distillers grain ⁽¹⁾	0.69	0.23
Grass forage ⁽³⁾	0.20	0.07
Legume forage ⁽³⁾	0.26	0.07
Sorghum ⁽¹⁾	0.14	–
Soybean hulls ⁽¹⁾	0.14	0.01
Soybean meal ⁽¹⁾	0.46	0.11
Wheat midds ⁽¹⁾	0.24	0.02

Notes: SD = Standard deviation; CDS = condensed distillers solubles.
Sources: (1) Kerr *et al.*, 2008. (2) Wagner, 2008, range of 1.0–2.23.
(3) Adams, 1975.

TABLE 2
Maximum recommended water sulphate concentrations for cattle

Water sulphate level (ppm (mg/L))	Comment
Less than 600	Safe
600–1 000	Generally safe. Slight performance reductions in confined cattle may occur with high water intakes.
1 000–2 000	Grazing cattle not likely to be affected. Performance may be decreased, particularly in confined cattle consuming dry feed. May result in diarrhoea. May cause slight decrease in copper availability.
2 000–3 000	Performance likely to be decreased, particularly in confined cattle consuming dry feed. Grazing cattle may also be affected. Likely to result in diarrhoea. May cause substantial decrease in copper availability. Sporadic cases of S-induced PEM possible.
3 000–4 000	Performance will likely be reduced in all classes of cattle. Likely to result in diarrhoea. May cause substantial decrease in copper availability. Sporadic cases of S-induced PEM likely.
Greater than 4 000	Potentially toxic. Should be avoided.

Source: Adapted from Wright, 2007, with modifications based on NRC (2005) recommendations.

A 1999 survey of 263 United States feedlots in 10 states with greater than 1000 animal capacities (NAHMS, 2000) demonstrated that approximately 77 percent of water samples contained less than 300 ppm sulphate, 15 percent of water samples contained 300 to 999 ppm sulphate and 8 percent of water samples registered greater than 1000 ppm sulphate. Effects of different concentrations of water sulphate on animal performance are reported in Table 2. NRC (2005) recommends that water for feedlot cattle should contain less than 600 ppm sulphate, although Wright (2007) reported that water sulphate concentrations less than 1000 ppm are generally safe. Water sulphate concentrations between 1000 and 2000 ppm will probably have no effect on grazing cattle growth and reproductive performance, but may decrease growth performance in confined cattle. In addition, these water sulphate concentrations may result in diarrhoea and a slight reduction in copper bio-availability (Wright, 2007). Water sulphate and S concentrations should be assessed in combination with dietary S levels to determine total S intake. The consumption of water containing 1000 ppm of sulphate can contribute 0.10 to 0.27 percent S to the diet. Thus, even with moderately elevated S content in water, the practical ration for ruminants may easily exceed 0.40 percent total dietary S (Olkowski, 1997).

MECHANISM OF ACTION OF EXCESS DIETARY SULPHUR

High S intake can adversely affect ruminants in two ways: decreased bio-availability of other trace minerals; and production of H₂S, that can reach toxic concentrations. High dietary S can decrease the bio-availability of trace minerals through formation of insoluble complexes within the rumen. One such interaction is that of copper, S and molybdenum, which combine to form copper tetrathio-molybdate. This complex renders copper unavailable to the animal (NRC, 2005). Suttle (1991) reported a 50 percent decrease in copper absorption when dietary S concentration increased from 0.2 to 0.4 percent of the diet DM. This secondary copper deficiency can result in

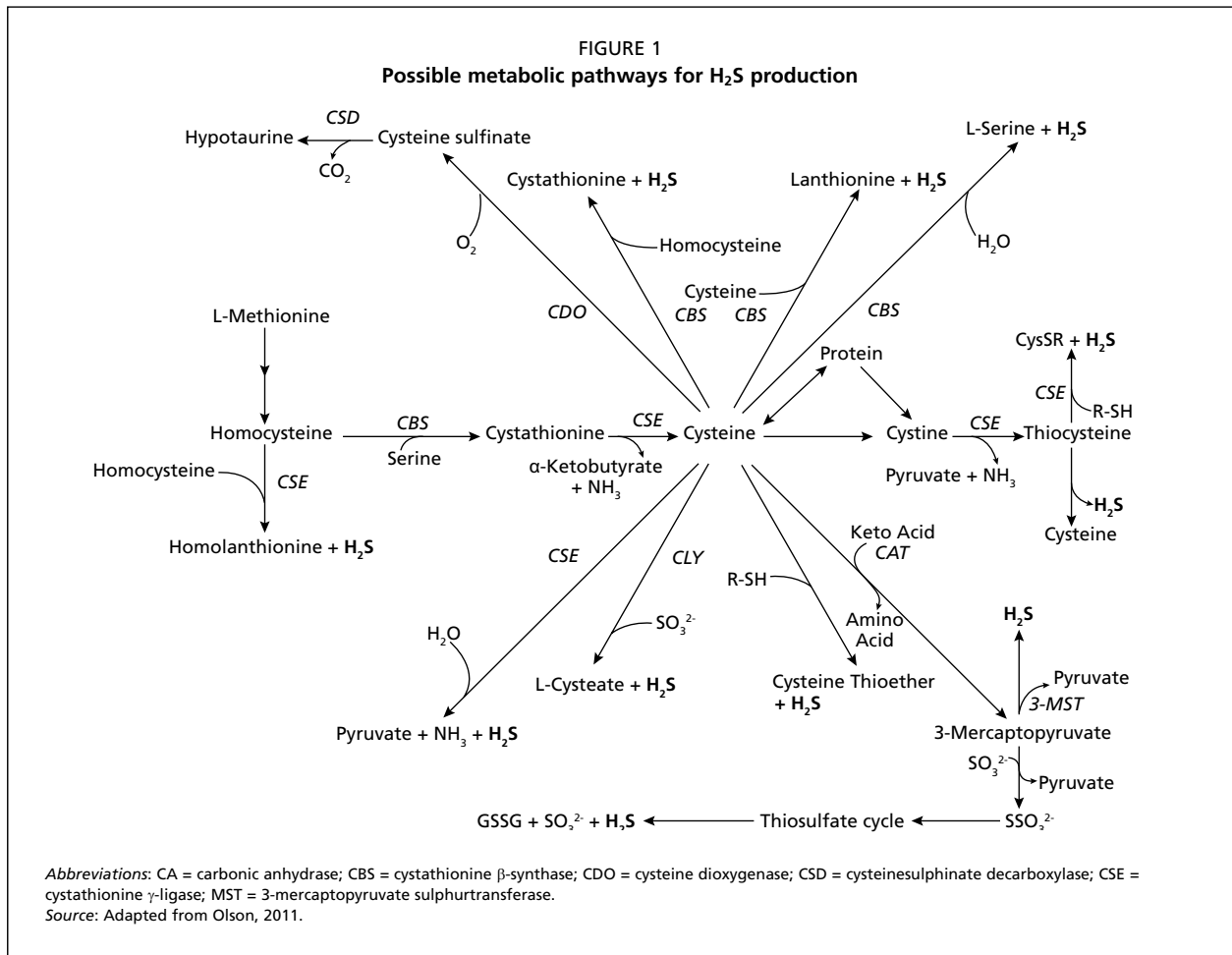
impaired reproduction and performance (NRC, 1996). Gould (1998) also reported that the bio-availability of other minerals, particularly iron and zinc, may be limited because of the formation of insoluble salts with sulphide. Availability of selenium also may be limited due to S, because Ivancic and Weiss (2001) reported decreased true digestibility of selenium as dietary S content increased, and Ganther and Bauman (1962) reported increased urinary excretion of selenium with excess dietary S concentrations.

More extreme effects of excess S involve reduction of sulphate and other non-toxic forms of S by ruminal microbes to H₂S and its ionic forms, which are highly toxic substances that interfere with cell respiration (Beauchamp, Bus and Popp, 1984; Bray, 1969; Kandyliis, 1984) and may lead to the central nervous system disorder known as PEM. Hydrogen sulphide is a colourless, flammable, water-soluble (0.25 g/100 mL) gas. Sulphide is also soluble in plasma (1 g in 242 ml at 20 °C) and it can penetrate cells of all types by simple diffusion (Pietri, Roman-Morales and Lopez-Garriga, 2010). It is this property that makes H₂S a broad-spectrum toxicant. Sulphide is lipophilic (5 times more soluble in lipophilic solvents than in aqueous solvents) and can pass plasma membranes. A typical concentration of H₂S in blood plasma is 50 µM and may be three times higher in brain (Olson, 2011).

SOURCES OF HYDROGEN SULPHIDE

Endogenous synthesis of hydrogen sulphide by mammalian cells

The amino acid cysteine is central to the endogenous production of most H₂S (Figure 1; Olson, 2011). Cysteine may be catabolized by several biochemical pathways involving transsulphuration or oxidation reactions to generate H₂S. As shown in Figure 1, the cysteine may be derived from methionine as a donor of the S. The biogenesis of H₂S has been proposed to be a promiscuous by-product of three pyridoxal phosphate-dependent enzymes (Kabil and Banerjee, 2010): cystathionine β-synthase (CBS), cystathionine γ-ligase



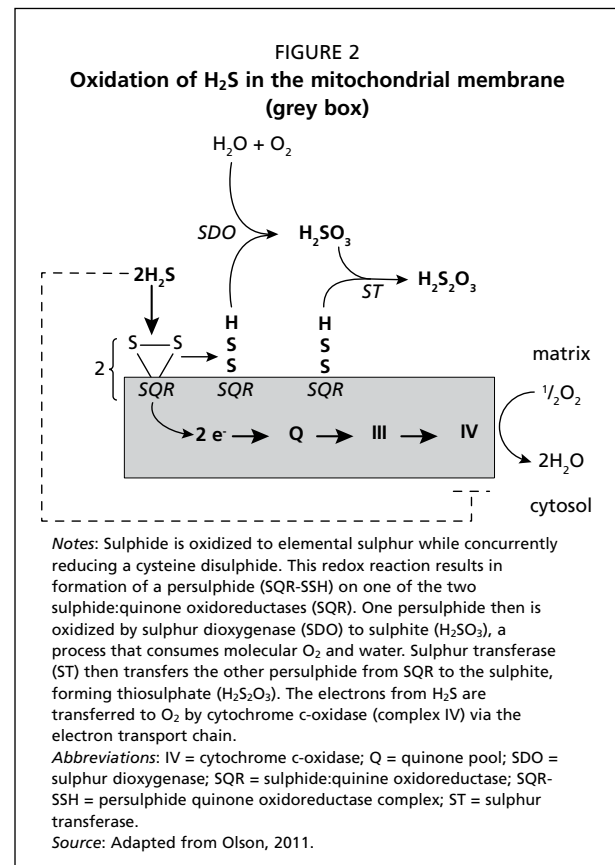
(CSE) and 3-mercaptopyruvate sulphurtransferase (MST). Cystathionine β-synthase and CSE catalyze several transsulphuration reactions of a multitude of substrate combinations, whereas MST deaminates cysteine to form mercaptopyruvate, which is subsequently converted to pyruvate and H₂S. The prevalence of CBS, CSE and MST in the different tissues of the animal body varies. For example, CBS was shown to be the predominant enzymatic pathway for H₂S in brain and CSE was the major pathway in the vasculature (Olson, 2011). Hydrogen sulphide also is produced in the vascular smooth muscle by the pathway involving MST. Generally considered the major catabolic pathway for cysteine, cysteine dioxygenase (CDO) catalyzes the addition of O₂ to cysteine to form cysteinesulphinatase that is subsequently decarboxylated to hypotaurine (Stipanuk and Ueki, 2010). The action of CDO is considered the major physiological regulator of intracellular cysteine availability. By oxidizing excess cysteine, the CDO may be an important physiological regulator of endogenous H₂S production. Future research is needed to associate the pathway for synthesis of H₂S in the myriad of tissues of an animal for association of this signal molecule to specific physiological functions.

Sulphate reduction to H₂S by ruminal bacteria

Although sulphur amino acids can be catabolized by mammalian cells into H₂S, it is well established that reduction of inorganic sulphate to H₂S does not occur in mammalian cells. Sulphate reduction to H₂S does occur in sulphate-reducing bacteria, which are present in both the ruminant and non-ruminant digestive tracts (NRC, 2005). Sulphur-reducing bacteria in the rumen utilize anaerobic respiration pathways for bio-energetic processes. Bacteria in the rumen can metabolize S as elemental, inorganic or organic S. Two metabolic pathways have been proposed for dietary S in the rumen: the assimilatory and dissimilatory pathways (Cummings *et al.*, 1995). The assimilatory pathway is the reduction of sulphate to sulphide and its incorporation into S-containing compounds (e.g. cysteine and methionine) destined for use in microbial proteins. Assimilatory bacteria include bacteria from the *Bacteroides*, *Butyvirio* and *Lachnospira* genera (Cummings *et al.*, 1995). The dissimilatory pathway is used by some rumen microbes to derive energy from the reduction of sulphate to H₂S; H₂S then is released into the rumen gas cap. Both assimilatory and dissimilatory sulphate reductions are carried out by anaerobic ruminal bacteria. However, reduction to H₂S predominates in the rumen (Cummings *et al.*, 1995). Although

many bacteria can produce sulphides, organisms from the *Desulfovibrio* and *Desulfotomaculum* genera are most likely the predominant sulphate-reducing bacteria in the rumen (Cummings *et al.*, 1995). Recent research (Sarturi *et al.*, 2011) suggests that rumen “available S” is important in determining production of H₂S. Organic forms of sulphur, such as those found in amino acids, are not readily available in the rumen for production of H₂S, whereas inorganic forms of sulphur (e.g. sulphuric acid and sulphur salts) are more readily available for production of H₂S. Calculating rumen degradable sulphur intake was able to explain 64.9 percent of the H₂S production, whereas total sulphur intake explained only 24.4 percent (Sarturi *et al.*, 2011). Accounting for area below rumen pH 5.6 increased accuracy of predicting H₂S production (Sarturi *et al.*, 2011).

In the rumen, the extent of dissimilatory sulphate reduction is proportional and limited to the amount of S-containing compounds. The concentration of the S metabolites HS⁻, HSO₃⁻, S²⁻ and S⁰ within the rumen fluid and gas are not static and are greatly affected by rumen pH (Beauchamp, Bus and Popp, 1984; de Oliveira *et al.*, 1997; Gould, 1998; Kung *et al.*, 1998). The acidic nature of the rumen favours the formation of H₂S, which has a pKa value for first and second dissociation steps of 7.04 and 11.96, respectively. One third of H₂S exists undissociated at a pH of 7.4, with two-thirds in the form of the hydrosulphide ion (Beauchamp, Bus and Popp, 1984). When rumen acidity increases, the amount of H₂S present in the rumen also increases. With a change of pH from 6.8 to 5.2, the percentage of H₂S in the rumen gas cap increased from 46.8 to 97.2 percent (Gould, 1998). Thus, high-concentrate diets (high in readily fermentable carbohydrates) that are high in sulphate and low in long fibre have been shown to increase ruminal H₂S concentrations in the gas phase and induce clinical symptoms of H₂S toxicity (Gould *et al.*, 1991; Sager, Hamar and Gould, 1990). Rather than relieving ruminal acid load by replacing starch-containing grains, maize milling co-products such as DG may actually increase acid load because it carries substantial quantities of acidity. Distillers grain has been shown to have a pH of 3.76–4.50 (Felix and Loerch, 2011; Uwituzze *et al.*, 2011a). It is unclear what causes the pH of DG to be so low, but sulphuric acid is a standard fermentation treatment in the ethanol production industry (McAloon *et al.*, 2000). Adding sulphuric acid to DG significantly decreases its pH and increases H₂S production in the rumen, although rumen pH is actually slightly increased when sulphur content of the diet is increased (Uwituzze *et al.*, 2011b). This may have been attributable to the fact that dietary sulphur decreases feed intake and VFA production and increases ruminal ammonia concentrations (Uwituzze *et al.*, 2011b). Further H⁺ ions, in the form of H₂S, are eructated, which further relieves rumen acidity. As such, strategies that buffer H⁺,



such as addition of forage or monensin, have been shown to competitively inhibit H₂S production and improve feed intake (Felix and Loerch, 2011).

Sulphide is readily absorbed through the rumen wall into the blood stream (Bray, 1969). Protonated H₂S, however, is not absorbed across the rumen wall (NRC, 2005). Catabolism of H₂S seems to be ubiquitous in animal tissues with the exception of brain (Lagoutte *et al.*, 2010). Oxidation of H₂S occurs in the mitochondria through action of two inner membrane-bound enzymes (Figure 2; Olson, 2011): sulphide:quinone oxidoreductase (SQR) and sulphur dioxygenase (SDO). It is clear from a number of studies that the major metabolic and excretory pathway for H₂S is oxidation to sulphate and subsequent excretion by the liver and kidney (Anderson, 1956). Further, sulphide absorbed from the rumen may be detoxified by oxygenated haemoglobin in the blood and *in vivo* reduction of oxyhaemoglobin is reversible (Evans, 1967). Hence, it is unlikely that much free sulphide would reach the brain after being absorbed from the rumen into the portal system (Bird, 1972). Detoxifying mechanisms, however, could be overwhelmed in cases where blood H₂S is high (Loneragan *et al.*, 1998). In ruminants, eructation (belching of gases) is a normal process and as much as 60 percent of eructated gasses are inhaled and enter the respiratory tract (Bulgin, Stuart and Mather, 1996). Thus, inhalation of H₂S from diets high in S has been implicated as a potential cause of PEM in ruminants.

In a classical demonstration of this process, Dougherty, Mullenax and Allison, 1965 infused H_2S into the rumen of sheep and reported that sheep with an open trachea collapsed after several eructations, whereas sheep with a blocked trachea produced no clinical signs of S toxicosis. As such, Bird (1972) stated that “the direct and shorter route to the heart and brain is afforded by the inspiration of H_2S and transfer into the pulmonary vein, which effectively bypasses the liver and enables H_2S to exert its toxic effect on the respiratory-circulatory systems.”

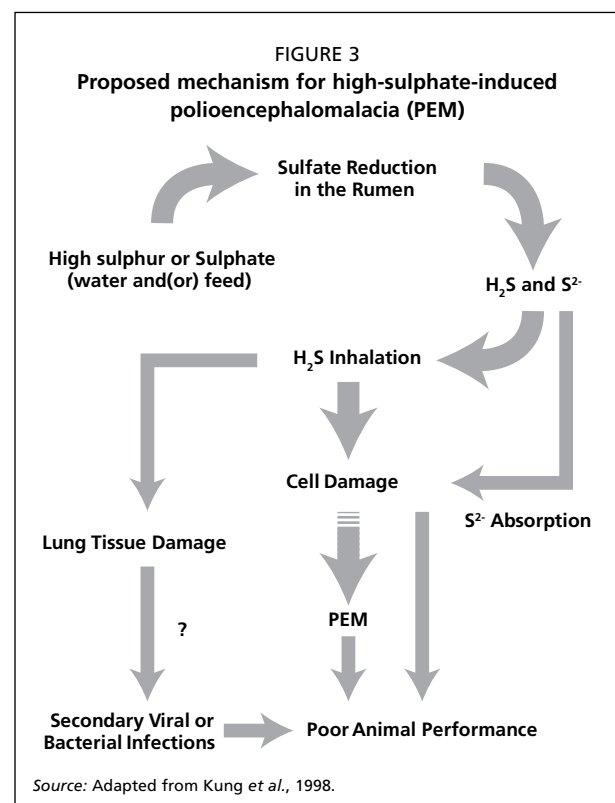
Manifestation of S toxicity

On the basis of other gas sensors and gas-based signalling pathways, metalloproteins, particularly haem-containing proteins serve as target molecules and probably mediate effects of H_2S . Because of its small size relative to other thiols, H_2S has easy access to the metal centres of metalloproteins. The H_2S may ligate reversibly to the ferric ion of haem. At higher concentrations (e.g. 20 μM), the H_2S reduces the ferric ion to ferrous and becomes oxidized to persulphide (HS-SH). Above-normal concentrations of H_2S favour production of sulphhaemoglobin and sulphmyoglobin, both of which have lesser abilities to carry O_2 than haemoglobin. High concentrations of H_2S also reduce methaemoglobin (Pietri, Roman-Morales and Lopez-Garriga, 2010).

Sulphide inhibits the functions of carbonic anhydrase, dopa oxidases, catalases, peroxidases, dehydrogenases and dipeptidases, thus affecting oxidative metabolism and the production of ATP (Short and Edwards, 1989). Specifically, H_2S is also thought to block the enzyme cytochrome c oxidase (Collman *et al.*, 2009). Blockage of oxidative processes becomes particularly evident in the brain because of the numerous oxidative processes, low concentrations of antioxidants and the inability of the brain to repair itself (Olkowski *et al.*, 1992). At submicromolar concentrations, H_2S seems to have a protective effect in nervous tissue because it can protect neurons against hypoxic injury, inhibit oxidative damage, increase glutathione production, scavenge reactive oxygen species and suppress mitochondrial oxidative stress (Bouillaud and Blachier, 2011). In fact, deficiency of H_2S production may be associated with Alzheimer's disease in humans. At high concentrations, H_2S decreases cellular respiration and can substantially limit the amount of O_2 delivered to the brain and the rate of ATP generation in the brain. Such a severe restriction in ATP generation in the brain causes necrosis of the cerebral cortex and softening of the brain tissue (Gould, 1998). Mild cases of H_2S toxicity in ruminants do not always, but can, result in decreased DM intake and average daily gain. Manifestations of S toxicosis include anorexia, weight loss, constipation, diarrhoea and depression. Severe cases of H_2S toxicity may result in PEM (Gould, 1998). Polioencephalomalacia literally means

softening (malacia) of the gray matter (polio) of the brain (encephalo). Signs of PEM include separation from the group, head pressing, “star gazing” in which cattle stand with their head held back and upward, teeth grinding and have a staggered gait. More extreme and advanced signs may include seizures, blindness and coma, and may eventually lead to death.

In the cardiovascular system, H_2S apparently exerts vasodilation and vasoconstriction effects depending on oxygen concentrations and interaction with other gasotransmitters such as NO (Leschelle *et al.*, 2005). At low concentrations, H_2S can positively decrease blood pressure (Olson, 2011), however, at toxic concentrations, H_2S has a paralyzing effect on the carotid body, further inhibiting normal respiration (Bulgin, Stuart and Mather, 1996). Thus, elevated pulmonary arterial pressure with increasing S intake has been observed (Loneragan *et al.*, 1998) and others (Bulgin, Stuart and Mather, 1996; Coghlin, 1944) have noted pulmonary oedema and respiratory distress as a feature of H_2S poisoning. Because H_2S is so toxic (Truong *et al.*, 2006), damage to lung tissue could result even if clinical signs of PEM do not exist. Decreases in intake and gain have been reported for cattle fed diets containing as little as 0.22 percent S (Zinn *et al.*, 1997, 1999), and continued linear decreases have been observed up to 0.46 percent S by numerous authors (Bolsen, Woods and Klopfenstein, 1973; Loneragan *et al.*, 2001; Spears and Lloyd, 2005). Potential mechanisms of S toxicity in ruminants are illustrated in Figure 3.



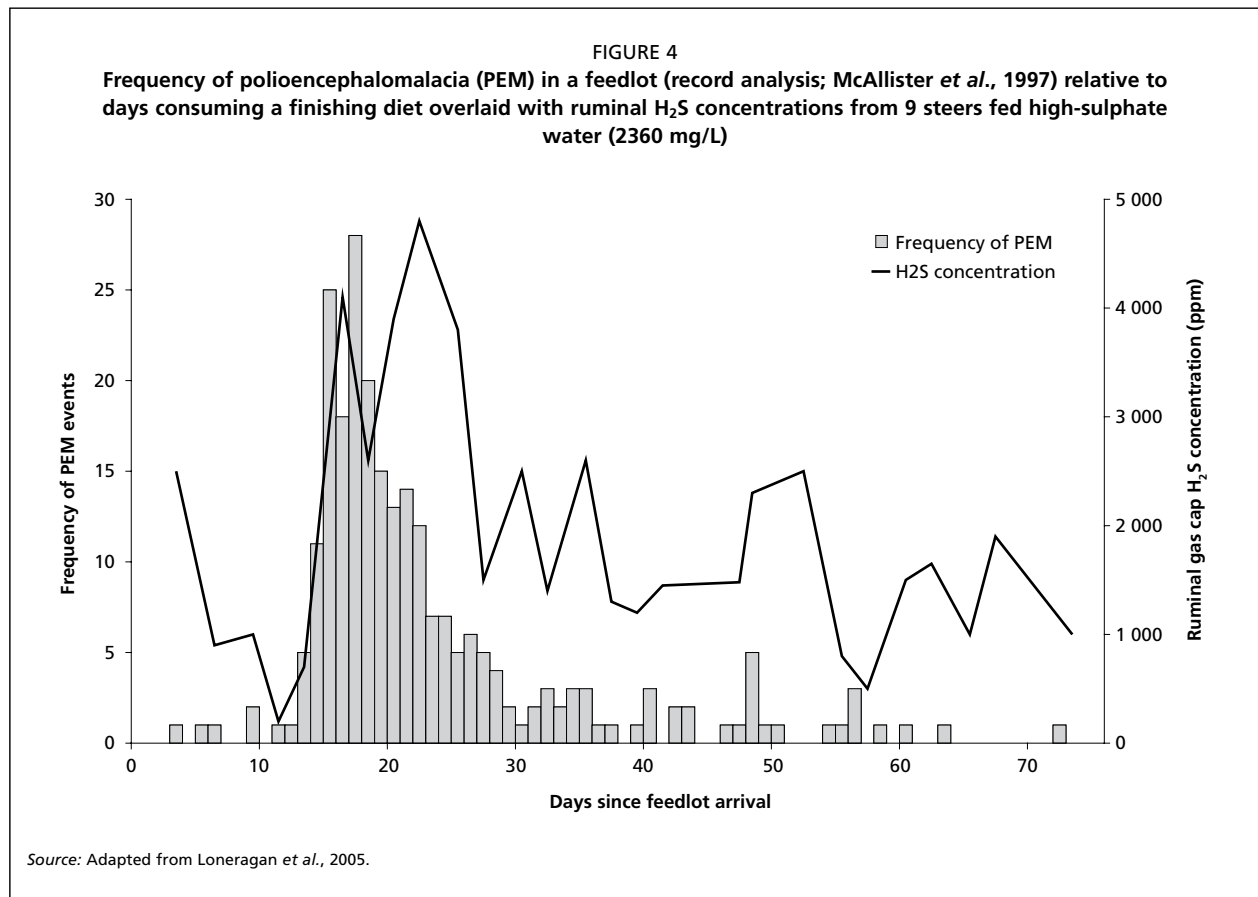
Hydrogen sulphide relaxes smooth muscles from the stomach through the colon (Olson, 2011). Moreover, H₂S is thought to have anti-inflammatory effects in the colon because it enhances ulcer healing independent of nitric oxide synthase and ATP-sensitive K channel involvement (Olson, 2011). Further, in model systems, H₂S protects against and promotes healing in colitis (Olson, 2011). In contrast, excessive sulphate entering the lower gastrointestinal tract can cause osmotic diarrhoea as the most significant observable clinical finding (NRC, 2005). This pro-inflammatory effect, in addition to cell cycle regulation effects, explains why H₂S can contribute to colo-rectal cancer in humans. Generally, non-ruminants respond to excessive S by decreasing feed intake (NRC, 2005).

Variability in PEM incidence

Incidence of PEM can be highly variable and is not always associated with dietary S or measurable H₂S. Signs of PEM have been induced in ruminants consuming diets with 0.4 percent S (Gould *et al.*, 1991), but in some studies animals have been fed more than 1.7 percent S without signs of toxicity (Chalupa *et al.*, 1971; Slyter *et al.*, 1986). Ruminal H₂S concentrations over 2000 ppm can precede the development of PEM (Gould, Cummings and Hamar, 1997). However, Drewnoski *et al.* (2011a) demonstrated that steers fed high S diets (0.60 percent) consistently

produce H₂S above 2000 ppm, peaking between 6 and 10 hours post-feeding, without incidence of PEM. The biological availability of the S source, ruminal pH and interactions with dietary nutrients, such as divalent cations, may explain some of the conflicting results. However, duration of feeding a high S diet, variability in S concentrations of feed, development of rumen microflora, and size of the rumen and rumen gas cap may affect responses to high S as well.

Cattle consuming high S diets seem most susceptible during the first 15–30 days of being fed a full high concentrate finishing diet (Drewnoski, Richter and Hansen, 2011). Sager, Hamar and Gould, 1990 and Low *et al.* (1996) both observed clinical signs of PEM beginning on day 15 after adaptation to a high-concentrate diet with excess S. During this time, ruminal pH became increasingly more acidic. Increased incidence of PEM early on in the feeding period has been postulated to coincide with a spike in ruminal concentrations of H₂S (Figure 4; McAllister *et al.*, 1997; Loneragan *et al.*, 2005). After this peak, H₂S concentrations decreased and no further cases of PEM developed. Variability in S content of the diet, as is possible when receiving multiple batches of co-products from various plants, is also a factor in susceptibility of cattle to PEM. Spiehs, Whitney and Shurson (2002) reported a range for S content of DG from 12 ethanol plants of 0.33 to 0.74 percent and a within-plant coefficient of variation ranging



from 6.4 to 40.8 percent. Buckner *et al.* (2011) reported a range for S content of DG from 6 ethanol plants of 0.71 to 0.84 percent and a within-plant coefficient of variation ranging from 2.2 to 12.9 percent. Loads of DG can be fed quickly in large feedlots, such that multiple batches could be fed in one day or could vary from day to day. When diets are high in S and vary significantly in S content from day to day (coefficient of variation of 15.7 percent), PEM incidence can increase (Domby *et al.*, 2011). Domby *et al.* (2011) observed that although performance and carcass characteristics were not affected by random changes in dietary S (a switch every 1–4 days between 0.48 and 0.60 percent S; sulphuric acid added to increase dietary S), mortality due to PEM was significantly increased (5.21 vs 0.67 percent) compared with diets that maintained a constant S concentration of 0.48 percent.

Previous research revealed a swift adaptation of sulphate-reducing bacteria to increased ruminal sulphate concentration (Lewis, 1954; Bird and Hume, 1971; Bird and Moir, 1971). Although ruminal organisms, in general, have a greater capacity to produce sulphide (Cummings *et al.*, 1995) and have a faster rate of sulphate reduction (de Oliveira *et al.*, 1997) after several days or weeks of high dietary S, changes in the dynamics of the ruminal microbial population may actually inhibit H₂S production and contribute to variability in PEM incidence. Development of a more stable combination of assimilatory and dissimilatory activities of sulphate-reducing bacteria (Huisingh, McNeill and Matrone, 1974) may decrease H₂S production and effectively incorporate more S into bacterial protein. Moreover, it has been suggested that dietary S increases propionate production by converting lactate to acryloyl-CoA, an S-containing intermediate (Russell, 2002), through the acrylate pathway (Whanger and Matrone, 1967). Increasing dietary concentration of DG (and S) will increase ruminal propionate concentrations in dry-rolled maize-based diets (Leupp *et al.*, 2009; Uwituzi *et al.*, 2011b), which may compete with H₂S for H⁺, effectively lowering ruminal H₂S concentrations. Taken together, these reports are evidence that adaptive mechanisms for the increased activity by sulphate-reducing bacteria exist. Adaptation to high dietary S by other ruminal microorganisms, however, is unclear.

Thiamine and PEM

The lack of adequate dietary thiamine will inhibit thiamine-dependent reactions of glycolysis and the trans-carboxylic acid cycle (Brent and Bartley, 1984) and can induce PEM. This activity seems to be caused by ruminal thiaminase production as a result of a shift in the ruminal environment from Gram-negative to Gram-positive bacteria, which commonly will occur during adaptation to a high-concentrate diet (Brent, 1976). The link between thiamine status and

PEM, and the dramatic effect that intravenous thiamine administration can have has led to the often incorrect assumption that outbreaks of PEM are the result of altered thiamine status (Gould, 1998). Subsequently, the addition of 100 to 200 mg of thiamine per head daily is often added to diets of cattle perceived to be at risk of developing PEM. However, the results from efforts to treat or prevent PEM with thiamine are mixed. Much of the confusion surrounding thiamine therapy may be attributed to the fact that high sulphate intake may induce PEM through multiple mechanisms. High sulphate intake has been shown to decrease duodenal thiamine flow (Goetsch and Owens, 1987), and sulphite, a transient product of sulphate reduction, can destroy thiamine in the rumen resulting in thiamine deficiency (Brent and Bartley, 1984). These forms of sulphate-induced PEM may respond to thiamine therapy or may be prevented by thiamine supplementation. Olkowski *et al.* (1992) suggested that although sulphite is transient, it may be a significant contributor because the sulphite produced is absorbed, oxidized to sulphate and then recycled back to the rumen and available to be reduced again. It also has been suggested that sulphite could have a direct impact on the brain tissue itself, as sulphite-derived radicals have been postulated to cause lipid peroxidation and damage to biological membranes (de Oliveira *et al.*, 1996; Brent and Bartley, 1984; Olkowski *et al.*, 1992). Although ruminal thiamine status may not be affected by the occurrence of S-induced PEM, dietary thiamine concentrations should be monitored to ensure that adequate thiamine is available to cattle and supplemental thiamine should be considered to avoid thiaminase-induced PEM. Further, thiamine is the primary method of treatment for animals afflicted with PEM. An intravenous injection of thiamine (10 mg/kg of body weight; Cebra and Cebra, 2004) is suggested.

Managing high-S diets

Possible strategies to manage high S concentrations include limiting the amount of high-S feedstuffs or water consumed, adapting cattle to high-S feeds in the diet, or offering feed additives that may combat high S intakes. Use of antibiotics that inhibit the Gram-negative bacteria responsible for H₂S, and adding dietary minerals that bind sulphide in the rumen are potential strategies that have been investigated. Kung, Bracht and Tavares (2000) analysed the effects of molybdenum, the antibiotics avoparcin, bacitracin, bambarmycin, lasalocid, chlortetracycline and oxytetracycline, as well as an experimental compound, anthraquinone, on sulphide production *in vitro*. Anthraquinone, bambarmycin, chlortetracycline, oxytetracycline and lasalocid all decreased *in vitro* H₂S production, with the greatest decreases occurring with anthraquinone, chlortetracycline and oxytetracycline (Kung, Bracht and Tavares, 2000). The effect of these compounds on *in vivo* H₂S production are

unclear. *In vitro* studies evaluating the effect of monensin on H₂S production have been inconclusive. Some researchers observed no change in *in vitro* H₂S production when 5 mg/L monensin was added to rumen fluid cultures containing 0.20–0.80 percent S (Quinn *et al.*, 2009; Smith *et al.*, 2010), whereas Kung, Bracht and Tavares (2000) found an increase in *in vitro* H₂S production with 5 mg/L monensin added to rumen fluid containing 1.09 percent S. *In vivo*, however, monensin supplementation at 33 mg/kg of feed (approximately 6.6 mg/L of rumen fluid) tended to decrease post-feeding ruminal H₂S and S²⁻ concentrations when diets containing 0.5 percent S were fed (Felix *et al.*, 2011).

Inclusion of molybdate successfully inhibits H₂S production *in vitro* (Kung, Bracht and Tavares, 2000), but molybdate binds copper and can result in decreased copper bio-availability *in vivo* (Loneragan *et al.*, 1998). The use of copper salts in addition to molybdenum salts may increase copper availability, while decreasing H₂S production. Cross, Rust and Powers (2010), however, demonstrated that the addition of 60 ppm copper and 6 ppm molybdenum did not decrease *in vivo* H₂S emissions when high-S diets were fed. Dietary manganous oxide also has been investigated and may initially maintain higher ruminal pH, in cattle fed high-S diets, resulting in cumulative ruminal H₂S concentration in feedlot cattle (Kelzer *et al.*, 2010). Ferric ions also show promise as a strategy to decrease ruminal H₂S production, potentially through competitive inhibition of ruminal sulphate reduction. Addition of 200, 300 or 400 mg iron/kg diet DM as ferric ammonium citrate to the diet of steers produced a linear decrease in ruminal H₂S concentration without affecting DM intake or ruminal pH (Drewnoski, Doane and Hansen, 2011).

Preliminary research has demonstrated that feeding high amounts of ammonium nitrate, molybdenum, or the zeolite clinoptilolite, often decreased H₂S concentration in the rumen gas cap but did not improve feedlot performance by steers consuming high-sulphate water (≥ 2000 ppm) in experiments conducted at Colorado State University (Wagner, 2008). Subsequent research, however, has demonstrated that clinoptilolite is ineffective at 2.5 or 5.0 percent of the diet DM at preventing or ameliorating PEM, or reduced nutritional status in feedlot steers fed a forage diet (Cammack *et al.*, 2010).

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Because some physiological roles of H₂S have only recently been elucidated, much information regarding the biochemistry and biology of H₂S remains to be determined. The typical concentrations of H₂S in the variety of tissues where it is synthesized needs to be determined so that associations with kinetic parameters of enzymes involved with synthetic and degradative pathways can be calculated.

Moreover, nutritional and other environmental factors that control the concentration of H₂S need to be studied to provide basic information for determining the physiological functions of it as a metabolic signal molecule. Intracellular chemical regulators of synthetic and degradative reactions remain to be defined. Much information also is needed on the mechanism by which H₂S binds to target molecules to promote its cellular and physiological effects.

With regard to the livestock industry, substantial general information is available on effects of excess sulphate and other sulphate-containing compounds on feed intake, efficiency of growth, and indicators of development of toxicity because of excess H₂S production in the rumen. Much research, however, is needed to characterize the role of diet composition, and other environmental strategies to mitigate H₂S production in the rumen remain to be discovered. Moreover, better methods to diagnose, treat and prevent PEM are needed.

CONCLUSIONS

Hydrogen sulphide has been shown to be a signal molecule in animal tissues and thus to have physiological effects on cellular and tissue functions. The question remains of whether cellular concentrations of H₂S are sufficient to exert the demonstrated effects. Metalloproteins and oxidized cysteine residues of proteins are postulated to serve as the target molecules for H₂S action in a cell. In fact, H₂S is suggested to be a third gasotransmitter in addition to NO and CO. Expansion of the maize ethanol industry and, to a lesser extent, the use of soybean for biodiesel production, has resulted in an unprecedented increase in costs of traditional feeds, leaving livestock producers searching for alternatives to maize and soybean. Maize ethanol co-products are exceptionally high in energy and protein and are economical and practical alternative feedstuffs. Because S toxicity is now recognized as having a major impact on health and performance of ruminants, one must consider not only the reported sulphate content in these co-products, but also the variability associated with batches of feed among plants as well as variability within a plant. In addition to accounting for S in feedstuffs, the importance of sulphate concentrations in water must also be recognized. For ruminants, total S intakes should not exceed 0.40 percent of DM. For feedlot cattle consuming diets with greater than 40 percent forage, total S intakes should not exceed 0.50 percent of DM. Cattle will vary considerably in their ability to handle excess S intake. Mild cases of H₂S toxicity may result in decreased average daily gain and feed efficiency; severe cases of H₂S toxicity may result in PEM, which can cause seizures, blindness and coma and may eventually lead to death. For sulphide to have toxic effects, it must bypass hepatic detoxification (oxidation to sulphate). Hepatic detoxification is bypassed when sulphide

is absorbed through the rumen wall and hepatic oxidation systems are potentially overwhelmed, or when eructated H₂S is absorbed through the lungs, effectively bypassing hepatic circulation. Cattle fed high-concentrate diets are most susceptible and susceptibility is also increased when cattle are adapted to a high-concentrate diets and when diets are highly variable in S content. Through analysis of sulphate content and careful selection of feeds and batches of feed with acceptable S concentrations, diets can be formulated to limit the impact of variation in feedstuff S concentration. In addition to management practices specifically designed to combat high S, such as antibiotic and mineral supplementation, normal management practices such as proper feed mixing and feed-bunk management also may assist in preventing negative effects because of excess S intake.

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Chapter 7

Feeding biofuel co-products to dairy cattle

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ABSTRACT

The expansion of ethanol and biodiesel production as bio-renewable fuel sources has resulted in increased availability of numerous co-products as livestock feeds. The growth of the bio-ethanol industry in the United States over the past decade has been rapid and has resulted in large quantities of distillers grain and other ethanol co-products becoming available for dairy cattle diets. While many types of grains can be used for ethanol production, maize grain is the grain most commonly used in the United States. Distillers grain is often added to dairy cow diets to provide a source of rumen-undegraded protein, energy and minerals. Distillers grain can be provided dried, wet, or in a modified wet form. In addition to distillers grain, condensed distillers solubles is a product of the ethanol industry. Pre-fermentation fractionation and post-fermentation processes produce other co-products, such as high-protein distillers grain, maize germ, maize bran and reduced-fat distillers grain, all which can be utilized in dairy cattle diets. From the biodiesel industry, glycerol has been investigated to determine its use in dairy cattle diets. Storage of wet co-products on the farm is challenging because wet feedstuffs will spoil quickly unless they are stored anaerobically. Ensiling co-products alone or in combination with complementary feedstuffs have been successful. In the near future it is likely that new biofuel products will become available in the market as a result of separation of the different nutrient fractions. Ruminant nutrition research will need to parallel these new product developments to ensure maximum economic return to livestock producers.

INTRODUCTION

In 1797, and just before retiring from office, George Washington had a farm manager from Scotland who started a distillation plant (DISCUS, 2007). The byproduct of this distillery, called "slop", was already considered a valuable food source for livestock, allowing the president to feed cattle and pigs with it. The advantages of using distillers grain with solubles (DGS) as a feedstuff for dairy cattle were already being tested halfway through the 20th century. Loosli and Warner (1957) studied the effects of maize [corn] and sorghum [milo] DGS on milk production. In their experiment, they compared the value of maize dried distillers grain with solubles, maize dried distillers solubles, sorghum dried distillers grain with solubles and sorghum dried solubles. They found no significant differences between DGS sources, although diets that contained DGS products resulted in greater 4 percent-fat-corrected milk (FCM) production, as well as a greater milk fat percentage.

Any grain that stores starch in its endosperm can be used to produce ethanol. The advantages of one cereal crop over the next rely on its alcohol yield per unit area, which depends upon the adaptation of that plant to its environment. Regardless of the grain, the process is basically the same. Ground cereal grain is fermented in water by the yeast *Saccharomyces cerevisiae*, with added co-factors. The starch-spent mash is separated from the liquid, and ethanol

is extracted from the supernatant liquid by distillation. The nutrients remaining in the mash are concentrated to an extent determined by the amount of starch removed. The three energy-yielding nutrient fractions that remain for digestion by livestock are protein, structural carbohydrates and fat. Each of the first two yields essentially the same amount of energy as the starch removed; fat in contrast yields 2.25 times more energy by weight than either of the other two fractions. The net result of starch removal is a feedstuff that releases more energy when catabolized in the organism.

Of the United States bio-refineries that use cereal grain as substrate, maize is used as the sole cereal in 95.4 percent of them (Table 1). In the European Union and Canada, however, maize is used exclusively by only 34.6 and 50 percent of the plants, respectively (RFA, 2011; ePURE, 2010; CRFA, 2010). Because of its more intense agricultural practices, maize is a less sustainable cereal as substrate for ethanol production in

TABLE 1
Number of operational ethanol plants that use grain as substrate

	Maize	Wheat	Other grain	Total
USA	186	0	9	195
EU	9	9	8	26
CANADA	8	5	3	16
Total	203	14	20	237

Sources: Adapted from: RFA, 2011; ePURE, 2010; CRFA, 2010.

MAIN MESSAGES

- With the expansion of the biofuel industry, numerous biofuel coproducts have become available. The primary co-products from ethanol production include dried distillers grain with solubles, wet distillers grain with solubles, and condensed distillers solubles. The primary co-product from biodiesel production is glycerol.
- Ethanol can be produced from any of the cereal grains, but is predominantly produced from maize in the United States. The resulting co-product, distillers grain with solubles, is recognized as a good source of ruminally-undegraded protein, energy (from fat and fibre) and minerals for dairy cow diets.
- Nutrient composition of biofuel co-products can vary. Diets that are not properly formulated can result in reduced dry matter intake, milk production losses and altered milk composition. It is highly recommended to obtain a nutrient composition profile of the co-product when formulating diets for dairy cattle.
- Wet co-products are challenging to store on the farm because they can spoil rapidly. If used fresh, they should be used within a few days of arrival, or they can be stored anaerobically. They can be stored for months if ensiled, either alone or in combination with other feeds.
- Glycerol can be included in dairy cow diets as an energy source or as a preventative for ketosis. Recommended inclusion levels as an energy source in lactating dairy cow diets is 15 percent of the diet.
- Maximum recommended levels of distillers grain with solubles for pre-weaned calves, growing heifers, dry cows and lactating dairy cows are 25, 30, 15 and 20 percent of the diet on a DM basis, respectively.

many parts of the world. In parts of the world where the cool weather is not adequate for maize production, wheat is the main grain used for ethanol production. Cyclic fluctuations in the price of wheat also create opportunities for other starch sources for ethanol production, such as barley, triticale and rye (Mustafa *et al.*, 2000).

The economic viability of a bio-refinery depends on factors such as ethanol yield, efficiency of fermentation and DGS quality (Wang *et al.*, 2008). This efficiency of fermentation, calculated as the ratio between expected and actual ethanol yields, usually varies between 90 and 95 percent (Wu *et al.*, 2006).

Linn and Chase (1996) suggested that the major factors that affect DGS variability are grain type and quality, milling and fermentation processes, drying temperature, and proportion of solubles added back to the DGS. There is less information available about the nutrient content of DGS produced from the fermentation of other crops such as wheat, barley or sorghum. However, data available indicate that composition usually reflects the nutrient content of the original grain once starch is fermented to ethanol. Thus, the concentrations of all remaining nutrients in DGS from different grain sources should increase proportionally to the amount of starch removed (Schingoethe, 2006). For example, if the grain has approximately 66 percent starch on a dry basis, nearly 2/3 of its constituents will be removed during fermentation and the remaining nutrients will be concentrated threefold.

NUTRIENT COMPOSITION OF BIOFUEL CO-PRODUCTS

As can be observed from Table 2, the low variability observed in the concentrations of CP in sorghum, wheat

and barley (standard deviation (SD) = 0.7, 1.1 and 0.5, respectively) translated into larger variations when the CP concentration of DGS from these same grains was compared (Table 3; SD = 5.3, 6.7 and 6.9, respectively). These results demonstrate that table values published in the literature often do not reflect actual values. Therefore, it is advisable to formulate diets based on chemical analysis of the product being used rather than on table values (Pritchard, 2006; Holt and Pritchard, 2004).

Sorghum distillers grain

Sorghum is a tropical heat- and drought-tolerant grass grown primarily in parts of the world that are too dry to grow maize (Corredor *et al.*, 2006). According to the Renewable Fuel Association, of the 195 United States ethanol bio-refineries that use grain as their main substrate, only 6 use sorghum-maize blends (RFA, 2011). As of 2011, there are no plants that utilize exclusively sorghum to produce ethanol. A report published by the United States Sorghum Checkoff Program (Agri-energysolutions, 2009) stated that 43 percent of the sorghum produced in Kansas and 23 percent of that produced in Texas is used for ethanol production. The report also noted some advantages of sorghum, including that sorghum requires less water and input costs than growing maize, that it can be grown in marginal lands, that yield per hectare can potentially be similar to maize, and that ethanol plants paid only slightly less for sorghum than for maize.

Sorghum grain is 84 percent endosperm, half of it flinty, characterized by smaller starch granules, tightly enveloped by a continuous protein matrix composed of highly insoluble glutelin and prolamin. As a result, sorghum is the grain

TABLE 2
Composition of different cereal grains

	Maize	Sorghum	Wheat	Barley	Triticale	Rye
Nutrients (% of DM)						
NDF	9.9 ± 1.3	13.8 ± 6.2	12.9 ± 1.1	19.8 ± 1.6	14.3 ± 0.2	16.5 ± 2.3
ADF	3.5 ± 0.4	5.2 ± 0.9	4.0 ± 0.4	6.9 ± 0.4	3.9 ± 0.2	5.2 ± 2.4
Lignin	0.7 ± 0.4	1.1 ± 0.3	1.2 ± 0.4	1.8 ± 0.4	1.1 ± 0.2	1.1 ± 0.1
Starch	73.8 ± 1.0	67.7 ± 9.9	66.9 ± 2.3	57.0 ± 2.8	64.9 ± 3.4	59.8 ± 1.9
CP	9.1 ± 0.3	10.9 ± 0.7	13.2 ± 1.1	12.3 ± 0.5	12.4 ± 1.2	11.1 ± 1.8
Crude fat	4.5 ± 0.6	3.3 ± 0.3	2.0 ± 0.2	2.1 ± 0.1	1.7 ± 0.1	1.6 ± 0.2
Ash	1.50 ± 0.08	1.8 ± 0.26	1.9 ± 0.1	2.9 ± 0.3	2.0 ± 0.1	1.9 ± 0.2
Ca	0.04 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.03
P	0.24 ± 0.13	0.34 ± 0.01	0.40 ± 0.03	0.41 ± 0.03	0.37 ± 0.03	0.36 ± 0.02
S	0.13 ± 0.02	0.11 ± 0.01	0.26 ± 0.19	0.14 ± 0.02	0.16 ± 0.01	0.14 ± 0.04
Energy parameters (Mcal/kg)						
NEM	2.13 ± 0.05	1.98 ± 0.04	2.11 ± 0.06	2.15 ± 0.18	2.01	2.02
NEG	1.45 ± 0.04	1.31 ± 0.01	1.45 ± 0.03	1.35 ± 0.01	1.37	1.34
NEL	1.97 ± 0.15	1.85 ± 0.09	1.91 ± 0.16	1.80 ± 0.17	1.85 ± 0.20	1.89 ± 0.12

Notes: Nutrients: NDF = neutral-detergent fibre; ADF = acid-detergent fibre; CP = crude protein. Energy parameters: NEM = net energy for maintenance; NEG = net energy for gain; and NEL = net energy for lactation. Data are reported as means ± the standard deviation. Sources: Adapted from NRC, 2001; FEDNA, 2003; INRA, 2004; CNCPS, 2009.

most resistant to microbial fermentation (FEDNA, 2003). Consequently, this results in the lowest effective protein degradability of all cereal grains (INRA, 2004). Its concentration of starch and fat (67.7 and 3.3 percent, respectively) are slightly less than that of maize, which, together with greater fibre concentration, results in a lower net energy for lactation (NEL) content (1.85 Mcal/kg NEL) compared with maize DGS (1.97 Mcal/kg NEL).

Although with great variability between experiments, there has been more CP reported in sorghum DGS compared with maize DGS (34.1 vs 31.2 percent; Table 3), with a range between 24.4 and 45 percent. These variations were also observed among DGS produced in the same plant, albeit with different moisture contents. Debenbusch *et al.* (2009) reported CP contents of 45 percent for dried (92 percent DM) and 34 percent wet (36 percent DM) sorghum DGS. In the same experiment, however, the CP concentration between wet and dried maize DGS varied by only 3 percent.

The average fat values in sorghum DGS studied were similar to that of maize (11.3 vs 11.9 percent) in spite of a 26 percent lower fat content in sorghum than maize, which resulted in similar NEL values between both types of DGS (2.24 Mcal/kg). The percentage of starch and acid-detergent fibre (ADF) of sorghum DGS were greater than in maize DGS as a result of the greater resistance to degradation of the protein matrix that encapsulates the starch granules of the sorghum endosperm.

Wang *et al.* (2008) compared ethanol production characteristics of 70 sorghum varieties with different nutrient composition and physical properties. The average starch content varied between 64 and 74 percent, and had an efficiency of conversion to ethanol of 86 to 93.8 percent.

The authors also determined that variations in ethanol yields could be as high as 7.4 percent, particularly due to negative effects on fermentation efficiency caused by high amylose concentration in some varieties. There were no significant differences due to grain colour, except for brown tannin-containing varieties. Results with those varieties confirmed that high-tannin genetic lines are not suitable for ethanol production. These experiments demonstrate the importance for ethanol plants of adequate selection of the substrate to be used for fermentation. The darker colour of sorghum DGS, often mistakenly identified with excessive heating during drying (Maillard reaction), is frequently due to the darker colour of the variety of sorghum, which can reduce their acceptability in the market.

Wheat distillers grain

Wheat is considered as one of the main ethanol-producing crops in the EU (FAOSTAT data) with almost one-third of the bio-refineries using it as the sole substrate (Table 1). In North America, western Canada produces over 500 million litres of ethanol each year from over 1.3 million tonnes of wheat or wheat-maize blends (University of Saskatchewan, 2009). Half of the bio-refineries located in Canada, use wheat as the sole substrate or together with other cereal grains (Table 1). In contrast, the United States has only one ethanol plant that uses wheat as part of its substrates, located in Texas (RFA, 2011).

Wheat is classified as hard or soft, depending on the physical hardness of the endosperm and its resistance to grinding (Hruskova and Svec, 2009; Saunders, 2009). Grain hardness does not affect ethanol yield (Swanston *et al.*, 2007), but wheat varieties with harder endosperm are associated with processing problems (Dexter and Edwards,

TABLE 3
Composition of different distillers grains

Item	Maize DDGS	Sorghum DDGS	Wheat DDGS	Barley DDGS	Triticale DDGS	Rye DDGS
Nutrients (% of DM)						
NDF	40.1 ± 26.1	38.3 ± 10.7	43.4 ± 17.3	60.9 ± 17.9	40.6 ± 17.4	70.0
ADF	18.9 ± 7.5	22.7 ± 7.9	18.0 ± 4.7	28.5 ± 3.8	15.6 ± 3.2	19.8 ± 0.1
Lignin	3.8 ± 4.8	NA	5.8 ± 1.5	7.0 ± 1.6	5.5 ± 0.3	7.10
Starch	5.0 ± 3.5	8.8 ± 2.0	3.6 ± 1.7	1.1 ± 0.7	5.1 ± 3.4	9.70
CP	31.2 ± 1.1	34.1 ± 5.3	38.6 ± 6.7	24.7 ± 6.9	30.6 ± 0.5	29.3 ± 0.7
ADICP (%CP)	9.9 ± 1.0	NA	7.1 ± 2.3	16.5 ± 2.0	10.7 ± 6.4	6.5
Crude fat	11.9 ± 2.1	11.3 ± 2.1	5.4 ± 1.8	5.7 ± 0.5	7.0 ± 2.0	3.9 ± 0.9
Ash	4.9 ± 3.8	2.3 ± 0.5	4.3 ± 1.4	4.2 ± 0.2	4.0 ± 1.9	3.4 ± 1.7
Ca	0.10 ± 3.46	0.10	0.18 ± 0.03	0.20	0.10 ± 0.90	0.16
P	0.78 ± 0.06	0.78 ± 0.31	0.96 ± 0.10	0.80	0.81	0.80
S	0.59 ± 0.18	0.66	0.44 ± 0.06	NA	NA	NA
Energy parameters (Mcal/kg)						
NEM	2.38	2.49	2.23 ± 0.21	1.87	2.48	2.26
NEG	1.69 ± 0.01	1.73	1.56 ± 0.21	1.24	1.75	1.58
NEL	2.28 ± 0.02	2.24	2.08 ± 0.20	1.73	2.10	2.12

Notes: Nutrients: NDF = neutral-detergent fibre; ADF = acid-detergent fibre; CP = crude protein; ADICP = acid-detergent-insoluble CP. Energy parameters: NEM = net energy for maintenance; NEG = net energy for gain; and NEL = net energy for lactation. Data are reported as mean ± the standard deviation. NA= not available.

Sources: For maize distillers grain data adapted from Shelford and Tait, 1986; Weiss *et al.*, 1989; Lodge *et al.*, 1997; Al-Suwaiegh *et al.*, 2002; Greter *et al.*, 2008; Urriola *et al.*, 2009; Depenbusch *et al.*, 2009; Nuez-Ortin and Yu, 2009; May *et al.*, 2010; McKeown *et al.*, 2010; Oba *et al.*, 2010; Van De Kerckhove, 2010. For sorghum distillers grain data adapted from Lodge *et al.*, 1997; Al-Suwaiegh *et al.*, 2002; Urriola *et al.*, 2009; Depenbusch *et al.*, 2009; and May *et al.*, 2010. For wheat distillers grain data adapted from Boila and Ingalls, 1994; Ojowi *et al.*, 1997; Mustafa *et al.*, 2000; Beliveau and McKinnon, 2008; Gibb, Hao and McAllister, 2008; Nuez-Ortin and Yu, 2009; Penner, Yu and Christensen, 2009; Au *et al.*, 2010; McKeown *et al.*, 2010; Zhang, 2010; Van De Kerckhove, 2010. For barley distillers grain data adapted from Wu, 1986; Weiss *et al.*, 1989; Sosulski *et al.*, 1997; Mustafa, McKinnon and Christensen, 2000; and Mustafa *et al.*, 2000. For triticale distillers grain adapted from Mustafa *et al.*, 2000; Greter *et al.*, 2008; Au *et al.*, 2010; McKeown *et al.*, 2010; and Oba *et al.*, 2010. For rye distillers grain data adapted from Shelford and Tait, 1986; Wang *et al.*, 1998; Mustafa *et al.*, 2000.

1998). Higher starch content (not hardness) in soft compared with hard wheat (even greater than 6 percent; INRA, 2004), is the main reason for higher ethanol yield. This was further demonstrated by Zhao *et al.* (2009) in research with 30 American wheat cultivars of different types. These authors found that soft had proportionately greater starch content than hard wheat, with averages of 65.2 and 61.1 percent, respectively, resulting in greater ethanol yields for soft compared with hard wheat (433 vs 408 litres per ton, respectively). In addition, yields per hectare are generally greater for soft wheat varieties (ERS, 2008), making them ideal substrates for ethanol production.

The lower concentration of fat in wheat DGS compared with maize and sorghum has resulted in lower NEL values in several experiments (Table 3). The average CP concentration was variable, with a range between 26.4 and 45.8 percent. This reflects differences in protein concentration of the original grain. From analyses of seven commercial soft wheat cultivars, Zhao *et al.* (2009) found CP values between 9.6 and 14.7 percent. These differences in protein concentration of the original grain were carried over to the resultant wheat DGS, where CP content ranged from 28.2 to 37.6 percent.

In addition to the grain texture (soft or hard), there are other factors that influence the CP content of wheat, such as season (winter or spring) and amount of nitrogen fertilizer used. Slaughter, Norris and Hruschka (1992) evaluated the

differences between United States Hard Red Winter (HRW) and United States Hard Red Spring (HRS) wheat over a three-year period, and found that HRS had less CP (12.7 percent) than HRW (15.4 percent). Kindred *et al.* (2008) noted that protein concentration in the grain increased by 4.9 percent and starch decreased by 2.3 percent when the cultivar was fertilized with 240 kg N/ha, and this is the main reason why CP concentration in wheat DGS is highly variable.

Barley distillers grain

Barley ranks fourth among all cereal grains produced in the world, with nearly 6 percent of the total (FAOSTAT data). In spite of its importance, there are no bio-refineries that use it as the single grain (Table 1). Of the 237 ethanol plants in North America and the EU that use cereal grains, only five use barley as part of their substrate (RFA, 2011; ePURE, 2010; CRFA, 2010).

Barley has an external fibrous coating (pericarp) that constitutes 18 percent of the total weight of the grain, and is three times greater than the fibrous coating in maize or sorghum (FEDNA, 2003). Barley's pericarp is lignified and abrasive because of the presence of silica in the epidermis. The high fibre concentration of barley results in lower starch and NEL concentration than most other cereals. Most of the fibre in barley is bound by β -glucans in concentrations that vary between 3 and 7 percent (Griffey *et al.*, 2010) depending on the cultivar, region of origin and climate. The average

concentration of β -glucans in barley is higher than in wheat, maize and rye (FEDNA, 2003). In ethanol production, while the mash is being prepared, β -glucans solubilize and increase viscosity considerably. A combination of two enzymes, β -glucanase and β -glucosidase, has been used to reduce this problem (Nghiem *et al.*, 2010). The former hydrolyses soluble β -glucans into oligosaccharides and reduces the overall viscosity of the mash. The latter converts non-fermentable oligosaccharides formed during β -glucans hydrolysis to glucose, allowing an ethanol yield of 402 litres per ton.

Barley genotypes can be classified as hull-less or hulled based on the ease of removal of the outer coating. Hull-less or "naked barley" differs from traditional hulled barley in that the loose outer protective cover (husk) is easily removed during combine threshing and cleaning of the grain (Griffey *et al.*, 2010). The use of "hulled barley" as an alternative to maize in ethanol production has been limited due to its low starch content, high fibre content, abrasive nature of its hull due to high silica content, and the presence of β -glucans (Hicks *et al.* 2005). In fact, of the five research studies from which information on DGS from barley was obtained (Table 3), none utilized DGS produced exclusively from barley in commercial bio-refineries. Mustafa, McKinnon and Christensen (2000) and Weiss *et al.* (1989) used barley DGS originating in commercial bio-refineries but blended them with other cereals to avoid flow problems at the ethanol plant. The remaining studies (Mustafa *et al.*, 2000; Wu, 1986; Sosulski *et al.*, 1997) used laboratory-scale fermenters. The lack of homogeneity in the fermentation process resulted in high variability in DGS composition, particularly for fibre and protein, with values ranging from 38.0 to 79.2 percent for NDF and 15.2 to 32.6 percent for CP.

The high fibre content in "hulled barley" dilutes the overall starch concentration to between 50 and 55 percent (Sohn *et al.*, 2007). The removal of the fibre coating in the "hull-less varieties" results in a greater starch content (60–75 percent; Bhatti, 1999), making them more profitable for the ethanol industry. Similarly, their protein and β -glucans contents are also greater. Ingledew *et al.* (1995) showed that the DGS from hull-less varieties had 34.5–36.4 percent CP, while the hulled had 24.2 percent and wheat DGS had 34.3 percent. Unfortunately, when the hull-less varieties lose the hull, they yield less tonnage of grain per hectare, reason enough to be viewed less favourably by grain producers (Hicks *et al.* 2010).

One alternative when using hull-less barley is to process the grain to eliminate hull and bran before fermentation, and by doing so reduce the non-fermentable components. Sosulski *et al.* (1997) obtained a 10 percent increase in the starch concentration of the flour and a reduction in more than 17 percent in the production of DGS by eliminating 21.7 percent of the hull and bran. In addition, the CP con-

centration increased by 24 percent in DGS obtained from unprocessed barley, and up to 32.3 percent in DGS derived from pre-processed grain. These experiments demonstrated that barley could be considered as a potential substrate for ethanol if high-starch varieties are used, together with pre-processing and enzyme addition during the process. Thus, barley could result in DGS with higher protein concentration and with an amino acid profile different from maize DGS.

Triticale distillers grain

First bred experimentally in Europe during the late 19th century, triticale is a hybrid of wheat (*Triticum*) and rye (*Secale*). The initial objective was to combine the high energy and protein concentration of wheat grain with the agronomic rusticity and protein quality of rye (FEDNA, 2003). The cultivars tolerate acid soils and drought, and have been grown with success almost any place where the parental species are cultivated (Varughese, Pfeiffer and Pena, 1997). World production of triticale is led by Poland, Germany and France, and is the least of all cereal grains under consideration, representing only 0.63 percent of the total (FAOSTAT data). Two of the four plants that use triticale are in Germany, and one each in Czechoslovakia and Sweden.

Similar to rye, triticale has high pentosan content, although the studies that evaluated its fermentation to ethanol (Wang *et al.*, 1997, 1998) did not include enzymes to reduce the viscosity of the fermentation mash. In spite of lower starch concentration, triticale's greater content of free sugars can make up for the difference during fermentation, with similar ethanol yields similar to wheat.

The average concentration of NDF, fat, starch and protein in triticale is midway between those for wheat and rye, although tending to be more similar to wheat (Table 2). The average protein concentration of triticale DGS obtained from the five experiments reported (Table 3) shows very little variability and is very close to rye DGS and quite different from wheat DGS. In spite of the crude fat concentration of triticale being intermediate between wheat and rye, the fat concentration of triticale DGS (7.0 percent) is higher than that of the DGS from these two grains.

Rye distillers grain

Rye originated in Asia, but due to its great resistance to frost and drought it has primarily been cultivated in northern Europe. World production of rye represents only 0.73 percent of the world production of cereal grain. This crop has always been important in countries such as Germany, which produced almost one-fourth of total world production (FAOSTAT data). In fact, two of the four plants in the world that use rye as part of its substrate for ethanol production are located in Germany, with the remaining two in Lithuania and Canada (RFA, 2011; ePURE, 2010; and CRFA, 2010).

Rye has 11–13 percent pericarp, so although not as much as barley it is nevertheless more than twice (6 percent) that of sorghum or maize (FEDNA, 2003). This results in NDF and starch concentrations of 16.5 percent and 59.8 percent, respectively (Table 1). Its protein content is higher than both sorghum and maize, although lower compared with the other grains. Compared with other grains, rye has a higher concentration of soluble and insoluble pentosans (8.7 percent vs 6 percent in barley, wheat and oats) and an average concentration of β -glucans (2.4 percent). In addition, rye pentosans differ from those of other grains in their chemical structure, such as greater proportions of soluble pentosans, β -1-3 links and molecular weights (FEDNA, 2003). This results in a greater tendency to form solutions of high viscosity in concentrated flour-water slurries, which leads to stirring and pumping problems during mashing and fermentation (Wang *et al.*, 1997, 1998).

There are very few studies where the viability of rye as a substrate for ethanol production has been tested. Four trials performed at the University of Saskatchewan (Wang *et al.*, 1997, 1998; Sosulski *et al.*, 1997; Sosulski and Sosulski, 1994) studied the parameters of fermentation of several cereal grains and published values for efficiency of fermentation and ethanol yields of rye and triticale similar to those obtained for wheat, and superior to those obtained for barley. The protein concentration of rye DGS is higher than that of barley DGS (29.3 vs 24.6 percent) but slightly lower than those obtained from triticale and maize DGS (Table 3). Rye DGS has the advantage of being very uniform. The value reported for NDF (Table 3) is derived exclusively from the work of Mustafa *et al.* (2000), where they analysed the composition of wheat, rye, triticale and barley DGS. The NDF concentration from triticale, wheat and barley DGS observed in this experiment were more than 20 percentage points lower than for rye DGS.

Maize distillers grain

Ethanol co-products commonly fed to dairy cattle include dried distillers grain with solubles (DDGS), wet distillers grain with solubles (WDGS), modified wet distillers grain with solubles (MWDGS), and condensed distillers grain solubles (CDS). When formulating diets for dairy cattle, accurate chemical composition analysis of ethanol co-products is critical. Laboratory testing of purchased DGS is highly recommended because nutrient profiles of DGS can vary considerably between and within ethanol plants.

Chemical composition of maize ethanol co-products can be influenced by factors such as grain quality, milling process, fermentation process, drying temperature and amount of solubles blended back into wet DGS before drying. The chemical composition of DDGS and CDS varies considerably (Table 4). Therefore, depending on the ratio of distillers grain to CDS in the final product, the nutrient profiles

of DDGS, WDGS and MWDGS can also vary considerably (Cao, Anderson and Kalscheur, 2009). In addition, ethanol has been produced from many types of grains (maize, barley, wheat, triticale and sorghum) and this can significantly alter the nutrient profile of the DGS produced, reflecting the nutrient profile of the original feedstock.

Currently, the DGS commonly fed has a greater protein concentration than what was reported 20 years ago (NRC, 1989). The latest edition (7th) of the Nutrient Requirements of Dairy Cattle (NRC, 2001) lists crude protein (CP) at 29.7 percent for maize DDGS, a number similar to values reported by commercial laboratories. According to data reported by Dairy One Forage Labs (Table 4), the average CP for DDGS is around 31 percent, but ranges from 27 to 35 percent.

Of particular interest to dairy nutritionists is that DDGS is a good source of rumen-undegraded protein (RUP). Rumen-undegraded protein values can vary depending on the method used to evaluate degradability, which needs to be considered when comparing RUP values of various feed sources. *In situ* reported RUP values for distillers grain ranged from 40 percent to 67 percent (Kleinschmit *et al.* 2007a; Cao, Anderson and Kalscheur, 2009; Mjoun *et al.*, 2010b). In these trials, DDGS had greater RUP than did WDGS (62.0 vs 46.9 percent), and RUP decreased as solubles inclusion in the final product increased (Cao, Anderson and Kalscheur, 2009). Kleinschmit *et al.* (2007a) evaluated five different sources of DDGS and found that RUP varied from 59.1 to 71.7 percent. Mjoun *et al.* (2010b) evaluated 3 types of DDGS and found RUP varied from 52.3 to 60.4 percent. Both studies (Kleinschmit *et al.* 2007a; Mjoun *et al.* 2010b) included DDGS and WDGS samples and both confirmed that WDGS had greater protein degradability. Some of the rumen degradable protein (RDP) in maize is altered in the fermentation process to produce ethanol; therefore the protein remaining in DDGS is expected to have greater RUP than the original maize. The lower RUP values observed for WDGS were probably due to the absence of drying and possibly greater quantities of solubles returned to the WDGS compared with DDGS.

Protein quality in DDGS can be good, although as with most maize products, lysine is the first limiting amino acid for milk production under many dietary situations. Very high RUP (e.g. >80 percent of CP) in DDGS usually results from heat damaged, indigestible protein. Heat damaged protein may be indicated by a high acid-detergent insoluble CP value, although in DDGS there is no clear relationship between acid-detergent insoluble CP and protein digestibility as in some other feeds. This is more than likely due to the fact that the Maillard reaction is a function not only of temperature and moisture, but also length of time during which the feed is exposed to high temperatures. Extensive heating creates darker DDGS and is believed to decrease

TABLE 4

Composition of dried distillers grain with solubles (DDGS), wet distillers grain with solubles (WDGS), modified wet distillers grain with solubles (MWDGS) and condensed distillers solubles (CDS)

	DDGS (1989) ⁽¹⁾	DDGS (2001) ⁽²⁾	DDGS ⁽³⁾	WDGS ⁽⁴⁾	MWDGS ⁽⁵⁾	CDS ⁽⁶⁾
Nutrients (% of DM)						
DM (% as is)	92	90.2	88.1 ± 6.18	33.4 ± 12.98	48.3	31.9
CP	25	29.7	31.2 ± 4.3	30.1 ± 9.4	28.2	20.2
SP (% of CP)	—	—	16.7 ± 7.1	22.4 ± 14.6	16.1	63.8
ADICP	—	5.0	4.4 ± 2.1	3.7 ± 2.1	1.3	0.6
NDICP	—	8.6	9.5 ± 2.9	8.3 ± 3.6	1.9	1.8
NDF	44	38.8	34.0 ± 4.7	31.2 ± 8.9	24.4	4.0
ADF	18	19.7	16.8 ± 3.5	15.4 ± 5.2	8.6	1.9
Lignin	4	4.3	5.1 ± 1.7	4.8 ± 1.6	5.3	0.4
Starch	—	—	5.3 ± 4.1	5.5 ± 8.5	7.3	5.3
Crude fat	10.3	10.0	12.6 ± 3.2	12.7 ± 3.8	12.0	17.9
Ash	4.8	5.2	5.9 ± 1.1	5.5 ± 1.6	5.9	9.6
Ca	0.15	0.22	0.08 ± 0.19	0.08 ± 0.17	0.06	0.10
P	0.71	0.83	0.88 ± 0.17	0.85 ± 0.18	0.88	1.55
Mg	0.18	0.33	0.32 ± 0.07	0.32 ± 0.09	0.41	0.68
K	0.44	1.10	1.05 ± 0.26	0.99 ± 0.30	1.25	2.23
Na	0.57	0.30	0.19 ± 0.20	0.17 ± 0.13	0.36	0.36
S	0.33	0.44	0.64 ± 0.18	0.58 ± 0.15	0.79	1.07
TDN	88	79.5	83.0 ± 5.0	84.8 ± 5.1	—	101.9
Energy parameters (Mcal/kg)						
NEL	2.04	1.97	2.06	2.10	—	2.58
NEM	2.18	2.07	2.17	2.22	—	2.78
NEG	1.50	1.41	1.49	1.53	—	1.99

Notes: Nutrients: DM = dry matter; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; CP = crude protein; SP = soluble protein; ADICP = acid-detergent-insoluble CP; NDICP = neutral-detergent-insoluble CP; TDN = total digestible nutrient. Energy parameters: NEM = net energy for maintenance; NEG = net energy for gain; and NEL = net energy for lactation. Data are reported as mean ± the standard deviation.

Sources: (1) NRC, 1989. (2) NRC, 2001. (3) Analysed by Dairy One Forage Lab (<http://www.dairyone.com>) from May 2000 to April 2011. Number of samples from 2501 to 6702 depending on nutrient analysed. (4) Analysed by Dairy One Forage Lab (<http://www.dairyone.com>) from May 2000 to April 2011. Number of samples of WDGS from 1035 to 2206 depending on nutrient analysed. (5) MWGS analysis is from two samples evaluated at South Dakota State University. (6) Analysed by Dairy One Forage Lab (<http://www.dairyone.com>) from May 2000 to April 2011. Number of samples of CDS from 103 to 757 depending on nutrient analysed.

the concentration of digestible lysine as this amino acid is very sensitive to high temperatures (Boucher *et al.*, 2009). It should be noted that the type of grain and the amount of solubles added back to distillers grain can also create darker products without necessarily reducing amino acid availability. Recently, Mjoun *et al.* (2010b) evaluated the intestinal digestibility of protein of four distillers grain products (conventional DDGS, reduced-fat DDGS, high-protein DDG and MWDGS) and found that, while these products were slightly less digestible than soybean products (92.4 and 97.7 percent, respectively), their digestibility values were greater than the 80 percent RUP digestibility used in feed formulation models such as NRC (2001). Intestinal digestibility of the essential amino acids exceeded 92 percent across all feedstuffs, with the exception of lysine, where distillers grain were less (84.6 percent) compared with soybean feedstuffs (97.3 percent) (Mjoun *et al.*, 2010b).

Neutral-detergent fibre (NDF) concentrations in maize DDGS are often between 30 and 40 percent of DM, but can vary considerably between individual ethanol plants. Some newer DDGS samples have been reported to have concentrations of NDF considerably lower than NRC val-

ues (NRC, 2001; Robinson, Karges and Gibson, 2008). Although DDGS contains a considerable amount of NDF, this fibre should not be considered a source of physically-effective fibre in diets. Because the maize is ground prior to fermentation to produce ethanol, the resulting DDGS has very small particle size (Kleinschmit *et al.*, 2007a). Replacing forage fibre with non-forage fibre provided by DDGS can create unfavourable fermentation in the rumen and potentially result in milk fat depression (Cyriac *et al.*, 2005). While fibre provided by DDGS is a good source of energy, it should not replace forage fibre in diets of high producing dairy cows.

Maximizing the fermentation of starch to ethanol is always the goal of ethanol production; however, there is usually some starch remaining in distillers grain. During the 1980s and 1990s, starch in DDGS was determined to be 10–15 percent (Belyea *et al.*, 1989; Batajoo and Shaver, 1998). Most samples from newer fuel ethanol plants contained 4–6 percent starch, with some samples greater than 8 percent (Mjoun *et al.*, 2010b). Improved processes to ferment starch to ethanol is most likely the reason for decreased starch concentrations in DDGS.

One concern of nutritionists is that the concentration of fat in distillers grain can vary greatly, and potentially exceed 12 percent, which is much greater than values reported in NRC (2001). The fat in DDGS is high in unsaturated fatty acids, predominantly linoleic acid (C18:2), reflecting the composition of maize oil (Elliot *et al.*, 1993). Dried or wet distillers grain that contain greater proportions of CDS result in greater concentrations of fat in the final product (Cao, Anderson and Kalscheur, 2009). Also, the method of analysis can significantly affect the crude fat value (Cao, Anderson and Kalscheur, 2009). A recent study that evaluated methods for crude fat analysis recommended the use of petroleum ether when analysing DDGS (Thiex, 2009).

High concentrations of unsaturated fatty acids are a concern when including DDGS in diets for lactating dairy cows because the presence of unsaturated fatty acids can increase incomplete biohydrogenation in the rumen, which has been related to observed milk fat depression. However, if diets are formulated to provide sufficient amounts of physically-effective fibre, increasing the concentration of polyunsaturated fatty acids will not necessarily result in milk fat depression (Ranathunga *et al.*, 2010).

Environmental concerns regarding excessive phosphorus (P) has increased the awareness of phosphorus concentrations in DDGS. Most DDGS contain between 0.65 and 0.95 percent P and this value increases with the amount of CDS added to the distillers grain with no solubles (Table 4). Even though DDGS protein is relatively undegraded in the rumen, phosphorus has been shown to be highly available (Mjoun *et al.*, 2008). Fortunately, high producing dairy cows often need some supplemental P, therefore inclusion of DDGS can replace more expensive inorganic sources. The greatest concern of feeding DDGS will be in regions of the United States where soils are already high in P. In order to minimize excess P in manure, diets should be formulated close to the animal's requirement (NRC, 2001). The other mineral that can be highly variable is sulphur (S). Although

an average S concentration in DDGS is about 0.64 percent (Table 4), it has exceeded 1.0 percent in some samples. Distillers grain products with greater concentrations of CDS often contain greater S concentrations (Cao, Anderson and Kalscheur, 2009). Though rarely reported in dairy cattle, excessive S concentrations in feed and water can result in central nervous system disorders, which can lead to poor performance or death.

Distillers grain available today usually contain more energy than indicated by the NRC reference values. Birkelo, Brouk and Schingoethe (2004) determined the energy value of WDG for lactating cows. In this study, digestible energy, metabolizable energy and NEL of WDG were 4.09, 3.36, and 2.27 Mcal/kg, respectively, which were 7 to 11 percent, and 10 to 15 percent higher than previously published values reported in NRC (1989) and NRC (2001) (Table 4). These higher energy values are probably attributable to increased fat concentration, as well as greater digestible fibre measured in DGS products than assumed by NRC (2001).

Amino acid composition of distillers grain from different grains

Tables 5 and 6 show the essential amino acid (EAA) composition of cereal grains and distillers grain obtained from them as a percent of the CP (values for rye distillers grain could not be found at the time of writing). The extent of heating during drying affects the availability of the amino acids in the co-products. Lysine is particularly affected because of the greater exposure and susceptibility to the Maillard reaction of the epsilon amino group of this amino acid. These effects were corroborated experimentally with greater total amino acid concentration (particularly lysine) in wet compared with dried distillers grain derived from both barley (Weis *et al.*, 1989) and maize (Kleinschmit *et al.*, 2007a).

The amino acid composition of milk protein can be used as an indicator of the ideal dietary amino acid balance for the

TABLE 5
Amino acid composition (% of CP) of different cereal grains

	TMP	Maize	Sorghum	Wheat	Barley	Triticale	Rye
Arginine	3.6	4.7	4.0	5.1	4.8	5.4	5.0
Histidine	2.7	2.9	2.2	2.3	2.2	2.4	2.1
Isoleucine	5.9	3.7	4.2	3.6	3.6	3.8	3.4
Leucine	9.7	12.5	13.6	6.8	6.8	6.4	6.0
Lysine	8.1	3.0	2.3	2.9	3.8	4.0	3.9
Methionine	2.6	2.1	1.6	1.6	1.7	1.8	1.6
Phenylalanine	4.9	4.9	5.3	4.7	4.9	4.3	4.4
Threonine	4.6	3.7	3.3	3.1	3.4	3.4	3.4
Valine	6.6	5.0	5.4	4.4	5.1	4.9	4.8
Total EAA	48.7	42.5	41.9	34.5	36.3	42.3	34.6
MPS		0.37	0.28	0.36	0.47	0.49	0.48

Notes and sources: Unless otherwise indicated, data are adapted from INRA, 2004. TMP = Total milk protein. Adapted from Jacobson, Van Horn and Sniffen, 1970. Total EAA = Total essential amino acids. MPS = Milk protein score (concentration of first AA in protein supplement / AA concentration in milk protein) from Schingoethe, 1996.

TABLE 6
Amino acid composition of dried distillers grain with solubles (% of CP) derived from different cereal grains

	TMP	Maize DDGS	Sorghum DDGS	Wheat DDGS	Barley DDGS	Triticale DDGS
Arginine	3.6	4.1	3.6	3.7	5.2	4.3
Histidine	2.7	2.6	2.3	1.9	0.9	2.6
Isoleucine	5.9	3.4	4.4	2.4	2.4	3.5
Leucine	9.7	8.6	13.6	5.9	6.0	8.8
Lysine	8.1	1.9	2.2	2.0	1.1	2.1
Methionine	2.6	1.7	1.7	1.8	0.8	1.8
Phenylalanine	4.9	4.6	5.5	4.3	3.3	4.6
Threonine	4.6	3.6	3.5	2.7	2.8	3.5
Valine	6.6	4.5	5.4	3.2	3.2	4.5
Total EAA	48.7	34.9	42.3	27.9	25.8	35.5
MPS	—	0.23	0.27	0.25	0.14	0.26

Notes and sources: (1) TMP = Total milk protein. Adapted from Jacobson, Van Horn and Sniffen, 1970. (2) Maize dried distillers grain with solubles data adapted from Greter *et al.*, 2008. (3) Sorghum dried distillers drains with solubles data adapted from Urriola *et al.*, 2009. (4) Wheat dried distillers grain with solubles data adapted from Boila and Ingalls, 1994. (5) Barley dried distillers grain with solubles data adapted from Weiss *et al.*, 1989, based on a mix 65% barley and 35% maize. (6) Triticale dried distillers grain with solubles data adapted from Greter *et al.*, 2008. (7) Total EAA = Total essential amino acids. (8) MPS = Milk protein score (concentration of first AA in protein supplement / AA concentration in milk protein) from Schingoethe, 1996.

high-producing cow. Following this thought, Schingoethe (1996) suggested the use of the milk protein score (MPS) as a good indicator of protein quality for high-producing cows. The MPS is calculated as the amino acid content of the most limiting amino acid in a protein supplement relative to that amino acid in milk. When calculating the MPS, both in the original grain and in the DDGS, the first limiting EAA is lysine. The second limiting amino acid with regards to milk protein both in cereal grain and their co-products is isoleucine. The exception is barley DGS, where methionine is second in MPS values. Similar to the total EAA value, the MPS value for the DDGS derived from cereal grains is lower than the MPS of the original grains. The greatest decrease in this index is observed for barley, which goes from being one of the cereal grains with the greatest MPS value (0.47; lysine = 3.8 percent of CP) to a barley DGS with very low MPS (0.14; lysine = 1.1 percent CP).

Sorghum DDGS has a greater concentration of total EEA (Table 6) than maize and triticale DDGS, which in turn have more than wheat and barley DDGS. However, with the possible exception of barley DDGS, the MPS values of all DDGS evaluated are similar, due to the similar lysine concentration (approximately 2 percent). These results suggest that sorghum DDGS has a more desirable EEA profile and MPS score, whereas barley DDGS would be the poorest for both parameters.

DEGRADABILITY OF DISTILLERS GRAIN FROM DIFFERENT CEREAL GRAINS

Tables 7 and 8 show there is very little relationship between protein degradability in the cereal grain of origin and the resulting DGS (sorghum DGS data not available at the time of writing). The effective protein degradability of the majority of DGS is lower than that of cereal grains, decreasing by 17.8, 18.4, 31.5 and 26.7 percentage points in wheat,

barley, triticale and rye DGS, respectively. One exception is maize, in which the effective protein degradability of the DGS increased by 5 percentage units (reaching 48 percent) compared with the kernels. Similar results were observed for the speed of degradation of the protein, which decreased in all DGS compared with the grain. In addition it can be observed that triticale DGS had less degradable protein (47.5 percent) and the lowest degradation rate (3.6 percent/hour).

Wet distillers grain with solubles or modified wet distillers grain with solubles

Wet distillers grain with solubles (WDGS) is sold for feeding without drying. Traditional wet distillers grain contains 30 to 35 percent DM (Table 4) and is similar in nutrient composition to DDGS. These wet co-products are often lower in price on a DM basis compared with DDGS, but the producer must determine if WDGS can be successfully used in their operation. There are benefits from using WDGS, particularly because of the high palatability, and because of how it can condition diets that are particularly dry. Total mixed rations that contain 10–20 percent WDGS on a DM basis maintain greater homogeneity as dry particles stick together. From a practical standpoint, this results in less particle separation and less sorting by livestock. Producers face two primary challenges: methods to conserve WDGS; and equipment to handle WDGS.

Modified wet distillers grain with solubles (MWDGS) is distillers grain that have either undergone partial drying or have been completely dried to DDGS and had CDS added back to achieve a higher moisture product. MWDGS DM is typically between 45 and 55 percent. Nutrient composition is typically similar to that reported for WDGS and DDGS (Table 4), but can vary depending on processing factors, especially the amount of solubles added back to the wet grain to make the final product. Nutrient composition of

TABLE 7
In situ ruminal protein kinetic parameters and effective degradability of different cereal grains

	Maize	Sorghum	Wheat	Barley	Triticale	Rye
a ⁽¹⁾	11.0	5.0	27.0	29.0	34.0	27.0
b ⁽²⁾	82.0	73.0	67.0	65.0	56.0	69.0
c ⁽³⁾	4.0	5.5	16.0	11.0	23.0	16.0
ED ⁽⁴⁾	43.0	39.0	76.0	71.0	79.0	77.0

Notes and sources: Adapted from INRA, 2004. The kinetics parameters were estimated according to the equation $P = a + b(1 - e^{-ct})$ from Ørskov and McDonald, 1979. (1) a = soluble fraction (%). (2) b = potentially degradable fraction (%). (3) c = rate of degradation (%/hour). (4) ED = Effective Degradability (%). The ED at assumed rates of passage $k = 0.06/h$ was calculated according to the equation $ED = a + bc/(k + c)$ from Ørskov and McDonald, 1979.

TABLE 8
In situ ruminal protein kinetic parameters and effective degradability of distillers grain products derived from different cereal grains

	Maize DDGS	Wheat DDGS	Barley DDGS	Triticale DDGS	Rye DDGS
a ⁽⁶⁾	18.4	27.2	17.3	17.4	14.6
b ⁽⁷⁾	75.2	66.5	68.5	80.3	78.6
c ⁽⁸⁾	3.9	5.6	6.4	3.6	5.0
ED	48.0	58.2	52.6	47.5	50.30

Notes and sources: The kinetics parameters were estimated according to the equation $P = a + b(1 - e^{-ct})$ from Ørskov and McDonald, 1979. (1) Maize distillers grain data adapted from Mjoun *et al.*, 2010b. (2) Wheat distillers grain data adapted from Boila and Ingalls, 1994; Ojowi *et al.*, 1997; Mustafa, McKinnon and Christensen, 2000; and Mustafa *et al.*, 2000. (3) Barley distillers grain data adapted from Mustafa, McKinnon and Christensen, 2000; and Mustafa *et al.*, 2000. (4) Triticale distillers grain data adapted from Mustafa *et al.*, 2000. (5) Rye distillers grain data adapted from Mustafa *et al.*, 2000. (6) a = soluble fraction (%). (7) b = potentially degradable fraction (%). (8) c = rate of degradation (%/hour). (9) ED = Effective Degradability (%). The ED at assumed rates of passage $k = 0.06/h$ was calculated according to the equation $ED = a + bc/(k + c)$ from Ørskov and McDonald, 1979.

MWDGS can vary significantly from plant to plant and within plant; therefore, nutrient analysis is highly recommended prior to use in specific diets.

Condensed distillers solubles

Condensed distillers solubles (CDS) is also sometimes referred to as “syrup”. It has a similar DM content to that of WDG (27–35 percent). Compared with other distillers products, CDS is higher in fat (and consequently energy), lower in fermentable carbohydrates (such as fibre), but much higher in minerals (Table 4). Minerals such as phosphorus, potassium and sulphur are proportionally greater in CDS compared with the solids portion of the grain. Thus, as more CDS is added back to the grain, fat and minerals increase, but CP decreases in the final co-product. This syrup can be sold separately, but often most ethanol plants add it back to the distillers grain during WDG and/or DDGS processing. CDS can also be dried to create dried distillers solubles.

Reduced-fat distillers grain with solubles

There has been interest in removing fat from DDGS for use in biodiesel production or as a feed-grade fat source. One such strategy is solvent extraction of DDGS. The resulting co-product, reduced-fat DDGS, has a much lower crude fat concentration (Table 9), but slightly greater concentrations of the remaining nutrients compared with conventional DDGS. Mjoun *et al.* (2010b) reported that RUP was higher in reduced-fat distillers grain with solubles compared with traditional DDGS (60.4 vs 52.3 percent).

Recently, ethanol plants have been installing centrifuges to remove fat from wet DGS. This process removed approximately 2 to 3 percentage units of fat from the final distillers grain product. This type of distillers grain has not yet been evaluated in dairy cow feeding studies, but it may allow a slightly greater dietary inclusion compared with traditional DDGS.

High-protein distillers grain

Until recently, most co-products resulted from either traditional maize dry-grind ethanol plants or from the maize wet-milling industry. As new processes have been developed, new co-products from these ethanol plants have resulted. In one such example, maize is milled into several fractions prior to fermentation such that the resulting products can be directed into different processing streams (Gibson and Karges, 2006). This fractionation results in new end products, such as high-protein DDG, dehydrated maize germ and maize bran. Furthermore, syrup can be added to the bran, resulting in a product being marketed as bran cake (Gibson and Karges, 2006). Examples of these feeds are shown in Table 9. These products are proprietary and therefore specific to individual companies. As a result, the nutrient composition of these streams may vary considerably and will be quite different from that of traditional DDGS.

High-protein DDG (HPDDG) is an example of a pre-fermentation fractionated DDG product. As a result of the fractionation process, HPDDG is higher in CP and lower in fibre compared with traditional DDGS (Table 9). The germ

TABLE 9
Chemical composition of feed products from fractionation technologies for production of ethanol and co-products

	RFDDGS	HPDDG	Product	Germ	Bran
Nutrients (% of DM)					
DM (% as is)	86.9	92.1 ± 1.35		94.1 ± 1.25	90.3
CP	34.3	43.4 ± 2.2		16.1 ± 1.0	15.3
SP (% of CP)	10.9	7.63 ± 2.67		53.4 ± 1.5	-
ADICP	4.5	2.75 ± 0.95		0.33 ± 0.05	0.30
NDF	43.8	26.5 ± 2.6		26.2 ± 3.2	21.4
ADF	12.7	12.5 ± 4.4		9.26 ± 3.63	7.36
Lignin	-	2.99 ± 1.55		2.23 ± 0.83	2.63
Starch	4.7	9.60 ± 1.61		23.8 ± 2.48	-
Crude fat	3.5	4.00 ± 0.77		19.0 ± 1.1	9.49
Ash	5.2	2.13 ± 0.28		5.90 ± 0.24	3.84
Ca	0.12	0.02 ± 0.01		0.02 ± 0.01	-
P	0.81	0.44 ± 0.05		1.21 ± 0.10	-
Mg	0.36	0.12 ± 0.02		0.50 ± 0.02	-
K	0.98	0.42 ± 0.06		1.49 ± 0.06	-
Na	-	0.13 ± 0.04		0.01 ± 0.001	-
S	0.78	0.80 ± 0.05		0.17 ± 0.01	-
Energy parameters (Mcal/kg)					
NEL	1.58	1.98		2.27	1.89

Notes and sources: Data are reported as means plus or minus the standard deviation. DM = dry matter; CP = crude protein; SP = soluble protein; ADICP = acid-detergent-insoluble CP; NDF = neutral-detergent fibre; ADF = acid-detergent fibre. (1) RFDDGS = reduced-fat dried distillers grain. Compilation of values reported by Mjoun *et al.*, 2010b, 2010c. (2) HPDDG = high-protein dried distillers grain. Dakota Gold HP Dried distillers Grain. Poet Nutrition, Sioux Falls, SD. Compilation of values reported by Robinson, Karges and Gibson, 2008; Abdelqader *et al.*, 2009b; Kelzer *et al.*, 2009; Mjoun *et al.*, 2010b; Tedeschi *et al.*, 2009; and Christen *et al.*, 2010. (3) Germ = Dakota Germ Maize Germ Dehydrated, Poet Nutrition, Sioux Falls, SD. Compilation of values reported by Robinson, Karges and Gibson, 2008; Abdelqader *et al.*, 2009a; Abdelqader *et al.*, 2009b; Kelzer *et al.*, 2009; and Tedeschi *et al.*, 2009. (4) Bran = Dakota Bran, Poet Nutrition, Sioux Falls, SD. Compilation of values from Janicek *et al.*, 2007; Tedeschi *et al.*, 2009; and Poet Nutrition, pers. comm. (5) NEL = Net energy for lactation, calculated from NRC, 2001, at 3× maintenance.

has been removed prior to grinding for ethanol production, so the HPDDG is much lower in fat and minerals. In addition, CDS is not added back to this product, making it a DDG rather than a DDGS.

Maize germ

Maize germ can be produced from traditional wet-milling practices or, more recently, by dry-milling fractionation processes. For wet milling, after the kernel is steeped, the germ and fibre fractions are removed by differences in density and particle size, respectively (Rausch and Belyea, 2006). However, for dry milling, the germ is not subjected to the steeping process and therefore retains more soluble protein, phosphorus, starch and fat from the kernel.

Maize germ from dry milling contains about 26 percent NDF and 24 percent starch on a DM basis, making it a highly fermentable feedstuff (Table 9). Tedeschi *et al.* (2009) found that maize germ has the fastest rate of fermentation compared with bran or DDGS. In addition, Abdelqader *et al.* (2009a) demonstrated greater DM degradation rates for maize germ compared with two different types of DDGS and soybean meal. When feeding dairy cows increasing amounts of maize germ, predicted NEL energy content of germ was calculated to be 2.39 Mcal/kg compared with an NRC (2001) prediction of 2.27 Mcal/kg (Abdelqader *et al.*, 2009c). The authors hypothesized that the greater energy prediction in the feeding study compared with the NRC

estimations was due to greater digestibility of the fibre fraction compared with values predicted by NRC (2001).

Dry maize grain contains most of the fat in the embryo or germ portion (Moreau, Johnston and Hicks, 2005). Therefore, maize germ will have greater fat concentrations compared with DDGS. Dry-milling maize germ is typically 17–20 percent fat compared with wet-milling maize germ, which is 40–50 percent fat (Rausch and Belyea, 2006). Small portions of the pericarp and endosperm remain attached to the germ in dry milling resulting in lower fat concentration. The amount of fat in the germ is 5 to 7 times greater than in maize grain and about double the fat of maize DDGS. The major fatty acids in maize germ are similar to other maize co-products (Abdelqader *et al.*, 2009b, c).

Because there is no steeping in the dry-milling process, soluble proteins are not lost. As a result, the maize germ is considerably higher in soluble protein (Table 9) compared with wet-milling maize germ. Using *in situ* methods, Abdelqader *et al.* (2009a) determined the RDP fraction of maize germ to be 71.8 percent compared with RDP of 44–48 percent for DDGS.

Maize bran

Maize bran is a co-product of the fractionation technology described above, and is currently produced by adding maize CDS to the bran fraction of the kernel. Most of the

fat and protein fractions are contributed by CDS whereas most of the fibre comes from the maize grain pericarp. Its high content of fibrous carbohydrates and very little starch makes maize bran a good fit for ruminant diets. The chemical composition of maize bran is presented in Table 9.

In vitro disappearance of the NDF fraction is approximately 87 percent (DeHaan, 1983). This suggests that in spite of its high fibre content, the energy supplied by this carbohydrate fraction is high. One of the advantages of high fibre supplements such as maize bran is that, although highly digestible, their pattern of rumen fermentation shifts towards more acetate rather than lactate, and as a result does not acidify rumen contents as much, and is less conducive to negative associative effects. Lignin in maize bran has a range of values from 1.60 to 3.66 percent of DM (Tedeschi *et al.*, 2009) which might suggest significant variation in this energy content. Tedeschi *et al.* (2009) suggested that the most influential variables that affect the rate of degradation of NDF also affect the predicted TDN values.

The relatively low protein concentration of maize bran has an advantage for nutritionists as the overall protein amino acid balance can be improved through the inclusion of other feeds with higher lysine concentration. Protein in maize bran has lower lysine concentrations than many other feeds, which needs to be taken into account at higher inclusion levels and when milk production amounts require limiting amino acids to be considered.

FEEDING DGS TO DAIRY CALVES

Distillers grain have not been widely examined as a feed-stuff for pre- and post-weaned dairy calves. Traditional concentrates contain easily digestible carbohydrates that promote rumen development in contrast to the low soluble carbohydrate and high fibre content in DDGS. Distillers grain with a protein concentration of 28–32 percent provides a good replacement for the traditional maize+soybean meal combination in calf starters. One concern may be the lower protein quality of DDGS compared with soybean meal when included in starter diets. Abe, Iriki and Funaba (1997) demonstrated that lysine is the first limiting amino acid in calves fed maize and maize gluten meal diets in the first 3 months. Because maize DDGS has a similar amino acid profile to other maize products, low lysine concentrations may be a concern. In calves less than 11 weeks old, soybean-based diets were first limiting in methionine, followed by lysine (Abe *et al.*, 1998). Consequently, DDGS and soybean meal may provide an ideal protein combination for young calves.

There is limited research investigating the use of DDGS in young calves. Thomas *et al.* (2006a, b) evaluated the inclusion DDGS at 0, 28 or 56 percent of the diet DM in starter diets of young dairy calves. Calves were weaned at 6 weeks and fed starter feeds for ad libitum consumption until 12 weeks old. Although calves fed starter containing

56 percent DDGS had greater dry matter intake (DMI) after weaning when compared with the 0 or 28 percent DDGS, body weight changes did not differ throughout the experiment (Thomas *et al.*, 2006a). Feed efficiencies of calves fed the 56 percent DDGS diet decreased slightly compared with calves fed 0 percent DDGS. The decreased feed efficiency may have been attributable to decreased concentrations of lysine in the starter as first limiting amino acid for support of lean body mass accretion. Development of the digestive tract and the rumen, specifically, was not different when comparing empty organ weights (Thomas *et al.*, 2006b). Calves fed DDGS, however, exhibited shorter, wider and denser rumen papillae, with less total surface area, indicating a shift in ruminal volatile fatty (VFA) patterns for calves fed DDGS. Though differences in pH were not observed, short papillae and mucosal proliferations are adaptive changes to low pH (Zitnan *et al.*, 2005). Darker colours of the papillae, greater papillae density and shorter papillae length in calves fed 56 percent DDGS may be indicative of parakeratosis, though the health status of calves did not differ between treatments.

Recently, Suarez-Mena *et al.* (2011) conducted a series of studies to determine the effect of DDGS in calf diets. When DDGS was included at higher levels (39–49 percent of the diet), average daily gain (ADG) was reduced by 6–10 percent and DM digestibility also fell. In a separate study, starter diets containing up to 20 percent DDGS had no effect on ADG and feed efficiency in calves less than 2 months old. It was also demonstrated that inclusion of DDGS at 20 percent of the diet DM had no effect on rumen development in 35-day old calves compared with the control diet. However, in calves 2 to 3 months old, the inclusion of DDGS at 20 percent of the diet decreased ADG by 4 percent. The authors concluded that DDGS could be included up to 20 percent of the starter diet in calves less than 2 months old, but less than 20 percent of the diet for calves 2 to 3 months old.

Overall, DDGS provides an excellent protein supplement in starter feeds for dairy calves. Feeding greater than 25–30 percent of DM as DDGS should be approached with caution. Data on feeding DDGS at greater concentrations is limited and there are indications of parakeratosis at higher inclusion levels. In addition, the amino acid profile of DDGS may well serve to balance the low methionine content typical of soybean meal-based supplement while decreasing cost of gain.

FEEDING DGS TO DAIRY HEIFERS

Zanton and Heinrichs (2005), based on a meta-analysis of heifer research, suggest body weight (BW) gains for large-breed dairy heifers should be around 0.8 kg per day. Excessive or restrictive weight gains caused by unbalanced rations may negatively affect the development of mammary

tissue and may compromise milk production. Since DGS has relatively high concentrations of protein and energy it can be a challenge to incorporate them at high inclusion rates in rations for growing heifers and maintain the recommended rate of gain. In order to accomplish this rate of gain with the inclusion of DGS, lower quality forages can be utilized to balance the diet. In this feeding scheme, DGS products complement high-fibre forages because of the high concentration in energy and protein in DGS products. Maintaining homogeneous mixes between dry forages and other dry feedstuffs is often challenging as smaller particles tend to separate and settle towards the bottom of the mixed ration. This leads to uneven intake of nutrients by growing heifers with resultant differences in growth. Instead of DDGS, inclusion of WDGS, due to its stickiness, reduces this problem and results in more uniform ration consumption (Klopfenstein, Erickson and Bremer, 2008). As previously mentioned, WDGS provide more protein, fat and P than is required by growing dairy heifers. Matching it with low quality, high-fibre feeds such as crop residues is a good low-cost feeding strategy that, when blended appropriately, meets the heifer's recommended nutritional requirements. Maize stalks or small grain straws are excellent alternatives to high protein- and high energy-containing forages such as maize silage and alfalfa hay. Also, ensiling crop residues with WDGS may improve nutrient digestibility of the crop residues and improve ease of feeding. For this reason, research at South Dakota State University with DGS in diets for growing dairy heifers has mostly used WDGS. Wet distillers grain has been evaluated in combination with other agricultural by-products such as soybean hulls (SH) and maize stalks (Anderson *et al.*, 2009, 2010).

Anderson *et al.* (2009) conducted a study to determine if the dietary energy supplied as fermentable fibre and fat from wet DGS with SH versus starch from maize grain would result in similar performance in growing heifers. WDGS was ensiled with SH at a ratio of 70 percent WDGS to 30 percent SH, on an as-fed basis, for 3.5 months in sealed silage bags before the start of the study. This blend (WDGS+SH) was used in heifer diets, replacing maize and soybean meal. Diets were: (1) control diet with 50 percent of the diet (dry basis) as grain mix, which was composed of maize, soybean meal and minerals; (2) low inclusion (24.4 percent) of the WDG+SH blend; and (3) high inclusion (48.7 percent) of the WDG+SH blend. The inclusion of the blend resulted in greater concentrations of NDF, ADF and ether extract (EE) and lesser concentrations of non-fibrous carbohydrates and starch in diets. Heifers were fed ad libitum. DMI decreased as the amount of the WDGS+SH blend increased in the diets, while average daily gain, which at 1.25 kg/day was much greater than recommended, did not differ among diets. Body frame measures such as wither and hip heights, body length and heart girth were also

similar across treatments. Studies reviewed by Klopfenstein, Erickson and Bremer (2008) also found improved feed conversion in growing beef cattle as WDGS increased in the diet. There is speculation that more fat and protein in the wet DGS bypass the rumen and are used to a greater extent in the small intestine. Conversely, maize and soybean particles are subject to greater degradation and fermentation in the rumen, resulting in a less efficient conversion of feed for growth (Klopfenstein, Erickson and Bremer, 2008). Anderson *et al.* (2009) concluded that a 70:30 (as fed) blend of WDGS and SH when fed in replacement of one-half or all of a traditional concentrate mix for growing dairy heifers maintained performance and improved feed efficiency.

In a second study, Anderson *et al.* (2010) evaluated the palatability and digestibility of WDGS ensiled with maize stalks. Mixes of 67 percent WDGS and 33 percent maize stalks (as-fed) were ensiled in silage bags and fed in the study. One bag was left untreated and the other was treated with a preservative. Treatment diets included: (1) Control with 30 percent (DM basis) as maize-soybean meal grain mix and 70 percent hay; (2) 99 percent untreated blend and 1 percent mineral mix; or (3) 99 percent treated blend and 1 percent mineral mix. No differences were detected for DM intakes or average daily gain (1.11 kg/day) of dairy heifers. Total tract digestibility was similar for the control and blend of WDGS and maize stalks treated with a silage preservative, but the untreated blend was less digestible compared with the other two treatments. Overall, the study demonstrated that when treated with silage preservative, ensiled WDGS mixed with maize stalks can be just as well-utilized as a traditional heifer ration. The study also demonstrated that, based on similar intakes to a control diet, WDGS ensiled with low quality forage is very palatable to heifers.

In a follow-up study, Anderson *et al.* (2011) evaluated the effect of dietary fat from DDGS in diets of growing heifers. It was hypothesized that feeding fat and fermentable fibre instead of starch as the energy source might affect heifer growth. Thirty-three Holstein heifers were used in a 24-week experiment, fed one of three diets: (1) control diet containing ground maize (15.9 percent of diet DM) and soybean products (17.9 percent); (2) low-fat diet containing low-fat, high-protein DDGS (21.9 percent) and ground maize (11.9 percent); and (3) high-fat diet using traditional DDGS (33.8 percent). All diets contained 33.8 percent concentrate mix, 39.8 percent grass hay, 24.8 percent maize silage, and 1.5 percent vitamins and minerals. Diets were formulated for 16.3 percent CP (DM basis) and balanced for 9.8 percent RDP and 6.5 percent RUP. The high-fat diet contained 4.8 percent fat compared with 2.8 percent in the control and low-fat diets, which were greater in non-fibrous carbohydrates. Diets were 1.0 Mcal/kg of DM and limit-fed at 2.45 percent of BW. Dry matter intakes, body weights,

and average daily gains (0.9 kg/day) were similar across all diets. Whereas heart girth was similar among treatments, hip height was less for heifers fed high-fat diet compared with those fed the control and low-fat diets. Wither height was greater for heifers fed the low-fat compared with high-fat diet, and tended to be greater compared with heifers fed the control diet. Body length was longest for heifers fed the control diet, shortest for heifers fed the high-fat diet, with heifers fed the low-fat diet in between. Feeding diets with additional fat from including DDGS compared with diets with low-fat DDGS or maize and soybean products to growing heifers may result in slightly greater body condition scores and slightly smaller body frame sizes.

Other groups have also conducted research on feeding distillers grain to growing heifers. Schroer *et al.* (2009) compared feeding a control diet that contained maize and soybean meal in the concentrate mix to diets with 20 percent DDGS, 20 percent de-oiled DDGS or 36 percent de-oiled DDGS to growing Holstein heifers. Average daily gain was similar among treatments (1.14 kg/day). Body growth measurements, DMI, and feed efficiency were also similar among treatments. The study demonstrated that high levels of deoiled DDGS as well as traditional DDGS can be included in growing heifer diets. Suarez-Mena, Lascano and Heinrichs (2011) fed four levels (0, 7, 14 and 21 percent) of DDGS in diets with high (75 percent) or low (50 percent) forage. They did not report growth parameters but focused on rumen and digestibility measures. Apparent digestibility was highest when DDGS was included at 14 percent of the ration. Ruminal ammonia and propionate increased, while acetate and protozoa numbers decreased with increasing DDGS inclusion levels.

In summary, distillers grain can be included in heifer diets under a variety of scenarios. Distillers grain can replace all or some of the maize and soybean meal from traditional heifer diets while maintaining growth performance. It can be combined as WDGS with low quality crop residues or forages that have complementary nutrient profiles, and be fed at high inclusion levels. However, it is recommended that because of the high energy and high palatability of distillers grain it should be limit-fed or diluted with high bulk-low energy feeds to prevent excessive body weight gains.

FEEDING DGS TO DRY COWS

Distillers grain has not been extensively evaluated as a feedstuff for dry dairy cows. Distillers grain is of lower cost relative to traditional protein and energy sources, allowing opportunities for including it into dry-cow diets. As described in the section on feeding distillers grain to dairy heifers, distillers grain nutritional profile is complementary to low energy, low protein forages commonly fed in dry-cow diets. The nutritional goals of diets for dry cows are to provide adequate energy for maintenance of body weight

and foetal growth, while avoiding overfeeding energy. The relatively high S content may be beneficial for balancing the dietary cation-anion difference in dry-cow diets.

The only research reported on the use of distillers grain in dry dairy cow diets was conducted by Mpapho *et al.* (2007a, b). This research investigated the use of WDGS at an inclusion rate of 15 percent of the diet DM. Cows were fed WDGS for 4 weeks prior to calving until 70 days in milk (DIM) replacing maize grain, soybean meal, and extruded and expeller soybean meal from the control diet. DM intake, both pre- and post-partum did not differ for cows fed WDGS compared with the control diet. During the subsequent lactation, feed efficiency and yields of milk, FCM and milk components were similar for the two diets. The percentage of protein in milk was, however, increased for cows fed WDGS. During the pre-partum period, concentrations of glucose, urea nitrogen, cholesterol, β -hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) in blood were not affected by treatments. However, post-partum cows fed WDGS had decreased concentrations of urea nitrogen, NEFA and BHBA, and tended to have greater concentration of glucose in blood than did cows fed the control diets. The authors concluded that feeding WDGS at 15 percent of the ration DM improved energy balance and resistance to ketosis and metabolic disorders post-partum as indicated by primary metabolic indicators in blood. Although research is limited, there is potential for the use of distillers grain in the diets of dry dairy cows.

FEEDING DGS TO LACTATING DAIRY COWS

More than 35 research trials with more than 140 treatment comparisons were conducted between 1982 and 2010 in which maize distillers grain, either wet or dried, was fed to lactating cows. Kalscheur (2005) conducted a meta-analysis of the data up to early 2005, which is similar to the recent meta-analysis of Hollmann, Allen and Beede (2011a) that summarized much, but not all, of the same data, but included only studies using maize DGS, and included some studies up to 2008. Other studies conducted since those summaries are also discussed, especially if results differ. The lactational response to feeding various amounts of DGS, as well as the response to feeding wet versus dried DGS, is covered below.

Amounts of DGS fed ranged from 4.2 percent of total diet DM (Broderick, Ricker and Driver, 1990) to 42 percent of DM (Van Horn *et al.*, 1985). Milk production was the same or higher for cows fed DGS compared with cows fed control diets in virtually all experiments, except when fed very large amounts (i.e. 30 percent or more of diet DM) as wet DGS (Kalscheur, 2005). A number of studies (Schingoethe *et al.*, 2009) and confirmed by the review of Hollmann, Allen and Beede (2011a) indicated that milk yield response was related to increasing concentrations of DGS in diets and peaked at approximately 21 percent DGS,

although Janicek *et al.* (2008) reported a linear increase in milk production when going from 0 percent to 30 percent DDGS in diets

Part of the additional production due to DGS may have been attributable to slightly more energy from a slightly higher fat content in DGS diets because fat contents of diets was not always balanced across diets in all experiments. However, in experiments such as by Pamp *et al.* (2006) that compared DGS to soybean protein as the protein supplement in isolipidic diets, production was similar or higher, even when DGS and soybean-based diets were formulated to be equal in RUP and fat. Birkelo, Brouk and Schingoethe (2004) indicated that new generation DGS contain more energy than older "book" values listed in the dairy NRC (2001).

Diet fermentability may be associated with responses to DGS. Hollmann, Allen and Beede (2011a) indicated that the greatest milk yield response to DGS was with 24 percent maize silage or 23 percent starch; concentrations greater than 47 percent maize silage or 32 percent starch resulted in negative milk yield responses. The recent report by Owens *et al.* (2009) supports this observation. In their study, when diets contained monensin – a compound known to slightly decrease milk fat percentages under some situations (Dubuc *et al.*, 2009) – feeding DGS in combination with high moisture maize decreased milk fat content and yield. Such decreases did not occur when the DGS was fed with dry maize or when high-protein dried distillers grain was fed with dry or high-moisture maize. Because all diets were balanced for fat content using a saturated fat source for the non-distillers diets, the milk fat depression with the high-moisture maize-DGS combination implies a possible interaction of increased ruminal starch fermentability with unsaturated fatty acids from the DGS, at least when in the presence of monensin. One must also be cognizant of the total fat in the diet, not just fat from DGS (NRC, 2001). Concentrations of maize silage and starch may need to be moderate to optimize lactational responses to DGS.

Milk production was higher when DGS products were fed than with the soybean meal-based control diet (Kleinschmit *et al.*, 2006). In that trial, two specially-processed DGS products intended to have higher quality were evaluated. Only small differences in response because of improved DGS quality were detected. The feeding of heat-damaged DGS can decrease production responses (Powers *et al.*, 1995); however, in general, the DGS available today is of better quality with less heat damage and other quality problems than the DGS used in some older research studies (University of Minnesota, Department of Animal Science, 2010).

Many research trials are of relatively short duration such as 3 or 4 week periods in Latin square designed experiments. Dairy producers are likely to be more concerned about long-term responses rather than shorter-term

research experiments that may not accurately reflect the response expected when feeding DGS continuously for long periods. Therefore, an experiment was conducted in which cows were fed wet DGS at 15 percent of diet DM for the entire lactation, during the dry period and into the second lactation. After the first year, there were no differences in production (31.7 and 33.6 kg/day for control and wet DGS diets, respectively), while fat percent (3.75 and 4.07), protein percent (3.29 and 3.41) and feed efficiency (1.30 and 1.57 kg FCM/kg DMI) were greater for cows fed wet DGS (Mpapho *et al.*, 2006). Reproductive efficiency and cow health were similar for both dietary groups; however, the response in feed intake and milk production tended to be more consistent when DGS was fed, possibly reflecting fewer digestive problems. Response during the dry period and first 70 days of the next lactation was similar for control and wet DGS fed cows (Mpapho *et al.*, 2007a).

Milk production responses to DGS are usually similar when fed with all forages (Kalscheur, 2005), although Kleinschmit *et al.* (2007b) observed slightly greater production when 15 percent dried DGS was fed in high alfalfa versus high maize silage diets. This probably reflected an improved amino acid status from the blend of alfalfa and DGS proteins versus a diet containing predominantly maize-based proteins. The summary by Hollmann, Allen and Beede (2011a) likewise showed a greater response to DGS with alfalfa-based than with maize silage-based diets. While there may be differences in protein quality of various sources of DGS (Kleinschmit *et al.*, 2007a), differences in yields of milk and milk protein are likely to be slight, unless a product is greatly heat-damaged.

Milk production is usually similar or higher when DGS replaces some of the starch in diets of dairy cattle. The starch content of diets is decreased from the typically 23 to 26 percent starch to sometimes less than 20 percent starch when fed DGS. Ranathunga *et al.* (2010) demonstrated that replacing incremental amounts of starch in diets from 29 percent starch in a diet that did not contain DGS to only 19.9 percent starch in a diet containing 21 percent dried DGS had no effect on milk production or composition but tended to improve feed efficiency. All diets contained 49 percent forage and were balanced for fat content (4.7 percent of DM) in that study such that the response measured was a response to DGS fibre versus maize starch.

Milk composition when feeding distillers grain with solubles

Milk composition is usually not affected by feeding DGS unless routinely recommended ration formulation guidelines are not followed, such as feeding sufficient amounts of functional (effective) fibre. Field reports of milk fat depression when diets contained more than 10 percent of ration DM as wet DGS are not supported by research

results. Research showed no decreases in milk fat concentration when diets contained wet or dried DGS at any level, even as high as 40 percent of DM intake (Schingoethe *et al.*, 2009). In fact, the milk fat concentration was usually numerically highest for diets containing DGS. Most of the research studies were conducted during early to mid-lactation, thus the milk fat data was typical for cows during these stages of lactation but may be lower than the average for the entire lactation. Studies that fed DGS throughout the lactation (Mpapho *et al.*, 2006), showed milk fat tests averaging 4.07 percent for both Holstein and Brown Swiss cows. Typical lower fat percentages occurred during times of greater milk production in early lactation, with higher fat tests in later lactation. Kleinschmit *et al.* (2006) and Pamp *et al.* (2006) observed fat percentages in Holstein cows of 3.54 to 3.60 percent during mid-lactation, whereas Kleinschmit *et al.* (2007b) observed an average of 3.72 percent fat during late-lactation. Partially replacing high-moisture maize with DGS increased milk fat concentration by 0.16 percentage units compared with that from dry maize (Hollmann, Allen and Beede, 2011a), and including monensin with the high-moisture maize may further aggravate the milk fat situation (Owens *et al.*, 2009). This may be due to increased fermentability of the diet and possibly partially due to the unsaturated fatty acids in the DGS.

Kalscheur's (2005) meta-analysis pointed out that milk fat content was lower only when cows were fed DGS in diets that contained less than 50 percent forage and 22 percent forage NDF. That result suggests why field observations of milk fat depression may have occurred. Because DGS contains an abundance of NDF, one may be tempted to decrease the amounts of forage fed when formulations indicate more than sufficient amounts of NDF. However, the small particle size of DGS means that its "effective fibre" – as measured by ability to stimulate chewing and/or rumination as well as measured by the ability to maintain milk fat (Grant, 1997) – is not as great as that of the forage fibre it replaced. Research by Leonardi, Bertics and Armentano (2005), Cyriac *et al.* (2005) and Hippen *et al.* (2010) supports observations from the meta-analysis by Kalscheur (2005). Cyriac *et al.* (2005) observed a linear decrease in milk fat concentration while milk production remained unchanged when cows were fed 0, 7, 14 and 21 percent of DM as dried DGS in place of maize silage, even though dietary NDF content remained unchanged at 32 percent of DM. The control diet contained 40 percent maize silage, 15 percent alfalfa hay and 45 percent concentrate mix. Thus, the key to maintaining good milk fat tests is to feed sufficient amounts of effective fibre. When diets contain insufficient forage fibre, and if the fermentability of the diet is high, some milk fat depression may occur.

The fatty acid content of milk fat when cows are fed DGS has been evaluated in a few studies. Because fat

in DGS, especially maize DGS, is quite unsaturated, with typically more than 60 percent linoleic acid, it is logical to expect a modest increase in concentrations of unsaturated fatty acids in milk as observed by Schingoethe, Brouk and Birkele (1999). Leonardi, Bertics and Armentano (2005) and Anderson *et al.* (2006) also reported modest increases in cis-9, trans-11 conjugated linoleic acid (CLA) and its precursor vaccenic acid (trans-11 C18:1) that are beneficial to humans for improved health status (Bauman *et al.*, 2006). Little change was observed though in fatty acids often associated with milk fat depression, such as trans-10, cis-12 CLA (Baumgard *et al.*, 2002).

Milk protein content is seldom affected by feeding DGS unless protein is limiting in the diet. Lower lysine concentrations in diets formulated with DGS may also cause a slight decrease in milk protein content (Nichols *et al.*, 1998; Kleinschmit *et al.*, 2007b). This effect may be more noticeable in diets that contain more than 30 percent DGS (Kalscheur, 2005), which reflects the high RUP and lysine limitation in DGS. In the meta-analysis of Hollmann, Allen and Beede (2011b), milk yield and milk true protein yield responses to added DGS were maximized when approximately 8.5 percent of the total dietary DM was non-maize-based CP. Milk yield response peaked for higher-producing cows (i.e. >30.0 kg/cow daily) at 4.3 percent dietary maize-based CP. This summary agrees with the meta-analysis of Kalscheur (2005), which indicated slightly higher milk protein percentages when blends of alfalfa and maize silage were fed with DGS than with either forage alone, but milk protein yields were the same for all forage combinations. Kleinschmit *et al.* (2007b) observed no differences in milk protein content or yield when feeding 15 percent dried DGS in diets where the forage varied from all alfalfa to all maize silage. However, amino acid balance was improved with the alfalfa diet indicating a more desirable blend of amino acids in the diet versus a high maize-based product diet with maize silage, DGS and maize, which was limiting in lysine. It may be logical to speculate that the energy in DGS may also stimulate milk protein synthesis by increasing EAA available to the mammary gland as the result of increased ruminal microbial protein synthesis; however, we are not aware of research testing this specific point.

One thing that cannot be evaluated by the meta-analyses cited above (Kalscheur, 2005; Hollmann, Allen and Beede (2011a, b) is the improvement in quality of the protein available in today's DGS versus DGS of only a few years ago. For instance, a recent survey of DDGS available from a large number of ethanol plants in the Midwest and elsewhere in the United States (University of Minnesota, Department of Animal Science, 2010) indicated higher concentrations of lysine (3.66 percent of CP) versus 2.24 percent of CP listed in the latest dairy recommendations (NRC, 2001). The higher lysine content in today's DGS may indicate

an overall improvement in the ethanol industry processing methods that minimize heat damage to DGS. This concept is supported by data of Kleinschmit *et al.* (2006, 2007a, b) that indicate that higher quality DGS products may contain more available lysine than do lower quality products.

Feeding distillers products probably does not affect milk flavour or processing parameters for the various dairy products. The authors are not aware of any research evaluating the effects of feeding DGS on milk quality; however, there is no reason to expect problems.

WET VERSUS DRIED DISTILLERS GRAIN WITH SOLUBLES

The response to wet or dried DGS is usually considered to be equal. However, few experiments actually compared wet versus dried DGS; most experiments simply compared DGS to a control diet. When Al-Suwaiegh *et al.* (2002) compared wet versus dried maize or sorghum DGS for lactating cows, they observed similar production for both wet and dried DGS but a tendency for more milk with maize versus sorghum DGS. Anderson *et al.* (2006) observed greater production when either wet or dried DGS were fed compared with the control diet (maize+soybean meal), a tendency for greater production with wet DGS instead of dried DGS, and a tendency for greater production with wet or dried DGS at 20 percent versus 10 percent of the ration DM.

The meta-analysis of Kalscheur (2005), which included 17 wet DGS treatment and 52 dried DGS treatment comparisons, showed absolutely no difference in milk fat content between wet DGS, dried DGS or control diets. In the two studies that directly compared wet versus dried DGS, milk fat percentages were not different (Al-Suwaiegh *et al.*, 2002), and actually higher (Anderson *et al.*, 2006) when fed wet versus dried DGS.

The main considerations regarding the use of wet versus dried DGS are handling and costs. Dried products can be stored for extended periods, can be shipped greater distances more economically and conveniently than wet DGS, and can be easily blended with other dietary ingredients. Feeding wet DGS avoids the costs of drying the product and will mix well directly into a total mixed ration (TMR). Wet DGS, though, will not remain fresh and palatable for extended periods; 5 to 7 days is the norm, possibly less in hot weather and a little longer in cooler weather. Some silage additives are claimed to extend the storage time of wet DGS (Schingoethe *et al.*, 2009).

Researchers at South Dakota State University and elsewhere have successfully stored wet DGS for more than six months in silo bags when the wet DGS was stored alone or blended with SH (Anderson *et al.*, 2009), with maize silage (Mjoun, Kalscheur and Garcia, 2011) or with beet pulp (Kalscheur *et al.*, 2004). Some field reports indicate successful preservation of wet DGS for more than a year in

silo bags. Storage of wet DGS will be discussed in greater detail later in the chapter.

FEEDING DIFFERENT CEREAL TYPES OF DISTILLERS GRAIN WITH SOLUBLES

There was no effect on milk production, DMI and rumen activity in eight research experiments with lactating dairy cows fed maize DGS substituted by other cereal DG. Weiss *et al.* (1989) compared the effect of partial or total substitution of soybean meal with barley DDGS in 60 mid-lactation cows. The authors did not find effects of the different protein meals on milk production, butterfat yield and DMI, but there was a trend towards a decrease in milk protein as DDGS increased in the diet. Digestibility coefficients of DM, NDF, ADF, lignin and CP of each diet were not affected by the protein meal in the diet.

Al-Suwaiegh *et al.* (2002) did not find significant differences in milk production, DMI, ruminal pH, rumen VFA and total ADF and NDF digestibility between early lactation diets that contained sorghum or maize DGS at an inclusion level of 15 percent of diet DM. Similar results were observed by Shelford and Tait (1986) with mid-lactation diets that included rye or maize DDGS at similar inclusion levels to Al-Suwaiegh *et al.* (2002).

When Greter *et al.* (2008) fed 21 percent of diet DM as triticale DDGS or maize DDGS as the sole protein supplement to mid-lactation cows, they observed that, although the plasma concentration of some EAAs and the milk urea nitrogen were higher in cows fed maize DDGS than those fed triticale DDGS, DM intake and milk yield were unaffected by DDGS type. These authors found significant interactions between parity and treatment for milk yield, milk fat concentration and 4 percent FCM. Multiparous cows fed triticale DDGS had greater milk fat concentration and FCM when compared with primiparous cows, but these differences were not found in cows fed maize DDGS. In another experiment (Oba *et al.*, 2010), diets evaluated triticale DDGS, maize DDGS, canola meal and soybean meal as the primary source of protein in lactating dairy cow diets. The type of DDGS (maize vs triticale) in the diets did not affect DMI, milk yield or composition, metabolites and plasma amino acids nor digestibility of DM, OM, CP, starch and NDF of the diet. Protein concentration in milk was less in cows fed either DDGS than in those supplemented with soybean meal. The diet with maize DDGS yielded less milk protein than the diet with canola meal. Plasma concentrations of arginine, lysine and threonine were greater in cows fed canola meal and soybean meal than those fed maize DDGS, however, the concentration in plasma leucine and phenylalanine was greater in cows fed maize DDGS. In general, the experiments suggest triticale DDGS can replace maize DDGS, canola meal and soybean meal in dairy cow diets without adverse effects on milk production.

Two recent experiments were conducted in Canada to evaluate the effect of a partial substitution of barley silage with wheat DDGS as a forage substitute. In the experiment by Zhang *et al.* (2010) three experimental diets were evaluated: (1) a control diet (50 percent barley silage + 50 percent concentrate; DM basis); (2) a diet where the barley silage was substituted with wheat DDGS at 20 percent of the diet DM; and (3) a diet where the barley silage was substituted with wheat DDGS and alfalfa hay (20 and 10 percent of the DM of the diet, respectively). Even though cows fed DDGS spent less time ruminating, had lower rumen pH and reduced acetate:propionate ratios than the cows fed the control diet, DMI, milk yield, milk protein and lactose were higher in cows fed DDGS. Milk fat concentration was higher for the control diet and lower for the diet that contained alfalfa hay; however, there were no differences in milk fat yield. Penner, Yu and Christensen (2009) found identical results in both production and rumen activity when comparing a control diet with a diet where they replaced 10 percent of the barley silage with a blend of wet maize and wheat DDGS. This study also tested two diets that included wheat or maize DDGS in substitution for 10 percent of the protein meal in the control diet. Partial substitution of the protein meal with DDGS had no effect on milk yield, components or rumen activity, and the type of DDGS – wheat or maize – had no effect on production parameters.

Urdl *et al.* (2006) also observed similar DMI, milk yield, and milk composition when maize or wheat DDGS replaced a blend of canola meal and soybean meal. Similar to Penner, Yu and Christensen (2009), they found no differences attributable to DDGS type.

Feeding distillers grain to grazing dairy cows

Investigation into the use of DDGS in grazing dairy cattle has been limited. Ideally, DDGS should be an excellent supplement to pasture grasses because of its RUP concentration and higher energy content. In a report by Shaver *et al.* (2009), DDGS was supplemented to dairy cows grazing ryegrass on Chilean dairy farms. On one farm, DDGS was fed at 2 kg/day in 5 kg of concentrate, replacing maize and soybean meal. Supplementation of DDGS varied by season of the year, but it tended to increase milk production by 1.8 to 1.9 kg/day across the year. Milk fat percentage decreased during the spring when grass quality was the highest. On 5 other farms where DDGS was mixed with maize silage to provide 2.5 kg/day and replaced a variety of concentrate feedstuffs, the inclusion of DDGS increased milk production 0.9 kg/day in the winter, but had no effect on milk production in the spring.

Distillers grain was also evaluated as part of a supplement for dairy cows grazing alfalfa pasture in a study by Nyoka, Hippen and Kalscheur (2007). Supplements were mixed with stored forages and concentrates to supply one-half of their daily requirements. The treatment sup-

plements were: (1) DDGS at 15 percent of estimated daily DMI; (2) DDGS replaced by soybean meal and extruded soybean; or (3) DDGS replaced by fishmeal and soy oil. Cows averaged 31.5 kg/day of milk and there were no differences because of supplement. Cows fed the fish meal and soy oil supplement produced milk with the highest milk fat concentrations and protein yields, with the DDGS-supplemented cows having the next greatest milk fat concentrations and protein yields. Results indicate that though protein quality of DDGS may limit production responses compared with fish meal when fed to grazing cattle, DDGS appear preferable as a protein supplement for grazing dairy cows compared with soybean meal.

Optimal inclusion amounts of distillers grain with solubles

The meta-analysis by Kalscheur (2005) indicated that milk production was maintained with increasing amounts of DGS in the diet and was actually numerically the highest when DDGS was fed as much as 30 percent of diet DM. This was further illustrated by the study of Janicek *et al.* (2008), which reported a linear increase in milk production when going from 0 percent to 30 percent DGS in diets. However, for inclusion of WDGS in dairy cow diets, the highest production was at 20 percent of diet DM (Hippen *et al.*, 2003; Kalscheur, 2005). Intake often declines when inclusion of WDGS is greater than 20 percent of the diet (Hippen *et al.*, 2003; Kalscheur, 2005).

Distillers grain is easily incorporated into diets at 10 percent of the diet DM and this is considered a safe inclusion rate. Numerous studies (Nichols *et al.*, 1998; Anderson *et al.*, 2006; Kleinschmit *et al.*, 2006) have demonstrated that dairy cows can easily consume 20 percent of their ration DM as distillers grain. With typical feed intakes of lactating cows, this is approximately 4.5 to 5.5 kg of dried DGS or 13.6 to 16.7 kg of wet DGS per cow daily (if WDGS is 33 percent DM). There have been no palatability problems and one can usually formulate nutritionally balanced diets with up to that proportion of DGS in the diet using most combinations of forages and concentrates. For instance, with diets containing 25 percent of the DM as maize silage, 25 percent as alfalfa hay and 50 percent as concentrate mix, the DGS can replace most – if not all – of the protein supplement, such as soybean meal, and a significant amount of the maize that would normally be in the concentrate mix. This was illustrated in the experiment by Anderson *et al.* (2006) in which feeding 20 percent of the diet DM as wet or dried DGS replaced 25 percent of the maize and 87 percent of the soybean meal that was fed in the control diet. This diet supported the highest milk production and feed efficiency of any of the diets evaluated in that study, while containing slightly more maize-based protein than Hollmann, Allen and

Beede (2011a) indicated as ideal. With diets that contain higher proportions of maize silage, even greater amounts of DDGS may be used; however, the need for some other protein supplement, protein quality (e.g. lysine limitation), total supplemented fat and phosphorus concentration may become factors to consider. With diets containing higher proportions of alfalfa, less than 20 percent DGS may be needed to supply the protein required in the diet. No strong nutritional advantages occur from feeding more than 20 percent distillers grain, but the possibility of feeding excess protein, fat or phosphorus may occur.

The economics of ration formulation often indicates that it is most profitable to feed as much DGS as possible. Indeed, beef cattle have been successfully fed 50 percent or more of diet DM as wet or dried DGS (Klopfenstein, Erickson and Bremer, 2008). Admittedly, feeding very large amounts of DGS may mean excessive amounts of nitrogen and phosphorus to dispose of in manure; however, this manure may be a cheaper source of these soil fertility nutrients than commercial sources of fertilizer.

FEEDING OTHER ETHANOL CO-PRODUCTS TO DAIRY CATTLE

In addition to wet and dried DGS, other co-products from the production of fuel ethanol have been evaluated in dairy cow diets. These are addressed in the following sections.

Condensed distillers solubles

Condensed distillers solubles (CDS) are usually blended with distillers grain to make distillers grain with solubles, which are marketed as WDGS or DDGS. CDS are a good source of protein, and fat (Rust, Newbold and Metz, 1990), and therefore energy when expressed on a dry basis. So far, only a few studies have been conducted evaluating the use of CDS in dairy cow diets. Udedibie and Chase (1988) showed that milk production increased slightly when cows were fed CDS processed from a mash blend of 60 to 70 percent maize, 16 to 18 percent rye and 12 to 14 percent barley. Huhtanen and Miettinen (1992) reported more protein but less fat content in Finnish CDS than generally measured in the United States product.

Huhtanen and Miettinen (1992) observed increased production when cows were fed 5.9 percent of their diet DM as CDS, but no difference when CDS was raised to 17.5 percent of the ration. It is likely that the increased milk production was a result of the added fat, as has been observed in previous research (Palmquist and Jenkins, 1980). Da Cruz, Brouk and Schingoethe (2005) investigated the inclusion of CDS in dairy cow diets at 0, 5 or 10 percent of total diet DM as substitution for a portion of rolled maize and soybean meal. DMI tended to decrease when cows were fed CDS compared with the control diet. Milk production was higher (34.1 vs 35.5 kg/day) when CDS

was fed at 5 percent of the diet compared with the control, but there was no advantage when CDS was increased to 10 percent. The milk fat profile was altered by the inclusion of CDS, resulting in milk with higher concentrations of stearic and oleic acids (Da Cruz, Brouk and Schingoethe, 2005). Unsaturated fatty acids in the milk of cows fed the 10 percent CDS diet increased compared with cows supplemented with 5 percent CDS, with a trend for more unsaturated fatty acids in CDS-supplemented diets compared with the control. Rumen acetate decreased in diets that contained CDS, and tended to be less for cows fed 10 percent CDS compared with 5 percent CDS. Butyrate concentration increased with increased CDS concentration in the diet. Lower acetate concentration in the rumen fluid in CDS-supplemented diets may be the result of long-chain unsaturated fatty acid inhibition of fibre digestion. Da Cruz, Brouk and Schingoethe (2005) concluded that CDS may be an economical source of energy and protein for lactating dairy cattle that can increase production, milk protein, and lactose. Although milk fat percentage was slightly decreased this was offset by the greater fat yield due to increased milk production.

In a more recent study, Sasikala-Appukuttan *et al.* (2008) fed CDS and DDGS in total mixed rations of lactating dairy cows to evaluate the optimal amount to include in diets, and determine whether CDS is better to be fed alone or in combination with DDGS. Their experimental diets were (1) 0 percent distillers grain products (control); (2) 18.5 percent DDGS; (3) 10 percent CDS; (4) 20 percent CDS; and (5) a combination diet of 18.5 percent DDGS with 10 percent CDS. In diets 2 and 3 there was 2 percent fat from DDGS or CDS, whereas diet 4 contained 4 percent fat from CDS and diet 5 contained 4 percent fat from the blend of DDGS and CDS. Although treatments did not affect DMI, milk production tended to be greater for the diets that contained maize co-products compared with the control. Concentrations of long-chain fatty acids as well as polyunsaturated fatty acids in milk were greater and medium-chain fatty acid concentrations less for the maize co-product diets compared with the control diet. Concentrations of *cis*-9, *trans*-11 CLA, as well as *trans*-10, *cis*-12 CLA, were greater for the maize co-product diets compared with the control diet. Molar proportions of rumen VFA were similar to those reported by Da Cruz, Brouk and Schingoethe (2005) for all diets that contained maize co-products. Sasikala-Appukuttan *et al.* (2008) concluded that CDS can replace up to 20 percent of the soybean meal and maize grain of the diet DM in the total mixed ration without adversely affecting milk production or DMI provided the overall diet has less than 7 percent total fat. Although not addressed by research in this study, another concern in research investigating the inclusion of CDS is the utilization of P. Because CDS contain about 1.5 percent P on DM basis, inclusion of

CDS in dairy cow diets may need to be limited so that P does not exceed the cow's requirement, resulting in excessive excretion of P in the manure.

Reduced-fat DDGS

Two feeding studies have evaluated reduced-fat DDGS (RFDGS) in dairy cow diets. Mjoun *et al.* (2010c) concluded that RFDGS could successfully replace soy-based ingredients at inclusions of 10, 20 or 30 percent of diet DM. Cows had similar DMI and milk production across inclusion levels. Milk from cows fed 30 percent RFDGS had the highest fat percentages, whereas milk from cows fed 10 and 20 percent had the greatest milk protein percentages. Mjoun *et al.* (2010a) also evaluated the inclusion of 20 percent RFDGS and 22 percent DDGS in early lactation diets. In this experiment, cows fed either DDGS diet had similar DMI and milk production to cows fed soybean meal diets. Cows fed the DDGS diets produced milk higher in protein percentage and yield even though lysine was determined to be limiting. These studies concluded that RFDGS are a good source of metabolizable amino acids and that, at 20 percent of the diet, RFDGS did not limit milk or milk protein production.

High-protein DDG

High-protein DDG (HPDDG) has been evaluated in three lactating dairy cow feeding studies (Hubbard *et al.*, 2009; Kelzer *et al.*, 2009; Christen *et al.*, 2010). Kelzer *et al.* (2009) evaluated diets formulated with 14.4 percent HPDDG and 15 percent traditional DDGS to a control, soybean-based diet. Cows fed HPDDG produced similarly to cows fed the soybean-based control or the DDGS-based diets. Hubbard *et al.* (2009) evaluated the inclusion of 20 percent HPDDG as replacement for soybean meal and soybean expeller meal. In this study, cows fed the HPDDG diet had greater milk, fat and protein yields than cows fed the soybean-based control diet. In addition, cows fed HPDDG had greater feed efficiency (milk/DMI) compared with control-fed cows. Christen *et al.* (2010) compared HPDDG at 12 percent of diet DM to three other protein supplement diets: soybean meal, canola meal or DDGS. Each supplement provided 38 percent of the protein fed in each diet. Diets were formulated to be deficient in CP (15.0 to 15.6 percent CP) to determine if amino acids provided by each supplement were limiting milk production. Cows had similar DMI and milk production regardless of the supplement. Fat and protein concentrations in milk of cows fed HPDDG was similar to that from cows fed soybean meal, but higher than for those fed DDGS. Although lysine was determined to be the first limiting amino acid for HPDDG, as with DDGS, it was concluded that HPDDG can successfully replace soybean meal and canola meal without reducing performance of lactating dairy cows.

Maize germ

Abdelqader *et al.* (2009c) investigated the inclusion of maize germ at 7, 14 and 21 percent of diet DM in dairy cow diets. Milk production and fat yields increased when maize germ from dry milling was fed at 7 and 14 percent of diet DM. Feeding at 21 percent of the DM, however, decreased the concentration and yield of milk fat and tended to decrease DMI. In this experiment, the diet with 21 percent germ had a total fat concentration of 8 percent because of inclusion of a basal amount of fat to the diet in addition to the germ. The negative effects of feeding 21 percent maize germ in the diet more likely resulted from total dietary fat rather than excessive contribution of fat from maize germ alone (NRC, 2001).

To determine the effects of fat contribution from germ on milk fat composition, Abdelqader *et al.* (2009b) evaluated four isolipidic diets formulated at 6 percent ether extract: (1) control diet with 2.5 percent supplemental fat from ruminally inert fat; (2) a diet containing 14 percent maize germ; (3) diet containing 30 percent DDGS; or (4) a diet containing 2.5 percent maize oil. DMI was greater for diets containing germ (27.2 kg/day) than for the control diet (24.8 kg/day), but similar to those that contained DDGS or maize oil (26.2 kg/day). In this experiment, milk fat concentration was not decreased when maize germ was fed, although milk fat concentration decreased for cows fed maize oil and tended to decrease for cows fed DDGS. Concentrations of trans-fatty acids and CLA, in particular cis-9, trans-11 CLA, in milk fat were significantly increased by feeding the DDGS or the maize oil diet compared with the control diet, whereas maize germ was not different from the control. These results indicate that the fat in the maize germ from dry milling has a degree of ruminal "inertness" compared with that in traditional DDGS or free maize oil. This is probably because the fat in maize germ is still located within the cell and cell has not been ruptured, thereby preventing ready access of the fat for ruminal biohydrogenation. As a result, a greater proportion of fat from maize germ escapes the rumen without being bio-hydrogenated.

A recent experiment (Kelzer *et al.*, 2009) compared a control diet (with soybean meal) with diets containing maize germ, DDGS or a high-protein DDG, all at 15 percent of diet DM. The greatest DMI and milk yield were observed when cows were fed the diet containing maize germ. Rumen fermentation parameters did not differ between maize co-product treatments; however, cows fed all maize co-products had lower concentrations of acetate in rumen fluid than those fed the control diet. Data to date indicate that maize germ from dry milling may be fed to lactating dairy cattle at concentrations of at least 15 percent of DM. Furthermore, Tedeschi *et al.* (2009) concluded that when energy is limiting, maize germ would be a preferable supplement to DDGS in dairy cattle diets.

Maize bran

Because maize bran has fat concentrations similar to DDGS, the inclusion of maize bran should be similar to that recommended for DDGS. When both DDGS and maize bran are included in the diet their combination should probably not exceed 20 percent of the diet DM to avoid milk fat depression. This is supported by results from Janicek *et al.* (2007) where maize silage and alfalfa was replaced with maize bran at 10, 17.5 and 25 percent of DM in lactating dairy cow diets. Milk yield also tended to increase, but no differences were observed on 3.5 percent FCM. When maize bran was increased from 10 to 25 percent of the diet DM, milk fat percentage decreased by 0.26 percent, but total fat yield was unaffected. Maize bran also increased milk protein by 0.12 kg/day when its concentration in the diet DM was increased from 10 to 25 percent. One important aspect of their findings was that feed conversion improved with the inclusion of maize bran in the diet reaching 1.55 kg of milk/kg of DMI at 25 percent inclusion rate. Inclusion of maize bran in dairy cattle diets will be limited by the total fat present in the diet. Its high fibre content together with the unfavourable amino acid profile suggests that it should be limited to diets for growing animals with functional rumens. As with some feeds with high fat content, it is possible that this product might undergo lipid oxidation after prolonged storage periods and possibly develop some palatability issues.

FEEDING GLYCEROL TO DAIRY CATTLE

Glycerol (glycerin) is a viscous liquid co-product of biodiesel production (Donkin and Doane, 2007) which is colourless, odourless, hygroscopic and sweet tasting. During biodiesel production, fatty acids are hydrolysed from the glycerol backbone of the triglyceride molecule by a transesterification process that uses methanol. After separation of the fatty acid esters, glycerol is removed, containing excess methanol and salts from the reactions. Separation or purification of the glycerol can be variable depending upon the plant and the processes used. Greater discussion on the nutritional composition and contaminants can be found in the chapter by Südekum in this volume.

Glycerin is generally recognized as safe when used in accordance with good manufacture and feeding practices (FDA, 2007, 21 C.F.R. 582.1320). Concerns have been expressed relative to contaminant levels in crude glycerol from residual methanol. The methanol content of crude glycerol should be less than 0.5 percent. A regulatory letter issued by FDA indicates that methanol levels higher than 150 ppm could be considered unsafe for animal feed (Donkin and Doane, 2007). The Office of the Texas State Chemist has established guidelines for labelling, with minimal levels of glycerol and maximal levels of moisture, sulphur, ash and methanol. Methanol is not to exceed 1 percent in crude glycerol targeted for ruminants (*Feedstuffs*, 2007).

Drenched glycerol has been used since the 1950s as an effective treatment for lactation ketosis in dairy cattle and it may even be more efficacious because it enters into the metabolic pathway much closer to glucose than other glucose precursors. Johnson (1954) reported 2000 grams of glycerol *per os* was the most effective means of supplying large quantities of glucose when compared with propylene glycol; however, its use was cost prohibitive until the recent availability of glycerol from biodiesel production. Data regarding the use of glycerol for treatment of ketosis was largely absent for many years because of its high cost.

Fermentation characteristics

Glycerol has been determined to be rapidly fermented by ruminal microbes. Garton, Lough and Vioque (1961) conducted *in vitro* incubations of glycerol and found that nearly 25 percent of the glycerol had disappeared at 2 hours, and by 8 hours nearly 90 percent was undetectable. Remond, Souday, and Jouany (1993) demonstrated that glycerol addition decreased pH more in fermenters fed starch when compared with those fed cellulose. Furthermore, the addition of glycerol led to a VFA mixture rich in butyrate, which became as high as 31 percent of the molar proportion of VFA. According to data from Remond, Souday and Jouany (1993), butyrate molar percentages were higher in fermenters fed starch versus those fed cellulose. Results of both *in vitro* and *in vivo* fermentation studies indicate glycerol is rapidly fermentable and, depending on the diet, will increase propionate and butyrate within ruminal fluid.

Glycerol feeding as a preventative for ketosis

Glycerol as a feed supplement preventative for ketosis in dairy cows was evaluated by Fisher *et al.* (1973). Fifty-two Holstein cows were randomly assigned at calving and over an 8-week period fed concentrates supplemented with 3 percent propylene glycol, 3 percent glycerol, 6 percent glycerol or a control containing no supplement. Cows fed glycerol supplemented at 6 percent lost less body weight and remained in a more positive energy balance than with the other treatments. Because treatment differences in metabolites and performance were quite minimal, Fisher *et al.* (1973) concluded that glycerol's effectiveness in the feed as an anti-ketogenic agent was questionable.

Researchers at South Dakota State University have been experimenting with glycerol in dairy cow diets since 2002. The first experiment was designed to test glycerol as a TMR top-dress for its ability to prevent ketosis (DeFrain *et al.*, 2004). Twenty-one multiparous and 9 primiparous Holstein cows were fed diets with top-dresses of: (1) 0.86 kg/day of maize starch; (2) 0.43 kg/day maize starch + 0.43 kg/day glycerol; or (3) 0.86 kg/day glycerol. Dosages of glycerol were selected based upon amounts shown to be effective

in drenching studies (Goff and Horst, 2001). Treatments were top-dressed and hand-mixed into the upper one-third of the daily ration from 21 days pre-partum until 21 days after calving. Pre-partum DMI was greater for control cows compared with those fed glycerol (13.3, 10.8 and 11.3 ± 0.5 kg/day for 0, 0.43 and 0.86 kg of glycerol, respectively). Rumen fluid collected post-partum showed cows fed glycerol had greater total VFA, greater molar proportions of propionate and a decreased ratio of acetate to propionate. Butyrate tended to be greater for cows fed glycerol post-partum. Glucose concentrations in plasma were actually greatest for cows fed the control diet compared with those fed glycerol, discounting the perception of the glucogenic effects of glycerol. DMI, body weight, body condition and liver lipid during the first 21 DIM were similar among treatments. There were no cows that exhibited signs of ketosis in any of the treatments. Yield of energy-corrected milk during the first 70 DIM tended to be greatest for cows fed the control diet. Cows fed glycerol had decreased milk urea nitrogen (MUN) concentrations. It was concluded that increased energy in glycerol supplemented diets may have been beneficial to the cows, but feeding glycerol did not provide an increase in gluconeogenic precursors.

In a transition cow experiment, a dry glycerol product (food grade, 65 percent glycerol) was fed from calving until 21 DIM in an experiment with 39 multiparous Holstein cows (Chung *et al.*, 2007) with 250 g of product, supplying 163 g/day of glycerol. Researchers observed no differences in feed intake or milk yield during the first 3 weeks of lactation. There was a tendency toward greater milk yield for dry glycerol-supplemented cows during week 6 of lactation (51.7 vs 45.8 kg/day) after the supplementation period had ended, suggesting a potential benefit of dry glycerol on energy status and subsequent milk production.

The effects of replacing high moisture maize with glycerol were determined in diets for transition dairy cows from 28 days pre-partum to 56 days post-partum (Carvalho *et al.*, 2011). Multiparous Holstein cows were fed diets containing either high-moisture maize or glycerol. Glycerol was included at 11.5 and 10.8 percent of the diet DM for pre- and post-partum diets, respectively. Feed intake, milk yield, milk composition and energy balance were not different with glycerol feeding. Blood glucose content was decreased and BHBA concentration was increased in cows fed glycerol during the pre-partum period. Cows fed glycerol had decreased acetate:propionate ratio at 56 DIM. These data indicate that glycerol is a suitable replacement for maize grain in diets for transition dairy cows.

Glycerol drenching as a treatment for ketosis

Goff and Horst (2001) evaluated an oral glycerol drench as an aid in the treatment of ketosis in two experiments. In the first, cows were administered 1, 2 or 3 L of glycerol via

esophageal pump. Thirty minutes after dosing, concentrations of blood glucose increased by 16, 20 and 25 percent for cows treated with 1, 2 or 3 L, respectively. Similar to observations by Schröder and Südekum (1999), Goff and Horst (2001) indicated that drenching with glycerol had no effect on ruminal pH. In the second experiment, two cows diagnosed with clinical ketosis were treated with 1 L of a glycerol drench. Both cows responded with higher concentrations of glucose in blood, decreased urinary ketone body excretion, and an increased milk production. These data further support the potential role glycerol could play as a glucose precursor in diets for transition dairy cows.

Researchers at Iowa State University have investigated the usefulness of drenching glycerol in combination with glucagon, a hormone to stimulate gluconeogenesis, in prevention of ketosis and fatty liver (Osman *et al.*, 2008), administering 400 mL of glycerol diluted with 100 mL of water for 14 days post-partum to 12 cows with or without glucagon treatment. Glucagon plus glycerol treatment increased plasma glucose concentrations on days 1, 7 and 13 post-partum by more than 40 mg/dL greater than that of the control group, and maintained it at an elevated concentration for longer than other treatments. Glycerol alone increased blood glucose on days 7 and 13. Plasma NEFA concentration was decreased by glucagon plus glycerol and glycerol treatments on all three sampling days. Glycerol treatment alone maintained lower plasma NEFA for longer than glucagon plus glycerol treatment on days 7 and 13 post-partum. However, no significant effect was observed for the glycerol-alone treatment in a later study using the same doses of glycerol for 14 days after calving in 8 cows with or without glucagon treatment (Osman *et al.*, 2010). Glycerol alone did not significantly affect plasma insulin, glucose, NEFA or BHBA concentration at any point during the treatment, except for a significant decrease in plasma BHBA concentration at day 9. However, co-administration of glucagon and glycerol increased plasma glucose and insulin and decreased plasma NEFA concentrations in both treatment weeks. Glycerol alone or in combination with glucagon did not significantly affect daily milk production, body condition score or liver composition. Researchers at Iowa State University determined drenching glycerol was an effective tool for prevention of fatty liver and ketosis, particularly when combined with hormonal therapy.

To better explain discrepancies in results obtained from feeding and drenching studies, Linke *et al.* (2004) at South Dakota State University used four high-producing Holstein dairy cows in a Latin square design with 1-week periods to evaluate the effect of methods of oral delivery versus feeding of glycerol on ruminal VFA and plasma concentrations of glucose, BHBA, NEFA and insulin. Cows were 132 DIM and producing an average of 59.9 kg of milk per day. To

create a mild negative energy balance, all cows were fed only grass hay for ad libitum consumption for 12 hours before the experiment. This regimen was successful at elevating plasma NEFA concentrations similar to that observed in cows during the first 2 days after calving. At 0800 the next morning (time 0) all cows were fed 5 kg of cracked maize. Re-feeding reduced NEFA concentrations in all cows. Treatments administered at time 0 were: (1) control, maize alone with no glycerol; (2) 1.0 kg of glycerol solution (80 percent glycerol) added to the maize; (3) 1.0 kg of glycerol solution in 0.5 L of water and delivered as oral drench with a drenching bottle; and (4) 1.0 kg of glycerol in 9 L of water and delivered into the rumen via a McGraff pump and an esophageal tube. Blood samples were collected at -1, -0.5, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours relative to administering glycerol. Rumen samples were collected at 0, 2, 4 and 6 hours. After administration of glycerol, concentrations of acetate decreased in rumens of all cows given glycerol, regardless of method of delivery. Likewise, propionate and butyrate were increased by glycerol in all forms, with peak concentrations at 4 hours. Glucose concentrations in plasma increased in cows that were drenched with glycerol or received tube delivery of glycerol into the rumen compared with both the control and glycerol-fed cows. For drenching and tubing, respectively, glucose reached peak concentrations at 1.5 and 3 hours. Compared with the control, glucose response, expressed as area under the curve over baseline, at 6 h was greater for drenching or tube delivery but not feeding glycerol. Insulin concentrations in plasma were also increased for drenching and tubing, reaching peak concentrations at 1.4 and 1.1 hours, respectively. Finally, BHBA was increased in plasma of all cows receiving glycerol, reaching peak concentrations at 2.5, 2.4 and 1.6 for drenching, tubing and feeding, respectively. Conclusions from this research are that to be glucogenic, glycerol must either be delivered in water to associate with the liquid fraction of the rumen content, or be able to "bypass" the rumen in some form to be absorbed as glycerol and converted to glucose by the liver.

Glycerol is an efficient glucogenic substrate because it enters the gluconeogenesis pathway at the triose phosphate level and therefore is not affected by two of the rate-limiting gluconeogenic enzymes. Logically, the dairy cow in negative energy balance has pathways activated for utilization of glycerol liberated from mobilization and hydrolysis of triglycerides from body fat. This activity is dependent upon absorption of glycerol rather than fermentation to propionate and butyrate, which is somewhat counterproductive in view of the ketogenic nature of butyrate. If absorbed intact, glycerol is a highly efficient glucogenic substrate. Glycerol that is available to rumen microbes will be converted to propionic and butyric acids.

The fraction converted to butyrate is metabolized to BHBA by the ruminal epithelium, thus glycerol that is fed in the diet instead of dosed is actually ketogenic rather than glucogenic.

Glycerol during lactation as an energy supplement

Schröder and Südekum (1999) determined the suitability of glycerol as an energy source in ruminant diets. Using wethers fed low- and high-starch concentrates, they added glycerol at 10, 15 or 20 percent of diet DM. With a low-starch concentrate diet they observed no effect on digestibility of organic matter, starch or cell-wall components. Feeding the same concentrations of glycerol in high-starch concentrate diets resulted in a decrease in cell-wall digestibility with no effect on the digestion of organic matter or starch. It appears that glycerol would act similarly to a carbohydrate (as opposed to a fat) in the rumen when formulated into typical high-forage, dairy diets. The authors determined the energy density of glycerol to be 1.98 to 2.27 Mcal/kg NEL.

Schröder and Südekum (1999) also used four rumen-cannulated steers to evaluate the effects of feeding glycerol. Steers consumed an average of 13.3 kg/day, of which 2.1 kg/day of starch for those fed control diets was substituted with 1.09 kg/day of glycerol of differing purities along with 1.4 kg/day of starch for steers fed the treatment diets. Feeding glycerol did not affect diet digestibility, but decreased the acetate:propionate ratio, increased ruminal butyrate concentrations and stimulated more water intake. These changes would be beneficial to the dairy cow because (1) increasing ruminal propionate would increase the supply of this gluconeogenic substrate to the liver; and (2) increasing ruminal butyrate would support the growth of the ruminal epithelial tissue and perhaps increase nutrient absorption from the rumen, as indicated by Dirksen, Liebich and Mayer (1985).

Because of results from the DeFrain transition cow experiment at South Dakota State University, it was decided to test glycerol at similar feeding amounts in mid-lactation cows as an energy supplement (Linke *et al.*, 2006). Six primiparous Holstein and six primiparous Brown Swiss cows (192 DIM; SD \pm 150), were assigned to one of three diets in a Latin square design with four-week periods. The diets were: (1) a control diet containing no glycerol; (2) low glycerol, with 0.5 kg/day of glycerol; and (3) high glycerol, with 1.0 kg/day of glycerol. Rumen VFA profiles showed that molar proportions of acetate were not changed in rumens of cows fed glycerol. Propionate tended to be increased for cows fed glycerol, and butyrate was increased linearly as the amount of glycerol fed increased. DMI intakes, milk yield and 4 percent FCM were not significantly changed by glycerol supplementation. Feed efficiency, however, was

increased by glycerol supplementation, with milk to feed ratios of 1.46, 1.59 and 1.60 kg of FCM/kg of DMI, for 0, 0.5 and 1.0 kg/day of glycerol, respectively. Milk composition was not changed except, as before, MUN concentrations were decreased with the addition of glycerol. We surmised by the increased feed efficiency and decreased MUN that the addition of glycerol may have improved rumen microbial efficiency. Based upon differences in feed efficiency, we calculated the energy value of glycerol to be about 20 percent greater than that of maize, yielding an NEL of about 2.31 Mcal/kg, similar to the estimate by Schröder and Südekum (1999).

More recently, Donkin *et al.* (2009) fed 0, 5, 10 and 15 percent glycerol (99.5% grade) of diet DM to lactating dairy cows replacing maize with glycerol and maize gluten feed. Feed intake was decreased with 15 percent glycerol during the first 7 days of the experiment, but recovered thereafter. Overall, feed intake was not affected by the addition of glycerol. Milk production and composition was not affected other than MUN, which decreased with the addition of glycerol. Cows fed 10 and 15 percent glycerol gained more weight after 8 weeks on the treatments than did cows fed other treatments. The researchers concluded that glycerol can be fed at up to 15 percent of diet DM to lactating dairy cows.

STORAGE OF BIOFUEL CO-PRODUCTS

At the present time, DGS is sold in either dried (DDGS) or wet (WDGS) form. Wet distillers grain is the main co-product by volume that remains after fermentation of grain starch to ethanol. After the fermentation process, the thin stillage is separated from the wet cake and condensed, resulting in a nutrient-dense syrup that is also known as CDS or the “solubles fraction”. This syrup is frequently sold locally for feeding purposes or it can be added back to the final product to obtain wet distillers grain with solubles (WDGS). An intermediate product, known in the ethanol industry as “modified WDGS”, consist of a partial water removal through centrifugation which results in a co-product with approximately 50 percent moisture. Water needs to be removed from these co-products to make long-distance transportation economically feasible. Heat-drying WDG and WDGS at the ethanol plant transform them into DDG or DDGS. It is the high nutrient density that results from water evaporation that makes DDG a feed in high demand. But this high nutrient content, when combined with this variable water activity remaining in the products, can pose different challenges for both products from a conservation standpoint. For all practical purposes, DDGS would have conservation problems similar to dried ground shelled maize, with the additional constraint of having three times as much fat. Conversely, WDGS (65 percent moisture) and modified WDGS (50 percent moisture)

have enough water activity to allow for mould and yeast growth.

Storage of dried distillers grain with solubles

Adequate storage and preservation of DDGS for moderate periods is possible provided certain environmental conditions are maintained. As mentioned earlier, with the exception of most of the starch that was fermented to ethanol, all the nutrients present in shelled maize grain are also present in DDGS, but concentrated approximately three-fold. Conditions for the conservation of DDGS are then going to be similar to that of maize grain. The difference is that DDGS has undergone significant processing, including heating, grinding, and fermentation, during the ethanol production process, which has basically transformed the original seed into a collection of inert particles loaded with nutrients without the protection of the cuticle present in unprocessed kernels. At the same time, intact kernels allow for minute inter-kernel air spaces, whereas ground DDGS does not. This small particle size modifies DDGS density and, when combined with other physical characteristics, can have a negative effect on particle flow inside containers. Aside from particle size, other factors which affect flow are temperature, pressure, fat content and bulk density (Ganesan, Muthukumarappan and Rosentrater, 2007). Fresh DDGS loaded warm at the ethanol plant can be difficult to remove from the railroad cars at destination. This also holds true for conservation of DDGS in vertical structures, because the higher the column of particles the greater the pressure at the bottom, which reduces flow. It is thus not recommended to store DDGS in feed bins or use auger systems to load and unload or to feed animals. This situation is further compounded if DDGS has more moisture than desirable.

Recent research suggests that flow rates for DDGS containing 9 and 12 percent moisture were 631 and 390 kg/min, respectively (Shurson, 2007). In this same study, calcium carbonate, zeolite and a commercial product were tested as flow-enhancing agents, but none was any different from the control (no additive). Density also influences degree of “caking” and flow ease. It is considered that DDGS should have an average density of $572 \pm 44.7 \text{ kg/m}^3$, but the range goes from 493 to 630 kg/m^3 (Shurson, 2007). Decreasing particle size in maize ground for fermentation increases the surface area of the particles in relation to their mass, and reduces the distance to the particle core, allowing a more rapid and efficient fermentation of the yeast used in ethanol production. This is the reason why plants tend to grind shelled maize as much as possible before adding it to the fermentation vats. This particle size will affect the degree of compaction and thus density of the co-products obtained. The mean particle size for DDGS was approximately $1282 \pm$ a standard deviation of $305 \mu\text{m}$ with a range of 612 to

2125 μm (Shurson, 2007). Particles on the lower end of the micron spectrum will be more prone to caking problems and reduced flow. In short, and in order to minimize “caking” problems, it is suggested to purchase DDGS from plants with particle size standardized towards the higher end of the spectrum (around 2000 microns), with fat contents not exceeding 10 percent, and that offer a co-product that consistently tests under 10 percent moisture.

Fat content in DDGS varies and it can be as high as 15 percent depending on the amount of solubles added back to the starch-expanded mash before being dried to DDGS. During the normal ethanol production process, maize kernels are ground prior to fermentation. This allows for greater access of the yeast's (*Saccharomyces cerevisiae*) enzymes to the nutrients previously protected by the grain cuticle. Once WDGS is dried to DDGS, these non-starch nutrients remain exposed. The germ in particular is very rich in lipids that, when exposed to air, can undergo auto-oxidation at varied speed depending on environmental conditions. This process can consume natural antioxidants present in the original grain, such as tocopherols (vitamin E). In the presence of air, the conjugated dienes combine with oxygen to produce peroxy radicals. These radical can further remove hydrogen from adjacent fatty acids, causing an autocatalytic chain reaction (propagation) to produce lipid peroxides. The termination stage requires the presence of an antioxidant such as α -tocopherol (vitamin E), which is the chain-breaking molecule.

In addition to auto-oxidation, the fat in DDGS can undergo photo-oxidation, which is even faster than auto-oxidation. Light acts on the oxygen molecule to form a radical called “singlet oxygen”, which reacts with double bonds of fatty acids in DDGS to produce hydroperoxides. From then on the propagation and termination stages will continue similar to the process described as auto-oxidation above (Cyberlipid Center, no date). After this process, the DDGS become rancid and the presence of these lipid peroxides leads to reduced palatability in ruminant animals. It is clear that exposure of DDGS and WDGS to sunlight and oxygen has to be reduced as much as practically possible.

One other aspect related to the conservation of distillers grain is the potential for mycotoxin contamination. Mycotoxins are not destroyed during the ethanol fermentation process or the distillers grain production processes, but instead augmented almost three-fold from their initial concentration in the original kernel. Inadequate storage conditions may also increase their concentration due to inoculation by mould spores present in the environment. The use of mycotoxin-contaminated distillers grain in dairy cattle diets poses a risk to human health because of the transfer to milk of the carcinogenic metabolite aflatoxin M1. Even when the toxin concentration is within acceptable standards for distillers grain, the additive nature of

the mycotoxins does not preclude the potential for toxicity when other slightly affected feeds are also included in the diet. In the presence of borderline-acceptable levels of aflatoxin B1 in DDGS, testing the TMR and/or individual feeds is recommended to ensure milk will not be contaminated.

If a feed ration has been found to have high mycotoxin concentration, the producer could include various feed additives to bind mycotoxins, and reduce absorption by the animals. For example, β -glucans, zeolyte and other binders have been reported to be effective. At the time of writing, the United States Food and Drug Administration does not recognize the potential “binding” properties of these additives, which can only be commercialized by the respective companies as “anti-caking” agents.

Storage of wet distillers grain with solubles

When ethanol plants are relatively close to the farms, WDGS is usually an attractive alternative. They are usually priced around one-third to one-quarter the price of DDGS and, on a dry basis, their nutrient content is practically the same as DDGS. However, WDGS has advantages other than just a competitive price, as WDGS helps improve the overall diet, increasing its palatability and reducing feed sorting, particularly when dry forages and concentrates predominate. These advantages are not such when other fermented feeds are included at high levels in the diet (e.g. maize silage, high-moisture maize, hay crop silage) as the inclusion of WDGS may result in excessively wet or acidic, or both, rations that may reduce intake. An additional drawback in the field is that the term WDGS or “wet cake” is applied loosely to any wet product coming from the ethanol plant that is not DDGS, regardless of its moisture content. The DM content of WDGS ranges in most cases between 30 and 40 percent.

Another product that has become quite popular among ethanol plants is the “modified” WDGS, with reduced water compared with WDGS. Modified WDGS has a DM concentration between 45 and 55 percent. On a dry basis, the nutritive quality of WDGS can be affected by processing, handling and storage. Mishandling between production at the plant and utilization on the farm can turn an excellent product into a lower quality or even health-threatening feedstuff.

From processing at the ethanol plant to delivery on the farm, there are critical time constraints that may challenge WDGS quality. Granted, WDGS does not remain for extended periods at the plant before being shipped. Oftentimes it leaves the plant still warm from the fermentation process. Temporary storage at the plant is usually done on concrete surfaces, so these surfaces should be maintained clean and protected from the weather. In addition, WDGS is not only palatable to livestock but also to birds, vermin and even companion animals (including dogs), whose faeces

can contaminate the product. Old material that remains from previous batches should be removed as it may have mould growth and can inoculate with spores fresh batches deposited on top. Similarly, WDGS that has been left outside with no cover can have been subject to precipitation, which modifies its moisture content and washes out soluble nutrients before inclusion in livestock diets.

If WDGS is not going to be fed to livestock within one week (3–4 days in summer), means of adequate storage need to be found. Covering with a weighted tarpaulin will protect WDGS from precipitation and exposure to light, but does not exclude air. Under these conditions WDGS will develop a dark crust approximately 5 cm thick on the surface, which it is advisable to discard upon feed-out. If WDGS is protected from air infiltration (e.g. in a silo bag), it will preserve well, either alone or blended with other feeds. Due to its high moisture content and density, it is not advisable to store WDGS in vertical structures such as silos. Storage can be easily accomplished in bunkers, covered piles, or in silo bags.

When storage is needed for prolonged periods (months), it is convenient to use silo bags to prevent extensive spoilage. Feeding out from bagged WDGS needs to follow similar guidelines as feeding silage from a bag. Removal needs to be approximately 30 cm in depth from the entire exposed surface at least every other day. Unloading WDGS at the farm on a firm surface, such as concrete or asphalt, prevents contamination with soil and seepage of minerals into the ground. The pile should be readily covered to protect it from precipitation and, eventually, seepage. Precipitation not only refers to rain but also snow in cold climates. When it leaves the ethanol plant, WDGS is usually still hot, with temperatures of around 60 °C not being unusual upon arrival at the farm. If during transportation or just after unloading WDGS is snowed-upon, this temperature will melt the snow and nutrients will be lost with the runoff.

The method of choice for preservation depends not only on the equipment available at the farm but also on the number of animals to be fed daily. Small- to medium-sized livestock operations benefit the most from silo bags because enough volume of WDGS can be removed from the exposed surface daily to keep ahead of potential spoilage losses. Producers need to be careful not to overstretch

the bag as the lateral pressure can tear the bag open during the filling process.

Storage of WDGS blended with forages

The low pH of WDGS arriving from the ethanol plant is a positive factor when mixing WDGS with other feeds (Table 10). Studies conducted at the Dairy Science Department of South Dakota State University have shown that the pH of these blends drops proportionally to the buffer effect and/or original pH of the companion feedstuff. The pH of most dry feeds is neutral at best, and mixing them 50:50 on a dry basis with WDGS reduces the pH of the blend to approximately pH 4. When compaction and air exclusion are adequate, this acidity supports adequate preservation. In fact, WDGS preserves perfectly well on its own without the need for such blends, but the blends help preserve other feeds that otherwise would require an additional storage structure.

Feeds that can ideally be mixed with WDGS are those deficient in the nutrients that WDGS supplies in excess. Feeds low in protein, fat and phosphorus are desirable companions because those nutrients are concentrated in WDGS. Examples of such feeds are soybean hulls, beet pulp, citrus pulp and crop residues such as maize stalks and small-grain straws. One additional advantage of the blend with dry residues is that the moisture in WDGS softens the structural carbohydrates, allowing for faster colonization by rumen bacteria. In addition, a practical advantage of blending WDGS with fibrous residues is that the blend is more easily reduced to smaller particles during the winter, when chunks of frozen DDGS can be difficult to incorporate into a total mixed ration.

Research conducted by the Dairy Science Department of South Dakota State University has demonstrated that to be able to achieve adequate air exclusion through compaction those blends should not exceed 50 percent DM. If this recommendation is to be followed then using “modified” WDGS (50 percent DM) would only work in blends with high moisture feedstuffs such as green chopped forages (e.g. maize plants). At the same time, the original WDGS with 60 to 70 percent moisture would work better in blends with drier feeds. From this perspective, the generic term “wet cake” is not descriptive enough and producers

TABLE 10
Wet distillers grain preserved in silo bags

Parameter	0	3	Day 7	14	129	SEM1
pH	3.1	3.1	3.2	3.2	3.1	0.04
			as % DM			
Acetic acid	0	0	0.11	0.30	0.23	0.16
Propionic acid	0.30	0.30	0.32	0.30	0.33	0.02
Lactic acid	0.90	0.95	0.97	1.02	0.98	0.02

Notes: SEM = Standard error of mean. Source: Mjoun, Kalscheur and Garcia, 2011.

need to be aware of which product they have purchased before even attempting to blend it with other feeds. Fibrous residues need to be chopped relatively finely, as particles in excess of 5 cm might not blend adequately, and will also allow for pockets of air to remain in the ensiled mass.

Research at South Dakota State University has also shown that blends of WDGS and fibrous residues (e.g. maize stalks, rye straw) stored in silo bags remained well preserved as long as the bag remained closed. However, when the bag was opened air rapidly infiltrated the ensiled mass, particularly in blends of modified WDGS, maize stalks and rye straw. This resulted in heating, yeast and mould growth, and non-enzymatic browning (Maillard reactions). These results have prompted producers to store blends of WDGS and roughages in silage piles rather than bag, with encouraging results. As with any ensiling procedure, the degree of compaction and air exclusion to be achieved is critical. When filling bags with bulky materials it is difficult to maintain enough pressure with the tractor without ripping the bag. In contrast, a pile can be driven over as often as needed to achieve adequate compaction.

Mjoun, Kalscheur and Garcia (2011) evaluated blends of whole-plant maize (WPC) and WDGS blends stored in silo bags. In this trial four experimental treatments were tested, which, on an as fed basis, were: (1) 100 percent WPC; (2) 75 percent WPC with 25 percent WDGS; (3) 50 percent WPC with 50 percent WDGS; and (4) 100 percent WDGS. Blend samples were analysed for fermentation parameters on days 3, 7, 14 and 129 of storage. Differences in the chemical composition among ensiled feeds were observed at day 129, but they were more related to differences in the initial chemical composition of WPC and WDGS than due to fermentation. After 3 days of fermentation in the bag the pH of 100 percent WPC was below 3.7, and without significant change thereafter. As mentioned earlier, the pH of the WDGS as it comes from the plant is low (typically 3.1 to 3.5). The low pH of WDGS is probably because of the sulphuric acid used to control fermentation. As a result, WDGS does not undergo a typical ensiling fermentation as the inherent acidity inhibits the growth of usual silage-fermenting bacteria (homo-fermentative), "pickling" the product right from the start. Lactic acid prior to ensiling was greatest for 100 percent WDGS (0.9 percent of DM) and decreased as WPC concentration increased. Blends of WPC with WDGS resulted in silages with more acetic than lactic acids. It was interesting to note that the pattern of fermentation was not typical of that of normal silage.

The relative absence of water soluble carbohydrates (spent during ethanol-production) resulted in lower concentrations of acetic acid in WDGS ensiled alone (Mjoun, Kalscheur and Garcia, 2011). As WPC was added at both 25 percent and 50 percent of the blend, acetic acid increased to concentrations above 43.6 g/kg of DM. Past

research has suggested that high acetic acid concentrations are associated with reduced animal performance. It is very likely for these observations to be somehow associated with fermented materials that have some sort of aerobic deterioration going on, with other fermentation products that reduce palatability and feed intake. Kung *et al.* (2003) reported that alfalfa silage fed at 16 percent of the diet DM and inoculated with *Lactobacillus buchneri* had high acetic acid concentration (57.0 g/kg of DM) and had no effects on DMI, but resulted in greater aerobic stability of the total mixed ration and milk production.

One thing that has to be considered is that when maize plants are ensiled, *Lactobacillus* organisms start to multiply and produce lactic acid until the decrease in the pH inhibits their proliferation. It is very likely that the low initial pH of WDGS inhibited the proliferation of homo-fermentative bacteria, which are responsible for lactic acid production (Woolford, 1984). When the pH of the feed is low from the start (such as with WDGS inclusion), homo-fermentative bacteria are inhibited, allowing for other groups (e.g. hetero-fermentative bacteria) to proliferate and produce ethanol and acetate. Although there was no ethanol detected prior to ensiling, it increased ($P < 0.05$) with time in all treatments (Mjoun, Kalscheur and Garcia, 2011). Ethanol concentration was highest (2.36 percent of DM) for 50 percent WDGS on day 129. There was no change in DM content, but ammonia-nitrogen increased over time ($P < 0.05$) in all silages. It is possible that ethanol was produced by the action of hetero-fermentative-type organisms in the presence of available fermentable substrates. It was concluded that ensiling WDGS with WPC can be used as an effective method of preserving both feeds. The low initial pH, coupled with the high acetic acid concentration on days 3 (2.77 percent), 7 (3.25 percent), 14 (3.34 percent) and 129 (4.32 percent), particularly for the 50:50 blend, suggested that preservation could be enhanced by combining both feedstuffs. The blend is easier to handle during the winter when compared with WDGS alone, the reason being that longer forage particles allow for easier breaking of the frozen mass. Aerobic stability of the blends was enhanced when compared with the original feedstuffs, particularly WPC. Aerobic stability was measured as the number of hours it took for the temperature in the feed to increase 4 °F above ambient temperature. The 50:50 WPC:WDGS blend had greater aerobic stability upon exposure to air in comparison with the other 3 treatments.

One of the advantages of high acetic acid in fermented feeds is the improvement of aerobic stability of the fermented material upon feed-out (Kleinschmit and Kung, 2006). In Mjoun, Kalscheur and Garcia's (2011) experiment, aerobic stability was enhanced in all silages that contained WDGS, despite acetic acid concentration being the lowest in silage with 100 percent WDGS. The authors could not

offer a definitive explanation as of why this happened, although they hypothesized that lower pH and higher propionic acid concentration in 100 percent WDGS may have been partly responsible for the improved aerobic stability. Acetic acid concentration was greatest at 25 and 50 percent WDG in the blends, which also resulted in more prolonged aerobic stability. Improved aerobic stability for 100 percent WDGS contrasted with the findings of Nishino, Harada and Sakaguchi (2003) who reported that ensiling wet brewers' grain alone decreased aerobic stability when compared with a multiple-ingredient TMR.

Ramirez-Ramirez *et al.* (2011) evaluated the nature of ensiling WDGS alone or in combination with 50, 75 or 100 percent maize silage or brome hay on a DM basis. Mixtures were ensiled for 60 days in polyethylene silo bags prior to analysis. The pH of WDGS ensiled alone was less than 4, in agreement to observations of Anderson *et al.* (2009) and Mjoun, Kalscheur and Garcia (2011). In all mixtures, the addition of maize silage or brome hay to WDGS increased the pH of the stored material, especially with the addition of brome hay. The authors concluded WDGS can be successfully preserved on the farm in combination with other common feeds.

Storage of WDG with soybean hulls or wet beet pulp

Among industry by-products, soybean hulls (SH) have nutrient characteristics that make them an ideal feed companion for WDGS. They also have excellent digestibility and contain less protein, fat, sulphur and phosphorus, providing excellent complementation with high concentrations of WDGS. The Dairy Science Department at South Dakota State University evaluated the fermentation of ensiled WDGS alone or combined with SH (Anderson *et al.*, 2009). Three treatments were evaluated, all on an as-fed basis: (1) 100 percent WDGS; (2) 85 percent WDGS+15 percent SH; and (3) 70 percent WDGS+30 percent SH. All straight feeds and feed blends were ensiled in laboratory silos opened at days 0, 3, 7 and 21 after ensiling. Feed samples were collected to evaluate fermentation characteristics.

DM concentration of the ensiled feeds increased from 35 to 43–49 percent as expected, through the treatments as WDGS inclusion was reduced (Table 11). As also expected, CP percentage declined as SH was added to the blend. The pH of 100 percent WDGS was the lowest (3.2; $P < 0.05$) and was higher as WDGS in the blends decreased. This could also be expected due to the higher pH (close to neutral) of the SH. Lactic acid concentration was highest for 100 percent WDG and tended to decline as SH was included in the treatments (Table 11.). There was no difference across treatments for acetic acid, propionic acid and ammonia-N. No changes were observed in the ensiled treatments over time for DM, CP, pH, lactic acid, propionic acid or ammonia-N ($P > 0.05$). In the treatments that combined WDGS with SH, acetic acid had increased by day 21. The production of ethanol increased with duration of ensiling, particularly when SH was added, which suggests that the blends supplied fermentation substrates. It could be speculated that the low pH in combination with the acetic acid observed by day 21 could have resulted in adequate preservation of the blends, even when SH was included at 30 percent.

Beet pulp (BP) is also a feedstuff oftentimes available to livestock producers. It is highly palatable due to its residual sugar content, and also rapidly fermented in the rumen, with a VFA pattern where acetate predominates. With a protein content that is relatively low (approximately 9 percent), it is nevertheless a good source of energy because of its highly fermentable fibre and remaining sugars. These nutrient characteristics make it an ideal feed companion for blends with WDGS, particularly when energy-dense diets are needed. Combining both feedstuffs results in blends easy to include in dairy cattle rations (Garcia *et al.*, 2004).

Kalscheur *et al.* (2004) evaluated the fermentation and preservation characteristics of ensiling WDGS with wet beet pulp (WBP). Different blends of WBP and WDGS were ensiled on an "as fed" basis as follows: (1) 100 percent WBP; (2) 67 percent WBP+33 percent WDGS; (3) 33 percent WBP+67 percent WDGS; and (4) 100 percent WDGS. Samples for analysis were collected at days 4, 8, 21 and 112 after ensiling. The pH of the WDGS+WBP blends decreased

TABLE 11
Composition of wet distillers grain (WDG), soybean hulls (SH) and their blends, and dietary recommendations for a typical dairy cow diet

Parameter	WDG	SH	WDG+SH Blend		Recommended dairy cow diet
			85:15	70:30	
DM (%)	32.0	91.0	40.9	49.7	50.0 to 60.0
NEL (Mcal/kg DM)	2.00	1.65	1.89	1.80	1.67 to 1.80
CP (% of DM)	32.0	11.0	25.0	20.5	16.0 to 20.0
Fat (% of DM)	12.0	1.10	8.36	6.01	5.0 to 8.0
P (% of DM)	0.70	0.21	0.54	0.43	0.38 to 0.42
S (% of DM)	0.33	0.09	0.25	0.20	0.18 to 0.22

Notes: DM = Dry matter; NEL = net energy for lactation; CP = crude protein. Source: Anderson *et al.*, 2009.

TABLE 12
Composition of ensiled blends of wet distillers grain (WDG) and wet beet pulp

Parameter	% WDG in the blend			
	100	67	33	0
DM (%)	33.0	30.2	26.5	23.1
pH	3.3	3.6	3.9	4.2
	as % of DM			
CP	30.5	25.4	18.4	8.6
NH ₃ -N	1.08	0.93	0.48	0.15
Total acids	6.53	4.81	2.98	2.37
Acetic acid	0	0.40	0.55	1.06
Propionic acid	0	0	0	0
Lactic acid	3.86	0.26	0.53	3.04

Notes: DM = Dry matter; CP = Crude protein. Source: Kalscheur *et al.*, 2004.

as the concentration of WDGS in the blends increased (Table 12). Lactic acid prior to ensiling was greatest for 100 percent WDG and decreased as WBP was included in the treatments (Table 12). Acetic acid was highest in 100 percent WBP prior to ensiling and decreased with the inclusion of WDGS in the treatments. By day 4 the pH of all feeds was below 4.0 and did not change thereafter. Acetic acid increased ($P < 0.05$) over time in all treatments and was highest for the 100 percent WBP. It was concluded that ensiling WBP and WDGS is an effective method of preserving both wet co-products.

Although WDGS alone can be ensiled without the need of any additives, the low initial pH for all blends and the increased acetic acid over time suggested that preservation was enhanced by combining both feedstuffs. Preservation losses were measured by using ash as a marker to determine organic matter (OM) disappearance according to the formula: percent OM loss = $1 - A/B \times 100$, where A = initial ash and B = final ash (Garcia *et al.*, 1988). OM losses were 10 percent or greater for all treatments with the exception of 67 percent WDGS, which was 51.6 percent lower than for 0 percent WDGS.

Recommendations for storing co-products.

Dairy cattle nutritionists are oftentimes reluctant to include agricultural by-products in their ration formulations because of the challenges inherent in balancing nutrient deficiencies with the requirements of animals of high genetic potential. To obtain balance rations it is necessary to make use of feeds that are nutritionally complementary to each other, so that nutrient deficiencies in one feed are strengths that allow for greater inclusion of another price-competitive feed that may have excess nutrients. This is oftentimes the situation with DDGS and WDGS, where the presence of high quality forages such as alfalfa can limit their inclusion in order not to exceed the overall protein content of the diet. Before choosing a preservation method for ethanol co-products, it is important to consider their individual characteristics as well as the ease of handling on the farm. The increased demand for ethanol has resulted in increased availability

of WDGS locally. The economic and practical feasibilities of transporting and storing WDGS on the farm need to be determined. The preservation of WDGS is excellent on its own due to the low initial pH, provided certain conditions are met. Similar to other ensiled feeds, the nutritive value can be maintained in time if air infiltration is avoided. When WDGS was bagged alone or in combination with soy hulls, beet pulp or green chopped maize, preservation went well. Advantages of the WDGS+green chopped maize blends are the improved aerobic stability at feed-out at higher WDGS inclusions, as well as the easier removal of WDGS during winter. These blends allow producers to stretch forage supplies during feed shortages, augment the energy density of the diet, and reduce the need for maize grain supplementation. In fact, with high maize prices and with feeding constraints described elsewhere in this chapter, producers are better off selling their maize for ethanol production and substituting it with DDGS in their cattle diets.

FUTURE BIOFUEL CO-PRODUCTS (NEXT GENERATION)

It is becoming feasible to fractionate DGS into products that are, for example, higher in protein, lower in fat and NDF, and higher or lower in phosphorous. The availability and use of co-products of DGS processing such as condensed maize distillers solubles, maize germ, maize bran and high-protein distillers grain will increase in the future. Several of these co-products were discussed earlier in this chapter, as well as biodiesel co-products. Innovations in processing technology are likely to result in additional distillers co-products from which to choose as livestock feeds. This may include new co-products from grain (especially maize) fermentation, but also totally new co-products from cellulosic ethanol production. Some questions to be answered about potential new products include: (1) Will the fat in DGS go to biodiesel or be utilized in animal feeds? (2) Will the fibre in DGS go to cellulosic ethanol? and (3) What about the feeding value of the cellulosic co-products from high-fibre sources?

Cellulosic ethanol is considered to be a leading alternative to fossil-fuel based liquid fuels because it is renewable

and can be produced worldwide. While research on cellulosic ethanol has been ongoing for several decades, its commercial viability has only been demonstrated recently. Fibre and storage carbohydrates within grasses can be converted to alcohol by yeast after enzymatic hydrolysis, but the protein cannot be utilized for ethanol production. Therefore, the use of grass to produce ethanol, especially species that contain appreciable amounts of protein, creates nitrogenous waste for bio-refineries. However, extraction of protein prior to enzymatic hydrolysis and concentrated as leaf protein can be utilized by livestock, thereby reducing protein costs and offsetting the land required for animal production (Dale *et al.*, 2009). Forage crops (e.g. reed canary grass, timothy and alfalfa, as well as barley, triticale, pearl millet and sweet sorghum hays) and crop residues (e.g. maize stover and bagasse, as well as wheat, barley, triticale and rice straws) have been identified as potential sources of lignocellulose for bio-ethanol production (Michaud, Bélanger and Surprenant, 1997).

Information concerning the feeding value of co-products from cellulosic ethanol or isobutanol production is currently quite limited. Isobutanol has potential because it can be produced in a similar manner to ethanol, but it can be directly blended with oil-based fuels, and efficiency of fermentation is identical to ethanol production. While the potential is great to develop a whole new series of possible feeds for animals, especially for ruminants, there remain a number of unknowns. To date, data available includes primarily *in vitro* or *in situ* data, with little animal performance data.

Treatment of fibrous materials is necessary to convert cellulose and other carbohydrates to forms that can be fermented to ethanol or to isobutanol. However, when cellulose-rich biomasses are used as an alternative to starch-rich maize grain as a source of sugars for ethanol production, large quantities of co-products need to be disposed of, preferably in a value-added process, possibly as animal feed. Fortunately, these cellulosic ethanol co-products are usually high in CP.

Ammonia fibre expansion (AFEX) is a pre-treatment process for cellulosic ethanol and may also be used to improve ruminant digestibility of feedstuffs not traditionally used as forages. During AFEX, concentrated aqueous ammonia is contacted with biomass under moderate temperatures (80–150 °C) and pressure (200–400 psi). After a short (5–30 minute) dwell time, the pressure is explosively released. This process has several physical and chemical effects on the lignocellulosic material that improve its digestibility. AFEX results in cellulose depolymerization and partial solubilization of hemicellulose. Solubilized hemicellulose and lignin components appear to be moved to the exterior of the cell walls during the process, opening up the structure to facilitate access to cellulose by ruminal microbes and enzymes. These changes dramatically increase the rate and extent of

both glucan and xylan release during enzymatic hydrolysis compared with untreated material. For cellulosic ethanol production, AFEX treatment can increase ethanol production from high fibre sources. For livestock producers, the important consideration is the feeding value of the remaining co-products, although AFEX treatment may also be a means of improving digestibility of high-fibre feed sources.

Bals *et al.* (2010) extracted 11 forages – including traditional forages, agricultural residues and dedicated energy crops (e.g. switchgrass) – using the AFEX process and digested *in vitro* with rumen inoculum. AFEX treatment improved 48-hour NDF digestion for several moderately indigestible forages compared with untreated samples, but showed no improvement for highly digestible samples such as alfalfa and maize silage. Of particular interest were maize stover and late-harvest switchgrass, as AFEX treatment improved digestibility by 52 percent and 128 percent over untreated material, whereas the improvement was 74 percent and 70 percent over conventional ammonia treatment, respectively. Weimer *et al.* (2003) included AFEX-treated rice straw at modest levels (70 g/kg DM) in a cattle diet and found improved milk yields and intake compared with untreated straw. An unknown at this time is whether feeding such products could support the high milk production needed to feed the world's future human population.

The CP content of all treated samples increased to more than 100 g/kg dry forage in the experiments by Bals *et al.* (2010). Scott *et al.* (2011) showed that AFEX + enzymatic hydrolysis of cellulose and hemicelluloses increased the N content and disappearance of plant constituents, but decreased the content of the major structural carbohydrates (ADF and NDF). The AFEX + enzymatic hydrolysis-treated forages could therefore be considered for use as a non-protein N supplement in combination with high-energy diets low in ruminally degradable protein.

A practical consideration may be to extract much of the leaf protein prior to AFEX or other treatments for cellulosic ethanol production (Dale *et al.*, 2009). Leaf protein properly processed to concentrate it and remove anti-nutritional factors will probably be at least as valuable in livestock diets as soybean meal protein. Leaf protein produced as a co-product of cellulosic ethanol production can be utilized by livestock (Kammes *et al.*, 2011). The effects of conservation method on protein extraction efficiency from orchardgrass (OG) and switchgrass (SG) were evaluated by Kammes *et al.* (2011). Two maturities of OG and SG were harvested with CP concentrations of 171 and 44 g/kg DM (immature) and 131 and 24 g/kg DM (mature) for OG and SG, respectively. Leaf juice was extracted with a screw press from fresh, stored or wilted chopped grasses. The liquid obtained was pH adjusted with HCl, treated with or without zinc salts (chloride), with or without heat, and then centrifuged to precipi-

tate leaf protein. Efficiencies of extraction were similar for fresh and stored grasses, which were both higher than for wilted grass. Leaf CP concentrations (g/kg DM) were approximately twice that of the original grass for all chemical and heat treatment combinations. *In vitro* degradation of OG leaf protein was evaluated using enzymes extracted from ruminants of lactating dairy cows. Fresh OG leaf protein treated with HCl + Zn salts at 140 °C had the greatest reduction in degradation compared with HCl control. There was an effect of conservation method on HCl + Zn salts 140 °C treated OG leaf protein, with similar degradability for stored (51.5 g/kg CP) and wilted (83.0 g/kg CP), which were higher than fresh (16.8 g/kg CP) after incubation for 4 hours. The authors concluded that leaf protein from fresh grass is most suitable because proteolysis during storage or wilting probably decreases its recovery and increases ruminal degradation, and both zinc salts and heat treatments decrease degradability of OG leaf protein within the rumen.

Scott *et al.* (2011) recently demonstrated similar results of increased nitrogen content, decreased NDF and improved fibre digestibility of reed canary grass hay, timothy hay, alfalfa hay, maize stover and barley straw with the AFEX, or AFEX followed by enzymatic hydrolysis and separation of the soluble sugars for ethanol production. The remaining solid co-product contained increased N and improved degradability of DM, NDF and total N in the rumen, as demonstrated via *in sacco* experiments.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Much research on new biofuel co-products has been conducted over the past decade. The goal of this research has been to determine optimal inclusion rates under various management and nutritional regimens. For practical application in the field, nutritionists and producers need additional information on how best to manage biofuel co-products for dairy cattle. These knowledge gaps and future research needs include:

- What is the optimal inclusion rate of biofuel co-products with different types of forages? Much of the research has been conducted with stored maize silage and alfalfa hay, but many other forage combinations exist. Distillers grain has been demonstrated as an excellent complement to fibrous residues (Anderson *et al.*, 2010) in growing dairy heifer diets. Further investigation is needed around the world to determine how biofuel co-products supplement fibrous residues in different production systems.
- What is the effect of biofuel co-products on milk composition? Past research has demonstrated that biofuel co-products can have a significant impact on milk composition. Much of this is related to ruminal fermentation and digestion. More research is needed to determine the effect of biofuel co-products on ruminal digestion, microbial protein synthesis and intestinal nutrient digestion, and how these affect milk composition with different types of diets.
- What is the impact of feeding biofuel co-products on nutrient digestion in dairy cattle? Limited research has been conducted to determine the impact on digestion of feeding biofuel co-products and subsequent excretion of nutrients to the environment. Excretion of certain minerals, such as phosphorus, is a concern in regions with intensive animal agriculture. Effects on greenhouse gas emissions also need investigation.
- Can variability of nutrient composition of co-products be reduced? Nutrient composition can vary considerably among different production plants. These differences can be attributed to factors such as the grain type, grain quality, milling process, fermentation process, water quality, drying temperature and the amount of solubles blended back to the distillers grain before drying. Lack of adjustment for changes in nutrient composition can result in diets not being formulated as intended. These changes can result in reduced animal performance.
- What is the effect on animal performance of interaction with other feeds of nutrients provided in ethanol co-products? High levels of polyunsaturated fat in combination with highly fermentable feeds and low effective fibre can negatively affect rumen fermentation. More work is needed to determine how biofuel co-products can be incorporated into diets without negatively affecting performance.
- What is the impact of feeding biofuel co-products on amino acid formulation? Diets high in maize co-products often result in a lysine deficiency. Further work is needed to determine amino acid availability from biofuel co-products for improving diet formulation for high-production dairy cows. Fast and reliable methods to determine lysine availability need to be perfected.
- There is limited research in feeding biofuel co-products to young calves, heifers and dry cows. More work is needed to define optimal and maximal inclusion rates for these categories.
- On-farm research of wet co-products storage is needed to best determine how small farms can store and utilize these co-products.
- Further work is needed to determine which feeds can be replaced by biofuel co-products to improve animal productivity, reduce environmental impact and reduce the cost of producing milk and meat. While many of the co-products are used currently as protein sources, it will become more commonplace to use them to replace energy feeds.
- What will be the nutrient composition of future biofuel co-products? Currently, many plants are removing a por-

tion of the oil by centrifugation, which is altering the composition of distillers grain. In addition, new biofuels will be developed, resulting in new co-products that potentially will be available for livestock feeding. Future work will be needed to determine how they best fit into dairy cattle diets.

CONCLUSIONS

Biofuel co-products, such as distillers grain with or without solubles, fractionated co-products and glycerol are excellent sources of protein and energy for dairy cattle. Research suggests that these co-products can replace more expensive sources of protein, energy and minerals. Because biofuel co-products can be highly variable, it is recommended they be tested to determine precise nutrient compositions and properly formulate diets. When balancing diets with various co-products, care must be taken to provide sufficient physically effective fibre to maintain normal rumen function and prevent milk fat depression in lactating cows. Nitrogen and phosphorus concentrations in biofuel co-product-based diets also need to be monitored to prevent excessive losses to the environment.

Maximum recommended levels of distillers grain for pre-weaned calves, growing heifers and dry cows are 25, 30 and 15 percent of the diet on a DM basis, respectively. Current recommendations for feeding distillers grain to dairy cows would be to include it up to 20 percent of the diet DM for either DDGS and WDGS. Diets with greater than 10 percent of the diet as DDGS or WDGS should be formulated using sound nutritional principles for dairy cattle respecting nutrient requirements. Glycerol can replace maize up to 15 percent of the diet for lactating dairy cows. As technology improves, new biofuel co-products will be developed and become available to livestock producers. These new co-products need to be evaluated individually with consideration of their unique nutritional profiles to determine optimal inclusion in diets of dairy cattle.

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Chapter 8

Utilization of crude glycerin in beef cattle

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ABSTRACT

Increased world demand for renewable fuels has prompted rapid expansion of the biodiesel industry, in which animal fats and plant oils are converted to combustible fuels and significant quantities of an edible byproduct, glycerin. Crude glycerin, which is approximately 75 to 85 percent glycerol, also contains water, minerals, and small amounts of residual methanol. Crude glycerin has a variety of applications in livestock feeding. Given its humectant properties, it is effective in agglomerating small feed particles, thereby reducing dust and maintaining homogeneity of mixed feeds. As a pelleting aid, it decreases energy expenditure associated with pelleting and improves durability and hygienic quality of pelleted feeds. In ruminants, the impact of glycerin on ruminal fermentation is well-documented. Acetate:propionate ratio generally decreases with addition of glycerin, presumably improving energetic efficiency. Inhibitory effects of glycerin on the fermentative activities of fibre digesting bacteria and fungi are evident, providing a plausible explanation for the decreases in fibre digestion often observed *in vitro* and *in vivo*. The deleterious effects of glycerin on fibre digestion are most evident when animals are fed diets containing higher levels of starch. It is apparent that the gut ecosystem adapts to the presence of glycerin, though it is not clear if this is a consequence of long-term population shifts or changes in capacity for substrate utilization by individual species of micro-organisms. Prior exposure to glycerin does enhance its utilization in cattle fed grain-based diets. Moreover, populations of pathogenic *E. coli* O157:H7 have been partially suppressed in the presence of glycerin when fed at low levels in the diet. Crude glycerin is a viable energy source for cattle, particularly when fed at less than 10 percent of the diet dry matter.

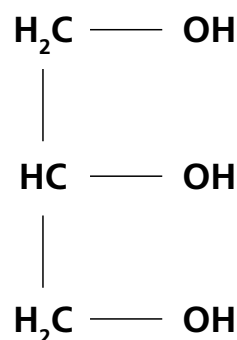
INTRODUCTION

Glycerin (glycerol) can be derived through production of alkyl esters (biodiesel) from plant oils or animal fats. Of the three processes available for alkyl ester production – oil conversion to fatty acids followed by acid-catalysed esterification; direct acid-catalysed esterification with methanol; and base-catalysed transesterification with methanol – the base-catalysed esterification is most economical, and therefore the most frequently employed process for biodiesel production (Van Gerpen, 2005). In base-catalysed esterification, fats and oils are reacted with methanol in the presence of potassium hydroxide, yielding glycerin (Figure 1) and alkyl esters. Residual methanol is reclaimed via distillation, and glycerin is recovered through evaporation following removal of methyl esters. Each 100 kg of oil or fat yields approximately 10 kg of glycerin (National Biodiesel Board, 2008).

Historically, glycerin has had a broad range of applications in human foods and pharmaceuticals, and has been used industrially for production of synthetic polymers, cosmetics and personal care products. It can be modified to yield mono- and diglycerides, which are important classes of emulsifying agents. Glycerin is a sweet (~60 percent the sweetness of sucrose), viscous liquid that has been used in beverages as a thickening agent, and exploited in food

systems as a result of its humectant properties (SDA, 1990). This latter attribute makes it attractive as an addition to animal feeds for texturing properties and dust control. Photo 1 illustrates the effect of glycerin when added at 12 percent of the diet dry matter in a typical feedlot ration. Levels of 4 percent or more are relatively effective in aggregating small feed particles, thus reducing dust and fines. In its pure form, glycerin is colourless; however, the colour of crude glycerin ranges from light amber to deep brown, and differences are largely attributable to varying concentrations of

FIGURE 1
Chemical structure of glycerin (glycerol)



MAIN MESSAGES

- Glycerin alters ruminal fermentation, increasing propionate production.
- Glycerin has a deleterious effect on fibre digestion in high-grain diets.
- Gut microorganisms can adapt to glycerin over time.
- Feed value of glycerin is greatest when it constitutes 10 percent or less of diet dry matter.

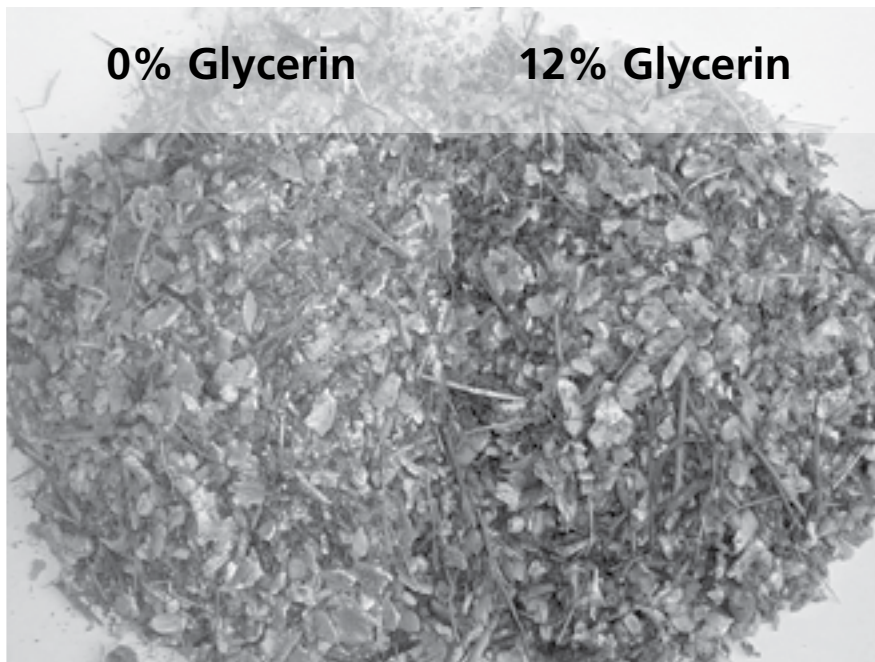


Photo 1
Effect of glycerin addition to a maize-based cattle finishing diet. Small particles are aggregated, minimizing segregation and dust

impurities within the co-product. Crude glycerin commonly contains 75-85 percent glycerol, with the balance of the crude liquid consisting of water, minerals, fatty acids and low [normally] concentrations of methanol.

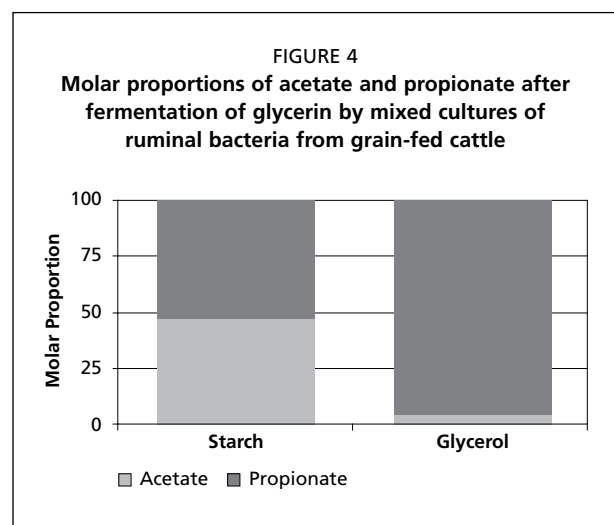
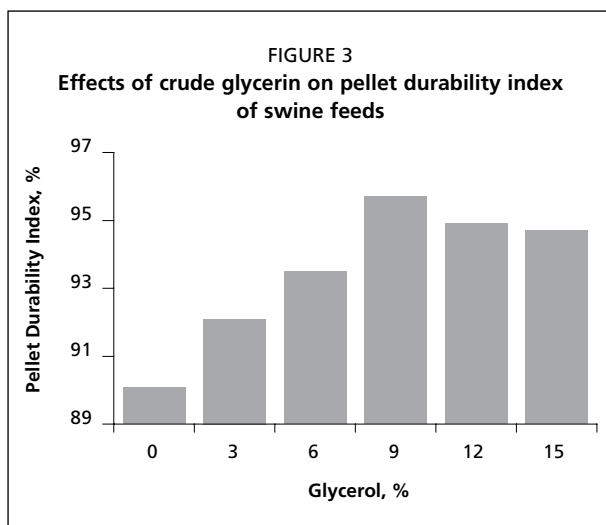
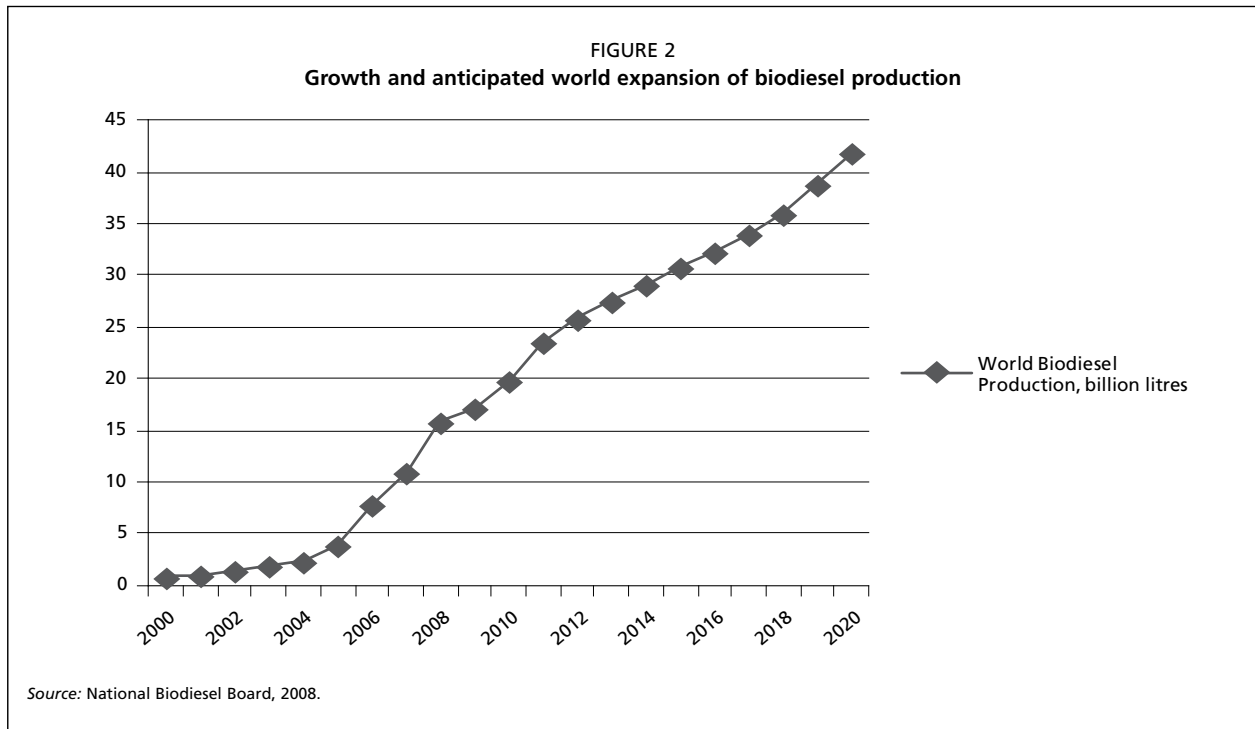
Figure 2 illustrates the recent dramatic growth in world biodiesel production and anticipated expansion through 2020 (OECD/FAO, 2011). Similar increases have been observed in the United States, resulting in large surpluses of crude glycerin that have caused market prices to plummet. The relatively low market value of glycerin has prompted interest in the co-product as a potential substitute for energy feeds in poultry and livestock diets. Given the large number of industrial applications for high purity forms of glycerin, it is probable that the price of crude glycerin will continue to increase as new markets are developed. Application rates in livestock and poultry diets will no doubt adjust over time in response to co-product prices in comparison with traditional energy sources.

Groesbeck (2007) evaluated crude glycerin as a pelleting aid in maize-based swine diets, and observed that energy costs associated with pelleting decreased linearly in response to adding glycerin to the mash at concentrations of 0 to 15 percent. The same author investigated the impact of glycerin addition on pellet durability indices (PDI) and found

that optimal PDI was achieved with approximately 9 percent glycerin (Figure 3). This ability to improve pellet durability while decreasing energy expenditure for feed processing has direct application to production of pelleted feeds for all types of livestock and poultry. Schröder and Südekum (2007) reported that the benefits to pellet stability were achieved only with high purity glycerin products. According to their observations, lesser qualities of glycerin, which may contain considerable quantities of water, are less apt to yield improvements. As a side benefit, Schröder and Südekum (2007) also noted that glycerin had a positive impact on hygienic quality of stored pellets, which they attributed to less fungal biomass in pellets containing glycerin.

FERMENTATION BY RUMINAL MICROBES

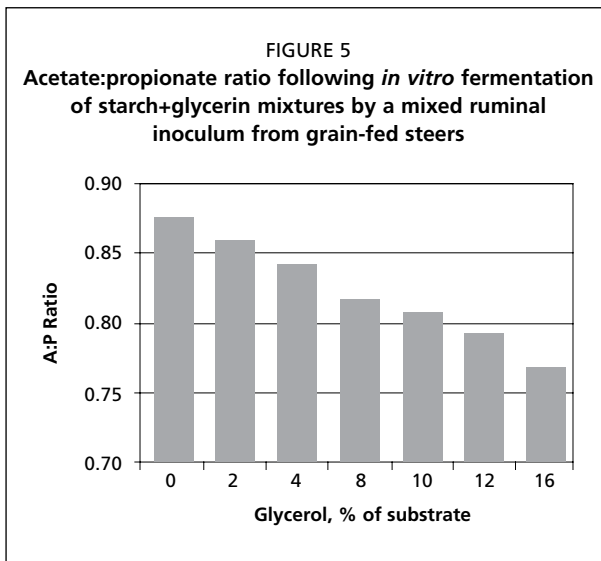
Our laboratory has conducted a series of *in vitro* experiments (unpublished) to evaluate the fate of glycerin when exposed to a mixed ruminal inoculum from grain fed animals. Figure 3 summarizes results of a study in which we compared maize starch and glycerin as substrates for fermentation. Starch yielded a fairly typical acetate:propionate ratio, whereas glycerin was fermented almost entirely to propionate. The conversion of glycerin to propionate by ruminal microorganisms is well-documented



in the scientific literature. Lee *et al.* (2011) reported decreases in the acetate-to-propionate (A:P) ratio as glycerol replaced alfalfa or maize silage in *in vitro* cultures of mixed ruminal microorganisms. We have noted similar effects in our laboratory for *in vitro* incubations in which maize starch was replaced by increasing proportions of pure glycerol (Figure 5; unpublished data). The A:P ratio decreased linearly as level of glycerin in the mixtures increased. Bergner *et al.* (1995) measured glycerin transformation by ruminal microorganisms using ¹⁴C-labeled glycerin, and observed that the majority of glycerin was converted to propionate, while no discernible amounts were converted to acetate. Similarly, Trabue *et al.* (2007) found that glycerol partially suppressed acetate production by ruminal microbes in inoculum taken from a dairy animal fed a

diet consisting of approximately 50 percent concentrate. In contrast, Wright (1969) determined that radio-labelled glycerin was converted to acetate, propionate and butyrate. The inoculum used in their study was extracted from cattle grazing clover-ryegrass pastures. Jarvis, Moore and Thiele (1997) utilized ruminal contents from red deer, and determined that a *Klebsiella planticola* strain transformed glycerin into approximately equimolar proportions of formate and ethanol. Collectively, these studies may suggest that metabolites of glycerin are influenced by the microbial milieu within the rumen, which obviously is a function of diet.

Digestion of fibre is of particular relevance in diets supplemented with glycerin. Roger *et al.* (1992) reported that cellulolytic activity was depressed by glycerol, noting that it



inhibited cellulolytic ruminal fungi far more than cellulolytic bacteria. Paggi, Fay and Faverin (2004) also reported deleterious effects of glycerin on cellulolysis, and suggested that the concentrations necessary for inhibition were consistent with levels capable of suppressing *Neocallimastix frontalis*, a ruminal fungus integrally involved in cellulolysis. Fungal colonization is an important step in the digestion of cellulose, especially for low quality forages. These observations could have important implications for diets that contain substantial amounts of cellulosic materials, including diets containing fibrous byproduct feeds derived from processed cereal grains, oilseeds, sugar cane and other agricultural commodities.

The impact of glycerin on fibre digestion has been the subject of studies conducted by several research groups, measuring fermentative end-products and concentrations of specific microbial populations often associated with fibre digestion. Abo El-Nor *et al.* (2010) investigated the impact of increasing proportions of glycerol (0, 3.6, 7.2 or 10.8 percent of substrate DM) on ruminal fermentation using continuous culture systems fed a substrate consisting of 60 percent alfalfa hay in combination with ground maize, soybean meal and soybean hulls. Total volatile fatty acid (VFA) production was greatest with the highest concentration of glycerol, A:P ratio declined linearly with increasing levels of glycerol addition, and digestibility of neutral-detergent fibre (NDF) decreased with the addition of 7.2 or 10.8 percent glycerol, perhaps suggesting that digestion of non-fibrous substrate was improved. Concentration of DNA from *Butyrivibrio fibrisolvens*, a key fibre-digesting organism in the rumen, decreased linearly in response to increasing levels of glycerol. Additionally, *Selenomonas ruminantium* and *Clostridium proteoclasticum* decreased with higher levels of glycerol, and total bacterial DNA decreased by nearly 32 percent with the highest level of glycerol addition. Based on these

observations, it is conceivable that high levels of glycerin affect not only fungi, but also may have deleterious consequences for ruminal bacteria. Krueger *et al.* (2010) reported decreases in A:P ratio with glycerol addition, though no negative effects on NDF digestibility were noted. Van Cleef *et al.* (2011a) found that the impact of glycerin on *in vitro* digestion was substantially influenced by prior exposure of donor animals to glycerin. *In vitro* digestibility of diets decreased in response to glycerin addition when ruminal digesta contents were obtained from cattle fed diets without glycerin, whereas diet digestion increased in response to glycerin addition when the ruminal inoculum was recovered from animals that had been adapted to a diet containing 15 percent glycerin (interaction, $P < 0.05$). Clearly, addition of glycerin to *in vitro* cultures can influence extent of digestion and end products formed, and these effects often are dependent on the levels of glycerin used in the *in vitro* systems.

IMPACT OF GLYCERIN ON *IN VIVO* DIGESTION

Given the impact of glycerin on ruminal microorganisms and *in vitro* digestion, changes in *in vivo* digestibility would be more-or-less expected. Parsons (2010) measured *in vivo* digestibility of grain-based diets in finishing cattle fed 0, 2 or 4 percent glycerin and determined that total tract digestion of dry matter was unchanged, while digestibility of NDF tended to decrease as the proportion of glycerin in the diet increased. Changes in NDF digestion were accompanied by decreases in ruminal concentrations of butyrate and valerate, but apparent total tract digestibilities of starch, protein and lipid were unaffected by glycerin addition to the diet. Schneider (2010) fed diets consisting of 60 percent maize silage and maize gluten feed, and noted that digestibility of organic matter and NDF decreased linearly when glycerin was substituted for maize gluten feed at 0, 4 or 8 percent of the diet. In contrast to these findings, Wang *et al.* (2009) actually observed improvements in digestibility of organic matter, NDF, protein and lipid (linear, $P < 0.01$; quadratic, $P < 0.01$) when glycerin was fed to steers at 0, 1.1, 2.2 and 3.3 g/kg DM in diets comprising 60 percent maize stover and 40 percent concentrate. Digestibility of nutrients in their study was optimized by feeding glycerin at 2.2 or 3.3 g/kg diet DM. The apparent differential effects of glycerin on fibre digestion in diets with and without starch are further supported by observations of Schröder and Südekum (2007), who reported improvements in fibre digestion in low-starch diets, while digestibility of fibre in high-starch diets was decreased with glycerin addition. It is conceivable that the deleterious effects of glycerin on fibre digestion are due to inhibition of specific populations of ruminal microorganisms that are important contributors to fibre digestion in animals fed starch-containing diets, but that are of lesser importance in roughage-based diets.

PERFORMANCE OF CATTLE SUPPLEMENTED CRUDE GLYCERIN

Use of glycerin as a component of cattle diets has been the subject of several recently published studies conducted in Europe, North America and Latin America. Pyatt, Doane and Cecava (2007) fed 0 or 10 percent crude glycerin in diets that were either 70 percent rolled maize with 10 percent distiller's grains, or 35 percent rolled maize with 30 percent distiller's grains and 15 percent soybean hulls. Glycerin decreased dry matter intake by approximately 10 percent, but improved conversion efficiency by 19 percent. Similarly, in a study by van Cleef *et al.* (2011b), feeding 7.5 or 15% glycerin to finishing cattle depressed feed intake, but improved efficiency of gain ($P < 0.01$). The authors also noted that intramuscular fat deposition was significantly less for cattle fed glycerin. Elam *et al.* (2008) also observed a linear reduction of dry matter intake ($P = 0.09$) in heifers fed 0, 7.5 or 15 percent crude glycerin, but efficiency was unchanged. Feeding glycerin also tended to decrease deposition of intramuscular fat within the *longissimus* muscle. The effects of glycerin feeding on intramuscular fat deposition are contrary to the popular belief that increasing proportion of glucogenic substrates in the diet will effect positive changes in the accumulation of intramuscular fat, which is the primary determinant of quality in beef grading systems used in United States, Canada, Australia and other countries. The absence of an improvement in intramuscular fat accretion, despite overwhelming evidence of increased propionate synthesis with glycerin supplementation, seemingly refutes this belief, and feeding glycerin may actually decrease value of carcasses as a result of suppression of intramuscular fat accretion. Parsons, Shelor and Drouillard (2009) conducted a dose titration of glycerin in flaked maize finishing diets for heifers, feeding concentration of 0, 2, 4, 8, 12 or 16 percent crude glycerin

(dry basis). Results of this study are shown in Table 1. Dry matter intake, daily gain and feed efficiency all responded in a quadratic manner to glycerin concentration. Optimal performance was achieved with 2 percent glycerin addition, and levels exceeding 10 percent of the diet depressed feed intake markedly.

As in the study by Elam *et al.* (2008), intramuscular fat deposition decreased linearly in response to increasing glycerin level in the diet ($P < 0.10$). Mach, Bach and Devant (2009) fed high-concentrate diets containing 0, 4, 8 or 12 percent glycerin to Holstein bulls and noted that performance was not statistically different among treatments, though the highest level of glycerin yielded numerically lower gain and carcass weight compared with other treatments. Gunn *et al.* (2011) replaced dry-rolled maize with a combination of glycerin, soybean hulls and maize gluten meal in fattening diets for early weaned steers, thus providing 0, 15 or 30 glycerin. Daily gains were 1.39, 1.33 and 1.07 kg/day for groups fed 0, 15 and 30 percent glycerin, respectively ($P < 0.01$), and feed intakes (dry basis) were 7.01, 6.06 and 5.05 kg/day, respectively. Efficiencies were not affected by amount of glycerin in the diet, however. Thus, it appears that excessive levels of glycerin are deleterious to growth of cattle, primarily as a result of the tendency to depress feed intake, while levels of 10 percent or less of the diet dry matter generally yield positive effects.

Given the impact of glycerin on microbial systems *in vitro*, it seems plausible that microbial adaptations will occur when glycerin is added to the diets of ruminants. Anecdotal observations would support this contention, as the differences in performance and feed intake of cattle fed high and low levels of glycerin appear more exaggerated during the early phases of feeding. Apercé *et al.* (2011b) reported a positive carryover effect of glycerin feeding, in which cattle fed glycerin during the

TABLE 1
Feedlot performance and carcass traits of heifers fed 0, 2, 4, 8, 12 or 16 percent crude glycerin in flaked maize finishing diets

Parameters	Percentage crude glycerin						SEM	Contrasts		
	0	2	4	8	12	16		Linear	Quadratic	Cubic
Number of heifers	62	62	61	63	63	62				
Final weight ⁽²⁾ (kg)	523 a	536 b	531 b	528 b	521 a	509 c	7.3	*	*	-
DMI (kg/day)	8.84 a	8.88 a	8.66 a	8.61 a	8.40 b	7.80 b	0.13	*	*	*
ADG (kg)	1.19 a	1.34 a	1.29 a	1.25 a	1.17 ab	1.03 b	0.09	*	*	-
Gain:feed ratio (g/kg)	136.2	151.6	149.6	145.8	139.7	132.4	7.0	-	*	-
Hot carcass weight (kg)	332	340	337	335	331	323	4.6	*	*	-
Dressing yield (%)	63.0	64.1	64.2	63.3	63.4	63.6	0.01	-	-	†
Longissimus muscle area (cm ²)	83	86	84	83	82	81	1.5	*	-	-
Marbling score ⁽³⁾	435	405	416	398	410	397	9.7	*	-	-
Subcutaneous fat over 12th rib (cm)	1.21	1.10	1.18	1.18	1.18	1.02	0.06	*	-	*

Notes: abc = Means in rows not having a common suffix differ $P < 0.05$. DMI = dry matter intake; ADG = average daily gain. (1) Contrasts: * = $P < 0.05$, † = $P < 0.10$. (2) Calculated by dividing Hot carcass weight by a common dressing percentage of 63.5 percent. (3) Marbling scores are indicative of intramuscular fat deposition. Higher scores indicate greater amounts of fat. Source: Parsons, Shelor and Drouillard, 2009.

growing period remained more efficient when fed diets without glycerin in the subsequent finishing phase. The finishing diets in this study comprised (dry basis) 30 percent maize gluten feed, 10 percent maize silage, dry-rolled maize and supplement. Maize gluten feed, which is a by-product of maize refining for production of sweeteners, contains appreciable amounts of glycerin. Wu (1996) determined that glycerol content of maize gluten feed was approximately 4.9 percent of dry weight. We have speculated that the carryover effect observed in the study by Aperce *et al.* (2011b) may reflect adaptation to glycerin by ruminal microorganisms, which when presented as a constituent of maize gluten feed is more readily fermented, as seen in the *in vitro* experiments conducted by van Cleef *et al.* (2011a). Interestingly, distillers grain, which is the principal by-product produced during fermentation of cereal grains for production of alcoholic beverages or fuel ethanol, also contain appreciable quantities of glycerol. We have measured levels of 10 percent or more of dry weight as glycerin, which is consistent with values reported by Wu (1994). In our experiments we have observed that relatively small quantities of glycerin can impair fibre digestion, though this effect seems less apparent in diets containing distiller's grain. This may be due to the fact that the glycerin that is an inherent component of distillers grain has itself suppressed fibre digestion, such that further additions of glycerin to the diet have only modest impact.

The ability to affect specific populations of gastrointestinal tract microorganisms may have other applications in cattle production systems, including control of food-borne pathogens. We previously reported that distillers grain, which now is used extensively in food animal production systems throughout North America, may increase shedding of food-borne pathogens, namely *E. coli* O157:H7 (Jacob *et al.*, 2008a, b, 2009). This may be a direct effect whereby some component of distillers grain stimulates growth of *E. coli* O157:H7, or indirectly as a result of substrate availability or other factors that influence competitiveness of the pathogen in the hind gut. In a recent study reported by Aperce *et al.* (2011a), the percentage of cattle testing positive for *E. coli* O157:H7 in faeces was decreased from 5.8 percent in cattle fed diets without glycerin, to 4.3 and 2.4 percent for cattle diets containing 4 or 8 percent glycerin, respectively (Linear, $P < 0.01$). The diets fed to these animals, including cattle in the control group, all contained 30 percent wet maize gluten feed, and therefore probably contained some base level of glycerin. It is unclear how crude glycerin derived from biodiesel production can decrease pathogen shedding, while feeding distillers grain, which itself contains glycerin, can increase pathogen shedding rates. Additional work is needed to corroborate these observations, not only for *E. coli* O157:H7, but also for other important shiga-toxin producing pathogens.

CONCLUSIONS

Crude glycerin is likely to increase in availability as a result of continued expansion of the biodiesel industry. Glycerin is an adaptable raw material suited to numerous industrial applications, perhaps suggesting that its use as a livestock feed may be quickly supplanted by higher value applications. As a feed resource, crude glycerin can be utilized effectively in diets for cattle to improve ruminal fermentation, rate of gain and growth efficiency. Glycerin consistently decreases acetate:propionate ratio, and may have inhibitory effects on fibre digestion, which is mediated via its inhibitory effects on some microbial populations. Concentrations less than 10 percent of the diet dry matter yield favourable biological responses in cattle, whereas levels in excess of 10 percent may have deleterious consequences for feed intake and growth of cattle.

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Chapter 9

Nutritional value and utilization of wheat dried distillers grain with solubles in pigs and poultry

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ABSTRACT

Dried distillers grain with solubles (DDGS) are a co-product of ethanol production from starch cereals (mainly maize in North America and wheat in Europe), which contains approximately the non-starch or non-fermentable fractions of the grain. As more becomes available with increasing bio-ethanol production, DDGS is being included not only in diets for ruminants but also in pig and poultry diets. This review paper considers the introduction possibilities of wheat DDGS in poultry and pig diets. Nutrients content in wheat DDGS and digestibility vary among ethanol plants, reflecting the starch extraction process and drying of the residues after starch extraction. Most of the variability concerns amino acid (AA) contents and their standardized ileal digestibility (SID), affected by the occurrence of Maillard reactions, reflected in the lightness score (L) of wheat DDGS. Samples with low L values (<50) are dark and have the lowest nutritional value, both in pigs and in poultry. Lysine is the most affected AA, with contents ranging between 0.9 (dark) and 3.0 percent of crude protein (CP; N×6.25) for wheat DDGS. In parallel, lysine SID is also variable, with the lowest values observed in DDGS with low lysine level in CP. For the darkest products, lysine SID is close to zero. Energy digestibility varies in parallel with changes in lysine content and L values, but is more related to other nutrients such as dietary fibre, fat and residual starch contents. In addition, wheat DDGS can supply significant amounts of available phosphorus. DDGS from wheat can be used in diets for poultry and pigs; however, in relation with nutritional value variability, practical use should take into account correct energy values or lysine SID content in order to prevent any performance deterioration. Finally, only the light-coloured products are recommended for non-ruminants, and their introduction at high inclusion rates will contribute to reducing the energy value of the diet.

INTRODUCTION

In recent years, ethanol production, as a partial substitute for petrol, has rapidly increased (Windhorst 2007; ePURE, 2010; RFA, no date). Ethanol is produced via enzymatic breakdown of starch and yeast-controlled fermentation of glucose into ethanol. Second-generation ethanol production, based on cellulose, is still in a development phase. The first generation production is mainly based on sugar cane in Brazil and maize in the United States, whereas in Canada and Europe wheat, triticale or barley are used in ethanol plants. Dried distillers grain with solubles (DDGS) is the primary co-product of this production when based on cereals. Mainly used initially in ruminant feeds, this co-product has become more available for non-ruminants, reflecting the increased supplies and also better nutritional information about this co-product. However, knowledge of its characteristics, its nutritional value and its acceptability and practical utilization in feeds for monogastric animals is

rather recent, especially for wheat DDGS. More abundant literature is available for maize DDGS, both for pigs (Stein and Shurson, 2009) or poultry (Batal and Dale, 2006). Thus, the objective of this review is to summarize recent results on wheat DDGS in poultry and pigs. It is partly based on the previous reviews of Cozannet *et al.* (2009, 2010d) and the results of a research project conducted in France on European wheat DDGS (Cozannet *et al.*, 2010a, b, c, 2011).

COMPOSITION AND CHEMICAL CHARACTERISTICS OF WHEAT DDGS

Ethanol production from wheat consists of extracting, hydrolysing and fermenting the starch fraction of the grain. It can then be assumed that the residue of that extraction that corresponds to the so-called wheat DDGS is more or less equivalent to the non-starch fractions of the grain. As for maize DDGS (Stein and Shurson, 2009), the wheat DDGS composition is then first dependent on the nutrient

MAIN MESSAGES

- Wheat DDGS, a co-product of the wheat ethanol industry, contains high levels of crude protein (ca 30 percent), but with a low and variable content of lysine. This variability in lysine level is dependent on the ethanol production process and the heat damage occurring during the DDGS drying process.
- In both pigs and poultry, the ileal digestibility of lysine in DDGS is lower than in wheat, and is also quite variable, with the lowest values in heat-damaged products.
- Overall, heat-damaged DDGS should not be fed to non ruminants; the dark colour of such products is an indicator of their poorer nutritional value.
- The energy value of wheat DDGS for pigs or poultry is lower than for wheat and is dependent mainly on their dietary fibre content.
- Standard or high quality wheat DDGS can be included at high levels in poultry or pig diets (up to 30 percent) without marked detrimental effects on performance, as far as they are included in diets meeting the animals' overall nutrient requirements.
- Overall, wheat DDGS represents a valuable source of energy and protein for non-ruminant animals, but attention should be paid to the variable composition and nutritional value of DDGS when formulating diets.
- Wheat (and maize) DDGS will evolve over the near future, with more fractionation of the nutrients other than starch; an increased use of enzymes; improvements and diversification of ethanol production technologies—all these contributing new opportunities for feeding pigs and poultry.

TABLE 1

Composition of wheat dried distillers grain with solubles (DDGS) and its comparison with wheat and maize dried distillers grain with solubles

	Wheat ⁽¹⁾	Wheat DDGS ⁽²⁾		Maize DDGS ⁽³⁾
		Mean	Min. – Max.	
Dry matter (DM)	86.8	92.7	89.3 – 94.4	88.9
Composition (as % of DM)				
Ash	1.8	5.0	4.6 – 5.7	5.8
Crude protein (N×6.25)	12.1	36.6	32.7 – 39.2	30.0
Crude fat	1.7	4.4	3.4 – 5.1	10.7
Crude fibre	2.5	7.6	6.1 – 9.0	8.6
Neutral detergent fibre (NDF)	14.3	30.1	25.4 – 35.3	41.5
Acid detergent fibre (ADF)	3.6	10.7	8.1 – 13.1	16.1
Acid detergent lignin (ADL)	1.2	3.2	2.1 – 4.5	
Starch	69.7	5.1	2.5 – 10.1	8.2
Sugars	2.8	4.0	2.4 – 7.2	
Gross energy (MJ/kg) ⁽⁴⁾	16.20	18.67	18.24 – 19.10	20.21

Notes: (1) Sauvant, Perez and Tran, 2004. (2) n = 7; products with luminance >50; Cozannet *et al.*, 2010a. (3) n=12, for dry matter, ash, protein, crude fat, crude fibre, NDF, ADF – Spiehs, Whitney and Shurson, 2002; n = 10, for gross energy and starch – Pedersen, Boersma and Stein, 2007. (4) Gross energy is standardized for a 89% DM content.

composition of the grain. Consequently, the nutrients, except starch, would be expected to be approximately three fold higher in wheat DDGS than those in the original grain (Table 1).

However, in practice, the chemical composition of wheat DDGS is much more variable than in the original cereals, with large differences among ethanol plants according to the method of grain preparation, namely with or without previous dehulling; the fermentation process; the amount of soluble fractions blended with distillers grain; the duration and temperature of drying; and possible further fractionation of the non-starch fractions (separation of proteins, etc.) (Belyea, Rausch and Tumbleson, 2004). There are two main technologies in use, resulting in co-products

with different starch content (Cozannet *et al.*, 2010a). The first involves entire grain grinding and fermentation, leaving a low-starch-content DDGS (<7%); whereas in the other process wheat bran is removed, leaving a higher-starch-content DDGS (>7%). In addition, reflecting the technical aspects among and within processes, the colour of DDGS can vary from light yellow to dark brown (Photo 1).

Measured with a Minolta colorimeter, luminance (L) values of 10 European wheat DDGS ranged from 43 (black products) to 63 (yellow products) in the study of Cozannet *et al.* (2010a). From their results it can be inferred that wheat DDGS with L values <50 have been overheated, with a high incidence of Maillard reactions. This agrees with previous recommendations for maize DDGS (Cromwell,



Photo 1

Range in colour of wheat DDGS; L*, a* and b* correspond to lightness, red index and yellow index, respectively

TABLE 2

Concentration of crude protein (CP) and amino acids (AA) in wheat and wheat dried distillers grain with soluble (DDGS)

	Wheat ⁽¹⁾	Wheat DDGS ⁽²⁾	
		Mean	Min – Max
Crude protein (as % of DM)	12.1	36.6	32.7 – 39.2
Essential AA (% CP)			
Arg	5.1	4.3	3.7 – 4.6
His	2.3	2.1	1.9 – 2.2
Lys	2.9	2.3	1.7 – 3.0
Phe	4.7	4.5	4.3 – 4.6
Leu	6.8	6.5	6.2 – 6.8
Ile	3.6	3.5	3.4 – 3.5
Val	4.4	4.3	4.2 – 4.4
Met	1.6	1.5	1.4 – 1.5
Thr	3.1	3.0	2.9 – 3.1
Trp	1.2	1.1	1.0 – 1.2
Total	35.7	33.0	31.2 – 34.4
Non-essential AA	61.9	56.3	53.9 – 57.7

Notes: (1) Sauvant, Perez and Tran, 2004. (2) n = 7; products with luminance > 50 – Cozannet *et al.*, 2010b.

Herckelman and Stahly, 1993; Pahm *et al.*, 2008a, b) defining an L value of 30 as the limit between overheated and standard products. In addition, only light-coloured DDGS have a sweet and fermented smell. Finally, the fermentation products correspond to 93 percent ethanol, 3 percent yeast and 4 percent glycerol (Hazzledine, 2008). Most of the non-ethanol components will be recovered in the DDGS residue and affect its composition.

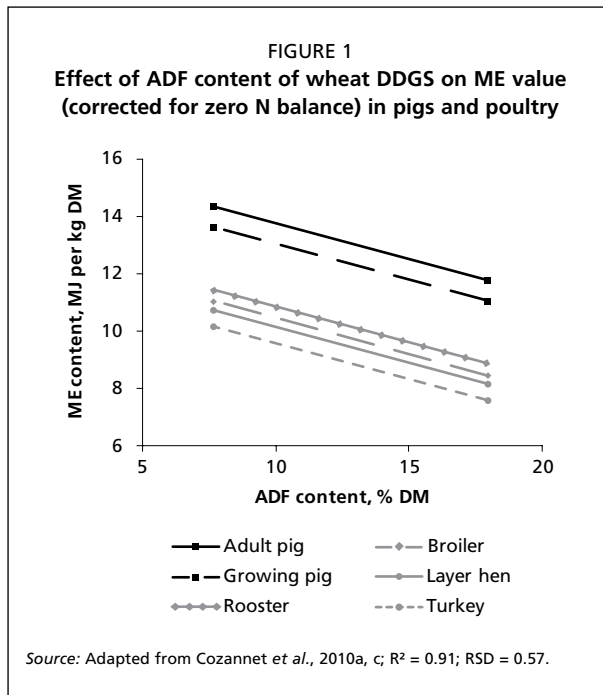
Average protein and essential amino acids (AA) contents for seven samples of European wheat DDGS are summarized in Table 2; these results agree with the data of Bandegan *et al.* (2009) obtained for Canadian wheat DDGS. According to the process, AA profiles (% N×6.25) should be in close agreement with those of the initial cereal. Nevertheless, yeasts used for starch fermentation represent an additional protein source, equivalent to about 5 percent of the total DDGS protein content (Ingledew, 1993). In addition, the level of soluble fractions added into distillers grain may be variable and influence the protein content and the AA profile. Despite these potential sources of variability, the AA profile is quite comparable in wheat and wheat DDGS, except for lysine and arginine, which are lower in DDGS (Table 2). In addition, even though crude protein (CP) con-

tents are rather constant between wheat DDGS samples, the lysine and arginine levels in CP are highly variable, even in light products: 1.7 to 3.0 percent and 3.7 to 4.6 percent, respectively (Cozannet *et al.*, 2010b). Consequently, unlike wheat or its milling co-products, poor correlations exist between lysine or arginine contents (as percentage of dry matter (DM)) and CP content. In other words, CP content cannot be used as a single indicator of lysine or arginine levels in wheat DDGS. These assumptions are more obvious when dark DDGS samples are included in the relationship, with lysine level being as low as 1 percent of CP (Table 5).

The sum of crude fat, CP, neutral detergent fibre (NDF) (or total dietary fibre – TDF; Prosky *et al.*, 1985), sugars, starch and ash is usually about 100 percent on a DM basis, especially for the grain. In the case of wheat DDGS, it is only 85 to 90 percent, and even less in low-L-value samples (Table 1). No clear interpretation of this situation is available: the presence of Maillard reaction components not included in the above chemical analyses may (partly) explain the difference. Some sources also indicate a sum higher than 100 percent, probably due to analytical mistakes and an overestimation of the dietary fibre fractions that can contain proteins (Stein *et al.*, 2006; Table 5). Sodium sulphite may be used in order to prevent this difficulty and to achieve lower NDF values (Kleinschmit *et al.*, 2006). This phenomenon is most important in the darkest samples with high rates of Maillard reactions, and nitrogen in NDF or acid detergent fibre (ADF) may then vary considerably between light and dark products (Table 5). For the same reasons, the analysis of lysine may be complicated and the interpretation of analytical results quite complex in connection with the blockage of a variable fraction of the lysine (Pahm *et al.*, 2008a, b; Cozannet *et al.*, 2011). Again, these difficulties are most important for low-L-value wheat DDGS. Overall, analytical difficulties are quite frequent for wheat and maize DDGS, and the interpretation of the results may be difficult. This also means that the DDGS chemical parameters measured cannot always be used for predicting accurately nutritional values such as net energy (NE) content of this co-product.

ENERGY VALUE OF WHEAT DDGS

Gross energy content is higher in wheat DDGS than in wheat (18.7 vs 16.2 MJ/kg; Sauvant, Perez and Tran, 2004; Table 1) due to the higher fat and CP contents. But, as for maize DDGS, and due to their variation in nutrient content and their high dietary fibre (DF) content, the metabolizability of energy in cockerels or the digestibility coefficient of energy in young or adult pigs are markedly lower for wheat DDGS than for wheat (minus 20 points) with digestible (DE) or metabolizable (ME) values lower for wheat DDGS than for wheat (minus 3 to 4 MJ ME/kg). The average energy values with European DDGS for pigs and poultry are reported in Tables 3 and 4. In addition, the energy values



of wheat DDGS are variable according to species and physiological stage, with most of the variation related to the DF content. In the case of ADF used as predictor of the DF content, Figure 1 indicates that the ME values are reduced by 0.24 MJ for each 1 percent increase in ADF; the coefficient does not differ significantly between the different groups of animals. This figure also illustrates that the ME values differ between animal species and physiological stages, with higher values in pigs than in poultry and also lower energy values in the growing animal (i.e. broilers or growing pigs) than in adults (i.e. cockerels or sows).

Overall, it can be concluded that the high DF content of DDGS penalizes their energy value, with a subsequent preferential use of these co-products in low-energy diets or in animals with greater ability to use the high DF feeds efficiently (adult pigs, for instance). These comparisons between species and physiological stages also illustrate that the relative energy values of ingredients are variable, with fibre-rich ones being better used in animals able to efficiently degrade the DF fractions of the feed or to tolerate higher DF levels in the feed (pigs vs poultry; adult vs young).

The DE or ME concepts have been used above for estimating the energy values of DDGS in order to compare the

TABLE 4
Energy digestibility and energy values of wheat dried distillers grain with solubles (DDGS) in growing and adult pigs

	Growing pig	Adult pig
Energy digestibility (%)	69.5	74.4
Energy values (MJ/kg)		
DE	12.96	13.86
ME	12.17	12.93
NE	7.89	8.77

Notes: DM content is standardized at 89%; n = 7; products with luminance > 50; DE = Digestible energy; ME = metabolizable energy; NE = net energy. Source: Cozannet *et al.*, 2010a. For comparison, the ME values of wheat average 13.7 and 13.9 MJ per kg (89% DM) in growing and adult pigs, respectively.

energy values on a common basis. There is no NE system available for poultry, while it is widely used in pigs with, as for any ingredient, a calculation of the NE value from DE value and crude fat, starch, CP and CF measurements (equation no. 4 in Noblet *et al.*, 1994; see also EvaPig, 2008). In connection with its high DF and CP contents, the NE/ME ratio in wheat DDGS is rather low (61 percent vs 78 percent for wheat; EvaPig, 2008) with a subsequent energy value expressed as a percentage of the energy value of wheat that is markedly lower in NE than in the DE or ME systems (Table 4). In practice, this means that for ingredients like DDGS, the NE system should be preferred, at least for pigs.

PROTEIN VALUE OF WHEAT DDGS

The protein value of ingredients for monogastric animals is usually estimated as the ileal digestibility of N and AA at the end of the small intestine, and the values are standardized to take into account the “basal” endogenous N and AA losses not related to the quantities of protein and AA included in the ingredients (Stein *et al.*, 2007). The so-called standardized ileal digestibilities (SID) of essential AA of wheat DDGS measured with caecotomized roosters and ileo-rectal anastomized pigs are presented in Table 5. Results indicate that most AA in wheat DDGS have a SID that is approximately 5 to 10 percentage units less than for wheat; that is mainly a consequence of the greater concentration of dietary fibre in DDGS than in cereals. But the difference is more accentuated for lysine (minus 20 points), reflecting presumably a loss in digestibility due to the drying of DDGS. In addition, the SID of

TABLE 3
Apparent metabolizable energy corrected for zero nitrogen deposition (AMEn) and AMEn/gross energy ratio in wheat dried distillers grain with solubles (DDGS) fed to cockerels, layers, broilers or turkeys

	Rooster		Layer		Broiler		Turkey	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
AMEn/GE, %	51.3	47.3–55.1	48.1	46.4–49.8	48.2	41.9–56.5	45.5	42.0–49.7
AMEn, MJ per kg	9.55	8.76–10.08	8.94	8.64–9.33	8.96	7.78–10.35	8.49	7.99–9.11

Notes: DM content is standardized at 89%; n=7; products with luminance > 50. Source: Cozannet *et al.*, 2010c. For comparison, the AMEn value of wheat in cockerels averages 12.8 MJ/kg at 89% DM; Sauvant, Perez and Tran, 2004).

TABLE 5
Standardized ileal digestibility of crude protein and amino acids (AA) of wheat dried distillers grain with solubles (DDGS) in caecotomized cockerels and ileo-rectal anastomized pigs

	Cockerel	Pig
Crude protein	82	82
Essential AA		
Arginine	78	88
Histidine	78	84
Lysine	61	69
Phenylalanine	88	89
Leucine	83	85
Isoleucine	79	76
Valine	81	79
Methionine	81	79
Threonine	73	80
Tryptophan	75	82
Total	78	82
Non-essential AA	84	84

Notes: n=7; products with luminance > 50. Sources: Cozannet *et al.*, 2010b, 2011.

lysine appears highly variable (Figure 2), since it ranged from 0 to 71 percent in roosters (Cozannet *et al.*, 2011) and from 9 to 83 percent in pigs (Cozannet *et al.*, 2010b) for 10 samples of wheat DDGS; the lowest values were observed in dark products with the probable occurrence of Maillard reactions (Table 6). These results suggest that colour determination might be a quick and reliable method for estimating the lysine digestibility of DDGS or, at least, identifying DDGS

sources with a poor AA digestibility. Nevertheless, Cozannet *et al.* (2010b; 2011) obtained a poor relationship between lysine digestibility and colour score for 10 samples of wheat DDGS, either in pigs or in roosters.

A better prediction was obtained with lysine content in CP according to a quadratic regression model (Figure 3) or a linear-plateau regression model with breakpoints of 1.9 percent lysine in CP either in roosters or in pigs, corresponding with 63 percent and 68 percent plateau SID values, respectively. The relationship between L values and lysine content of CP indicates that this breakpoint lysine percentage corresponds to an L value of 50. Overall, these data suggest that dark products with L values <50 have low and variable lysine content in CP and low and variable lysine SID values in both pigs and poultry. Consequently, they should not be used in feeds for non-ruminants. For light-coloured products, the situation is less critical, but attention should still be paid to lysine, which is less digestible than most other AAs, and to the lysine content of CP, which remains rather low in comparison with the requirements of growing birds or pigs.

MINERALS AND PHOSPHORUS VALUE OF WHEAT DDGS

As for the other nutrients, minerals are three times more concentrated in wheat DDGS than in wheat grain (Table 7). This is particularly true for potassium, calcium and phosphorus. However, the sodium content is greater than what could be expected from the inherent mineral content in

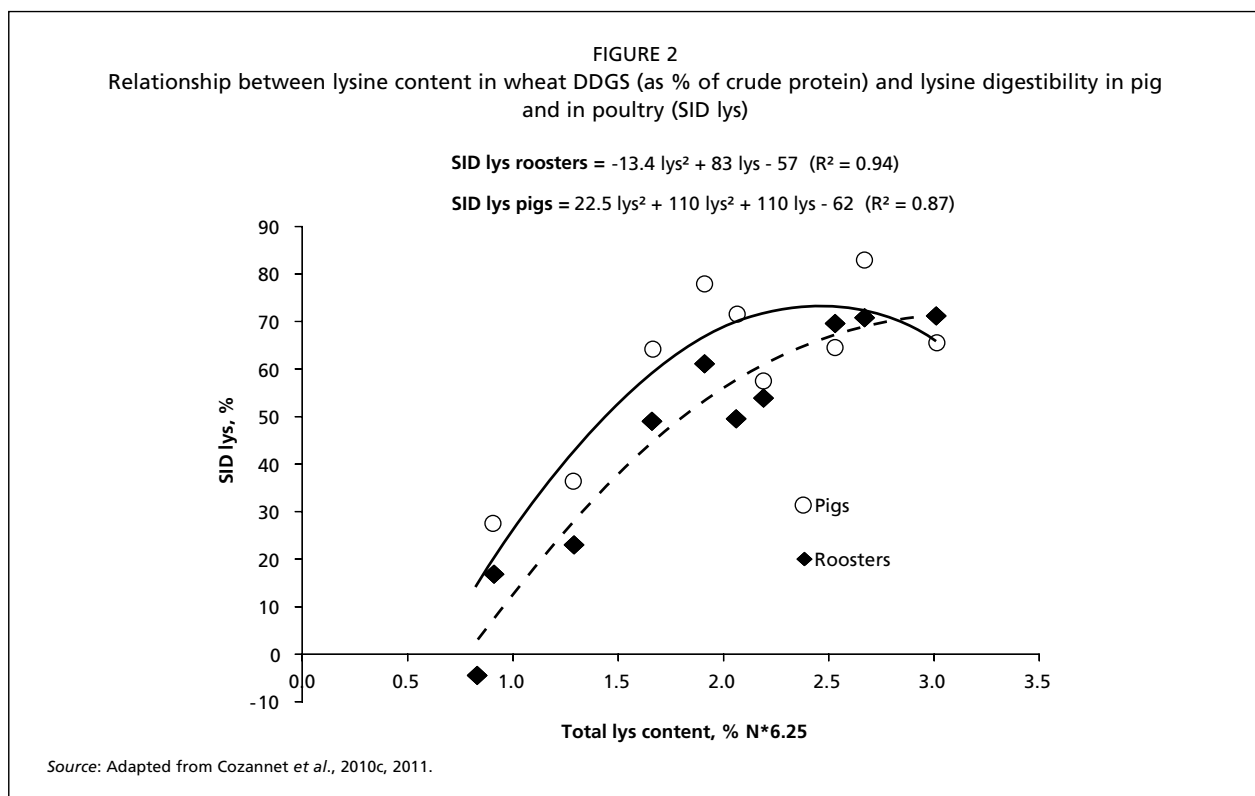


TABLE 6
Digestive utilization of nutrients of wheat dried distillers grain with solubles (DDGS) in poultry and pigs: impact of colour

	Dark ⁽¹⁾	Light ⁽¹⁾
Lightness (L value)	46.2	57.4
Dietary fibre (as % of DM)		
NDF	33.6	30.1
ADF	18.4	10.7
Crude protein in ADF (as% of DM)	6.8	1.1
Lysine content (% of N×6.25)	1.01	2.29
Amino acid digestibility in rooster (%)		
Crude protein	59.8	81.8
Non-essential AA	64.1	83.9
Essential AA	51.0	78.0
Lysine	12	61
Amino acid digestibility in pig (%)		
Crude protein	66.7	81.9
Lysine	24	69
AMEn value in poultry (MJ/kg) ⁽²⁾		
Rooster	8.38	9.55
Layer	7.62	8.94
Broiler	8.31	8.96
Turkey	7.25	8.49
DE value in pigs (MJ/kg) ⁽²⁾		
Growing pig	11.18	12.96
Adult pig	11.99	13.86

Notes: AA = amino acid; ADF = acid-detergent fibre; NDF = neutral-detergent fibre; AMEn = apparent metabolizable energy corrected for zero nitrogen deposition. (1) 3 dark products and 7 light products. (2) DM content is standardized at 89% for AMEn and DE values. Sources: Cozannet *et al.*, 2010a, b, c, 2011.

wheat grain. This extra source of sodium derives from sodium hydroxide (NaOH) used in the industrial ethanol process. For sulphur, even if no published data is available for

wheat DDGS, those published for maize DDGS (Waldroup *et al.*, 2007) show that extra sulphur is related to the addition of sulphuric acid (H₂SO₄) in the process. It would be similar in some plants producing wheat DDGS. For poultry, a sodium imbalance could lead to lower feed intake (low-sodium diet) or greater water consumption (high-sodium diet), which may increase the incidence of wet litter or dirty eggs. These values should also be taken into account when calculating the electrolytic balance of the diets.

Phosphorus is mainly present in the form of phytic phosphorus in wheat (Table 7; 65 percent) which is not digestible in pigs or poultry (no digestive phytase activity). As reported by several authors (Waldroup *et al.*, 2007), there could be a heat destruction of phytate during drying, but mainly a phytate hydrolysis by *Saccharomyces cerevisiae* during the fermentation stage (Martinez-Amezcu, Parsons and Noll, 2004.). Thus, Widyaratne and Zijlstra (2007) demonstrated a partial hydrolysis of inositol phosphate 6 (IP6) of wheat used for ethanol production into lower inositol phosphates (IP5, IP4, etc.) in wheat DDGS. The same result has been confirmed in 7 wheat DDGS samples (Table 7; P. Cozannet, unpublished data). Thus, wheat DDGS phosphorus digestibilities ranging from 50 to 62 percent were measured in pigs (Nyachoti *et al.*, 2005; Widyaratne and Zijlstra, 2007; Yañez *et al.*, 2011); these values are in agreement with those of Pedersen, Boersma and Stein (2007) for maize DDGS. However, there is a lack of data for poultry, even if we can hypothesize, in parallel with pig data, that wheat DDGS phosphorus availability should be at least 60 percent. When considering these data, one should be aware that several factors could affect phosphorus content and digestibility. Thus, in the case of maize DDGS, the extent of addition of solubles to the wet grain prior to drying affects the phosphorus

TABLE 7
Mineral composition and phosphorus digestibility of wheat dried distillers grain with solubles (DDGS) and comparison with wheat and maize dried distillers grain with solubles

Mineral content (as% of DM)	Wheat ⁽¹⁾	Wheat DDGS ⁽²⁾		Maize DDGS ⁽³⁾
		Mean	Range	
Sodium	0.01	0.36	0.24 – 0.63	0.22
Sulphur	0.17	0.65	–	0.84
Potassium	0.46	1.07	0.94 – 1.13	0.96
Calcium	0.08	0.22	0.14 – 0.39	0.08
Magnesium	0.12	0.29	0.26 – 0.31	–
Zinc	0.31	–	–	–
Copper	0.06	–	–	–
Total Phosphorus	0.37	0.86	0.80 – 0.97	0.70
Phytic Phosphorus	0.24	0.23	0.07 – 0.45	–
Phytic P/Total P (%)	65	27	8 – 54	–
Pig P digestibility ⁽⁴⁾ (%)	30	–	50 – 62	59
Poultry P availability (%)	58	–	–	62

Notes: (1) Data from Sauvont, Perez and Tran, 2004. (2) Unpublished data from Cozannet and co-workers; n = 7; products with luminance > 50; completed with average values for magnesium and sulphur from Sauvont, Perez and Tran, 2004, and Piron *et al.*, 2008. (3) Data from Waldroup *et al.*, 2007, based on a literature review. (4) Wheat P digestibility values 30% and 45% without and with endogenous wheat phytase – Sauvont, Perez and Tran, 2004; wheat DDGS values from Nyachoti *et al.*, 2005, Widyaratne and Zijlstra, 2007, 2008, and Yañez *et al.*, 2011; maize DDGS value from Pedersen, Boersma and Stein, 2007. n = 10.

content because the solubles contain three times more phosphorus than wet grain (Martinez-Amezcuca *et al.*, 2007). The drying temperature can also improve maize DDGS phosphorus bio-availability. For instance, Martinez-Amezcuca and Parsons (2007) showed an increase from 69 percent in the control DDGS to as much as 91 percent in the highest-heat treated DDGS sample. But, with these highest drying temperatures, lysine digestibility was markedly depressed. Finally, in the case of wheat DDGS, phytic phosphorus is mainly concentrated in the aleurone layer (Pointillart, 1994), which is one of the outer membranes of the grain, while phytic phosphorus is located mainly in the germ in maize. Some ethanol plants remove the bran from the wheat grain at the beginning and re-introduce it at the end of the process, thus leading to less hydrolysis of phytate phosphorus (52 and 54 percent Phytic P/Total P ratio for this type of process; P. Cozannet and co-workers, unpublished data, Table 7). We can then hypothesize that such wheat DDGS would have a lower phosphorus digestibility, probably close to the values for wheat or wheat gluten (30 and 28 percent P digestibility in pigs, respectively; Sauvante, Perez and Tran, 2004).

PERFORMANCE IN POULTRY AND PIGS FED WHEAT DDGS

Most results on performance of poultry and swine fed DDGS concern maize DDGS, and due to the relative similarity between wheat and maize DDGS, the expected performance and recommendations for wheat DDGS should be close to those for maize DDGS. However, it should be noted that the energy value for maize DDGS is higher than for wheat DDGS due to differences in fat content. For the same reason, the impact of feeding wheat DDGS on fat quality (i.e. hardness or fatty acids composition of fat) should be less than with maize DDGS. In a first series of experiments in which diet formulation did not take into account the actual nutritional values for digestible lysine content or for ME content, results in broilers or turkeys indicated an increase in feed conversion ratio (FCR) with increased levels of maize or wheat DDGS (Lumpkins, Batal and Dale, 2004; Métayer *et al.*, 2009). In addition, these effects were more pronounced in younger than in older birds (Robertson, 2003). One limitation in feeding such diets might be the reduced feed intake due to the high DF presence in the diets, with greater detrimental effects in young birds (Friesen *et al.*, 1991), but more probably a limitation might be the AA shortage or deficiency due to the low and variable availability of amino acids in DDGS associated with lower energy intake (Widyaratne and Zijlstra, 2007), all of which limit protein and body-weight gain. In contrast, a second series of trials indicated that rather high levels of DDGS can be included if the actual AA and energy values of DDGS are considered in the

formulation of diets with appropriate supplementations (of AA and/or energy) in order to meet animal requirements (Waldroup *et al.*, 1981). Based upon such results, Lumpkins, Batal and Dale (2004) suggested that a safe inclusion level of maize DDGS was 6 percent in the starter period and 12 to 15 percent in the grower and finisher periods for broilers, whereas Thacker and Widyaratne (2007) suggested that wheat DDGS could be incorporated safely up to 15 percent. Finally, more accurate lysine content estimates would allow higher incorporation levels. For instance, Wang *et al.* (2007) did not show any detrimental effect of maize DDGS inclusion levels up to 25 percent in broilers, in the grower and finisher periods, with low density diets formulated on levels of digestible amino acids. Similarly, for turkey hens, Robertson (2003) demonstrated that 10 percent maize DDGS can be fed in the growing-finishing phases with no detrimental effects on growth performance as long as the actual energy value or lysine levels are considered.

Corresponding data have been reported for swine (Avelar *et al.*, 2010). In the review of Stein and Shurson (2009), the inclusion of 10, 22.5 or 30 percent maize DDGS did not affect average daily gain (ADG) in 10 experiments conducted on piglets. Nevertheless, in 10 trials, the average daily feed intake (ADFI) was reduced in two trials and FCR reduced 5 trials. The analysis of data from 25 experiments with grower-finisher pigs fed diets containing maize DDGS suggests that performance is maintained up to 20 percent DDGS in the diet (Cromwell *et al.*, 1983; Stender and Honeyman, 2008). Similarly, the inclusion of 25 percent wheat DDGS (characterized for its energy and protein values) in a wheat and pea-based diet fed to pigs from 52 to 85 kg did not affect ADFI, ADG or FCR (Widyaratne and Zijlstra, 2007). In contrast, inclusion of 0, 5, 10, 15, 20 or 25 percent wheat DDGS in wheat-soybean meal-based diets fed to grower pigs (20 to 51 kg) linearly reduced ADG and ADFI, whereas FCR was not affected (Thacker, 2006). The low quality of wheat DDGS used in the Thacker (2006) study and the BW range of pigs might partly explain these results. Finally, it has been reported in young pigs that the inclusion of 10 percent of maize DDGS can positively affect gut health by reducing the prevalence and severity of lesions due to *Lawsonia intracellularis* challenge (Whitney *et al.*, 2002). Up to now, no similar effects have been demonstrated with wheat DDGS.

In summary, the performance achieved with wheat DDGS is usually maintained at rather high inclusion rates of DDGS, in either poultry or pigs, if the nutritional value of the diet is maintained. However, the performance of young animals may deteriorate due to primary effects on feed intake, with possible accentuation of the effects due to low availability of amino acids (particularly lysine).

TABLE 8
Digestibility of dried distillers grain with solubles (DDGS) and performance improvement of animals by exogenous enzyme addition

Source	Species	DDGS type	Parameter	Change	Enzyme activities
Yáñez <i>et al.</i> , 2011	pig	Wheat/Maize DDGS	Amino acids, energy and phosphorus digestibility	Phosphorus digestibility +13%	Phytase + xylanase
Jones <i>et al.</i> , 2010	pig	Maize DDGS	Performance	Average daily gain (ADG) -2.4%; Average daily feed intake (ADFI) -5.6% ADG -7.2%; ADFI -9.1% ADG +1.8%; ADFI +2.2%	α -Galactosidase + galactomannanase + β -glucanase + xylanase Galactomannanase + xylanase Xylanase
Emiola <i>et al.</i> , 2009	pig	Wheat DDGS	Nitrogen energy digestibility	Nitrogen digestibility +6.5%; Energy digestibility +12.3%	Xylanase + glucanase + cellulase
			Performance	Nitrogen digestibility +9.6%; Energy digestibility +12.6% ADG +6.6%; ADFI -1.3% ADG +14.4%; ADFI -1.6%	Xylanase + glucanase + cellulase (dose 2X) Xylanase + glucanase + cellulase Xylanase + glucanase + cellulase (dose 2X)
Wang <i>et al.</i> , 2009	pig	Maize DDGS	Nitrogen and energy digestibility	DM digestibility +7.9% DM, N and GE digestibilities +2.0%, +6.1% and +6.7% respectively	Mannanase Mannanase + galactosidase + mannosidase
			Performance (8 weeks)	ADG +9.5%; FCR -14.3% ADG +8.4%; FCR -16.4%	Mannanase Mannanase + galactosidase + mannosidase
Widiyaratne, Patience and Zijlstra, 2009.	pig	Wheat DDGS	Performance	No change	Xylanase
Péron and Plumstead, 2009.	pig	Maize DDGS	Ileal nitrogen and amino acids digestibility, faecal energy digestibility	Nitrogen and amino acids digestibilities from +4 to +8% and Energy digestibility +6%	Xylanase + phytase
Adeola <i>et al.</i> , 2010.	broiler	Maize DDGS	Energy digestibility	Energy digestibility +6.0%	Xylanase + amylase
Olukosi, Cowieson and Adeola, 2010.	broiler	Maize DDGS	Performance	ADG +4.6%	Phytase
			Energy and nitrogen digestibility	Nitrogen digestibility +11.7%	Phytase + xylanase
Oryschak <i>et al.</i> , 2010a.	broiler	Rice DDGS	Amino acids digestibility	No change	Xylanase + glucanase + amylase + protease + invertase
Péron, Plumstead and Moran, 2009.	broiler	Maize DDGS	Performance (low-energy diet)	ADG +12.0%	Xylanase + amylase + protease + phytase
			Performance (high-energy diet)	ADG +5.0%	
Pérez Vendrell <i>et al.</i> , 2009.	broiler	Wheat or Maize DDGS	Energy digestibility	Energy digestibility +7.0%	Xylanase + phytase
			Performance (high-energy diet)	Apparent metabolizable energy ADG +4.0%	
Ghazalah, Abd-Elsamee and Moustafa, 2011.	layer	Maize DDGS	Performance	Egg production 2.4%; Egg mass 3.0%; FCR -2.8%	Glucanase + xylanase + amylase + polygalacturonase + protease

FEED ADDITIVES POTENTIAL FOR WHEAT DDGS

The foregoing sections indicate that the high DF content in DDGS represents a limiting factor in DDGS utilization by non-ruminants, with consequent low DE or apparent metabolizable energy (AME) values and lowered amino acid SID values. It would then be logical to attenuate this effect by supplementing diets with enzymes (Adeola and Cowieson, 2011). Numerous trials have studied the effect of carbohydrases on digestibility and performance in pigs and poultry. The most important results are summarized in Table 8. Numerous different enzymes have been tested in these studies, coupled with DDGS variable in quality and nutritional values. Overall, inconsistent results were

observed among studies. For digestibility trials, no significant effect has been reported by Yáñez *et al.* (2010) regarding maize or wheat DDGS amino acids and energy digestibility in piglets. Only phosphorus digestibility has been improved by phytase addition. In contrast, studies by Wang *et al.* (2009) and Adeola *et al.* (2010) suggest a global improvement (+6 percent) of DE (pigs) or AME content (broilers) of maize DDGS supplemented with an exogenous enzyme mixture. Comparable improvements were also suggested in other trials (Pérez Vendrell *et al.*, 2009; Olukosi, Cowieson and Adeola, 2010.). Supplementation of a multi-enzyme complex to diets containing wheat DDGS improved the digestibility of nutrients for finisher pigs (Emiola *et al.*,

2009), although the barley and maize contained in the diets might have also interacted with the multi-enzyme complex to contribute to the positive response. These results agree with the positive effects of xylanase on nutrient digestibility of wheat (Barrera *et al.*, 2004) and wheat co-products from flour milling (Yin *et al.*, 2000; Nortey *et al.*, 2007, 2008).

For performance, enzyme effects depend on numerous parameters (Adeola and Cowieson, 2011). The study of Emiola *et al.* (2009) suggests a global improvement of performance in animals fed wheat DDGS diets. This positive effect of enzymes supplementation is consistent with the results of Wang *et al.* (2009), Jones *et al.* (2010) or Péron, Plumstead and Moran (2009) with pig diets and Olukosi, Cowieson and Adeola (2010) in poultry diets. In contrast, a meta-analysis carried out by Jacela *et al.* (2009) involving 4506 pigs (4 trials) and different enzyme types suggests no beneficial effect of enzymes in maize-soybean meal diets containing up to 60 percent maize DDGS. These latter results are corroborated by Widyaratne, Patience and Zijlstra (2009).

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Wheat and maize DDGS are produced after a series of operations, the last being drying the product for its conservation, transportation and inclusion in dry compound feeds. In these stages of the process, and especially during the last stage, proteins and carbohydrates interact with the production of Amadori compounds generated by Maillard reactions. A major impact concerns the lysine fraction of the proteins, which can be destroyed or, at least, blocked and become unavailable for digestion. A major area of research would consist in producing methods for characterizing these compounds, studying their impact on the physical and nutritional parameters of wheat (and maize) DDGS, and proposing methods for a rapid and simple prediction of the nutritional value of DDGS, in addition to the classical prediction methods based on crude nutrients. This would also help the ethanol plants to optimize and standardize their procedures, not only for ethanol yield but also according to the nutritional value of their co-products (Oryschak *et al.*, 2010b). The important effects of drying on product physical properties and nutritional value also suggest the potential of infrared technologies as a quick and reliable tool for DDGS evaluation. This work has been started and is promising, but it still requires additional data for its complete achievement. In addition, the full potential of this raw material should be evaluated according to its proper nutritional values under a least-cost formulation constraint for diets fed to different animal species and stages of production. Environmental impact of biofuels production requires further work, as anticipated by Jarret *et al.* (2011) and Jarret, Martinez and Dourmad (2011) in terms of slurry

properties, methane production and carbon footprint of DDGS used as animal feed. Further research would also be required for phosphorus evaluation (Widyaratne and Zijlstra, 2009), which review also pointed out a lack of references in the field of micronutrient and vitamin contents in wheat DDGS. The impact of wheat DDGS on the gut health of pigs and poultry should also be investigated in order to have a full overview of wheat DDGS potential in pig and poultry production. Finally, the production of ethanol from cereals will probably change in the near future due to fractionation of residues in order to produce protein-, fat-, DF- or micro-constituents-rich fractions, with consequent major changes in the composition of DDGS. This implies that DDGS characteristics for pigs and poultry nutrition will need to be defined precisely.

CONCLUSIONS

This review shows that wheat DDGS are a potential source of energy, protein and phosphorus for poultry and pig diets. However, nutritionists using DDGS in diets for monogastric species should be aware of the current variability in nutrient content and digestibility. Colour score appears to be a promising method for a rapid and reliable estimation of both energy and amino acids digestibility, or, at least, a rapid classification method of DDGS usable for non-ruminant animals. In practice, a better knowledge of product quality might prevent any detrimental effect in animals fed DDGS and allow higher inclusion levels. Our review also suggests that the processing of DDGS should be adapted and optimized in order to obtain a high quality co-product. Finally, quality and uniformity improvement might be expected for DDGS in the future, but there will also be diversification of the co-products with the production of more specific co-products (with or without hulls; protein concentrations; germ separation; etc.).

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Chapter 10

Feeding biofuels co-products to pigs

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ABSTRACT

Dried distillers grains with solubles (DDGS) and other co-products from the fuel ethanol industry may be included in diets fed to pigs in all phases of production. The concentration of digestible energy (DE) and metabolizable energy (ME) in DDGS and maize germ is similar to maize, but high-protein dried distillers grain (HPDDG) contains more energy than maize. In contrast, if the oil is removed from DDGS, the co-product will have a lower energy concentration than maize or conventional DDGS. Glycerin is a co-product from the biodiesel industry and also contains more energy than maize. Phosphorus in DDGS and HPDDG is highly digestible to pigs, and apparent total tract digestibility (ATTD) values of approximately 60 percent have been reported for these ingredients. In contrast, the digestibility of phosphorus in maize germ is much lower and similar to maize. The concentration of starch in DDGS is low (between 3 and 11 percent on an as-fed basis), but the concentration of fat in DDGS is approximately 10 percent and the concentration of acid-detergent fibre (ADF), neutral-detergent fibre (NDF), and total dietary fibre in DDGS is approximately three times greater than in maize (9.9, 25.3 and 42.1 percent, respectively). The ATTD of dietary fibre is less than 50 percent, which results in low digestibility values for dry matter (DM) and energy in DDGS. The concentration of most amino acids in DDGS is approximately three times greater than in maize, but the standardized ileal digestibility (SID) of most amino acids average approximately 10 percentage units less than in maize. The same is the case for maize germ and HPDDG. Nursery pigs, beginning at two to three weeks post-weaning, and growing-finishing pigs may be fed diets containing up to 30 percent DDGS without any negative impact on pig growth performance, if they are formulated on a SID amino acid basis using crystalline amino acids to ensure that all digestible amino acid requirements are met.

However, carcass fat in pigs fed DDGS-containing diets has a higher iodine value (unsaturated to saturated fatty acid ratio) than in pigs fed no DDGS. As a result, it may be necessary to withdraw DDGS from the diet of finishing pigs during the final three to four weeks prior to harvest to achieve desired pork fat quality. High-protein DDGS may be used in diets fed to growing-finishing pigs in quantities sufficient to replace all of the soybean meal, and at least 10 percent of maize germ. Up to 30 percent de-oiled DDGS can be included in diets fed to weanling pigs, but results from one experiment indicate that adding de-oiled DDGS at any level to growing-finishing pig diets results in reduced growth rate and feed conversion. Due to limited research on this co-product, it is unclear if this is a valid and repeatable finding. Crude glycerin can be included in diets fed to weanling and growing-finishing pigs in quantities of up to 6 and 15 percent, respectively, and lactating sows fed diets containing up to 9 percent crude glycerol perform similarly to sows fed a standard maize-soybean meal diet. Lactating sows can be fed diets containing up to 30 percent DDGS, and DDGS can replace all of the soybean meal in diets fed to gestating sows without negatively impacting sow or litter performance. Inclusion of DDGS in diets fed to pigs may improve intestinal health and the immune system activation, but more research is needed to elucidate the mechanism responsible for these effects. Manure volume will increase if DDGS is included in the diet because of the reduced dry matter digestibility. Nitrogen excretion may also increase, but this can be prevented by the use of crystalline amino acids in diets containing DDGS. In contrast, P excretion can be reduced in diets containing DDGS if the total dietary concentration of P is reduced to compensate for the greater digestibility of P in DDGS.

INTRODUCTION

Distillers co-products have been used in swine diets for more than 50 years, but the rapid growth of the United

States fuel ethanol industry in the past decade has dramatically increased the total quantities of distillers co-products available to the livestock and poultry industries. Distillers

MAIN MESSAGES

- **Maize DDGS is the predominant ethanol industry co-product available for use in swine diets, and can be added at levels up to 30% of diets in all phases of production, and up to 50% in gestating sow diets, to achieve acceptable performance.**
- **Maize DDGS is primarily an energy source but also contributes significant amounts of digestible amino acids and available phosphorus to swine diets.**
- **Limited quantities and information is available on the nutritional value, optimal dietary inclusion rates and benefits and limitations of feeding other maize co-products from the ethanol industry.**
- **Glycerin is a co-product of the biodiesel industry, has an energy value greater than maize for swine and can be added at levels of up to 6% for weanling pigs, 9% for lactating sows and 15% for growing-finishing pigs to achieve acceptable performance.**
- **Significant opportunities exist to use particle size reduction, hydrothermal processing and enzymes to enhance energy and nutrient digestibility of distillers co-products, but the application and potential benefits of these technologies are not well understood.**
- **Special consideration should be given to the methanol content of crude glycerin, as well as to the possible presence of mycotoxins in DDGS when using them in swine diets.**
- **Feeding diets containing increasing levels of DDGS to growing-finishing pigs reduces pork fat firmness, but reducing feeding levels, withdrawing it from the diet for a period of time before harvest and adding conjugated linoleic acid to the diet 3 to 4 weeks before harvest can minimize the negative effects of DDGS diets on pork fat quality.**

grain production increased from 2.7 million tonne in 2000 to 32.5 million tonne in 2010. In 2011, there were over 200 ethanol plants in the United States producing distillers co-products. The two main types of ethanol production processes are dry-grind ethanol plants (Figure 1) and wet mills (Figure 2). Both process maize and mix it with yeast to convert starch into ethanol and carbon dioxide. After distillation of ethanol, the residual co-products are centrifuged to remove water, and are often dried to produce co-products for the feed industry. The type of milling and further processing determines the nutritional value and composition of distillers co-products. Wet mills use maize to produce ethanol, maize gluten feed, maize gluten meal, steep water, maize germ meal, and crude maize oil. The majority of ethanol produced today is from dry-grind ethanol plants, and the maize co-products they produce include wet distillers grain, condensed distillers solubles (CDS), modified wet distillers grain, dried distillers grain (DDG), and dried distillers grain with solubles (DDGS). For swine diets, DDGS is the predominant form used.

New ethanol and co-product production technologies are being implemented and include “back-end” oil extraction, and, to a much lesser extent, “front-end” fractionation, which are creating an increasing number of nutritionally diverse maize co-products, including high-protein DDGS (from fractionation), de-oiled or de-fatted DDGS (from oil extraction), maize germ meal, maize bran, and crude maize oil. Furthermore, maize, wheat, barley, grain sorghum, or mixtures of these cereal grains, may be used in the production of ethanol, and the co-products produced from each grain source are distinctly different in nutrient composition and value.

The United States biodiesel industry grew from producing 424 million litres of biodiesel in 2005, to 2.616 billion litres in 2008, before declining to 1.192 billion litres produced by 140 biodiesel plants in 2010 (NBB, 2011). The recent decline in United States biodiesel production has been mainly due to excess production capacity, product surpluses, and poor profitability. The principal co-product of biodiesel production is crude glycerin¹ (Ma and Hanna, 1999; van Gerpen, 2005), with 0.3 kg of crude glycerin generated for every gallon of biodiesel produced. Glycerin has thousands of uses, with new uses being continually developed as new technologies are adopted. When United States biodiesel production increased from 2005 to 2008, crude glycerin supplies exceeded demand for industrial uses and more of it became available, at an economical price, for use in animal feeds. Although the quantity of crude glycerin is significantly less than the amount of distillers co-products currently being produced, it does have applications in swine diets as an energy source when adequate supplies are available and economics are favourable for its use.

In order for the swine industry to capture maximum value and dietary use of biofuels co-products, the nutritional value (energy, nutrient content and digestibility), maximum dietary inclusion rates and any limitations affecting their use must be determined for each co-product in each pig production phase.

¹ Use of the word “glycerin” refers to the chemical compound or feedstuff while “glycerol” refers to glycerin on a biochemical basis relative to its function in living organisms. In addition, because glycerin is marketed on a liquid basis, all data are presented on an “as is” basis.

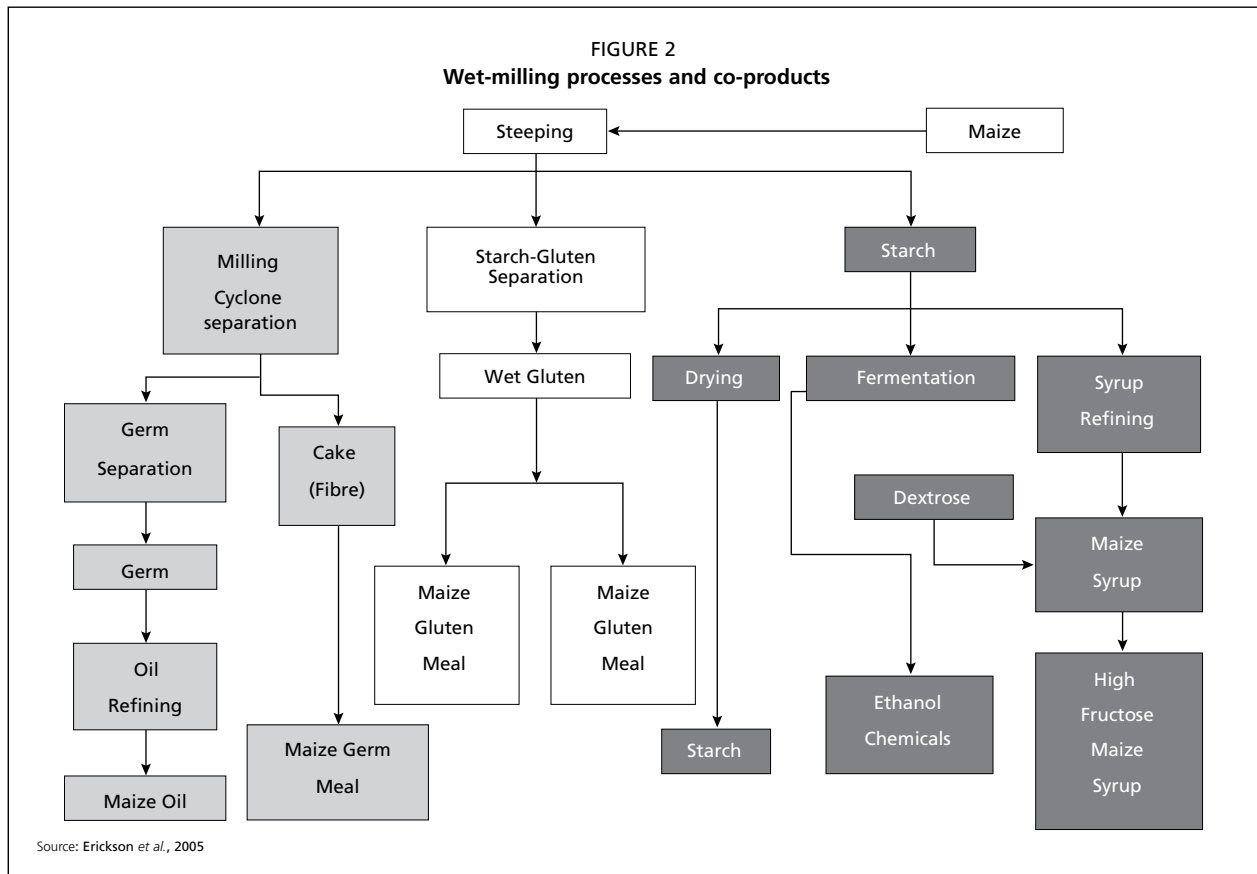
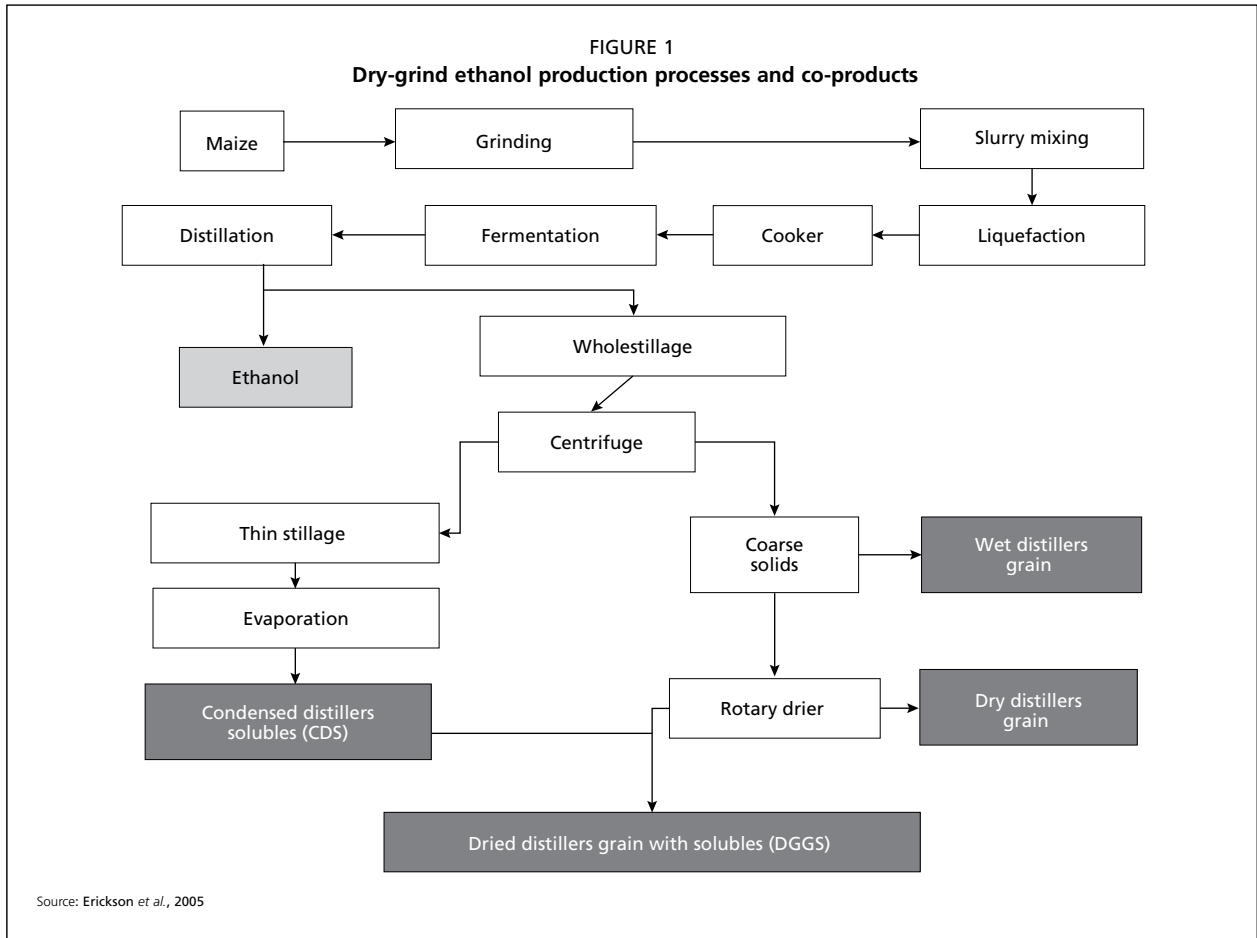


TABLE 1
Chemical composition of maize, sorghum and distillers co-products produced from maize and sorghum (as-fed basis)

Parameter	Maize	Sorghum	Maize DDGS	Sorghum DDGS	Maize DDG	Maize HPDDGS	Maize HPDDG	De-oiled maize DDGS	Enhanced maize DDGS	Maize germ
N	4	1	34	3	1	1	1	1	2	1
Gross energy, kcal/kg	3891	3848	4776	4334	–	–	4989	–	4742	4919
Crude protein, %	8.0	9.8	27.5	31.0	28.8	44.0	41.1	31.2	29.1	14.0
Calcium, %	0.01	0.01	0.03	–	–	–	0.01	0.05	0.27	0.03
Phosphorus, %	0.22	0.24	0.61	0.64	–	0.35	0.37	0.76	0.86	1.09
Crude fat, %	3.3	–	10.2	7.7	–	3.0	3.7	4.0	10.8	17.6
Crude fibre, %	–	–	–	7.2	–	7.0	–	–	–	–
Starch, %	–	–	7.3	–	3.83	–	11.2	–	–	23.6
Neutral-detergent fibre, %	7.3	7.3	25.3	34.7	37.3	–	16.4	34.6	29.7	20.4
Acid-detergent fibre, %	2.4	3.8	9.9	25.3	18.2	–	8.7	16.1	8.7	5.6
Total dietary fibre, %	–	–	42.1	–	–	–	–	–	25.2	–
Ash, %	0.9	–	3.8	3.6	–	–	3.2	4.64	–	3.3
Indispensable amino acids, %										
Arginine	0.39	0.32	1.16	1.10	1.15	–	1.54	1.31	1.34	1.08
Histidine	0.23	0.23	0.72	0.71	0.68	–	1.14	0.82	0.75	0.41
Isoleucine	0.28	0.37	1.01	1.36	1.08	–	1.75	1.21	1.04	0.45
Leucine	0.95	1.25	3.17	4.17	3.69	–	5.89	3.64	3.26	1.06
Lysine	0.24	0.20	0.78	0.68	0.81	1.03	1.23	0.87	0.93	0.79
Methionine	0.21	0.18	0.55	0.53	0.56	–	0.83	0.58	0.58	0.25
Phenylalanine	0.38	0.47	1.34	1.68	1.52	–	2.29	1.69	1.38	0.57
Threonine	0.26	0.29	1.06	1.07	1.10	–	1.52	1.10	1.03	0.51
Tryptophan	0.09	0.07	0.21	0.35	0.22	–	0.21	0.19	0.19	0.12
Valine	0.38	0.48	1.35	1.65	1.39	–	2.11	1.54	1.40	0.71
Dispensable amino acids, %										
Alanine	0.58	0.86	1.94	2.90	2.16	–	3.17	2.13	1.99	0.91
Aspartic acid	0.55	0.60	1.83	2.17	1.86	–	2.54	1.84	1.80	1.05
Cysteine	0.16	0.18	0.53	0.49	0.54	–	0.78	0.54	0.52	0.29
Glutamic acid	1.48	1.92	4.37	6.31	5.06	–	7.11	4.26	4.06	1.83
Glycine	0.31	0.29	1.02	1.03	1.00	–	1.38	1.18	1.11	0.76
Proline	0.70	0.77	2.09	1.40	2.50	–	3.68	2.11	1.99	0.92
Serine	0.38	0.37	1.18	2.50	1.45	–	1.85	1.30	1.25	0.56
Tyrosine	0.27	0.25	1.01	–	–	–	1.91	1.13	1.04	0.41

Notes: N = number of trials reported. Source: From Stein, 2008, whose review drew on data from Bohlke, Thaler and Stein, 2005; Feoli *et al.*, 2007a; Jacela *et al.*, 2007; Pedersen, Boersma and Stein, 2007a, b; Urriola *et al.*, 2009; Whitney, Shurson and Guedes, 2007; Pahn *et al.*, 2008; Soares *et al.*, 2008; Shurson and Alghamdi, 2008.

BIOFUELS CO-PRODUCTS USED IN SWINE DIETS

Dry-grind distillers co-products

The most common co-product from the fuel ethanol industry is dried distillers grain with solubles (DDGS), which, by definition, is a product that contains all the distillers grain and at least 75 percent of the condensed distillers solubles (CDS) produced after fermentation (Table 1). This co-product contains all parts of the maize kernel that are not converted into ethanol during fermentation. If condensed distillers solubles are not added back to the grain, the product is called dried distillers grain (DDG). This co-product has a lower concentration of fat and phosphorus than DDGS and it is produced in limited quantities compared with DDGS.

A few dry-grind ethanol plants in the United States have implemented “front-end” fractionation processes to enhance ethanol yield and produce a wider variety of co-products. However, the quantities of these co-products are

limited, resulting in limited use in swine diets. If the grain is de-hulled and de-germed prior to fermentation, a high-protein DDGS (HPDDGS) may be produced (Table 1). This co-product contains less fat and fibre, but more protein, than conventional DDGS because fibre and fat are removed during the de-hulling and de-germing process. If the CDS is not added back to the distilled grain produced from de-hulled and de-germed grain, HPDDG is produced (Whitney, Shurson and Guedes, 2007). The maize germ that is extracted from maize during de-germing can also be fed to pigs, but this product has a relatively high concentration of non-starch polysaccharides (Whitney, Shurson and Guedes, 2007).

In contrast, approximately 30 percent of the United States ethanol industry is currently using “back-end” oil extraction, with oil extraction projected to be occurring in 40 percent of the industry by 2012, and in 55 percent of the industry by 2013. Currently, the range in crude fat con-

TABLE 2
Composition of co-products from the maize wet-milling industry (as-fed basis)

Parameter	Maize germ meal	Maize gluten meal	Maize gluten feed	Glutenol
Crude protein, %	21.07	60.66	21.5	45.0
Calcium, %	0.03	–	0.22	–
Phosphorus, %	0.58	0.58	0.83	–
Crude fat, %	2.12	1.23	3.0	3.3
Crude fibre, %	9.53	1.32	–	3.8
Starch, %	13.63	10.14	–	1.5
Neutral-detergent fibre, %	54.41	11.21	33.3	–
Acid-detergent fibre, %	11.13	6.93	10.7	–
Total dietary fibre, %	42.57	8.45	–	–
Ash	2.41	3.65	–	4.0
Indispensable amino acids, %				
Arginine	1.49	2.18	1.04	–
Histidine	0.64	1.29	0.67	–
Isoleucine	0.75	2.59	0.66	–
Leucine	1.70	9.76	1.96	–
Lysine	1.04	1.27	0.63	–
Methionine	0.37	1.29	0.35	–
Phenylalanine	0.91	3.79	0.76	–
Threonine	0.78	1.94	0.74	–
Tryptophan	0.18	0.22	0.07	–
Valine	1.22	2.91	1.01	–
Dispensable amino acids, %				
Cysteine	0.33	0.99	0.46	–

Notes: Based on data from NRC, 1998; Shurson and Alghamdi, 2008; and unpublished data from University of Minnesota.

tent of DDGS sources is increasing (6 to 14 percent on a DM basis) compared with the typical range in crude fat content in DDGS only a few years ago (9 to 13 percent on a DM basis). However, depending upon the extraction equipment and methodology, crude fat levels in DDGS can be as low as 5 percent on a DM basis. Unfortunately, the effects of oil extraction on digestible, metabolizable and net energy content of DDGS for livestock and poultry are not known, but research is being conducted to obtain this information. This information will be essential for establishing price and value differentials among DDGS sources relative to crude fat content, as well as for accurate diet formulations using reduced-oil co-products.

If oil is extracted from the DDGS, a de-oiled DDGS is produced (Jacela *et al.*, 2007). De-oiled DDGS contains 2 to 4 percent oil, and therefore also contains less energy than conventional DDGS (Jacela *et al.*, 2007; Table 1). However, most of the dry-grind ethanol plants are extracting oil from the condensed solubles fraction, resulting in a semi-de-oiled DDGS containing approximately 7 percent oil. If fibre is removed from the DDGS after production, a co-product called enhanced DDGS is produced (Soares *et al.*, 2008). This co-product contains approximately 10 percent less non-starch polysaccharides than conventional DDGS.

WET-MILLING CO-PRODUCTS

Although the majority of ethanol produced in the United States is from dry-grind ethanol plants, some plants use

wet-milling technology. The major co-products produced from wet milling include maize germ meal, maize gluten meal and maize gluten feed (Table 2). The majority of these co-products are marketed to the ruminant feed industry, but they are also potential feed ingredients for swine. A new wet-milling technology that fractionates maize prior to fermentation has resulted in the production of a product called Glutenol (Shurson and Alghamdi, 2008). This product is equivalent to the HPDDGS produced from the dry-grind process after fermentation of de-hulled and de-germed maize, but contains slightly more protein and less fibre than HPDDGS.

Liquid co-products from the fuel ethanol industry

Two liquid co-products from the fuel ethanol industry – maize condensed distillers solubles (CDS) and maize steep water – may be fed to pigs (de Lange *et al.*, 2006). Maize CDS is a co-product from dry-grind fuel ethanol production, whereas maize steep water is a co-product produced from wet milling. Steep water contains approximately 50 percent CP and 3.3 percent P (DM basis), but only 0.5 percent oil (Table 3), whereas CDS contains 18.9 percent oil, but only 22.3 percent CP and 1.43 percent P (DM basis).

Co-products from the bio-diesel industry

Biodiesel is produced by a variety of esterification technologies, using new or used vegetable oils and animal fats as

TABLE 3
Composition of maize condensed distillers solubles (CDS)
and maize steep water (dry matter basis)

Item	Maize CDS	Maize steep water
N	5	3
Dry matter, %	30.5	45
Crude protein, %	22.3	50
Crude fat, %	18.9	0.5
Ash, %	8.4	18.0
Ca, %	0.04	–
P, %	1.43	3.3
Na, %	0.21	–
K, %	–	5.0
pH	3.7	4.3
Acetic acid, %	0.11	–
Propionic acid, %	0.63	–
Butyric acid, %	0.01	–
Lactic acid, %1	9.8	20.0
Total non-starch polysaccharides, %	6.1	–
Starch, %	9.9	–
Total sugars, %	3.5	–

Notes: N = number of trials reported. Source: Based on data from Braun and de Lange, 2004; Niven *et al.*, 2006.

the initial feedstock. In general, oils and fats are filtered and pre-processed to remove water and contaminants, followed by mixing with an alcohol (usually methanol) and a catalyst (sodium or potassium methylate). This causes the oil molecules (triglycerides) to be broken apart into methyl esters and glycerin, which are then separated from each other and purified (NBB, 2011). Biodiesel is the name given to these esters when they are intended for use as fuel. The biodiesel industry can use any fat or oil feedstock, including recycled cooking grease and algae oil, but historically the primary feedstock source has been soybean oil. However, current prices of soybean oil have accelerated the industry's interest in utilization of alternative oil or fat sources for their initial feedstock.

NUTRIENT AND ENERGY COMPOSITION AND DIGESTIBILITY IN DISTILLERS GRAIN CO-PRODUCTS

Concentration and digestibility of carbohydrates

Most cereal grains contain between 60 and 70 percent starch, which is easily digested by pigs and absorbed in the form of glucose. However, production of alcohol from grain requires that the grain is fermented, and most of the starch in the grain is converted to alcohol during this process. All distillers co-products therefore have a low concentration of starch, whereas the concentration of most other nutrients is increased compared with their content in the original grain (Tables 1 and 2). Therefore, the concentrations of carbohydrates in distillers co-products are lower than in cereal grains and most of the carbohydrates are non-starch

polysaccharides (fibre). The concentration of the different fibre fractions (neutral-detergent fibre - NDF, acid-detergent fibre - ADF, and total dietary fibre - TDF) is approximately three times greater in DDGS and DDG than in maize, but high-protein dried distillers grain (HPDDG), high-protein dried distillers grain with solubles (HPDDGS) and glutenol contain less fibre than DDG and DDGS because the maize was de-hulled before fermentation. The digestibility of fibre in DDGS and in DDG is less than 20 percent in the small intestine and less than 50 percent over the entire gastro-intestinal tract (Urriola, Shurson and Stein, 2010). Therefore, the fibre fraction contributes relatively little to the energy value of these products (Urriola, Shurson and Stein, 2010). It is expected that the digestibility of fibre in other distillers co-products is equally low, but fibre digestibility has not yet been reported for these co-products.

The low digestibility of fibre in distillers co-products results in increased quantities of manure being excreted from pigs fed these ingredients because the overall DM digestibility of diets containing distillers co-products is lower than in maize-based diets (Pedersen, Boersma and Stein, 2007a). Currently, much effort is directed towards developing feed additives such as enzymes or yeast products that can improve the digestibility of fibre in distillers co-products. If the digestibility of fibre in distillers co-products is improved, the energy value of these products will also improve.

Digestibility of amino acids

The digestibility of most amino acids in maize DDGS (Table 4) is approximately 10 percentage units lower than in maize (Fastinger and Mahan, 2006; Stein *et al.*, 2006; Pahm *et al.*, 2008). The lower digestibility of amino acids in maize DDGS compared with maize, may be a result of the greater concentration of fibre in DDGS than in maize, because dietary fibre reduces amino acid digestibility. Another reason for the variability and reduced digestibility of amino acids among maize DDGS sources compared with maize, is due to differences in production technologies and drying temperatures and duration among plants producing maize DDGS (Pahm *et al.*, 2008). Excessive heating during the drying process has been shown to result in the production of Maillard products, which reduce amino acid digestibility, particularly lysine (Urriola *et al.*, 2009). However, variability in digestibility of amino acids does not appear to be related to the region within the United States where the DDGS is produced (Pahm *et al.*, 2008).

The variability in the concentration and digestibility of lysine in maize DDGS is greater than the variability in digestibility of most other amino acids. Urriola *et al.* (2009) determined amino acid digestibility of 8 maize DDGS sources and showed that lysine standardized ileal digestibility (SID) ranged from 55.7 to 68.7 percent, and tryptophan digestibility ranged from 56.2 to 72.0 percent,

TABLE 4
Standardized ileal digestibility of amino acids in maize, sorghum, and distillers co-products produced from maize and sorghum

Item	Maize	Sorghum	Maize DDGS	Sorghum DDGS	Maize DDG	Maize HPDDG	Maize germ	De-oiled maize DDGS	Maize gluten meal	Maize gluten feed
n	2	1	34	1	1	1	1	1	1	1
Indispensable amino acids, %										
Arginine	87	70	81	78	83	83	83	83	89	87
Histidine	83	65	78	71	84	81	69	75	80	78
Isoleucine	81	66	75	73	83	81	57	75	84	80
Leucine	87	70	84	76	86	91	68	84	88	85
Lysine	72	57	62	62	78	64	58	50	80	66
Methionine	85	69	82	75	89	88	68	80	90	83
Phenylalanine	84	68	81	76	87	87	64	81	85	87
Threonine	74	64	71	68	78	77	53	66	84	71
Tryptophan	70	57	70	70	72	81	67	78	63	64
Valine	79	64	75	72	81	80	62	74	80	77
Dispensable amino acids, %										
Alanine	83	69	78	73	82	86	64	77	–	–
Aspartic acid	80	66	69	68	74	76	60	61	–	–
Cysteine	82	64	73	66	81	82	64	64	82	59
Glutamic acid	80	52	80	76	87	88	72	78	–	–
Glycine	84	71	63	67	66	75	76	53	–	–
Proline	96	50	74	83	55	73	84	73	–	–
Serine	83	72	76	73	82	84	65	73	–	–
Tyrosine	82	67	81	–	–	88	59	81	87	84

Notes: n = number of trials reported; HPDDG = high-protein dried distillers grain. Source: Adapted from Stein, 2008, based on data from Bohlke, Thaler and Stein, 2005; Jacela *et al.*, 2007; Pedersen, Boersma and Stein, 2007b; Stein, 2007; Urriola *et al.*, 2009; Whitney, Shurson and Guedes, 2007; Pahn *et al.*, 2008.

but standardized ileal digestibility of other amino acids was less variable among sources. The production of Maillard products results in a reduction in the total concentration of lysine as well as in the digestibility of lysine, but the concentration of crude protein is not changed. In non-heat-damaged maize DDGS, the concentration of lysine as a percentage of crude protein is between 3.1 and 3.3 percent, but in heat-damaged maize DDGS this percentage can be as low as 2.10 percent (Stein, 2007). Therefore, it is recommended that the lysine concentration is measured before maize DDGS is used in swine diets, and only sources that contain at least 2.80 percent lysine, expressed as a percentage of crude protein, be used in diets fed to swine (Stein, 2007). Some of the variability in amino acid digestibility, and lysine digestibility in particular, is caused by the addition of solubles to the distilled grain fraction before drying, because the solubles contain some residual sugars that were not fermented into ethanol. The presence of these sugars will increase the likelihood of Maillard reactions occurring when the mixture of distilled grain and condensed solubles is dried. As a result, the digestibility of amino acids in maize DDG is greater than in maize DDGS, because the solubles are not added to the distilled grain when DDG is produced (Pahn *et al.*, 2008).

The digestibility of amino acids in maize HPDDG is within the range of values measured for maize DDGS, but

data for only one source are available (Whitney, Shurson and Guedes, 2007). The digestibility of amino acids in maize germ is less than in maize DDG and maize DDGS. The reason for this observation may be due to the proteins in maize germ having different chemical properties compared with other proteins in the grain kernel (Whitney, Shurson and Guedes, 2007).

Although sorghum has a lower digestibility of amino acids than maize (Pedersen, Boersma and Stein, 2007b), sorghum DDGS has amino acid digestibilities that are within the range of values observed in maize DDGS (Urriola *et al.*, 2009). However, amino acid digestibility data have been reported for only one source of sorghum DDGS. Digestibility of amino acids was measured in one source of de-oiled maize DDGS and all values reported were within the range of values reported for conventional maize DDGS (Jacela *et al.*, 2007).

Digestibility of phosphorus

Fermentation results in release of a portion of the phytate-bound phosphorus in maize, which in turn results in a greater digestibility of P in fermented feed ingredients than in maize (Table 5). Therefore, the ATTD of phosphorus is much greater in maize DDGS and maize HPDDG than in maize, whereas the digestibility of phosphorus in maize germ is similar to maize (Stein, Pedersen and Boersma,

TABLE 5
Concentration and digestibility of phosphorus in maize and distillers co-products produced from maize (as-fed basis)

Parameter	Maize	Maize DDGS	Maize HPDDG	Maize germ
n	2	10	1	1
Total phosphorus (%)	0.22	0.61	0.37	1.09
Total phosphorus (as % of DM)	0.25	0.70	0.40	1.18
ATTD (%)	24.1	59.0	59.6	28.6
Digestible phosphorus (%)	0.05	0.36	0.22	0.31

Notes: n = number of trials reported; ATTD = Apparent total tract digestibility; HPDDG = high-protein dried distillers grain. Sources: Stein, 2008, based on data from Bohlke, Thaler and Stein, 2005; Pedersen, Boersma and Stein, 2007a; Whitney, Shurson and Guedes, 2007.

2005; Pedersen, Boersma and Stein, 2007a; Whitney, Shurson and Guedes, 2007). There are no data on the ATTD of phosphorus in other sources of distillers co-products produced from maize or in DDGS produced from sorghum.

Digestibility of lipid

The ATTD of lipid in DDGS has been reported only from one experiment, which showed that the ATTD of oil in DDGS is approximately 70 percent (Stein, Pedersen and Boersma, 2005). However, there is a need for more information on oil and fatty acid digestibility in distillers co-products because of the important contribution of the oil to co-product energy value, as well as the effects on carcass fat quality in pigs.

Digestibility of energy

The ATTD of energy in most distillers co-products is lower than in maize because of the greater concentration of fibre in the co-products than in maize (Table 6). The fibre in maize DDGS has a low digestibility in the small intestine, and the fermentation of fibre in the large intestine is less than 50 percent complete, resulting in low digestibility of energy in distillers co-products. In maize DDGS, the ATTD of energy is 82.9 percent compared with 90.4 percent in maize (Pedersen, Boersma and Stein, 2007a). However, because of the higher oil concentration in maize DDGS compared with maize, the concentration of gross energy (GE) is also greater in maize DDGS than in maize (5434 vs 4496 kcal GE/kg DM). As a result, the concentration of digestible energy (DE) in maize DDGS is similar to

maize (4088 vs 4140 kcal DE/kg DM; Stein, Pedersen and Boersma, 2005; Pedersen, Boersma and Stein, 2007a), but varies among DDGS sources (Pedersen, Boersma and Stein, 2007a; Anderson *et al.*, 2012; Mendoza *et al.*, 2010b). The concentration of DE in maize germ (3979 kcal DE/kg DM) is also similar to maize, but maize HPDDG has a greater concentration of DE (4763 kcal DE/kg DM) than maize (Whitney, Shurson and Guedes, 2007). The ME content of DDG containing 7.9 percent crude fat (2959 ±100 kcal/kg DM) was similar to that determined for DDGS containing 8.9 percent crude fat (2964 ±81 kcal/kg DM; Dahlen *et al.*, 2011). In contrast, de-oiled maize DDGS has a lower concentration of DE than maize (3093 kcal DE/kg DM; Jacela *et al.*, 2007). The concentration of DE in sorghum DDGS has been measured in one experiment and it was reported that sorghum DDGS contained approximately 220 kcal/kg (as-is basis) less than maize DDGS (Feoli *et al.*, 2007a), which may be a result of a lower concentration of oil in sorghum DDGS compared with maize DDGS.

IMPROVING NUTRIENT DIGESTIBILITY OF DDGS

Energy digestibility of DDGS is at least 10 percent lower than that of the feedstock grain from which it was produced, indicating that significant opportunities for improvement exist. The relatively high concentration of fibre in DDGS may be one of the main reasons for reduced nutrient digestibility in DDGS compared with the grain source from which it was derived (Stein and Shurson, 2009). The impact of feed processing and feed additives such as supplemental enzymes on nutrient digestibility of DDGS has not been extensively studied, but knowledge from recent studies will be useful for identifying strategies for improving nutrient digestibility of DDGS in feed processing plants.

Particle size reduction

Grinding grain is common in the feed industry to improve nutrient digestibility and feed processing, and in the ethanol industry to improve fermentation and ethanol production efficiency. Reducing mean particle size from coarse to fine (e.g. from 1000 to 400 µm) will improve nutrient digestibility of ground grain such as maize (e.g. Wondra *et al.*, 1995) and also of protein sources such as soybean meal

TABLE 6
Concentration of energy in maize and in distillers co-products produced from maize and sorghum (DM-basis)

Parameter	Maize	Maize DDGS	Sorghum DDGS	Maize HPDDG	Maize Germ	De-oiled maize DDGS	Maize gluten meal	Maize gluten feed
n	2	10	2	1	1	1		
Gross energy (kcal/kg DM)	4458	5434	4908	5399	5335	4655	–	–
ATTD (%)	90.0	76.8	76.0	88.2	74.6	–	–	–
Digestible energy (kcal/kg DM)	4072	4140	3459	4763	3979	3093	4694	3322
Metabolizable energy (kcal/kg DM)	3981	3897	–	4476	3866	2851	4256	2894

Notes: n = number of trials reported; ATTD = apparent total tract digestibility. Source: Stein, 2008, based on data from NRC, 1998; Feoli *et al.*, 2007d; Jacela *et al.*, 2007; Pedersen, Boersma and Stein, 2007a; Whitney, Shurson and Guedes, 2007; Widmer *et al.*, 2007.

(Fastinger and Mahan, 2003). The underlying mechanism is that large feedstuff particles provide less surface area per unit of mass for digestive enzymes to interact with their substrates (Goodband, Tokach and Nelssen, 2002). Nutrient digestibility for larger particles is therefore lower than for smaller particles, because nutrient digestion is limited to a specific time interval due to digesta transit through the gastrointestinal tract.

Opportunities may exist to grind DDGS to increase nutrient digestibility, because the mean particle size of DDGS varies widely among samples. For example, the mean particle size of unground maize DDGS ranged from 434 to 949 μm from dry-grind ethanol plants (Liu, 2008). Mendoza *et al.* (2010c) evaluated DDGS from 15 different sources and observed considerable variability in particle size among sources, but DE and ME content can be improved by grinding to a smaller particle size.

Reducing mean particle size from 517 to 383 μm in DDGS increased the apparent ileal digestibility and ATTD of energy in grower pigs by 2.3 and 1.3 percentage units, respectively (Yáñez *et al.*, 2011). Liu *et al.* (2011b) showed an even greater response for improving ME of DDGS by reducing particle size, where each 25-micron decrease in DDGS particle size (from 818 μm to 308 μm), resulted in a ME contribution from DDGS to the diet of 13.6 kcal/kg DM, but diet flowability was reduced. Combined, grinding of DDGS will have more of a positive impact on nutrient digestibility on the DDGS sources with a mean particle size greater than 660 μm (Liu, 2008), and mean particle size should be measured routinely in feed quality evaluation.

Hydrothermal processing

Unlike grinding, which is common for all dry feed, not all monogastric feed is subjected to hydrothermal processing (Hancock and Behnke, 2001). Steam pelleting of feed is common in some parts of the United States and Western Europe, whereas mash feeding is common in western Canada and Australia. The impact of pelleting on nutrient digestibility of maize co-products is not clear, but it appears to improve nutrient digestibility. Growth performance and nutrient digestibility was improved when nursery pigs were fed diets containing 30 percent maize DDGS (Zhu *et al.*, 2010). Pelleting of diets containing high levels of maize fibre (maize gluten feed) improved N balance, apparently due to the increased availability of tryptophan (Yen *et al.*, 1971).

Extrusion subjects feed to heat and pressure more extensively than steam pelleting, and can open the physical structure of the feedstuff matrix (Hancock and Behnke, 2001). Extrusion processing is common for aquaculture and pet feed, because fish and companion animals have generally much lower nutrient digestibility of plant-based feeds than swine and poultry. Therefore, extrusion is required to achieve suitable feed management character-

istics. However, very little is known about the effects of extruding maize and maize co-products on nutritional value for swine (Muley *et al.*, 2007). In broiler chicks, extrusion of DDGS from triticale, wheat and maize improved energy and amino acid digestibility (Oryschak *et al.*, 2010a, b). In contrast, extrusion of DDGS from wheat and maize increased energy digestibility for both in pigs, perhaps, in part, by enhancing nutrient digestibility of residual starch in DDGS, but also by improving amino acid digestibility in maize DDGS (Beltranena *et al.*, 2009). These results indicate that effects of extrusion processing on nutrient digestibility will be specific to source of DDGS and species targeted.

Supplemental enzymes

The addition of exogenous enzymes to animal feeds to improve nutrient digestion is not a new concept, and responses have been reviewed in detail (Chesson, 1987; Bedford, 2000). The majority of commercial enzyme products have been targeted toward poultry (Annison and Choct, 1991; Cowan, 1993) and are typically added to diets containing barley, oats, peas, rye or wheat (Aimonen and Nasi, 1991; Thacker, Campbell and GrootWassink, 1992; Viveros *et al.*, 1994; Hubener, Vahjen and Simon, 2002), with only limited research evaluating enzyme use in maize-soybean meal diets (Saleh *et al.*, 2005).

The introduction of larger quantities of co-products, such as DDGS, into swine diets will increase the dietary content of fibre. The negative effects on energy and nutrient digestibility, and ultimately animal performance, from feeding such diets may be reduced partly by using supplemental enzymes (Zijlstra, Owusu-Asiedu and Simmins, 2010). Detailed chemical characterization of fibre components in DDGS indicates that it contains arabinoxylan constituents, which is one potential substrate for supplemental fibre-degrading enzymes, and that some intact phytate remains as substrate for supplemental phytase (Widyaratne and Zijlstra, 2007; Liu, 2011). However, results from a recent study by Kerr, Weber and Shurson (2011) showed minimal effects on nutrient digestibility, and no improvement in growth performance, from supplementing with ten different commercial enzyme products and additives in nursery or finishing pig diets containing 30 percent DDGS.

Phytase

Plant-based phytate is well known for its ability to bind P and other nutrients and thereby reduce digestibility of these nutrients (Oatway, Vasanthan and Helm, 2001). The phytate contained in the grain is partly transformed during the fermentation process to produce ethanol and co-products. Intact phytate (inositol hexaphosphate) does, unlike nutrients other than starch, not concentrate 2 to 3 fold in the DDGS, but is instead partially hydrolyzed into inositol phosphates, which contain 5 or fewer P molecules

(Widyaratne and Zijlstra, 2007). Digestibility of P is therefore higher in DDGS than in the feedstock grain. Still, sufficient phytate in DDGS remains to hinder P digestibility. Indeed, the addition of 500 FTU (phytase units) of phytase to a maize starch diet containing 44 percent DDGS increased the ATTD of energy of P in the diet by 10.5 percentage units, but did not affect energy and amino acid digestibility (Yáñez *et al.*, 2011). However, data on the impact of phytase, with or without other enzymes, on nutrient (and energy) digestibility in maize co-product diets is lacking and inconsistent. While addition of 500 units phytase improved P digestibility in diets containing 20 percent DDGS in starter or finisher pigs, it did not improve DM digestibility (Xu, Whitney and Shurson, 2006a, b). In contrast, Lindemann *et al.* (2009) reported that pigs fed diets containing 20 percent DDGS supplemented with 250 or 500 U/kg phytase exhibited greater DM, energy, and N digestibility than unsupplemented pigs, but there were no further improvements in faecal DM, energy or N digestibility with additional xylanase supplementation. Therefore, even though DDGS has a higher P digestibility than grain and protein meals, supplemental phytase may provide additional benefits in diets containing DDGS.

Fibre-degrading enzymes

The negative impact of fibre or non-starch polysaccharides has been described for cereal grains, including barley and wheat (Fairbairn *et al.*, 1999; Zijlstra *et al.*, 2009). The positive effects of fibre-degrading enzymes on energy digestibility of wheat have been defined, as long as the supplemental enzyme matches with a substrate that limits nutrient utilization or animal performance (e.g. Mavromichalis *et al.*, 2000; Cadogan, Choct and Campbell, 2003; Barrera *et al.*, 2004). Thus, not surprisingly, diets containing wheat co-products from flour milling (co-products that have been subjected to limited processing during production) have a drastically increased non-starch polysaccharide content and hence arabinoxylan content, and supplemental xylanase improved energy digestibility in swine (Nortey *et al.*, 2007, 2008). Combined, these results indicate that wheat fibre in its native form is a good substrate for supplemental xylanase in swine diets.

Interestingly, the relationship between co-products from ethanol production (maize or wheat DDGS) and the potential benefits from supplemental xylanase is less clear. Studies have shown no improvement in growth performance from adding enzymes to maize DDGS diets for nursery pigs (Jones *et al.*, 2010), while studies by Spencer *et al.* (2007) and Yoon *et al.* (2010) showed improvements from the use of enzymes in nursery and in grower-finisher diets, respectively. Additional studies have also shown improvements in nutrient digestibility when enzymes are added to DDGS diets (Jendza *et al.*, 2009; Yoon *et al.*, 2010; Feoli *et*

al., 2008d), but improvements in nutrient digestibility do not always result in improvements in growth performance (Kerr, Weber and Shurson, 2011). Because DDGS has been subjected to extensive periods in solution, followed by drying, adding supplemental xylanase to DDGS diets does not always seem to improve energy digestibility of wheat DDGS (Widyaratne, Patience and Zijlstra, 2009; Yáñez *et al.*, 2011) or maize DDGS (Mercedes *et al.*, 2010), although positive examples exist (Lindemann *et al.*, 2009). Furthermore, xylanase supplementation did not improve growth performance in nursery pigs fed diets containing 30 percent maize DDGS (Jones *et al.*, 2010), although xylanase improved growth performance and digestibility of diet components in broilers (Liu *et al.*, 2011a). Finally, supplementation of a multi-enzyme complex to diets containing wheat DDGS improved growth performance and nutrient digestibility in finisher pigs (Emiola *et al.*, 2009), although the barley and maize contained in the diets used might have also interacted with the multi-enzyme to provide the positive response, and the multi-enzyme complex may be required to open the fibre matrix.

The more extensive processing used during ethanol production compared with flour milling might thus have caused changes in the feedstuff matrix that may make supplemental enzymes less advantageous for improving nutrient digestibility. These differences in enzyme responses may be due to fibre-degrading enzymes that can be added during the ethanol production process to enhance ethanol yield, making the regular substrate for these supplemental enzymes not the limiting factor for nutrient digestibility. Feedstuffs and enzyme selection require proper characterization to ensure that the substrates and enzymes match, and that the substrate is indeed the critical factor that hinders nutrient digestibility.

IN VITRO ENERGY DIGESTIBILITY IN DDGS

Nutritional value of DDGS is known to vary substantially among sources (Nuez Ortín and Yu, 2009; Stein and Shurson, 2009; Zijlstra and Beltranena, 2009). Specifically, the ATTD of energy ranged from 74 to 83 percent for maize DDGS (Pedersen, Boersma and Stein, 2007a) and from 56 to 76 percent for wheat DDGS (Cozannet *et al.*, 2010). Prediction of quality of DDGS prior to feed processing is thus an important component of reducing the risk of less predictable animal performance when using DDGS in animal feeds. *In vitro* energy digestibility techniques can be used to screen ranges in energy digestibility among feedstuff samples and thereby support the development of feedstuff databases and rapid feed quality evaluation systems such as near-infrared reflectance spectroscopy (Zijlstra, Owusu-Asiedu and Simmins, 2010).

In vitro digestibility techniques using enzymes and incubation periods that mimic *in vivo* digestion can predict with

reasonable accuracy the ATTD of energy among feedstuffs in swine (Boisen and Fernández, 1997). However, variation within feedstuffs such as DDGS is a greater concern for processing complete feed with an accurate DE content, and should be explored thoroughly for individual feedstuffs or feedstuff combinations.

Using *in vitro* digestibility techniques, the ATTD among samples of the same cereal grain can be predicted accurately for barley (Regmi, Sauer and Zijlstra, 2008) and wheat (Regmi, Ferguson and Zijlstra, 2009a). However, similar efforts were not successful in predicting the ATTD for protein feedstuffs with a more complex fibre and protein matrix, such as DDGS (Regmi *et al.*, 2009; Wang *et al.*, 2010).

In vitro fermentation has been used recently as a tool in feedstuff characterization, based on the hypothesis that gas produced and fermentation kinetics reflect the same kinetics as *in vivo* fermentation of fibre in the large intestine of swine. Although *in vitro* fermentation characteristics have been measured in an array of feedstuffs, only recently has *in vitro* fermentation of maize DDGS been compared with other feedstuffs, and its fermentation rate is similar to wheat bran and lower than field pea and sugar beet pulp (Jha *et al.*, 2011).

ENERGY PREDICTION EQUATIONS FOR DDGS

Because of variability in DE and ME values among DDGS sources, several prediction equations have been developed to estimate ME content using various chemical analysis measures (Mendoza *et al.*, 2010b; Anderson, Shurson and Kerr, 2009; Pedersen, Boersma and Stein, 2007a). However, there are several challenges in accurately predicting ME content of DDGS sources:

- Accuracy has not been validated.
- May not represent the wide range in nutrient variability among sources.
- Some analytes required by equations (e.g. GE, TDF) are not routinely measured or are expensive to analyse.
- Analytical variability among labs and procedures affects accuracy (e.g. NDF).
- Adjustments for fat and fibre in some equations seem counterintuitive.

NUTRIENT AND ENERGY COMPOSITION AND DIGESTIBILITY IN MAIZE CO-PRODUCTS FROM WET-MILLING

The majority of the research with energy and nutrient digestibility has been conducted with products from the dry-grind fuel ethanol industry, and only limited data are available on the digestibility of nutrients and energy in co-products from the wet-milling process for swine. For maize germ meal and glutenol, no data on energy and nutrient digestibility have been published, and for maize

gluten meal and maize gluten feed, only data for amino acid digestibility have been published (Table 4). Both maize gluten meal and maize gluten feed have amino acid digestibility values that are greater than in maize DDGS, and for most amino acids the digestibility in maize gluten meal is similar to the values measured in maize (Table 4), whereas the values in maize gluten feed generally are intermediate compared with those measured in maize and maize DDGS. Values for DE and ME in maize gluten meal are greater than in maize and maize DDGS, and similar to values reported for maize HPDDG, but DE and ME in maize gluten feed are lower than in maize and similar to values measured for de-oiled DDGS (Table 6).

CRUDE GLYCERIN

Energy composition and digestibility

During digestion in non-ruminants, intestinal absorption of glycerin has been shown to range from 70 to 90 percent in rats (Lin, 1977), to more than 97 percent in pigs and laying hens (Bartlet and Schneider, 2002). Glycerin is water soluble and can be absorbed by the stomach, but at a rate that is slower than that of the intestine (Lin, 1977). Absorption rates are high, which is probably due to glycerin's small molecular weight and passive absorption, rather than going through the process of becoming part of a micelle that is required for absorption of medium- and long-chain fatty acids (Guyton, 1991). Once absorbed, glycerol can be converted to glucose via gluconeogenesis or oxidized for energy production via glycolysis and the citric acid cycle, with the shuttling of protons and electrons between the cytosol and mitochondria (Robergs and Griffin, 1998). Glycerol metabolism largely occurs in the liver and kidney, where the amount of glucose carbon arising from glycerol depends upon metabolic state and level of glycerol consumption (Lin, 1977; Hetenyi, Perez and Vranic, 1983; Baba, Zhang and Wolfe, 1995). With gluconeogenesis from glycerol being limited by the availability of glycerol (Cryer and Bartley, 1973; Tao *et al.*, 1983), crude glycerin has the potential of being a valuable dietary energy source for monogastric animals.

Pure glycerin is a colourless, odourless and sweet-tasting viscous liquid, containing approximately 4.3 Mcal GE/kg on an as-is basis (Kerr *et al.*, 2009). However, crude glycerin can range from 3 to 6 Mcal GE/kg, depending upon its composition (Brambilla and Hill, 1966; Lammers *et al.*, 2008a; Kerr *et al.*, 2009). The difference in GE between crude glycerin and pure glycerin is not surprising, given that crude glycerin typically contains about 85 percent glycerin, 10 percent water, 3 percent ash (typically Na or K chloride), and a trace amount of free fatty acids. As expected, high amounts of water negatively influence GE levels, while high levels of free fatty acids elevate the GE concentration. The ME of glycerin has been assumed to be approximately 95%

of its GE (Brambilla and Hill, 1966; Lin, Romsos and Leveille, 1976; Rosebrough *et al.*, 1980; Cerrate *et al.*, 2006), but there have been no empirical determinations of the ME of crude glycerin in swine until recently.

Bartlet and Schneider (2002) reported ME values of refined glycerin in 35-kg pigs and determined that the ME value of glycerin decreased as the level of dietary glycerin increased (4189, 3349 and 2256 kcal/kg at 5, 10 and 15 percent inclusion levels, respectively) with an average value of 3292 kcal/kg on an as-is basis. Because pre-caecal digestibility of glycerin was determined to be approximately 97 percent (Bartlet and Schneider, 2002), the observed decrease in ME value may be a result of increased blood glycerol levels following glycerin supplementation (Kijora *et al.*, 1995; Kijora and Kupsch, 2006; Simon, Bergner and Schwabe, 1996), suggesting that complete renal re-absorption is prevented and glycerol excretion in the urine is increased (Kijora *et al.*, 1995; Robergs and Griffin, 1998).

In nursery and finishing pigs, Lammers *et al.* (2008a) determined that the ME content of a crude glycerin co-product containing 87 percent glycerin was 3207 kcal/kg, and did not differ between pigs weighing 10 or 100 kg (Table 7). Based strictly on its glycerin content, this equates to 3688 kcal ME/kg on a 100 percent glycerin basis (3207 kcal ME/kg/87 percent glycerin), which is slightly lower than the 3810 kcal ME/kg (average of the 5 and 10 percent inclusion levels) reported by Bartlet and Schneider (2002), but similar to the 3656 kcal ME/kg as reported by Mendoza *et al.* (2010a) using a 30 percent inclusion level of glycerin.

Similar to data reported by Bartlet and Schneider (2002), increasing crude glycerin from 5 to 10 to 20 percent in 10-kg pigs (Lammers *et al.*, 2008a) quadratically reduced ME content (3601, 3239 and 2579 kcal ME/kg, respectively), suggesting that high dietary concentrations of crude glycerin may not be fully utilized by 10-kg pigs. In contrast, dietary concentrations of crude glycerin had no effect on ME determination in 100-kg pigs (Lammers *et al.*, 2008a). The ratio of DE:GE is an indicator of how well a crude glycerin source is digested, and for the crude glycerin source evaluated by Lammers *et al.* (2008a), it equalled 92 percent, suggesting that crude glycerin is well digested,

being only slightly lower than the 97 percent of glycerin digested before the caecum, as reported by Bartlet and Schneider (2002). In addition, the ratio of ME:DE indicates how well energy is utilized once digested and absorbed. For the crude glycerin source evaluated by Lammers *et al.* (2008a), the ratio was 96 percent, which is identical to the ME:DE ratio for soybean oil, and is comparable to the ratio of ME:DE (97%) for maize grain (NRC, 1998), all of which support the assertion that crude glycerol is well utilized by the pig as a source of energy.

Chemical composition variability

Similar to other co-products used to feed livestock, the chemical composition of crude glycerin can vary widely (Thompson and He, 2006; Kijora and Kupsch, 2006; Hansen *et al.*, 2009; Kerr *et al.*, 2009). The consequences of this variable chemical composition in crude glycerin relative to its energy value for animals have not been well described. Recently, 10 sources of crude glycerin from various biodiesel production facilities in the United States were evaluated for energy utilization in growing pigs (Table 8). The crude glycerin sources originating from biodiesel plants using soybean oil averaged 84 percent glycerin, with minimal variability noted among 6 of the sources obtained. Conversely, crude glycerin sources obtained from biodiesel plants using tallow, yellow grease or poultry oil as initial lipid feedstock ranged from 52 to 94 percent glycerin. The crude glycerin co-products derived from either non-acidulated yellow grease or poultry fat had the lowest glycerin content, but also had the highest free fatty acid concentrations. The high fatty acid content of the non-acidulated yellow grease product was expected because the acidulation process results in greater separation of methyl esters, which subsequently results in a purer form of crude glycerin containing less free fatty acids (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). In contrast, the relatively high free fatty acid content in the crude glycerin obtained from the biodiesel plant utilizing poultry fat as a feedstock is difficult to explain because details of the production process were not available. Moreover, these two crude glycerin co-products (derived from non-acidulated yellow grease and poultry fat) had higher methanol concentrations than

TABLE 7
Digestible and metabolizable energy of crude glycerin fed to pigs, as-is basis

Trial	Pigs	Initial BW (kg)	DE (kcal/kg)	SEM	ME (kcal/kg)	SEM
1	18	11.0	4,401	282	3,463	480
2	23	109.6	3,772	108	3,088	118
3	19	8.4	3,634	218	3,177	251
4	20	11.3	4,040	222	3,544	237
5	22	99.9	3,553	172	3,352	192

Notes: All experiments represent data from 5-day energy balance experiments following a 10-day adaptation period (Lammers *et al.*, 2008a); BW = body weight; DE = digestible energy, ME = metabolizable energy; SEM = Standard Error of the Mean. Trial 1 included pigs fed diets containing 0, 5 and 10% crude glycerin. Trial 2 included pigs fed diets containing 0, 5, 10 and 20% crude glycerin. Trials 3, 4 and 5 included pigs fed diets containing 0% and 10% glycerin.

TABLE 8
Chemical analysis of crude glycerin, percentage as-is basis

Sample ID	Glycerin	Moisture	Methanol	pH	NaCl	Ash	Fatty acids
USP	99.62	0.35	ND	5.99	0.01	0.01	0.02
Soybean oil	83.88	10.16	0.0059	6.30	6.00	5.83	0.12
Soybean oil ⁽¹⁾	83.49	13.40	0.1137	5.53	2.84	2.93	0.07
Soybean oil	85.76	8.35	0.0260	6.34	6.07	5.87	ND
Soybean oil	83.96	9.36	0.0072	5.82	6.35	6.45	0.22
Soybean oil	84.59	9.20	0.0309	5.73	6.00	5.90	0.28
Soybean oil	81.34	11.41	0.1209	6.59	6.58	7.12	0.01
Tallow	73.65	24.37	0.0290	3.99	0.07	1.91	0.04
Yellow grease	93.81	4.07	0.0406	6.10	0.16	1.93	0.15
Yellow grease ⁽²⁾	52.79	4.16	3.4938	8.56	1.98	4.72	34.84
Poultry fat	51.54	4.99	14.9875	9.28	0.01	4.20	24.28

Notes: Samples analysed as described in Lammers *et al.* (2008a), courtesy of Ag Processing Inc., Omaha, NE 68154, USA. Glycerin content determined by difference as: 100 -% methanol -% total fatty acid -% moisture -% ash. Data obtained from Kerr *et al.*, 2009. ND = not determined. USP = United States Pharmacopeial Convention grade glycerin or initial feedstock lipid source. (1) Soybean oil from extruded soybeans. All other soybean oil was obtained by hexane extraction of soybeans. (2) Crude glycerin that was not acidulated.

the other glycerin sources. Recovery of methanol is also indicative of production efficiency because it is typically re-used during the production process (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). The high amount of methanol content in crude glycerin from non-acidulated yellow grease was expected because this co-product had not been fully processed at the production facility. The reason crude glycerin obtained from the plant utilizing poultry fat contained relatively high methanol is unclear because no processing information was available from the plant. However, this higher level of methanol may be due to lower overall efficiency of the production process at this plant (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006).

The average ME of the 11 sources of glycerin described in Table 9 was 3486 kcal/kg (Kerr *et al.*, 2009), with little difference among the sources, with the exception of the two sources with high levels of free fatty acids (co-products obtained from non-acidulated yellow grease and poultry fat). These sources high in free fatty acid content had higher ME values than the other crude glycerin co-products, which was not surprising given that these two co-products also had a higher GE concentration than the other co-product sources. The ME:GE ratio among all glycerin co-products was similar, averaging 85 percent, which is similar to ratios reported by others (88%, Lammers *et al.*, 2008a; 88%, Bartlet and Schneider, 2002; 85%, Mendoza *et al.*, 2010a). Because the GE of the crude glycerin can vary widely among co-product sources, comparison of ME as a percentage of GE provides valuable information on the caloric value of crude glycerin for swine. A high ME:GE ratio indicates that a crude glycerin source is well digested and utilized.

Because more than one chemical component can influence energy content of feed ingredients, stepwise regression was used to predict GE and ME values, and to predict ME as a percentage of GE among glycerin sources.

TABLE 9
Energy values of crude glycerin co-products in swine, on an as-is basis

Sample	GE (kcal/kg)	ME (kcal/kg)	% of GE
USP	4325	3682	85.2
Soybean oil	3627	3389	93.4
Soybean oil ⁽¹⁾	3601	2535	70.5
Soybean oil	3676	3299	89.9
Soybean oil	3670	3024	82.5
Soybean oil	3751	3274	87.3
Soybean oil	3489	3259	93.5
Tallow	3173	2794	88.0
Yellow grease	4153	3440	92.9
Yellow grease ⁽²⁾	6021	5206	86.6
Poultry fat	5581	4446	79.7

Notes: USP = United States Pharmacopeial Convention (USP) grade glycerin or initial feedstock lipid source. (1) Soybean oil from extruded soybeans. All other soybean oil was obtained by hexane extraction of soybeans. (2) Crude glycerin that was not acidulated. Source: Kerr *et al.*, 2009.

If the GE of a crude glycerin source is unknown, it can be predicted by using the following equation: GE kcal/kg = $-236 + (46.08 \times \% \text{ of glycerin}) + (61.78 \times \% \text{ of methanol}) + (103.62 \times \% \text{ of fatty acids})$, ($R^2 = 0.99$). Metabolizable energy content can subsequently be predicted by multiplying GE by 84.5% with no adjustment for composition (Kerr *et al.*, 2009). Additional research is needed to refine and validate these equations relative to glycerin, methanol, ash and total fatty acid concentrations for all body weights.

SPECIAL CONSIDERATIONS FOR CO-PRODUCTS FROM THE ETHANOL INDUSTRY

Mycotoxins

Like all feed ingredients, maize co-products may contain mycotoxins that can negatively affect animal performance, or might be stored under conditions that cause co-product deterioration. Mycotoxins can be present in maize co-products if the grain delivered to the ethanol plant is contaminated with them. Mycotoxins are not destroyed during the

ethanol production process, nor are they destroyed during the drying process to produce distiller co-products. In fact, if they are present in maize used to produce ethanol, their concentration will be increased by a factor of approximately three in DDGS. However, the risk of mycotoxin contamination in United States distillers grain by-products is very low because it is uncommon for most of the major maize growing regions in the United States to have climatic and weather conditions that lead to mycotoxin production in maize on a regular basis. Furthermore, most ethanol plants monitor grain quality and reject sources that exceed acceptable (very low) levels of mycotoxins.

Recently, Zhang *et al.* (2009) conducted surveys to assess the prevalence and levels of aflatoxins, deoxynivalenol, fumonisins, T-2 toxin and zearalenone in 235 DDGS samples. The samples were collected between 2006 and 2008 from 20 ethanol plants in the mid-western United States and from 23 export shipping containers, and analysed using state-of-the-art analytical methodologies. Their results indicated that (1) none of the samples contained aflatoxins or deoxynivalenol levels higher than the U.S. Food and Drug Administration (FDA) guidelines for use in animal feed; (2) no more than 10 percent of the samples contained levels of fumonisins higher than the recommendation for feeding equids and rabbits, and the remaining bulk of the samples contained fumonisins lower than FDA guidelines for use in animal feed; (3) no samples contained detectable levels of T-2 toxins; (4) most samples contained no detectable zearalenone; and (5) the containers used for export shipping of DDGS did not contribute to mycotoxin production.

The prevalence and levels of deoxynivalenol (vomitoxin) in the 2009 United States maize crop were unusually high, resulting in production of deoxynivalenol-contaminated DDGS in 2010. As a result, researchers (Früge *et al.*, 2011a, b; Barnes *et al.*, 2011) evaluated the effectiveness of commercial products for mitigating the negative effects of feeding diets containing DDGS contaminated with deoxynivalenol, and some benefits were observed.

Sulphur

Sulphur levels can be highly variable among DDGS sources and can range from 0.31 to 1.93 percent (average 0.69 percent) on a DM basis (University of Minnesota data; www.ddgs.umn.edu). Sulphuric acid is commonly added during the dry-grind ethanol production process to keep pH at desired levels for optimal yeast propagation and fermentation in order to maximize the conversion of starch to ethanol, and is less costly compared with other acids. According to AAFCO (2010), sulphuric acid is generally recognized as safe according to U.S. Code of Federal Regulation (21 CFR 582) and is listed as an approved food additive (21 CFR 573). In addition, maize naturally contains about 0.12 per-

cent sulphur, and is concentrated by approximately three-fold, like other nutrients, when maize is used to produce ethanol and DDGS. Yeast also contains about 3.9 g/kg sulphur and naturally creates sulphites during fermentation.

Sulphur is an essential mineral for animals and serves many important biological functions in the animal body. However, when excess sulphur (greater than 0.40 percent of diet DM) is present in ruminant diets, neurological problems resulting from polioencephalomalacia (PEM) can occur. In contrast, sulphur content of DDGS does not appear to be a concern in swine diets. Kim, Zhang and Stein (2010) conducted four experiments to determine the effects of dietary sulphur level on feed palatability and growth performance of weanling and growing-finishing barrows. Their results showed that inclusion of 20 to 30 percent of DDGS in diets fed to weanling and grow-finishing pigs reduced palatability of the diets and negatively affected growth performance. However, the concentration of sulphur in the DDGS-containing diets had no impact on feed palatability or growth performance.

Lipid oxidation

Some sources of DDGS may contain high levels of oxidized lipids due to the high drying temperatures used in some ethanol plants. Song, Saari Csallany and Shurson (2011) reported that the thiobarbituric acid reactive substances (TBARS; a measure indicative of lipid oxidation) level can vary considerably (1.0 to 5.2 malondialdehyde (MDA) equivalent ng/mg oil) among 31 DDGS sources. The highest TBARS level measured in one DDGS source was 26 times higher than that of maize (0.2 MDA equivalent ng/mg oil). As a result, the use of supplemental dietary antioxidants may be warranted in order to minimize metabolic oxidation. Harrell *et al.* (2010) and Harrell, Zhao and Reznik (2011) reported that the dietary addition of a commercial antioxidant can improve growth performance of pigs fed diets containing oxidized maize oil or 20 to 30 percent DDGS, and in a subsequent study showed that supplementing nursery pig diets with another commercially available antioxidant improved growth performance of pigs when fed diets containing 60 percent DDGS. However, no research has been conducted to determine the efficacy of these synthetic antioxidants relative to common forms of vitamin E.

SPECIAL CONSIDERATIONS FOR CRUDE GLYCERIN

Because glycerin varies in energy content, salt content and methanol concentration, modifications in diet formulation may be required. Depending on the salt level in the crude glycerin, supplemental levels of dietary salt may need to be limited, depending upon the animal species and stage of production where it is fed. It is generally well accepted that

feeding diets containing up to 3 percent dietary NaCl will have no adverse effects on pig performance as long as adequate water is freely available (adapted from NRC, 1980). However, the impact of increased water intake on increased manure volume and changes in composition (Sutton *et al.*, 1976) needs to be considered.

Adding 10 to 20 percent crude glycerin to swine mash diets may also affect the ability of feed to flow in bulk bins and automatic feeding systems, as indicated by Cerrate *et al.* (2006), Hansen *et al.* (2009), Lammers *et al.* (2008a) and Kerr *et al.*, (2009), especially in feeds containing dried whey. Because no quantitative measurements to assess feed flowability were taken in any of these experiments, the potential interactions among levels of glycerin supplementation, diet type and feed handling system affecting feed flowability are yet to be characterized.

Methanol levels in crude glycerin warrant special consideration. Methanol is a potentially toxic compound and has been reviewed in detail by others (Roe, 1982; Medinsky and Dorman, 1995; Skrzydlewska, 2003). Methanol can be introduced orally, by respiration or through the skin, and is distributed by the blood to all organs and tissues in proportion to their water content (Liesivuori and Savolainen, 1991). Metabolic elimination of methanol is much slower than that of ethanol. Small amounts of methanol are excreted in the kidney and lung, but the majority is metabolized by the liver and released as CO₂. Acute methanol intoxication is manifested initially by signs of narcosis followed by a latent period in which formic acid accumulates causing metabolic acidosis (reduced blood pH, depletion of blood bicarbonate and visual degeneration, with abdominal, leg and back pain). Chronic exposure to methanol causes headache, insomnia, gastrointestinal problems and blindness. Animals differ widely in their ability to metabolize methanol, depending upon enzyme activity and hepatic folate levels (Roe, 1982; Black *et al.*, 1985; Medinsky and Dorman, 1995; Skrzydlewska, 2003). Little research on methanol metabolism or toxicity has been conducted in pigs. Makar *et al.* (1990) reported that pigs, compared with all other species studied, have extremely low levels of folates and very low levels of a key enzyme (10-formyl H₄folate dehydrogenase) in the folate pathway, suggesting the ability of the pig to dispose of formate is limited, and slower than that observed in rats or monkeys. However, Dorman *et al.* (1993) indicated that methanol- and formate-dosed minipigs did not develop optic nerve lesions, toxicologically significant formate accumulation or metabolic acidosis, indicating that minipigs do not appear to be overtly sensitive to methanol toxicity.

When considering the potential for methanol and formate toxicity, it is interesting to note that in some countries, formaldehyde, a methanol metabolite, can be used as a silage preservative, and formic acid can be used in finished

feeds to reduce bacterial loads. Formic acid or formate salts have also been used safely in diets for swine (Overland *et al.*, 2000; Canibe *et al.*, 2005) and formaldehyde in diets for laying hens (Khan, Hussain and Khan, 2006). It is also interesting to note that calcium formate has been used as a dietary calcium supplement for humans (Hanzlik, Fowler and Eells, 2005).

As a general-purpose feed ingredient, glycerin is regulated in the United States under 21CFR583.1320, requiring that levels of methanol in methyl esters of higher fatty acids should not exceed 0.015 percent. Recently, however, crude glycerin has been defined by the Association of American Feed Control Officials (AAFCO, 2010) and can be fed to non-ruminants up to 10 percent of the complete feed as long as it contains not less than 80 percent glycerin, not more than 15 percent water, not more than 0.15 percent methanol, up to 8 percent salt, up to 0.1 percent sulphur, and not more than 5 ppm heavy metals. German regulations (Normenkommission für Einzelfuttermittel im Zentralausschuss der Deutschen Landwirtschaft, 2006) allow 0.5 percent (5000 ppm) methanol in crude glycerin.

FEEDING DISTILLERS CO-PRODUCTS TO SWINE Sows

Maize DDGS is the only maize co-product that has been evaluated for use in sow diets and for which published reports are available. Feeding diets containing 50 percent maize DDGS to gestating sows resulted in no negative effects on lactation feed intake, litter weight gain, and weaning to oestrus interval (Wilson *et al.*, 2003). In fact, sows fed maize DDGS in gestation (50 percent) and lactation (20 percent) for two consecutive parities had increased litter size in the second parity compared with those fed a maize-soybean meal diet. The reason for this observation is unknown, but it may be a consequence of the increased fibre concentration in diets containing maize DDGS because litter size is sometimes improved if sows are fed high-fibre diets during gestation (Ewan *et al.*, 1996; Grieshop, Reese and Fahey, 2001). More research needs to be conducted to verify if the increase in litter size is a common response to including maize DDGS in diets fed to gestating sows.

Results of four experiments in which maize DDGS was fed to lactating sows have been reported, and dietary inclusion rates in these experiments were: up to 15 percent (Hill *et al.*, 2008b); 20 percent (Wilson *et al.*, 2003) or 30 percent (Song *et al.*, 2010; Greiner *et al.*, 2008) of the diet. No negative performance effects were reported in any of these experiments, and milk composition, apparent nitrogen digestibility or nitrogen retention were not affected by feeding DDGS diets. However, sows fed diets containing 20 or 30 percent maize DDGS had lower values for blood urea nitrogen than sows fed a maize-soybean meal diet (Song *et al.*, 2010), which indicates that these sows were fed diets

with a better amino acid balance compared with sows fed the control diet. Greiner *et al.* (2008) observed that sows fed a 30 percent maize DDGS diet had improved weight gain in lactation and reduced wean to oestrus intervals, but these effects were not reported in the other experiments. There is, however, no information on the performance of pigs farrowed by sows fed maize DDGS, but there are no indications that the growth performance of these pigs would be affected.

Therefore, maize DDGS can be included in sow diets at levels up to 50 percent in gestation and up to 30 percent in lactation if diets are formulated on a ME, digestible amino acid and available phosphorus basis. It is possible that the inclusion rate of DDGS in diets fed to gestating sows can be greater than 50 percent, and for lactating sows, greater than 30 percent, but no research has been reported concerning this hypothesis.

Weanling pigs

Growth performance responses (Table 10) from inclusion of maize DDGS at levels up to 30 percent in weanling pig diets have been reported from 10 experiments (Whitney and Shurson, 2004; Linneen *et al.*, 2008; Gaines *et al.*, 2006; Spencer *et al.*, 2007; Barbosa *et al.*, 2008; Burkey *et al.*, 2008). Growth rate was not affected in any of these experiments by feeding DDGS diets, beginning as early as 4 days post-weaning (Whitney *et al.*, 2004). Average daily feed intake was reduced in two experiments when DDGS was included in the diet (Gaines *et al.*, 2006; Barbosa *et al.*, 2008), but the Gain:Feed (G:F) ratio was improved when DDGS was added to the diet in 5 of the 10 experiments (Gaines *et al.*, 2006; Spencer *et al.*, 2007; Barbosa *et al.*, 2008). Nursery pig mortality was reported in only two experiments, and no negative effects were observed from feeding DDGS diets.

Palatability, feed preference and growth performance of nursery pigs have been evaluated when various levels and qualities of distillers co-products were added to the diet (Hastad *et al.*, 2005; Seabolt *et al.*, 2008). Nursery pigs

prefer diets without DDGS or HPDDGS, but colour differences among sources appear unrelated to feed preference.

Effects of introducing DDGS-containing diets to weanling pigs at different times post-weaning was investigated (Spencer *et al.*, 2007) by offering pigs a 4-phase nursery programme in which DDGS was introduced either in phase 1 (7.5 percent), phase 2 (15 percent) or phases 3 and 4 (15 percent). There were no differences in growth performance among treatments, which indicated that DDGS may be introduced immediately after weaning without compromising pig growth performance. However, this result was not observed by Burkey *et al.* (2008), who reported that inclusion of DDGS in diets fed to pigs before day 21 post-weaning resulted in a reduction in growth performance.

Inclusion of sorghum DDGS in diets fed to weanling pigs at levels up to 60 percent of the diets has been investigated in three experiments (Senne *et al.*, 1995, 1996; Feoli *et al.*, 2008d). No differences in average daily gain (ADG), average daily feed intake (ADFI) or G:F ratio were observed when feeding diets containing levels up to 20 percent of sorghum DDGS (Senne *et al.*, 1995), but the inclusion of 30 percent sorghum DDGS in diets reduced growth performance compared with pigs fed diets containing no DDGS (Feoli *et al.*, 2008d). When weanling pigs were fed diets containing 0, 15, 30, 45 or 60 percent sorghum DDGS from day 7 to day 29 post-weaning (Senne *et al.*, 1996), quadratic reductions in ADG and G:F were observed, with growth performance of pigs fed up to 30 percent DDGS being similar to that of pigs fed control diets, but inclusion of 45 or 60 percent DDGS reduced ADG and G:F. It is possible that differences in DDGS quality or diet formulation methods may have contributed to these different responses.

De-oiled maize DDGS can be included in diets fed to weanling pigs in concentrations of up to 30 percent, with no changes in ADG, ADFI or G:F (Jacela *et al.*, 2008a). No experiments have been conducted to investigate the effects of including distillers co-products other than DDGS and de-oiled DDGS in diets fed to weanling pigs. As a result, it is unknown if any of the other maize co-products can be used effectively in weanling pig diets.

TABLE 10
Effects of including maize dried distillers grain with solubles (DDGS) in diets fed to weanling pigs

Item	n	Response to dietary maize DDGS		
		Increased	Reduced	Unchanged
ADG	10	0	0	10
ADFI	10	0	2	8
G:F	10	5	0	5
Mortality	2	0	0	2

Notes: n = number of trials reported; ADG = Average daily gain; ADFI = Average daily feed intake; G:F = Gain:Feed ratio.

Source: Stein and Shurson, 2009, derived from data calculated from experiments by Whitney and Shurson, 2004; Gaines *et al.*, 2006; Linneen *et al.*, 2006; Spencer *et al.*, 2007; Barbosa *et al.*, 2008; and Burkey *et al.*, 2008.

Growing-finishing pigs – growth performance

In the last decade, results from at least 25 experiments have been reported on growth performance of growing-finishing pigs fed diets containing up to 30 percent maize DDGS (Table 11). In 23 of these experiments, DDGS was included in maize- and soybean-meal-based diets, and wheat-field pea-based diets were used in two experiments. There are also reports from eight experiments in which sorghum DDGS was included in diets, with two experiments using wheat DDGS in growing-finishing pig diets.

Results from early research showed that adding up to 20 percent maize DDGS to growing-finishing pig diets

TABLE 11
Effects of including maize dried distillers grain with solubles (DDGS) in diets fed to growing-finishing pigs

Parameter	n	Response to dietary maize DDGS		
		Increased	Reduced	Unchanged
Average Daily Gain	25	1	6	18
ADFI	23	2	6	15
Gain:Feed (G:F)	25	4	5	16
Dressing percentage	18	0	8	10
Backfat (mm)	15	0	1	14
Lean meat (%)	14	0	1	13
Loin depth (cm)	14	0	2	12
Belly thickness (cm)	4	0	2	2
Belly firmness	3	0	3	0
Iodine value	8	7	0	1

Notes: ADFI = Average daily feed intake. Based on experiments (n is number of trials involved) published after 2000 and where a maximum of 30% DDGS was included in the diets. The primary source was Stein and Shurson, 2009, whose data derived from experiments by Gralapp *et al.*, 2002; Fu *et al.*, 2004; Cook, Paton and Gibson, 2005; DeDecker *et al.*, 2005; Whitney *et al.*, 2006; McEwen, 2006, 2008; Gaines *et al.*, 2007a, b; Gowans *et al.*, 2007; Hinson *et al.*, 2007; Jenkin *et al.*, 2007; White *et al.*, 2007; Widyaratne and Zijlstra, 2007; Xu *et al.*, 2010a, b; Augspurger *et al.*, 2008; Drescher *et al.*, 2008; Duttlinger *et al.*, 2008b; Hill *et al.*, 2008a; Linneen *et al.*, 2008; Stender and Honeyman, 2008; Weimer *et al.*, 2008; and Widmer *et al.*, 2008.

would be acceptable for maintaining growth performance, but performance was reduced if 40 percent was used (Cromwell *et al.*, 1983). Average daily gain was improved in one experiment, reduced in six experiments, and not affected by DDGS level in 18 experiments when up to 20 percent maize DDGS was added to diets adequately fortified with amino acids (McEwen, 2006, 2008; Augspurger *et al.*, 2008; Drescher *et al.*, 2008; Duttlinger *et al.*, 2008b; Widmer *et al.*, 2008) and studies where up to 30 percent maize DDGS was added (Cook, Paton and Gibson, 2005; DeDecker *et al.*, 2005). In contrast, data from other experiments in which 10, 20 or 30 percent maize DDGS was included in diets fed to growing-finishing pigs showed a linear reduction in ADG (Fu *et al.*, 2004; Whitney *et al.*, 2006; Linneen *et al.*, 2008; Weimer *et al.*, 2008). A linear reduction in ADFI was also observed in two of these experiments (Fu *et al.*, 2004; Linneen *et al.*, 2008). Xu *et al.* (2010b) showed that ADG was not affected, but ADFI was reduced and G:F was linearly improved in pigs fed diets containing 0, 10, 20 or 30 percent DDGS. Results from two additional experiments in which performance of finishing pigs fed diets containing 0 or 30 percent DDGS were compared showed no differences in ADG and ADFI, but G:F was reduced in pigs fed the DDGS-containing diets (Gaines *et al.*, 2007a, b). The reduction in G:F in the latter experiments and the increase in G:F in the experiment by Xu *et al.* (2010b) suggests that the energy concentration may have varied among the sources of DDGS used in these experiments.

A linear increase in ADG and G:F was also observed when a barley-wheat-field pea-based diet was fortified

with 0, 5, 10, 15, 20 or 25 percent maize DDGS and fed to growing-finishing pigs (Gowans *et al.*, 2007). However, inclusion of 25 percent DDGS in a wheat-field pea-based diet reduced ADG and ADFI compared with results obtained for pigs fed a diet containing no DDGS (Widyaratne and Zijlstra, 2007).

Data for ADFI were reported only in 23 experiments: increasing in two experiments, decreasing in six experiments, and unaffected by dietary DDGS inclusion in 15 experiments. G:F was improved in 4 experiments, reduced in 5 experiments and unaffected by dietary treatments in 16 experiments.

Based on the data provided from these 25 experiments, it is not possible to determine the reasons why pig performance was maintained in most, but not in all, experiments in which DDGS was included in the diets. It is possible that the maize DDGS used in the experiments in which performance was reduced may have been of a poorer quality (lower nutrient digestibility) than expected. In some of the experiments in which performance was reduced by feeding increasing levels of maize DDGS, dietary CP levels were also increased. In such diets, DDGS inclusion rate is confounded by CP level and it is not possible to determine if the reduced performance is caused by the increase in maize DDGS concentration or by the increase in CP concentration. However, in most of the experiments in which ADG was reduced, a reduction in ADFI was also observed. It is therefore possible that the poorer performance was due to reduced palatability of the maize DDGS used in those diets. It has been demonstrated that, if given a choice, pigs prefer to consume diets containing no maize DDGS (Hastad *et al.*, 2005; Seabolt *et al.*, 2008).

Results from the eight experiments in which sorghum DDGS was included in diets fed to growing-finishing pigs demonstrated that if sorghum DDGS is used at concentrations of 30 percent or less, no differences in pig performance are observed (Senne *et al.*, 1995, 1996). However, if greater dietary inclusion rates are used, ADG will be reduced (Senne *et al.*, 1996; 1998; Feoli *et al.*, 2007b, c; 2008a, b, c). Likewise, G:F is not affected if the inclusion of sorghum DDGS is limited to 30 percent (Senne *et al.*, 1995; 1996), but G:F may be reduced if 40 percent is used (Senne *et al.*, 1998; Feoli *et al.*, 2008a), although this is not always the case (Feoli *et al.*, 2007c, 2008b, c). Average daily feed intake is not affected by sorghum DDGS if 30 percent or less is included in the diet (Senne *et al.*, 1995), but ADFI may be reduced at greater inclusion levels (Senne *et al.*, 1996; Feoli *et al.*, 2007c, 2008b).

Inclusion of 25 percent wheat DDGS in a wheat-field pea-based diet fed to growing-finishing pigs did not affect ADG or G:F (Widyaratne and Zijlstra, 2007), but adding up to 25 percent wheat DDGS in wheat-soybean meal-based

diets for growing pigs linearly reduced ADG and ADFI, whereas G:F was unaffected (Thacker, 2006). However, when the dietary inclusion of DDGS was reduced to 0, 3, 6, 9, 12 or 15 percent during the finishing phase in this experiment, no differences in growth performance were observed during this period (Thacker, 2006). The diet used by Widyaratne and Zijlstra (2007) was formulated based on concentrations of digestible amino acids measured in the batch of DDGS that was fed to the pigs, whereas the diets used by Thacker (2006) were formulated based on a total amino acid basis. This may explain why different responses were obtained in these experiments because it has been shown that wheat DDGS sometimes has a very low lysine digestibility (Nyachoti *et al.*, 2005; Lan, Opapeju and Nyachoti, 2008).

The addition of up to 40 percent high-protein maize DDG to diets fed to growing-finishing pigs was evaluated by Widmer *et al.* (2008), where maize HPDDG replaced all of the soybean meal in the maize-based diets. Overall growth performance was not different for pigs fed the maize HPDDG diets compared with pigs fed the maize-soybean meal control diets, but ADFI and ADG were reduced during the growing phase when 40 percent maize HPDDG was fed (Widmer *et al.*, 2008). These results indicate that maize HPDDG may be included in maize-based diets fed to growing-finishing pigs at levels needed to replace all the soybean meal, but it is necessary to include relatively large concentrations of crystalline amino acids in HPDDG diets to compensate for the low concentrations of lysine and tryptophan in this ingredient, and diets should always be formulated on the basis of standardized ileal digestible amino acids.

Widmer *et al.* (2008) also determined the effects of adding 5 or 10 percent maize germ to maize-soybean meal diets for growing-finishing pigs and observed a linear increase in the final weight of the pigs as the level of maize germ increased in the diets, and a tendency for increased average daily gain. Therefore, feeding diets containing 10 percent maize germ improves growth performance compared with typical maize-soybean meal diets, and it is possible that higher dietary inclusion rates can be used, but research to investigate this possibility is needed.

De-oiled DDGS was evaluated in diets fed to growing-finishing pigs in one experiment (Jacela *et al.*, 2008b). Results from this experiment showed that inclusion of 5, 10, 20 or 30 percent de-oiled maize DDGS linearly reduced ADG and ADFI. Based on the data from this experiment, it is concluded that de-oiled DDGS should not be included in diets fed to growing-finishing pigs. However, more research is needed to verify if the results from this experiment are repeatable or if it is possible to change diet formulations in such a way that de-oiled DDGS can successfully be included in diets fed to growing-finishing pigs.

Growing-finishing pigs – carcass composition and quality

The effects of feeding maize DDGS diets on carcass dressing percentage have been reported from 18 experiments (Table 11). In ten of these experiments, no difference in dressing percentage was observed (Fu *et al.*, 2004; McEwen, 2006, 2008; Xu *et al.*, 2007; Augspurger *et al.*, 2008; Drescher *et al.*, 2008; Duttlinger *et al.*, 2008b; Hill *et al.*, 2008a; Stender and Honeyman, 2008; Widmer *et al.*, 2008), whereas reduced dressing percentage of DDGS-fed pigs was observed in eight experiments (Cook, Paton and Gibson, 2005; Whitney *et al.*, 2006; Gaines *et al.*, 2007a, b; Hinson *et al.*, 2007; Xu *et al.*, 2010b; Linneen *et al.*, 2008; Weimer *et al.*, 2008). For pigs fed sorghum DDGS, the dressing percentage increased in one experiment (Senne *et al.*, 1996), was unaffected by dietary DDGS inclusion in one experiment (Senne *et al.*, 1998), and was reduced in five experiments (Feoli *et al.*, 2007b, c, 2008a, b, c). For pigs fed wheat DDGS, dressing percentage also was reduced (Thacker, 2006) and this was also the case for pigs fed de-oiled maize DDGS (Jacela *et al.*, 2008b). It has been suggested that the inclusion of fibre-rich ingredients in diets fed to pigs may reduce the dressing percentage of pigs because of increased gut fill and increased intestinal mass (Kass, van Soest and Pond, 1980). This may explain the reduced dressing percentage observed in DDGS-fed pigs in some experiments, but it is unknown why this effect has not been observed in other experiments.

Backfat thickness of pigs fed maize DDGS was reduced in one experiment (Weimer *et al.*, 2008), but in 14 other experiments no difference in backfat thickness was observed (Table 11). Loin depth was not affected by the dietary inclusion of maize DDGS in 12 experiments, but in two experiments loin depth was reduced (Whitney *et al.*, 2006; Gaines *et al.*, 2007b). A reduction in loin depth was also reported when wheat DDGS was included in the diet (Thacker, 2006). The reduced loin depth may be a result of pigs fed DDGS having lower ADG in these experiments and therefore being marketed at a lighter weight. Of the 14 experiments that reported lean percentage of pigs fed diets containing maize DDGS, only one experiment (Gaines *et al.*, 2007b) reported a reduction in lean percentage, whereas no differences were reported in the remaining experiments. Carcass lean percentage was also reported for pigs fed sorghum DDGS (three experiments) and wheat DDGS (one experiment), but no changes due to dietary DDGS inclusion were observed in these experiments.

Belly thickness was reported to be linearly reduced if maize DDGS was included in the diet (Whitney *et al.*, 2006; Weimer *et al.*, 2008), and also if sorghum DDGS was used (Feoli *et al.*, 2008c). However, pigs fed DDGS-containing diets also had reduced ADG in these experiments, and as a result they were marketed at a lighter weight than the

control pigs, which may explain the reduction in belly thickness. In the experiments by Widmer *et al.* (2008) and Xu *et al.* (2010a, b), no differences in the final bodyweight of pigs were observed, and in these experiments no differences were observed in belly thickness between pigs fed control or DDGS-containing diets.

The adjusted belly firmness of pigs fed diets containing maize DDGS is reduced compared with pigs fed maize-soybean meal diets with no DDGS (Whitney *et al.*, 2006; Xu *et al.*, 2010a; Widmer *et al.*, 2008). This observation is in agreement with data showing that the iodine value of the belly fat is increased in pigs fed DDGS (Whitney *et al.*, 2006; White *et al.*, 2007; Xu *et al.*, 2010a, b; Hill *et al.*, 2008a; Linneen *et al.*, 2008; Stender and Honeyman, 2008). An increase in iodine value of carcass fat also occurs when pigs are fed sorghum DDGS diets (Feoli *et al.*, 2007c; 2008b, c). The increase in carcass fat iodine values in pigs fed DDGS-containing diets is a result of the relatively large quantities of unsaturated fatty acids, particularly linoleic acid (C18:2), in maize and sorghum DDGS because increases in dietary unsaturated fatty acid concentrations will increase carcass fat iodine values (Madsen *et al.*, 1992).

Carcass fat iodine values are important measures of carcass quality because high iodine values result in soft and potentially less valuable bellies and loins. As a result, several studies have been conducted to evaluate alternative nutritional strategies in an attempt to reduce the negative effects of DDGS on iodine values. The dietary inclusion of up to 5 percent tallow in diets containing 40 percent sorghum DDGS did not reduce the iodine value in jowl fat (Feoli *et al.*, 2007c), even though tallow contains a high proportion of saturated fatty acids. Similarly, the addition of 5 percent tallow to 30 percent DDGS diets did not improve backfat or belly fat iodine values (Pomerence *et al.*, 2011). In contrast, the addition of one percent conjugated linoleic acid to diets containing 20 or 40 percent maize DDGS for ten days prior to pig harvest reduced fat iodine values and the n6:n3 ratio (White *et al.*, 2007). This observation is consistent with the observation that conjugated linoleic acids may reduce the activity of the delta-9 desaturase enzyme that is responsible for desaturation of de novo synthesized fatty acids (Gatlin *et al.*, 2002). Thus, addition of conjugated linoleic acids to DDGS containing diets fed during the late finishing phase may be used to reduce iodine values in carcass fat. Removal of DDGS from the diet during the final three to four weeks prior to harvest will also reduce the negative impact of DDGS on carcass fat iodine values, and will result in pigs that have acceptable iodine values (Hill *et al.*, 2008a; Xu *et al.*, 2010b). Evans *et al.* (2010) conducted a study to evaluate the effects on pork fat quality of feeding diets containing 0 or 0.6 percent conjugated linoleic acid, 0 or 20 percent DDGS, and 0 or 7.4 ppm ractopamine to finishing pig 27 days prior to harvest. Iodine

value increased in belly fat and jowl fat with diets containing DDGS and ractopamine, and decreased when finishing pigs were fed diets containing conjugated linoleic acid. Similarly, Gerlemann *et al.* (2010) evaluated the effects of feeding 0 or 20 percent DDGS, 0 or 7.4 ppm ractopamine, and 0 or 0.6 percent conjugated linoleic acid to finishing pigs 27 days prior to harvest on growth performance and carcass characteristics. Their results indicated that feeding diets containing ractopamine and conjugated linoleic acid improved growth performance and carcass quality, and the responses of DDGS, ractopamine and conjugated linoleic acid are independent of each other. Overall consumer acceptance of bacon and cooked pork loins from pigs fed diets containing up to 30 percent DDGS was evaluated by Xu *et al.* (2010b) and no differences were observed compared with pork from pigs fed maize-soybean meal diets.

There is no information on the effect of feeding diets containing wheat DDGS on belly firmness and iodine values, but wheat DDGS contains less fat than DDGS produced from maize or sorghum. Therefore, it is expected that inclusion of wheat DDGS in diets fed to finishing pigs will have less of an impact on carcass iodine values than if maize or sorghum DDGS is used.

Pigs fed diets containing maize HPDDG or de-oiled maize DDGS may also have softer bellies and increased iodine values compared with pigs fed maize-soybean meal diets (Jacela *et al.*, 2008b; Widmer *et al.*, 2008), but pigs fed diets containing maize germ have firmer bellies and reduced iodine values (Widmer *et al.*, 2008). There are no reports of the effects of other distillers co-products on carcass composition and quality. Overall consumer acceptance of pork from pigs fed maize DDGS, maize HPDDG, and maize germ was not different from that of pigs fed maize-soybean meal diets. It is therefore unlikely that consumers will be able to tell whether or not the pork they are eating was from a pig that was fed distiller's co-products or not.

Only one experiment has been conducted to evaluate the effects of feeding diets containing DDGS to gestating and lactating sows on pork (bratwurst) quality (White *et al.*, 2008). These researchers fed diets containing 30 percent DDGS during gestation and 15 percent DDGS during lactation, with or without an omega-3 feed supplement. Bratwurst from sows fed DDGS and the omega-3 dietary supplement had the highest overall quality score and a lower calculated iodine value compared with sows fed DDGS diets without the supplement, but higher iodine values than bratwurst from sows fed the control diet and the control diet supplemented with omega-3 fatty acids.

Feeding liquid distillers co-products to growing-finishing pigs

Squire *et al.* (2005) fed diets containing 0, 7.5, 15.0 and 22.5 percent CDS to growing pigs and showed that feed

TABLE 12
Growth performance, nutrient digestibility and carcass quality of pigs fed liquid diets containing maize and soybean meal with either non-fermented or fermented maize condensed distillers solubles (CDS) at 15% of DM

Parameter	Diet		
	Control	Non-fermented CDS	Fermented CDS
Initial BW (kg)	23.5	23.3	23.4
Final BW (kg)	50.1 a	47.5 b	48.6 ab
ADG (g)	952 a	858 b	898 ab
ADFI (kg)	1.62 a	1.49 b	1.61 a
Feed:gain	1.70	1.73	1.80
Energy digestibility (%)	81.6 ab	82.5 a	79.9 b
Protein digestibility (%)	72.5 a	73.2 a	69.3 b
Fat digestibility (%)	80.9 b	85.4 a	85.4 a
Final BW (kg)	106.5	107.0	–
Carcass dressing (%)	82.1	82.6	–
Backfat depth (mm)	16.6	17.1	–
Loin depth (mm)	54.3	53.7	–
Carcass lean yield (kg)	61.1	60.9	–
Loin pH	5.74 b	5.80 a	–
Loin drip loss (%)	9.63	8.83	–

Notes: ADG = average daily gain; ADFI = average daily feed intake; BW= body weight; a,b = Means within rows lacking a common letter are different ($P < 0.05$). Data for growth performance are expressed on a diet DM basis. Source: Based on data from de Lange *et al.*, 2006.

palatability was reduced when more than 15 percent CDS was included in the diet (Table 12). Feeding the non-fermented CDS diet resulted in reduced growth rate, feed intake and feed conversion compared with pigs fed the maize-soybean meal control diet, while growth performance of pigs fed the fermented CDS diet was not different from pigs fed the control diet (Table 12). Energy and protein digestibility were reduced when feeding the fermented CDS diet compared with pigs fed the non-fermented CDS and the control diet. However, fat digestibility of the non-fermented and fermented CDS diets was greater than when pigs were fed the control diet. In this study, only pigs on the control and non-fermented CDS diets were fed to slaughter weight. Feeding the non-fermented CDS diet resulted in similar carcass dressing percentage, backfat depth, loin depth and carcass lean yield compared with pigs fed the control diet, indicating that acceptable carcass quality can be achieved when feeding liquid non-fermented CDS diets to growing-finishing pigs. Loin pH was greater from pigs fed the CDS diet compared with pigs fed the control diet, which probably resulted in a trend toward reduced loin drip loss. Reduced drip loss is a significant benefit to meat processors.

Niven *et al.* (2006) reported results from a preliminary study that showed that growth rate and feed conversion were numerically improved when pigs were fed liquid diets containing 5 percent maize steep water, but adding 10 percent maize steep water numerically reduced pig performance. In a larger subsequent study, de Lange *et al.*

TABLE 13
Growth performance and carcass characteristics of pigs fed liquid diets containing increasing levels of phytase-treated maize steep water

Parameter	Inclusion of maize steep water (%)			
	0	7.5	15.0	22.5
Initial BW (kg)	69.1	68.8	68.8	69.3
Final BW (kg)	108.3	104.6	107.7	103.1
ADG (g)	1191 a	1080 a	1063 a	899 b
ADFI (kg)	2.76 a	2.49 ab	2.58 ab	2.29 b
Feed:gain	2.33 a	2.30 a	2.42 ab	2.55 b
Carcass weight (kg)	86.3	82.7	83.4	80.5
Loin depth (mm)	58.2	58.9	56.4	58.3
Backfat depth (mm)	18.1	18.7	18.0	17.1
Lean yield (%)	60.3	60.3	60.5	60.1

Notes: ADG = average daily gain; ADFI = average daily feed intake; BW= body weight; a,b = Means within rows lacking a common letter are different ($P < 0.05$). Source: Based on data from de Lange *et al.*, 2006.

(2006) showed that ADG, ADFI and F:G were not changed when pigs were fed liquid diets containing 0, 7.5 or 15 percent phytase-treated maize steep water, but adding 22.5 percent maize steep water to the diets resulted in reduced performance (Table 13). No effects were observed for dietary inclusion level of maize steep water for carcass weight, loin depth, backfat depth and lean yield.

In summary, feeding diets containing 15 percent fermented maize CDS results in growth performance comparable to when typical liquid maize-soybean meal diets are fed, but feeding diets containing 15 percent non-fermented maize distillers solubles results in reduced performance due to reduced palatability. However, feeding liquid diets containing 15 percent non-fermented CDS results in similar carcass composition compared with pigs fed liquid maize-soybean meal diets. Similarly, feeding liquid maize-soybean meal diets containing up to 15 percent maize steep water treated with phytase results in acceptable growth performance and carcass composition comparable to feeding a typical liquid maize-soybean meal diets. Maize CDS and steep water can successfully be used in liquid feeding systems for growing-finishing pigs to achieve satisfactory growth performance and carcass quality at a substantial savings in feed cost.

FEEDING CRUDE GLYCERIN TO SWINE

Growth performance and carcass characteristics

In swine, German researchers (Kijora and Kupsch, 2006; Kijora *et al.*, 1995, 1997) have suggested that up to 10 percent crude glycerin can be fed to pigs with little effect on pig performance. Likewise, Mourou *et al.* (1994) indicated that growth performance of pigs from 35 to 102 kg was not affected by the addition of 5 percent glycerin (unknown purity) to the diet. The impact of dietary glycerin on carcass quality in pigs has been variable. Kijora *et al.* (1995) and

Kijora and Kupsch (2006) showed no consistent effect of 5 or 10 percent crude glycerin addition to the diet on carcass composition or meat quality parameters, while in an additional study, pigs fed 10 percent crude glycerin exhibited a slight increase in backfat, 45-minute pH, flesh colour, marbling and leaf fat (Kijora *et al.*, 1997). Although they did not note any significant change in the saturated fatty acid profile of the backfat, there was a slight increase in oleic acid, accompanied by a slight decrease in linoleic and linolenic acid concentrations, resulting in a decline in the polyunsaturated to monounsaturated fatty acid ratio in backfat. Likewise, Mourot *et al.* (1994) reported no consistent change in carcass characteristics due to 5 percent crude glycerin supplementation of the diet, but did note an increase in oleic acid and a reduction in linoleic acid in backfat and *Semimembranosus* muscle tissue. Kijora and Kupsch (2006) found no effect of glycerin supplementation on water loss in retail pork cuts. However, Mourot *et al.* (1994) reported a reduction in 24-hour drip loss (1.75 versus 2.27 percent) and cooking loss was also reduced (25.6 vs 29.4 percent) from the *Longissimus dorsi* and *Semimembranosus* muscles due to dietary supplementation with 5 percent glycerin. Likewise, Airhart *et al.* (2002) reported that oral administration of glycerin (1 g/kg BW) 24 hours and 3 hours before slaughter tended to decrease drip and cooking loss of *Longissimus dorsi* muscle.

Recently, there has been increased interest in utilization of crude glycerin in swine diets due to the high cost of feedstuffs traditionally used in swine production. For newly weaned pigs, it appears that crude glycerin can be utilized as an energy source up to 6 percent of the diet, but crude glycerin does not appear to be a lactose replacement (Hinson, Ma and Allee, 2008). In 9 to 22-kg pigs, Zijlstra *et al.* (2009) reported that adding up to 8 percent crude glycerol to diets as a wheat replacement improved growth rate and feed intake, but had no effect on G:F. In 28 to 119-kg pigs, supplementing up to 15 percent crude glycerol to the diet quadratically increased ADG and linearly increased ADFI, but the net effect on feed efficiency was a linear reduction (Stevens *et al.*, 2008). These authors also reported that crude glycerin supplementation appeared to increase backfat depth and Minolta L* of loin muscle, but decreased loin marbling and the percentage of fat-free lean with increasing dietary glycerin levels. In 78 to 102-kg pigs, increasing crude glycerin from 0 or 2.5 percent to 5 percent reduced ADFI when fat was not added to the diet, but had no effect when 6 percent fat was supplemented (Duttlinger *et al.*, 2008a). This decrease in feed intake resulted in depressed average daily gain, but had no effect on feed efficiency. In contrast, Duttlinger *et al.* (2008b) reported supplementing up to 5 percent crude glycerin to diets had no effect on growth performance or carcass traits of pigs weighing 31 to 124 kg.

Supplementing 3 or 6 percent crude glycerin in pigs from 11 to 25-kg body weight increased average daily gain even though no effect was noted on feed intake, feed efficiency, dry matter, nitrogen or energy digestibility (Groesbeck *et al.*, 2008). Supplementing 5 percent pure glycerin did not affect pig performance from 43 to 160 kg, but pigs fed 10 percent glycerin had reduced growth rate and feed efficiency compared with pigs fed the control or 5 percent glycerin supplemented diets (Casa *et al.*, 2008). In addition, diet did not affect meat or fat quality, or meat sensory attributes. In 51 to 105-kg pigs, including up to 16 percent crude glycerin did not affect pig growth performance or meat quality parameters (Hansen *et al.*, 2009). Lammers *et al.* (2008b) fed pigs (8 to 133-kg body weight) diets containing 0, 5 or 10 percent crude glycerin and reported no effect of dietary treatment on growth performance, backfat depth, loin eye area, percentage fat-free lean, meat quality or sensory characteristics of the *Longissimus dorsi* muscle. In addition, dietary treatment did not affect blood metabolites or frequency of histological lesions in the eye, liver or kidney, and only a few minor differences were noted in the fatty acid profile of loin adipose tissue. Likewise, Mendoza *et al.* (2010a) fed heavy pigs (93 to 120 kg) up to 15 percent refined glycerin and reported no effect on growth performance, carcass characteristics or meat quality. Schieck *et al.* (2010b) fed pigs either a control diet (16 weeks, 31 to 128 kg), 8 percent crude glycerin during the last 8 weeks (45 to 128 kg) or 8 percent crude glycerin for the entire 16 week period (31 to 128 kg), and reported that feeding crude glycerin during the last 8 weeks before slaughter supported similar growth performance, with little effect on carcass composition or pork quality, except for improvement in belly firmness, compared with pigs fed the maize-soybean meal control diet. Longer-term feeding (16 weeks) resulted in a slight improvement in growth rate, but a small depression in feed efficiency. Some minor differences in carcass composition were noted, but there was no impact on pork quality. When considering the results from all of these studies (Table 14), there appears to be no consistent (positive or negative) effect of feeding up to 15 percent crude glycerin on growth performance, carcass composition or pork quality in growing-finishing pigs compared with typical cereal grain-soybean meal-based diets.

Sows

Only one study has been reported relative to feeding crude glycerin to lactating sows. In that study, lactating sows fed diets containing up to 9 percent crude glycerin performed similar to sows fed a standard maize-soybean-meal diet (Schieck *et al.*, 2010a).

EFFECTS OF DDGS ON PIG HEALTH

Distiller's by-products contain residual yeast cells and yeast cell components and approximately 3.9 percent of the

TABLE 14
Relative performance of pigs fed supplemental glycerin⁽¹⁾

Glycerin equivalency ⁽²⁾	ADG	ADFI	G:F ratio	Base feed	Pig size	Source
4.0 ⁽³⁾	105	109	98	Wheat-soybean meal-fish meal-	9–22 kg	Ziljstra <i>et al.</i> , 2009
8.0 ⁽³⁾	108	105	104	lactose		
5.0	98	100	99	Maize- soybean meal	10–22 kg	Hinson, Ma and Allee, 2008
2.7	107	103	103	Maize- soybean meal	11–25 kg	Groesbeck <i>et al.</i> , 2008
5.4	108	104	103			
4.8	105	108	97	Barley- soybean meal	31–82 kg	Kijora <i>et al.</i> , 1995
9.7	112	112	100			
19.4	96	103	94			
29.4	82	105	78			
2.9	103	108	97	Barley- soybean meal	24–95 kg	Kijora and Kupsch, 2006
4.9	102	106	97			
7.6	102	101	101			
8.3	102	107	97			
10.0	103	104	100			
10.0	106	110	96	Barley- soybean meal	27–100 kg	Kijora <i>et al.</i> , 1997
4.6	114	110	103	Barley- soybean meal	32–96 kg	Kijora <i>et al.</i> , 1995
9.7	119	113	106			
5.0	97	101	96	Wheat- soybean meal	35–102 kg	Mourot <i>et al.</i> , 1994
4.2	101	102	97	Maize- soybean meal (whey in	8–133 kg	Lammers <i>et al.</i> , 2008b
8.5	100	103	97	Phase 1)		
4.2	103	103	100	Maize- soybean meal	28–119 kg	Stevens <i>et al.</i> , 2008
8.4	103	104	99			
12.6	100	108	92			
2.5	99	99	99	Maize- soybean meal	31–124 kg	Duttlinger <i>et al.</i> , 2008b
5.0	99	101	98			
3.0	98	104	93	Wheat-barley-lupin, soybean	51–105 kg	Hansen <i>et al.</i> , 2009
6.1	87	93	95	meal -blood meal-meat meal		
9.1	96	102	94			
12.2	91	98	93			
6.6	104	105	98	Maize-soybean meal	31–127 kg	Schieck <i>et al.</i> , 2010b
2.5	97	99	98	Maize-soybean meal	78–102 kg	Duttlinger <i>et al.</i> , 2008a
5.0	95	97	98			
5.0	101	100	101	Maize-barley-wheat bran- soybean	43–159 kg	Casa <i>et al.</i> , 2008
10.0	96	100	95	meal		
5.0	106	105	101	Maize- soybean meal	93–120 kg	Mendoza <i>et al.</i> , 2010a
10.0	100	101	98			
15.0	95	100	95			

Notes: ADG = average daily gain; ADFI = average daily feed intake; BW= body weight. (1) Percentage relative to pigs fed the diet containing no supplemental glycerin. Percentage difference does not necessarily mean there was a significant difference from pigs fed the diet containing no supplemental glycerin. Main dietary ingredients and weight range of pigs tested are also provided with each citation. (2) Represents a 100% glycerin basis. In studies utilizing crude glycerin, values adjusted for purity of glycerin utilized. (3) Unknown purity, but product contained 6.8% ash and 15.6% ether extract.

dry weight of DDGS is contributed by yeast cell biomass (Ingledew, 1999). Beta-glucans, mannan-oligosaccharides, chitin and proteins are biologically important fractions of yeast cell walls and many of these compounds are capable of stimulating phagocytosis (Stone, 1998). Yeast cells also contain nucleotides, glutamate and other amino acids, vitamins and trace minerals, which may also affect the activity of the immune system when fed to pigs (Stone, 1998).

Whitney, Shurson and Guedes (2006a, b) conducted two experiments to investigate if adding 10 or 20 percent DDGS to the diet of young growing pigs was effective in reducing the prevalence, length or severity of intestinal

lesions produced by porcine proliferative enteropathy (ileitis) after pigs were challenged with *Lawsonia intracellularis*. These results indicated that dietary inclusion of DDGS may aid in resisting a moderate ileitis challenge similar to an approved antimicrobial regimen, but under more severe challenges, DDGS may not be effective.

Knott *et al.* (2005) studied the effects on weaned pigs of feeding spray-dried CDS, a spray-dried, high lipid fraction of CDS and a residual solubles fraction of CDS after the lipid was removed. Pigs fed diets containing either dried condensed distillers soluble or the residual soluble fraction had growth performance that was similar to that of pigs fed diets containing carbadox, but lower ADG and ADFI

than pigs fed diets containing spray-dried porcine plasma. Feeding the diet containing residual solubles and the positive control diet containing spray-dried porcine plasma resulted in greater villi height and villi height: crypt depth ratio compared with pigs fed diets containing carbadox.

More recently, Perez and Pettigrew (2010) showed that feeding diets containing up to 20 percent DDGS does not prevent pigs from bearing an *E. coli* infection or showing clinical signs of the disease. However, feeding DDGS diets appears to delay the shift from commensal to β -haemolytic coliforms in faeces, speed the excretion of β -haemolytic bacteria and recovery, as well as promote more stable and uniform gut microbiota.

In conclusion, results from one study indicate that feeding a diet containing DDGS may be effective in reducing the incidence, severity, and length of lesions caused by a moderate *Lawsonia intracellularis* infection. The mode of action of this response is unknown, but it seems that there are compounds in a fraction of CDS that may improve villi height: crypt depth ratio in the proximal portion of the small intestine. It also appears that feeding DDGS diets has beneficial effects in modulating the gut microbiota when weaned pigs are challenged with β -haemolytic coliforms.

EFFECTS OF DDGS ON NUTRIENT CONCENTRATION AND GAS AND ODOUR EMISSIONS OF SWINE MANURE

Odour and gas characteristics of swine manure, and energy, N and P balance were measured in pigs fed a maize-soybean meal diet or a diet containing DDGS (Spiehs *et al.*, 2000). Dietary treatment had no effect on H₂S, NH₃ or odour detection levels over the 10-week experimental period. Pigs fed the DDGS-containing diets had greater N intake, but ADFI and percentage N retention were not different between treatments. Feeding DDGS-containing diets tended to increase N excretion, but P retention did not differ between dietary treatments. Gralapp *et al.* (2002) fed diets containing 0, 10 or 20 percent DDGS to finishing pigs to determine the effects on growth performance, manure characteristics and odour emissions. There were no differences in total solids, volatile solids, chemical oxygen demand or total N or P concentration of manure among dietary DDGS levels. However, there was a trend for increasing odour concentration with increasing dietary levels of DDGS. More recently, Li, Powers and Hill (2010) compared the effects of feeding three diets (maize-soybean meal-based control diet, diet containing 20 percent DDGS with inorganic trace mineral sources, and a diet containing 20 percent DDGS with organic trace mineral sources) on ammonia, hydrogen sulphide, nitrous oxide, methane and non-methane total hydrocarbon emissions from growing-finishing pigs. Emissions of hydrogen sulphide, methane and non-methane total hydrocarbon emissions increased

when pigs were fed DDGS diets, but adding organic sources of trace minerals to diets alleviated the adverse effects of DDGS on hydrogen sulphide emissions.

Inclusion of DDGS in diets fed to lactating sows also reduced the concentration of P in the faeces (Hill *et al.*, 2008b), but it is unknown if total P excretion was reduced, because DM digestibility of the diets was not determined. Feeding diets containing 40 percent DDGS to gestating sows reduced apparent DM digestibility of the diet and increased faecal output, but did not affect the total volume of slurry produced or N, P or K output in slurry (Li, Powers and Hill, 2010; Li *et al.*, 2011).

The effects of extrusion and inclusion of DDGS on nitrogen retention in growing pigs has also been determined by Dietz *et al.* (2008). As DDGS increased in the diet, faecal N concentration increased but the concentration of N in the urine decreased. Extrusion and inclusion of DDGS in the diet reduced the amount of N digested per day, but N digestibility as a percentage of N intake decreased when DDGS was included in the diet but was not affected by extrusion. Nitrogen retention also tended to be reduced by dietary inclusion of DDGS and was reduced by extrusion, resulting in a trend for reduced net protein utilization from extrusion. These results suggest that extrusion of diets containing DDGS may reduce N retention in growing pigs.

Four experiments were conducted to evaluate effects of diet formulation method, dietary level of DDGS and the use of microbial phytase on nutrient balance in nursery and grower-finisher pigs (Xu *et al.*, 2006a, b; Xu, Whitney and Shurson, 2006a, b). Nursery pigs were fed a maize-soybean meal control diet or a diet containing 10 or 20 percent DDGS and formulated on a total P basis or on a relative bio-available P basis, using a relative P bio-availability estimate of 90 percent for DDGS (Xu, Whitney and Shurson, 2006a). Phosphorus digestibility, retention and faecal and urinary excretion were similar for pigs fed the control diet and pigs fed the DDGS containing diets. Within dietary DDGS levels, pigs fed diets formulated on a total P basis had greater P retention and urinary P excretion than pigs fed diets formulated on a relative bio-available P basis. No differences were observed among treatments in the concentration of soluble or insoluble P in the manure. It was also shown that pigs fed a DDGS-containing diet without or with phytase had lower DM digestibility compared with pigs fed a maize-soybean meal diets without or with phytase, which resulted in the excretion of greater manure volume (Xu *et al.*, 2006b). However, N digestibility and excretion were not affected by dietary treatment, but phytase improved P digestibility and reduced P excretion.

Diets without DDGS or with 20 percent DDGS and phytase were formulated to contain Ca:available P ratios of 2.0:1, 2.5:1 and 3.0:1 to determine the optimal Ca:available P ratio in nursery diets (Xu *et al.*, 2006a).

Dietary DDGS and phytase resulted in greater P digestibility and reduced P excretion compared with maize-soybean meal diets containing no DDGS or phytase. Nitrogen and Zn digestibility were not affected by dietary treatments, but Ca digestibility was greater for maize-soybean meal diets than for DDGS diets. There were no interactions between dietary DDGS and phytase and the Ca:available P ratio, suggesting that the range of Ca:available P ratios (2:1 to 3:1) established by NRC (1998) are acceptable when 20 percent DDGS and phytase are added to nursery diets to minimize P excretion in the manure.

The effects of feeding maize-soybean meal diets containing 20 percent DDGS and phytase on DM, N and P digestibility in growing-finishing pigs have also been measured (Xu, Whitney and Shurson, 2006b). Unlike for nursery-age pigs, feeding diets containing DDGS without or with phytase resulted in no change in DM digestibility and DM excretion. Although N digestibility was not affected by dietary treatment, there was a trend for reduced N excretion when phytase was added to the diets.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Much has been learned over the past decade about the nutritional value, optimal dietary inclusion rates, benefits and limitations of using DDGS in swine diets. However, current record high feed prices, as well as the abundant supply and cost competitiveness of DDGS, requires more evaluation of diet formulation approaches to further increase its use in swine diets without the risk of reduced performance. As high dietary inclusion rates of DDGS continue to be used, new feed formulation strategies and the use of additives effective in reducing the negative effects of DDGS on pork fat quality need to be developed. Nutritional tools need to be developed to provide accurate assessments of value differences among DDGS sources and provide accurate estimates of nutrient loading values (energy and digestible amino acids) for use in more accurate diet formulation as a means to manage variability in nutrient content and digestibility among sources. Further research is also needed to evaluate feed processing technologies and exogenous enzyme applications that can enhance energy and nutrient digestibility by focusing on the fibre component on distillers co-products. There appear to be potential health and immune system benefits from feeding distillers co-products to swine, which need to be further explored and understood. Finally, nutritional value and feeding applications for new distillers co-products need to be defined if they are to be used successfully in swine diets.

CONCLUSIONS

Dried distillers grain with solubles is the predominant maize distillers co-product used in swine diets. Although nutrient

content and digestibility varies among DDGS sources, it is considered to be primarily an energy source (approximately equal to that of maize), but also contributes significant amounts of digestible amino acids and available phosphorus to swine diets in all phases of production. Energy digestibility of DDGS can be improved by grinding to reduce particle size, but other feed processing technologies need to be further evaluated for their potential benefits in improving nutrient digestibility, with particular focus on the insoluble fibre fraction. The use of exogenous enzymes and other additives have potential for also improving the nutritional value of DDGS, but their responses have been inconsistent. Mycotoxin levels in United States maize DDGS are typically low and reflect the prevalence in the grain used to produce ethanol and DDGS. Although sulphur levels in DDGS are variable, and some sources may contain levels exceeding one percent, there is no evidence that sulphur levels in DDGS are detrimental to pig health and performance. Research is underway to determine the impact, if any, of lipid oxidation in DDGS on pig health and performance, although initial evidence indicates that supplemental dietary antioxidants may be warranted to achieve optimal growth performance.

If high quality maize DDGS is used, approximately 30 percent can be included in diets fed to lactating sows, weanling pigs, and growing-finishing pigs, whereas 50 percent can be included in diets fed to gestating sows. Dietary inclusion of sorghum DDGS should be limited to 20 percent in weanling pig diets, but 30 percent may be included in diets fed to growing-finishing pigs. Maize HPDDG may be included in diets fed to growing-finishing pigs in quantities sufficient to substitute all soybean meal, but there are no data on the inclusion of maize HPDDG in diets fed to sows or weanling pigs. Maize germ can be included in diets fed to growing-finishing pigs in concentrations of at least 10 percent.

Carcass composition and eating characteristics of pork products are not influenced by the inclusion of DDGS, HPDDG or maize germ in diets fed to growing-finishing pigs. However, belly firmness is reduced and fat iodine values are increased by the inclusion of DDGS and HPDDG in these diets. It may therefore be necessary to reduce the dietary inclusion levels of these co-products in the diets fed during the final 3 to 4 weeks prior to slaughter, or to supplement diets with conjugated linoleic acid to minimize negative effects on pork fat quality.

There is some evidence that feeding DDGS diets may enhance gut health of growing pigs, but more research is needed to determine if this response is repeatable. Formulating DDGS-containing diets on a digestible P basis reduces manure P concentration, but, due to lower DM digestibility, manure volume is increased in pigs fed diets containing DDGS. Adding DDGS to swine diets seems to

have minimal, if any impact on gas and odour emissions from manure, and with the exception of the concentration of P, the chemical composition of manure is not changed if pigs are fed DDGS containing diets. The use of crystalline amino acids to balance the amino acid profile in DDGS diets is essential not only for achieving optimal performance but also for minimizing excess nitrogen excretion.

Crude glycerin is a co-product from the biodiesel industry and contains more energy than maize for swine. When available and economical, glycerin may be included in diets for sows by up to 9 percent, in weanling pig diets by at least 6 percent, and in diets for growing-finishing pigs by up to 15 percent. At these inclusion levels, no change in pig performance or carcass composition will be observed, but feed flowability may be reduced. However, it is important to measure sodium and methanol content of the sources to be fed to swine in order to adjust dietary inclusion rates if necessary.

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Chapter 11

Co-products from biofuel production for farm animals – an EU perspective

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ABSTRACT

The first part of this chapter presents a brief history of co-products from bio-ethanol production. Co-products, such as distillers grain, are well known for their beneficial nutrient composition and have been used in animal nutrition since the early 1900s. Recent animal trials have shown that wheat-based dried distillers grain with solubles (DDGS) can replace protein supplements like soybean or rapeseed meals in dairy cow diets up to about 200 g/kg dry matter (DM). In contrast to maize-based DDGS in North America, which is higher in fat, European wheat-based DDGS has not influenced milk fat content negatively. Moreover, trials with fattening bulls showed that DDGS as a main protein source is able to sustain high productive performance. Trials with grower-finisher pigs suggested that DDGS up to 200 g/kg diet did not influence growth performance, fattening and slaughtering variables. Similarly, laying intensity of hens as well as egg quality and health were not affected by inclusion levels ranging from 150 g/kg to 300 g/kg diet. Trials with broilers suggest that diets that contain more than 100 g/kg DDGS may reduce performance. Hence, it is recommended to add non-starch polysaccharide (NSP)-degrading enzymes (e.g. xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS.

In the second part, a brief review and summary of data is presented on the use of glycerol for farm animals, with emphasis on ruminants, which will cover quality criteria for glycerol, rumen events and effects on feed intake and performance of dairy cows. As a fail-safe usage for glycerol in diets of all farm animals, methanol should be removed from the glycerol as far as technically possible. Glycerol at different purities may help to stabilize the hygienic quality of pelleted compound feeds without compromising pellet physical quality. Glycerol is a versatile feedingstuff, in particular for ruminants. Data on ruminal turnover of glycerol would suggest that it could replace rapidly fermentable carbohydrates and thus is not a direct competitor of propylene glycol. Previous studies have shown that glycerine may help to prevent ketoacidosis in high yielding dairy cows by increasing glucose precursors. Mature cattle can consume considerable quantities of glycerol (1 kg/day). However, greater dry matter intakes by cows supplemented with glycerine often did not result in increased milk or milk component yields. Further effort is thus required to fully explore the potential of glycerol in dairy cow diets, but type of diet and route of glycerol administration seem to play important roles.

In the third part, again putting an emphasis on ruminants, the feeding value of rapeseed products such as rapeseed meal (solvent-extracted) and rapeseed cake (mechanically extracted) is reviewed. Rapeseed meal compares well with soybean meal for dairy cows if fed on an isonitrogenous basis. Milk and milk component yields were similar for diets containing soybean meal or rapeseed meal. The value of rapeseed cake would benefit from standardization of the composition, because varying crude fat and crude protein concentrations makes the feeding value difficult to predict and could also affect storage stability of the cake. Even though the amino acid composition in rapeseed products is quite well balanced and favourable to non-ruminant animals, the sensitive reaction of pigs and poultry to glucosinolates in rapeseed meal and cake are still of concern. Therefore, it is recommended to add iodine, since glucosinolates act as antagonists. However, if glucosinolates are present in high concentrations, the negative effects may not be compensated, even if iodine is supplemented at high levels. Concluding, it is evident that a more widespread use of rapeseed meal and rapeseed cake in diets for pigs and poultry requires further reduction of glucosinolate levels.

Finally, energy utilization efficiency and sustainability of co-products from biofuel are addressed. To date, no definite regulations exist in order to assign emissions either to the main product or the co-product(s). Applying a causation principle, the producer or the responsible party should be accountable for all emissions. However, drying of DGS is only of interest if the products will be utilized as feedstuffs for animals, and thus emissions associated with processing of co-products are not of interest or necessity for biofuel producing companies.

MAIN MESSAGES

- **Efficient utilization of biofuel co-products is a key tool towards more sustainable biofuel production.**
- **Future research should quantify all activities in the processing of biofuel co-products in order to be able to evaluate carbon footprints.**
- **DDGS is a valuable protein supplement for ruminants and non-ruminants.**
- **Glycerine is a valuable energy supplement for ruminants and non-ruminants.**
- **Rapeseed meal and cake are valuable protein supplements for ruminants and non-ruminants.**

INTRODUCTION

Road transport fuels are considered to contribute about 18 percent of greenhouse gas (GHG) emissions in the EU (EEA, 2008; The Royal Society, 2008; Pinkney, 2009), with a consistent increase of about 1.6 percent per year (IEA, 2008a). Apart from more efficient vehicles and new transportation technologies, politics considered the use of biofuels as an essential element to reduce the emissions from fossil fuel and to decarbonize transport fuels. Some expert groups assessed the GHG reduction potential of biofuel as being at least 50 percent of fossil fuel emissions (e.g. CONCAWE, EUCAR and JRC, 2007; RFA, 2011). Estimations by IEA (2008a) expect an increase in world biofuel consumption from 24.4 million tonne oil equivalent (Mtoe) in 2006 to 94 Mtoe in 2020; 125 Mtoe in 2030; and approximately 210 Mtoe in 2050 (about 6 percent of the global need; IEA, 2008a). In 2020, about 55 Mtoe of biofuel will be consumed in the United States and the EU.

Fischer (2009) analysed the relationships among emerging biofuel development, food security and climate change, concluding that the additional non-food use of crops will have a significant impact on the world food system. Therefore, higher plant yields and the continuous development of the second generation of biofuels, produced from woody or herbaceous non-food plant materials, will receive increasing interest in the future (IEA 2008b).

The CO₂-saving effect or the carbon footprints (CF) of biofuel of the first generation depends on many factors, such as proper manufacturing, using the most appropriate feedstock, efficiency of feed production for fermentation, processing of co-products (e.g. drying), and further use of co-products. The utilization of co-products from biofuel production of the first generation, such as glycerine, oilseed cakes, meals and distillers grain with solubles in wet (DGS) or dried (DDGS) form is an important and controversial issue (see Windhorst, 2008; Fischer, 2009; Pinkney, 2009) that encompasses:

- contribution to the reduction of GHG emissions;
- pressure on land use; and
- competition between feed, food and fuel for crop yields.

Co-products may contribute to mitigate this conflict. They contain less fat and starch than oilseeds and cereal

grains, respectively, but more fibre, proteins and minerals. The crude protein (CP) concentration of the co-products varies between 300 and 400 g/kg dry matter (DM) and is similar to some traditional feed protein sources. All environmental and nutritional aspects and calculations (e.g. CF) should consider the whole processing chain and all final products. Crutzen *et al.* (2008) estimated the N₂O release from agro-biofuel production without considering co-products and their utilization. They concluded that use of cereal grains and rapeseed for biofuel production is very ineffective and environmentally unfriendly. However, in a more recent publication on this subject the same authors performed a life-cycle analysis and came to a similar conclusion, namely that biofuel production may trigger a net increase in global warming (Mosier *et al.*, 2009).

The objective of this chapter is to analyse and summarize results of studies dealing with co-products from biofuel production in farm animal nutrition under European conditions.

CO-PRODUCTS FROM BIO-ETHANOL PRODUCTION History

Distillers grain with solubles in wet and dry forms are the most important co-products of alcohol production from cereal grains. The starch of the raw material is mainly fermented to alcohol. The co-product comprises all the other components of the original substrate, such as CP, ether extract, fibre and ash as well as the CP from yeast used for fermentation. Traditionally, DGS at DM concentrations of 40–90 g/kg has been fed to ruminants, horses and pigs in close proximity to the distilleries.

At the end of the nineteenth century considerable data were available on the composition and the feed value of distillers grain (e.g. Schulze and Maerker, 1872, and Behrend and Morgan, 1880, both noted in Kellner, 1905). Already at that time it was known that the raw materials had the ability to influence the composition of DGS, with Maercker (1908) describing that the fermentation of cereal grains resulted in co-products (i.e. DGS) with the highest concentration of nutrients, while molasses fermentation gave the lowest nutritive value. On the basis of the composition of the original substrate and the alcohol output,

TABLE 1
Composition (g/kg dry matter unless stated) of distillery co-products (fresh and dried) of various origins

Source of co-product	Water (g/kg)	Crude protein	Crude fat (Ether extract)	Crude fibre	N-free extractives	Ash
Cereal grains, unspecified, dried	75	235	75	134	415	66
Maize grain, fresh	913	20	9	8	45	5
Dried	86	285	107	102	401	22
Molasses, fresh	922	19	–	–	40	19
Rye grain, fresh	922	17	4	7	46	4
Dried	100	165	82	162	478	13
Potatoes, fresh	943	12	1	6	31	7
Dried	100	243	37	95	408	117

Source: Kellner, 1905.

TABLE 2
Mean digestibility coefficients (ranges in parentheses) of distillery co-products for ruminants and pigs

Source of co-product	Organic matter	Crude protein	Crude fat (Ether extract)	N-free extract	Crude fibre
Ruminants					
Cereals grains, general	0.710 (0.600–0.810)	0.640 (0.490–0.800)	0.940 (0.920–0.940)	0.800 (0.540–0.850)	0.610 (0.410–0.920)
Maize grain	0.690 (0.660–0.720)	0.640 (0.610–0.670)	0.930 (0.910–0.950)	0.700 (0.700–0.710)	0.670 (0.640–0.700)
Rye grain	0.570 (0.450–0.680)	0.590 (0.520–0.650)	0.620 (0.600–0.640)	0.490 (0.440–0.540)	0.500 (0.370–0.620)
Pigs					
Cereal grains, general	0.580	0.780	0.560	0.510	0.360

Source: Kellner, 1905.

the same author calculated the composition of DGS. In his famous textbook *The Nutrition of Domestic Animals*, Kellner (1905) summarized the composition (Table 1), digestibility (Table 2) and starch units for different co-products of ethanol production.

Developments in distilling technology with consequences for composition and nutritive value of DGS during the last century were reported in several scientific publications (e.g. Naesi, 1985; Askbrant and Thomke, 1986), in animal feeding (e.g. Jensen, Falen and Chang, 1974; Firkins, Berger and Fahey, 1985), as substrate for ensiling (e.g. Abrams *et al.*, 1983, Flachowsky *et al.*, 1990) and were summarized in various textbooks in Germany (e.g. Kling, 1928; Nehring, 1949; Becker and Nehring, 1967; Kling and Wöhlbier, 1983; Menke and Huss, 1987; Jeroch, Flachowsky and Weißbach, 1993).

Due to the high demand for liquid fuels throughout Europe and the decreasing availability of fuels from fossil sources, the production of biofuel, including bio-ethanol, has gained more importance. The increased production capacity and the increasing number of large biofuel plants has resulted in large amounts of DGS. It is unrealistic to distribute large quantities of DGS beyond the immediate vicinity of a biofuel plant. Due to the short shelf life of DGS, a large proportion is dried and used as dried distillers grain with solubles (DDGS). The nutritional quality of DGS and DDGS varies considerably, reflecting the variability of

the feedstocks, the diversity of the production processes and the proportion of solubles that are included in the final product (Belyea, Rausch and Tumbleson, 2004; Losand *et al.*, 2009; Zijlstra and Beltranena, 2009). Intensive research on the use of distillers grain—mostly maize-based—in livestock has been conducted in North America over the past years (reviewed i.a. by Klopfenstein, Erickson and Bremer, 2008; Schingoethe *et al.*, 2009). However, experiments that examine the nutritional value of DDGS common in Europe, based on wheat, barley or rye, or mixtures of these grains, are rare (Franke, Meyer and Flachowsky, 2009; Aldai *et al.*, 2010; Meyer *et al.*, 2010; Noblet *et al.*, this volume).

Nutritive value and feeding to ruminants

The chemical composition and energy concentration of DGS and DDGS from different grains are presented in Table 3. Distillers grain with solubles is high in CP, with considerable variation between the different types of grain used in the production process. The highest average CP content, 370 g/kg DM, was reported for DDGS produced from a mix of 90 percent wheat and 10 percent barley (Franke, Meyer and Flachowsky, 2009; Losand *et al.*, 2009; Meyer *et al.*, 2010). Mustafa, McKinnon and Christensen (2000) reported that the ruminal escape of CP was lower for wheat- than barley-based DGS (490 versus 415 g/kg CP). Generally, distillers grain has a relatively high fibre concentration, with highest cell-wall (neutral-detergent fibre –

TABLE 3
Chemical composition and net energy (NE) concentration (g/kg of dry matter unless stated) of distillers grain with solubles in wet (DGS) or dried (DDGS) form, as reported by various sources

	Grain source and form					
	Barley, wheat and rye-triticale DGS ⁽¹⁾	Wheat unspecified ⁽²⁾	Wheat and barley DDGS ⁽³⁾	Wheat and barley DDGS ⁽⁴⁾	Rye DGS ⁽⁵⁾	Wheat and barley DDGS ⁽⁶⁾
Dry matter (DM) (g/kg)	289	n.a. ²	923	934	n.a.	923
Crude protein	154	362	367	370	153	367
Ether extract	60	67	62	50	67	64
Ash	42	54	58	54	28	58
NDF	743	414	496	305	n.a.	490
ADF	311	173	159	155	n.a.	162
Starch	110	n.a.	n.a.	n.a.	54	n.a.
Sugar	n.a.	n.a.	n.a.	n.a.	45	n.a.
Calcium	n.a.	3.0	n.a.	n.a.	n.a.	n.a.
Phosphorus	n.a.	10.5	n.a.	n.a.	n.a.	n.a.
Sodium	n.a.	2.3	n.a.	n.a.	n.a.	n.a.
Magnesium	n.a.	6.0	n.a.	n.a.	n.a.	n.a.
Sulphur	n.a.	5.7	n.a.	n.a.	n.a.	n.a.
NE maintenance (MJ/kg)	n.a.	9.13	n.a.	n.a.	n.a.	n.a.
NE gain (MJ/kg)	n.a.	6.28	n.a.	n.a.	n.a.	n.a.
NE lactation (MJ/kg)	n.a.	8.46	n.a.	n.a.	n.a.	n.a.
NE lactation (MJ/kg DM)	n.a.	n.a.	n.a.	7.3	n.a.	n.a.

Notes: n.a. = not analysed; NDF = neutral-detergent fibre; ADF = acid-detergent fibre. Sources: (1) Mustafa, McKinnon and Christensen, 2000; (2) Schingoethe *et al.*, 2009; (3) Franke, Meyer and Flachowsky, 2009; (4) Losand *et al.*, 2009; (5) Engelhard, 2011; (6) Meyer *et al.*, 2010.

TABLE 4
Digestibility coefficients of nutrients measured in sheep according to GfE (1991) and estimated concentrations of metabolizable energy (ME) of distillers grain with solubles in wet (DGS) or dried (DDGS) form from rye, wheat or wheat+barley

Grain source + supplement	Rye + DGS	Wheat or wheat+barley, + DDGS ⁽¹⁾	Wheat+barley, +DDGS ⁽²⁾
n	6	15	4
Organic matter	0.568 (±0.038)	0.758 (±0.048)	0.780 (±0.021)
Ether extract	0.598 (±0.302)	0.839 (±0.107)	0.914 (±0.010)
Crude fibre	0.515 (±0.100)	0.517 (±0.259)	
n		4	
NDF		0.650 (±0.131)	
ADF		0.544 (±0.110)	
ME (MJ/kg DM)	9.1	12.1	12.6

Notes: n = number of sheep in trial; NDF = neutral-detergent fibre; ADF = acid-detergent fibre. (1) Means with standard deviation in parenthesis. (2) Least squares means with standard error in parenthesis. Sources: Alert, Losand and Priebe, 2007; Losand *et al.*, 2009; Meyer *et al.*, 2010.

NDF) values found for barley-based distillers grain, probably due to a greater hull proportion in grain DM.

Nutrient digestibility coefficients can be used to calculate metabolizable energy (ME) for ruminating animals (GfE, 1995). Therefore a number of experiments were carried out with adult wethers in order to evaluate the nutrient digestibility of rye DGS as well as wheat- or wheat+barley-based DDGS. The experimental diets consisted of grass hay, grass silage or straw supplemented with DDGS ranging from 15 to 75 percent of diet DM. The apparent total tract digestibility of organic matter, ether extract, crude fibre, NDF and acid-detergent fibre (ADF) is shown in Table 4

The digestibility of ether extract and fibre fractions showed the most variation. When compared with rapeseed meal, wheat- and barley-based DDGS had similar organic

matter and ether extract digestibilities (Meyer *et al.*, 2010). Organic matter digestibility of the rye-based DGS was notably lower and ranged from 0.531 to 0.619 (Alert, Losand and Priebe, 2007). This is reflected in a lower concentration of ME for rye DGS, for which no obvious explanation exists. The ME concentrations of wheat- and barley-based DDGS compared well with ME of rapeseed meal (RSM; Meyer *et al.*, 2010).

Table 5 shows results of experiments with lactating dairy cows conducted in Germany and Austria that compared DDGS or DGS (mainly based on wheat) with other protein supplements, like RSM or soybean meal (SBM). The aim of these studies was to investigate whether the different kinds of distillers grain can adequately replace RSM or SBM in diets of high-yielding cows. Most of the rations comprised a con-

TABLE 5
Comparison of four trials with distillers grain with solubles in wet (DGS) or dried (DDGS) form, mainly from wheat fermentation, in diets for lactating dairy cows

Parameter	Trial 1		Trial 2		Trial 3			Trial 4	
Duration (days)	147		50		n.s.			60	
Cows (n)	16		36		126			123	
Basal diet	MS, GS		MS, GS		MS, GS			MS, GS, Hay	
Protein supplement (kg DM/day)	Wheat DDGS	RSM	Rye DWG	BG	Wheat DDGS	SBM, RSM	Maize DDGS	Wheat DDGS	SBM, RSC
	3.5	3.6	ca. 3.8	ca. 1.9	ca. 1.8	ca. 1.5	ca. 1.1	ca. 1.0	ca. 1.2
DM intake (kg/day)	20.8	21.9	ca. 24.0	ca. 23.6	n.s.			20.8	20.9
Milk (kg/day)	34.9	34.0	42.1	42.5	35.8	37.0	26.4	25.9	26.2
Fat (g/kg milk)	32.6	35.3	38.9	39.7	41.0	42.0	44.6	44.8	44.3
Protein (g/kg milk)	31.1	32.9	32.3	32.4	35.1	35.3	33.3	33.4	33.9

Notes: MS = maize silage; GS = grass silage; RSM = rapeseed meal; BG = brewers grain; SBM = soybean meal; RSC = rapeseed cake. Sources: Trial 1 – Franke, Meyer and Flachowsky, 2009, working at Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Institute for Animal Health, Braunschweig, Germany. Trial 2 – Engelhard, 2011, working at Centre for Livestock Husbandry and Equipment, Regional Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt (LLFG), Iden, Germany. Trial 3 – Dunkel, 2011, working at Agricultural Research Centre of Thuringia (TLL), Jena, Germany. Trial 4 – Urdl *et al.*, 2006, working at Institute of Livestock Research, Agricultural Education and Research Centre Raumberg-Gumpenstein (LFZ), Irdning, Austria.

TABLE 6
Comparison of dried distillers grain with solubles (DDGS) in diets for bulls during the whole fattening period and growing male calves before the beginning of the fattening period

	Trial 1			Trial 2				Trial 3			
Animals (n)	44		42	15		14	15	21			
Final live weight (kg)	710	712	720	556	560	557	558	162	164	153	157
Basal diet	MS			MS				MS + Hay			
Protein supplement	DDGS	SBM	RSM	DDGS	SBM	RSM	RSM + DDGS	DDGS	RSM	DDGS	RSM
Supplement intake (kg DM/day)	ca. 1.3	ca. 1.0	ca. 1.4	1.44	0.96	1.30	0.72 + 0.74	0.42	0.44	0.59	0.58
DM intake (kg/day)	9.37	9.37	9.51	7.66	7.54	7.59	7.97	2.4	2.4	2.9	3.0
Crude protein intake (kg/day)	1.110	1.116	1.102	1.118	1.103	1.078	1.155	0.412	0.423	0.469	0.476
Energy intake (MJ ME/day)	108.3	109.3	111.0	86.2	84.9	84.7	89.3	31.0	30.3	35.5	36.2
Live weight gain (kg/day)	1.493 b	1.602 a	1.549 ab	1.310 b	1.390 ab	1.440 ab	1.460 a	1.008	1.039	1.003	1.053

Notes: MS = maize silage; RSM = rapeseed meal; SBM = soybean meal; RSC = rapeseed cake; ME = metabolizable energy. a, b = Different suffixes in a row within a trial indicate significant differences ($P < 0.05$). Sources: Trial 1 – Ettle *et al.*, 2009, working at Institute for Animal Nutrition and Feed Management, Bavarian State Research Centre for Agriculture (LfL), Poing, Germany. Trial 2 – Meyer *et al.*, 2010, working at Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Institute for Animal Health, Braunschweig, Germany. Trial 3 – Preißinger, Spiekens and Obermaier, 2009, working at Institute for Animal Nutrition and Feed Management, Bavarian State Research Centre for Agriculture (LfL), Poing, Germany.

siderable portion of grass silage and maize silage. The proportion of distillers grain in the diets ranged from 50 g (Urdl *et al.*, 2006) to 170 g/kg DM (Franke, Meyer and Flachowsky, 2009). The feed intake in all experiments varied between 21 and 24 kg DM/day and was not influenced by protein source. Mean milk yield and milk fat concentration across studies ranged from 26 to 43 kg/day and from 33 to 45 g/kg milk. However, no significant differences were detected within the experiments. Only one study showed a lower milk protein concentration yet no lower protein yield for cows fed DDGS compared with RSM (Franke, Meyer and Flachowsky, 2009). In accordance with recommendations of Schingoethe *et al.* (2009) the outcome of the different experiments suggest that distillers grain can replace other protein supplements up to about 200 g/kg DM in dairy cow rations.

The results of trials with male calves and fattening bulls are presented in Table 6. Primarily wheat-based DDGS

replaces RSM or SBM in maize silage or maize silage- and hay-based rations. The animals were fed DDGS from 140 g (Ettle *et al.*, 2009) up to 200 g/kg DM (Preißinger, Spiekens and Obermaier, 2009) of the diets. No differences between protein sources were detected in DM, CP and ME intake, nor in liveweight gain in both experiments with Simmental calves (Preißinger, Spiekens and Obermaier, 2009). Due to the higher final live weight, the mean feed intake of Simmental bulls (Ettle *et al.*, 2009) was higher (9.4 versus 7.7 kg DM/day) than that of Holstein bulls (Meyer *et al.*, 2010). Simmental and Holstein bulls showed good growth performance, and liveweight gain averaged about 1.55 and 1.40 kg/day, respectively. However, liveweight gain differed significantly within experiments. Ettle *et al.* (2009) found differences between bulls fed DDGS (1.49 kg/day) and SBM (1.60 kg/day), which might be a result of the higher energy concentration of SBM, as DM intakes were not different

across treatments. Feeding a mixture of DDGS and RSM resulted in the highest weight gain (1.46 kg/day) compared with SBM, RSM or DDGS (1.31 kg/day; Meyer *et al.*, 2010). The results of the experiments with fattening bulls showed that DDGS as the main protein source compares well with other protein supplements and is able to sustain high productive performance. This also indicates that differences between CP sources regarding the amino acid pattern of the ruminally undegraded CP (RUP) was not a constraint for intensive growth.

Nutritive value and feeding to non-ruminants – pigs

Co-products from biofuel production, such as DDGS, have also been fed to non-ruminant animals, particularly pigs (e.g. Lindermayer, 2004; Richter *et al.*, 2006a; Berk, 2007; Hackl *et al.*, 2007; Berk, Lebzien and Flachowsky, 2008; Kluge and Kluth, 2008) and poultry (e.g. Damme and Pegeanova, 2006; Richter *et al.*, 2006b; Trautwein *et al.*, 2008). Patience *et al.* (2007) summarized mainly North American results from feeding studies with DDGS in pigs.

Some authors investigated the amino acid pattern of DDGS and its praecaecal digestibility in pigs (e.g. Richter *et al.*, 2006a; Hackl, Pripke and Henning, 2007; Hackl *et al.*, 2007; Kluth, Wolf and Rodehutsord, 2009). Hackl, Pripke and Henning (2007) and Hackl *et al.* (2007) studied a wheat DDGS with 386 g CP per kg DM. Compared with wheat (32 g lysine per kg CP), DDGS contained only 17 g lysine per kg CP. The low concentration and the low praecaecal digestibility coefficient of lysine in wheat-DDGS (0.69 compared with 0.872 for wheat) underline the significance of lysine as the first limiting amino acid in DDGS for pigs. Although DDGS contains about 2.5–3 times more CP than wheat, it has only 1–1.5 times the concentration

of praecaecally digestible lysine. Very low praecaecal digestibilities have been reported by Hackl, Pripke and Henning (2007) and Hackl *et al.* (2007) only for sulphur-containing amino acids (0.67–0.69), but not for most of the other essential amino acids. In broilers, however, Kluth, Wolf and Rodehutsord (2009) measured a praecaecal digestibility coefficient for lysine in DDGS of 0.79.

In a feeding trial with 80 growing-finishing pigs (40 females and 40 castrated males) from 35 kg initial live weight up to 115 kg slaughter weight, Berk (2007) partially replaced SBM and/or RSM by DDGS or a DDGS/RSM mix (Table 7). The feed in mash form and drinking water were offered for ad lib intake. Feed intake, total weight and slaughtering results were not influenced ($P > 0.05$) by protein source. From this data it can be concluded that DDGS can partially replace SBM in diets for growing-finishing pigs in intensive production systems.

Richter *et al.* (2006a) carried out four feeding trials with piglets (0–100 g/kg DDGS in the diet; Table 8) as well three trials with growing-finishing pigs (0–250 g/kg DDGS in the diet; Table 9). The authors concluded that piglets below 10 kg live weight should not consume DDGS, and diets of heavier animals could receive DDGS up to 100 g/kg diet.

The results suggest that DDGS up to 200 g/kg in the diet of grower-finisher pigs did not influence performance. The lower recommended inclusion level for piglets is most likely due to the low lysine content of the DDGS. Hence, higher inclusion levels may be possible if lysine levels are adjusted accordingly. Kluge and Kluth (2008), Punz, Windisch and Schedle (2010) and Schedle, Mair and Windisch (2010) replaced SBM in grower-finisher diets completely by DDGS, and observed no adverse effect on fattening and slaughtering variables. Additional non-starch

TABLE 7
Protein sources for grower or finishers, feed intake, daily weight gain and some slaughter data for pigs

Protein source	Animal	Soybean	Soybean/RSM	Soybean/DDGS	SBM+RSM +DDGS
Soybean meal	Grower	15.0	6.0	8.0	6.0
	Finisher	11.0	–	5.0	3.0
Rapeseed meal	Grower	–	10.0	–	5.0
	Finisher	–	15.0	–	6.0
DDGS	Grower	–	–	8.0	5.0
	Finisher	–	–	10.0	6.0
Crude protein (g/kg DM)	Grower	178	176	178	175
	Finisher	163	166	166	169
Feed intake (kg/animal/day)	total	2.83	2.81	2.83	2.76
Weight gain (g/animal/day)		1010	959	998	940
Lean meat (%)		54.4	55.6	54.7	55.7
Backfat thickness (mm)		29.0	28.0	28.4	25.1
Backfat fatty acids (% of total)					
SFA		40.5	40.1	41.1	39.2
MUFA		47.4	49.5	46.8	48.8
PUFA		12.1	10.4	12.0	12.4

Notes: SBM = soybean meal; RSM = rapeseed meal; DDGS = dried distillers grain with solubles; SFA = short-chain fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids. Source: Berk, 2007.

TABLE 8
Average liveweight gain (g/day) of piglets (18–65 animals per treatment; initial age: 28–48 days; final age: 70 days) fed with various amounts of wheat-based dried distillers grain with solubles (DDGS)

Trial	DDGS (g/kg of diet)				
	0	30	50	80	100
1	480 a	440 bd	448 bc	417 d	–
2	518	–	–	–	505
3	445 a	–	408 ab	–	346 c
4	364	–	353	–	361

Notes: a,b,c,d = different suffixes indicate significant differences ($P < 0.05$). Source: Richter *et al.*, 2006a.

TABLE 9
Average liveweight gain (g/day) of pigs (15–36 animals per treatment; initial live weight: 27–32 kg; final live weight: 112–121 kg) fed with various amounts of wheat-based dried distillers grain with solubles (DDGS)

Trial	DDGS (g/kg of diet)				
	0	100	150	200	250
1	791	784	787	–	–
2	834 a	–	827 a	–	745 b
3	932	905	–	939	–

Notes: a,b different suffixes indicate significant differences ($P < 0.05$). Source: Richter *et al.*, 2006a.

polysaccharide (NSP) enzyme supplementation did not improve animal performance.

Another important aspect of DDGS incorporation in pig diets is P excretion, which is a major concern for the swine industry due to its potential impact on the environment. There are no European studies on this subject reported so far. A Canadian study evaluated the effect of wheat-based DDGS on P excretion patterns of grower-finisher pigs. Intake, excretion and retention of P were influenced by DDGS. Total tract P digestibility of DDGS was 40 percentage units higher than that of wheat. Similarly, daily P excretion of pigs fed DDGS was higher than that of pigs fed the wheat control diet (Widyaratne and Zijlstra, 2007). Another study conducted in North America measured, *inter alia*, P in maize-based DDGS fed to growing pigs. Apparent total tract digestibility for P in DDGS was measured at 59.1 percent while the control group fed a maize-based

diet had apparent total tract digestibility of 19.3 percent. It was concluded that with DDGS a greater proportion of the organic P will be digested and absorbed, thus reducing the need to add inorganic P to pig diets (Pedersen, Boersma and Stein, 2007).

Nutritive value and feeding to non-ruminants – poultry

Richter *et al.* (2006b) included up to 200 g/kg of wheat-based DDGS in diets for chicks, pullets, laying hens and broilers. No effect of DDGS inclusion level on growth performance of chicks and pullets was observed (Table 10).

Laying intensity of hens as well as egg quality were not affected ($P > 0.05$) by 150 g/kg DDGS in diets of laying hens (Damme and Peganova, 2006; Richter *et al.*, 2006b). Askbrant and Thomke (1986) observed no negative effect on egg yield and health of laying hens fed diets with 300 g/kg DDGS.

Richter *et al.* (2006b) carried out three feeding studies with 276 broilers per treatment (unsexed). The diets contained 0, 50, 100, 150 or 200 g/kg DDGS and was offered in pelleted form from days 1–14; mash feed was fed from days 15–33. The final live weight of the broilers amounted to 1995, 1987, 1953, 1884 and 1842 g per animal for DDGS inclusion levels of 0, 50, 100, 150 and 200 g/kg, respectively. These results suggest that diets that contain more than 100 g/kg DDGS may reduce performance, which is in agreement with Chidothe, Acamovic and McDevitt (2002), Chidothe, McDevitt and Acamovic (2002) and Trautwein *et al.* (2008).

Other authors added NSP-degrading enzymes (e.g. xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS. In addition to an improved energy supply due to partial degradation of NSP and subsequent absorption of its constituent sugars (reviewed by Dänicke, 1999), the supplementation of xylanase is supposed to change the composition and metabolic potential of bacterial populations and may also influence fat absorption in younger animals (Hübner, Vahjen and Simon, 2002). Dalibard, Gadi and Kratz (2008) added an NSP-enzyme produced by *Penicillium funiculosum* to diets of layers containing 100 or

TABLE 10
Influence of dried distillers grain with solubles (DDGS) on live weight and feed conversion ratio (FCR) of chicks and pullets (average of two trials; 168 animals per treatment)

	DDGS in diet (g/kg)				
	0	50	100	150	200
Live weight (g) at:					
8 weeks	654	654	658	644	656
18 weeks	1432	1439	1448	1429	1435
FCR (kg/kg, feed/gain)					
0–8 weeks	3.16	3.18	3.17	3.17	3.16
0–18 weeks	5.12	5.13	5.08	5.09	5.10

Source: Richter *et al.*, 2006b.

200 g/kg maize-based DDGS. Enzyme supplementation did not increase nutrient digestibilities and energy concentration, but enzyme-supplementation of diets with 100 and 200 g/kg DDGS increased apparent ME concentration by 0.24 and 0.18 MJ/kg DM, respectively. Richter *et al.* (2006b) measured higher final live weight of chicks and pullets after enzyme supplementation in a diet with 150 g/kg DDGS. However, laying hens did not respond to enzyme supplementation. Chidothe, Acamovic and McDevitt (2002) and Chidothe, McDevitt and Acamovic (2002) measured higher liveweight gain in broilers fed with 100 and 200 g/kg enzyme-supplemented DDGS, but the gain was still below the level of the control group without DDGS. Similar results have been reported by Trautwein *et al.* (2008) after feeding diets with 100 g/kg DDGS.

Another important aspect which needs to be considered is the availability of P. Studies referring to wheat-based DDGS, the most common DDGS source in Europe, is reviewed in another chapter in this document, which provides a more in-depth account of wheat DDGS in poultry (Noblet *et al.*, this volume). Studies on maize-based DDGS reported a substantial variability in relative P bio-availability among different batches, which seems mainly due to different heating conditions employed during processing. During the process of fermentation for bio-ethanol production, small quantities of phytase are produced by the yeast, converting the P into better available forms (Martinez Amezuca, Parsons and Noll, 2004).

CO-PRODUCTS FROM BIODIESEL PRODUCTION

Glycerine

Biofuel production in the European Union is mainly based on biodiesel production from rapeseed oil, basically in the form of rapeseed oil methylester, leaving glycerine as a co-product. During biodiesel generation, fatty acids are hydrolyzed from the glycerine backbone of the triglyceride molecule by a transesterification process using methanol. Subsequent to separation of the fatty acid esters, glycerine still contains methanol and salts from the reactions. Separation or purification of glycerine can fluctuate depending on the plant and the process applied (Schröder and Südekum, 1999). Yield of glycerine from this process is approximately 1 unit per 10 units of biodiesel produced (Friedrich, 2004).

Starting around 60 years ago, researchers have shown that glycerine may help prevent ketoacidosis in the high-yielding dairy cow by increasing glucose precursors (Forsyth, 1953; Johnson, 1954; Fisher, Erfle and Sauer, 1971; Fisher *et al.*, 1973). Around 40 years ago, glycerine was registered as a feed additive (E 422) in the European Union (Anonymous, 1970) with no restrictions as to animal species and quantity added to feeds. Today, glycerine is listed as a feedstuff in the “Positive List” of authorized feed materials (Standards

Commission for Straight Feeding Stuff, 2011). Meanwhile, research expanded not only for dairy cattle but also other farm animals to elucidate the conditions under which glycerine could be used advantageously. The reader is referred to two other chapters in this book, which provide a more in-depth account of inclusion of glycerine in transition and lactating cow diets (Kalscheur *et al.*, this volume) and of swine energy value, metabolism, contaminants, feeding levels, performance and carcass composition (Shurson *et al.*, this volume).

Glycerine quality

Glycerine varies in quality, depending on the degree of refinement. Schröder and Südekum (2002) analysed the chemical composition of glycerine at different stages of the rapeseed oil methylester production process (Table 11). Important to notice is that the impure quality with elevated methanol concentrations (267 g/kg DM) was not a commodity but an intermediate product that was used for experimental purposes only. For the benefit of a fail-safe usage of glycerine in diets for all farm animals, methanol should be removed as far as is technically possible. Table 12 presents two different glycerine qualities according to the German “Positive List” (Standards Commission for Straight Feeding Stuff, 2011). Crude glycerine is the quality currently used in farm animal feeding and it is strongly recommended that at least the specifications listed should

TABLE 11
Chemical composition of glycerine representing different stages of the rapeseed oil methylester production process

Parameter	Purity of glycerine		
	Low	Medium	High
Water (g/kg)	268	11	25
Dry matter composition (g/kg unless stated)			
Glycerine	633	853	998
Crude fat	7.1	4.4	n.a.
Phosphorus	10.5	23.6	n.a.
Potassium	22.0	23.3	n.a.
Sodium	1.1	0.9	n.a.
Lead (mg/kg)	3	2	n.a.
Methanol	267	0.4	n.a.

Notes: n.a. = not analysed; analyses were omitted because the glycerine content was close to 1000 g/kg. Source: Schröder and Südekum, 2002.

TABLE 12
Standardized composition (g/kg) of two different glycerine qualities according to the German “Positive List”

Parameter	Glycerine	Glycerine, crude
Glycerine	Minimum 990	Minimum 800
Water	5–100	100–150
Ash	Maximum 1.0	Maximum 100
Methanol	ND	Maximum 2.0
Other	–	NaCl, K, P, S

Notes: ND = not detected. Source: Standards Commission for Straight Feeding Stuff, 2011.

be declared on each batch of crude glycerine. Due to legal restrictions on the use of animal products in farm animal feeding and because crude glycerine may contain some residual fat, the source of the glycerine must also be known and stated.

Südekum *et al.* (2008) investigated physical, chemical and hygienic quality characteristics of pelleted compound feeds with varying quality glycerine inclusion levels of 50, 100 and 150 g/kg concentrate DM. The quality of the concentrates was assessed under two environmental conditions (15 °C and 60 percent relative humidity; 20 °C and 70 percent relative humidity) and storage durations of four and eight weeks. The chemical composition was only slightly affected by concentration and purity of glycerine or by storage and duration. Moreover, the data indicated that glycerine of different purities had a preserving effect and the physical quality of the pellets was not affected by purity or concentrations of glycerine. However, Löwe (1999) noted that when pellets were produced with molasses and glycerine concentrations greater than 50 g/kg, pellets showed a rough and scaly surface. This author also remarked that when feeds are stored in meal form, concentrations greater than 50 g glycerine/kg may result in lump formation, and therefore suggested restricting glycerine concentration in pelleted compound feeds to 60–70 g/kg based on general storage behaviour, including storage in large silos.

In conclusion, glycerine of different purities as a co-product from rapeseed oil methylester production may help stabilize the hygienic quality of pelleted compound feeds without compromising physical quality of the pellets.

Rumen events when feeding glycerine

Previous studies on ruminal metabolism of glycerine indicated that glycerine is rapidly and extensively fermented in the rumen, with propionic acid as the major product of fermentation (Bergner *et al.*, 1995; Kijora *et al.*, 1998). However, there is controversial information regarding the exact biochemical pathway and the end products of glycerine fermentation by ruminal microbes. Ferraro *et al.* (2009) measured *in vitro* gas production from glycerine, lucerne and maize silage. Results indicated that glycerine has a long lag time and a slow rate of degradation. Moreover, glycerine fermentation resulted in reduced acetate and increased butyrate concentration. Krueger *et al.* (2010) evaluated the *in vitro* effect of two levels of glycerine (20 or 200 g/kg) on their inhibitory effect against ruminal lipolysis by mixed rumen microbes, as well as the effect of feeding various amounts of glycerine on fermentation kinetics of lucerne hay. They concluded that an inclusion rate of up to 200 g/kg decreased the rate of free fatty acid accumulation and decreased fermentation rate, but appeared to have no negative effect on NDF digestibility. The authors suggested that utilizing glycerine as a short-term feed ingredient in

cattle diets can potentially inhibit bacterial fat degradation.

Schröder and Südekum (2002) evaluated *in vivo* effects of glycerine in compound feeds on nutrient turnover in the rumen and digestibilities in the whole tract of cattle. Four ruminally cannulated steers were used in a 4×4 Latin square design, and received a mixed diet consisting of 400 g/kg DM forage and 600 g/kg DM concentrate. Concentrate in pelleted form comprised either no glycerine or 150 g/kg glycerine of different purities (630, 850 or >995 g/kg glycerine). Feeding glycerine resulted in a slight shift towards a reduced ratio of acetic acid versus propionic acid. Rumen fill was slightly higher when diets contained glycerine. Furthermore, glycerine appeared to have an impact on water turnover since the proportion of bailable liquids of total ruminal contents was higher when diets contained glycerine, irrespective of quality. No effect on fermentation of fibre components was observed *in vivo*, although when glycerine was supplemented to a medium containing cellobiose as the sole energy source (Roger *et al.*, 1992) it inhibited the growth and cellulolytic activity of two rumen cellulolytic bacterial species (*Ruminococcus flacefaciens* and *Fibrobacter succinogenes*). The growth of the anaerobic fungal species, *Neocallimastix frontalis*, was inhibited as well, and its cellulolytic activity almost completely disappeared. Another study by Abo El-Nor *et al.* (2010) measured the effects of substituting maize grain with glycerine at different levels (36, 72, 108 g/kg DM) on deoxyribonucleic acid (DNA) concentration of selected rumen bacteria using continuous fermenters. The DNA concentration for *Butyrivibrio fibrisolvens* (fibre degradation) and *Selenomonas ruminantium* (starch and sugar degradation) were reduced when glycerine at levels of 72 and 108 g/kg DM was incorporated. However, the implications of this data concerning the inhibition of bacterial and fungal growth are that it could be caused both by specific *in vitro* conditions, such as the single species, and by sole substrate conditions.

The *in vivo* data indicated that there should be no negative effects on ruminal turnover and digestibilities of organic matter constituents in the total tract when glycerine is used as a substitute for rapidly-fermentable starch sources like wheat or maize grain. Further, possible effects of glycerine on rumen microbial protein metabolism may require more detailed investigations. Paggi, Fay and Fernandez (1999) investigated the *in vitro* effect of increasing levels of glycerine (50, 100, 200 or 300 mM) on the proteolytic activity of bovine rumen fluid and found that all concentrations of glycerine reduced proteolytic activity by 20 percent. Kijora *et al.* (1998) infused 400 g glycerine per day (corresponding to 100 g/kg DM intake) into the rumen of growing bulls fed on a hay-grain diet. They observed lower concentrations of isobutyric and isovaleric acid in the rumen and concluded that fewer branched-chain amino acids had been degrad-

ed. A slower rumen microbial crude protein and amino acid degradation would primarily increase the protein value of fermented forages.

Dairy cow performance in response to glycerine

Previous studies have shown that glycerine may help to prevent ketoacidosis in high yielding dairy cows by increasing glucose precursors (Forsyth, 1953; Johnson, 1954; Fisher, Erfle and Sauer, 1971; Fisher *et al.*, 1973; Sauer, Erfle and Fisher, 1973). In the majority of these trials, glycerine was applied as an oral drench. Recent research has focused on using glycerine either as a dietary supplement or as a partial replacement for starchy dietary ingredients.

Khalili *et al.* (1997) fed grass silage for ad libitum consumption and 7 kg per day of a barley-based concentrate to mid-lactation Friesian cows. Barley was partially replaced with either glycerine, a fractionated vegetable fatty acid blend or a 1:1 mixture of glycerine and free fatty acids. Glycerine intakes (150 g/day) had no effects on intake or performance, although the combination of glycerine and free fatty acids tended to increase milk yield. DeFrain *et al.* (2004) fed complete diets to Holstein cows from 14 days pre-partum to 21 days post-partum. Diets were top-dressed with 860 g maize starch (control), 430 g maize starch and 430 g glycerine, or 860 g glycerine (per day per cow). Rapidly fermentable glycerine replaced a slowly and incompletely fermentable carbohydrate source. Pre-partum dry matter intake was greater for cows fed the control when compared with the two glycerine-supplemented diets. Rumen fluid collected post-partum from cows who received a glycerine supplemented diet had greater total volatile fatty acids, greater molar proportions of propionate and a decreased ratio of acetate to propionate. Furthermore, concentrations of butyrate seemed to be greater in rumens of cows fed glycerine-supplemented diets. Yield of energy-corrected milk during the first 70 days post-partum tended to be greatest for cows fed the control diet. Since the only observed effect of glycerine-supplemented diets pre-partum was on dry matter intake, the authors suggested that glycerine should be delivered as a drench in hypoglycaemic dairy cows and not fed as a component of transition dairy cow diets. Bodarski *et al.* (2005) observed an increase in β -hydroxybutyrate in blood serum as well after adding 500 mL glycerine per day for the first 70 days post-partum. However, glycerine supplementation decreased total non-esterified fatty acid levels when compared with the non-supplemented controls. Bodarski *et al.* (2005) observed that cows that consumed the glycerine diet exhibited a higher dry matter intake and gave 13 to 18 percent more milk than the control groups.

Recently, two German groups investigated glycerine in diets for dairy cows in direct comparison with propylene glycol. Engelhard *et al.* (2006) supplemented the same calculated amounts per cow of both glycerine and propylene

glycol pre-partum (150 g/day) and post-partum (250 g/day). Energy-corrected milk yields as well as concentrations of milk fat and protein were not different between cows fed propylene glycol or glycerine. Nevertheless, the authors observed that older cows (>second lactation) that received the glycerine-supplemented diet consumed more DM, and hence energy. Blood level indices of ketosis such as β -hydroxybutyrate and non-esterified fatty acids were not different between groups.

Rapeseed meal and rapeseed cake – ruminants

Rapeseed meal is still considered to be an important source of high-quality protein for all farm animal species, and especially for ruminants. Approximately 4.4 million tonne of RSM was produced in Germany in 2008, of which 3 million tonne was used for domestic consumption exclusively (Weiß and Schwarz, 2010). It can be assumed that the main part was utilized as protein supplements in ruminant nutrition. One of the main reasons for this may be the low cost of RSM in comparison with imported SBM. Moreover, techniques to extract RSM, including physical pressure and high temperatures, result in an increased fraction of CP protected from ruminal degradation.

Protein values of SBM and RSM published in feeding value tables and research papers differ markedly. The concentration of RUP is considered to be 350 g/kg CP for SBM and 250 g/kg CP for RSM (Universität Hohenheim – Dokumentationsstelle, 1997). Similarly, mean values calculated from data reported in the feed composition table of the AFRC (1993) resulted in 280 g RUP/kg CP for RSM and 370 g RUP/kg CP for SBM at a rumen outflow rate of 5 percent per hour.

However, more recent experiments indicate that the considerable differences between the tabulated ruminal degradability values of the two meals in favour of SBM no longer reflect the current situation. A cross-sectional study conducted by Südekum *et al.* (2003; Table 13) covered all oil mills processing rapeseed and soybean in Germany, and in addition encompassed some imported SBM samples.

A total of 15 studies published between 1983 and 1997 could be identified (Rooke, Brookes and Armstrong, 1983;

TABLE 13
Protein value of contemporary qualities of rapeseed (RSM) and soybean (SBM) meals as compared with feeding table values

Parameter	RSM	SBM
Mean RUP (g/kg CP)	300	300
DLG Table values	250	350
Mean uCP (g/kg DM)	231	288
DLG Table values	219	298–308

Notes: RUP = ruminally undegraded crude protein; uCP = utilizable crude protein at the duodenum (sum of microbial and RUP). Sources: Südekum *et al.*, 2003. DLG Table is from Universität Hohenheim – Dokumentationsstelle, 1997.

Mir *et al.*, 1984; Voigt *et al.*, 1990; Kendall, Ingalls and Boila, 1991; Tuori, 1992; Zinn, 1993; Khorasani, Robinson and Kennelly, 1994; Liu, Steg and Hindle, 1994; Moss and Givens, 1994; Vanhatalo, Aronen and Varvikko, 1995; Stanford *et al.*, 1995, 1996; Gralak *et al.*, 1997; Mustafa *et al.*, 1997; Zebrowska *et al.*, 1997). Nine studies observed greater RUP values (g/kg CP) for SBM than RSM, three studies reported the opposite, and three studies noticed no differences between RUP values for SBM and RSM. Moreover, RUP values varied greatly in all studies; more precisely, results for SBM ranged between 200 and 500 g/kg CP and RSM from 120 to 560 g/kg CP. Thus, data reported by Südekum *et al.* (2003) appear acceptable and may more closely mimic recent and current SBM and RSM qualities than historical tabular values. In conclusion, it can be stated that it is currently recommended to state a mean RUP concentration of 300 g/kg CP for RSM and SBM (Südekum and Spiekers, 2002).

Other recent experiments tested the hypothesis that SBM can be fully replaced by RSM in dairy cow diets when fed on an approximate isonitrogenous and isocaloric basis (without considering differences in ruminal degradation or amino acid pattern, or both. Table 14 summarizes the data and indicates that milk yield and milk component concentrations were similar for diets containing SBM or RSM, and thus the hypothesis can still be sustained. The energy concentration of the whole diet seems to be a key factor for the successful replacement of RSM for SBM, as lower energy concentrations generally mean insufficient DM intakes, and this may be further aggravated if RSM (moderate energy density) is included at the expense of SBM (high energy density).

Steingass *et al.* (2010) tested at what concentrations rapeseed cake could replace SBM. A feeding trial, with 60 dairy cows and 7 time periods (4 control + 3 periods with rapeseed cake or rapeseed cake+RSM) revealed higher DM

intake and milk yield, as well as lower milk fat and protein values, when rapeseed cake was fed. The authors suggested that even though rapeseed cake and RSM differ widely in their protein values, both feedstuffs can be regarded as suitable full protein supplements in diets for dairy cows.

Moreover it should also be pointed out that the overall quality of RSM and rapeseed cake depends also on the concentration of glucosinolates and, in case of rapeseed cake, the content and quality of the lipid proportion. Generally, average glucosinolate concentrations of RSM are low while glucosinolate concentrations of rapeseed cake are considerably higher. However, there is great variation for both feedstuffs. In addition, crude fat in rapeseed cake fluctuates, making ration formulation a difficult task. Increasing crude fat content lowers CP concentrations and vice versa. Hence, grouping of rapeseed cakes according to crude fat concentration (g/kg) appears necessary. Additionally, storage stability should also be considered, since the fat is in a non-protected form after the mechanical extraction of the seed. It has also been reported by farmers and consultants that physical characteristics resulting from plaque forming during oil extraction may handicap rapeseed cake handling, e.g. a homogenous distribution in complete diets or silage mixtures is difficult to achieve.

Rapeseed cake and meal – pigs and poultry

Pigs and poultry react more sensitively than ruminants to the glucosinolate content in rapeseed meal and cake. Even though the amino acid composition in rapeseed products is well balanced and favourable for monogastric animals, there are two limiting factors: the concentration and structural type of glucosinolates, and the dietary fibre. There are two different types of glucosinolate: aliphatic glucosinolate derived from methionine, and indole glucosinolate derived from tryptophan. Aliphatic glucosinolate, which has the most negative antrnutritive

TABLE 14

Comparison of rapeseed (RSM) and soybean (SBM) meals in diets for high-producing dairy cows – summary of German trials

Location, duration of trial and diet	Protein supplement (kg/day/cow)	Milk (kg/day)	Fat (g/kg milk)	Protein (g/kg milk)
LWZ Haus Riswick; lactation weeks 5–35. Basal diet of 1/3 MS + 2/3 GS	SBM 2.3 kg	31.1	39	31
	RSM 3.1 kg	31.3	39	32
LWZ Haus Riswick; lactation weeks 2–44. TMR with 50% MS + 25% GS	SBM 1.6 kg	25.2	42	34
	RSM 2.2 kg	25.8	41	34
TMR with 40% (MS + EMS) + 25% GS	SBM 4.0 kg	40.0	38	33
	RSM 4.3 kg	40.5	39	33
LVA Köllitsch; 17 weeks. Basal diet of 50% MS + 50% GS	SBM 1.6 kg	31.2	39	34
	RSM 2.0 kg	32.7	40	34
Universität Hohenheim; duration not specified. TMR with 22% MS + 21% GS	SBM 1.2 kg	30.9	45	35
	RSM 1.8 kg	32.4	43	35

Notes: MS = maize silage; GS = grass silage; TMR – totally mixed ration; EMS – ear-maize silage. Locations: LWZ = Chamber of Agriculture of North Rhine-Westphalia, Landwirtschaftszentrum (LWZ) Haus Riswick, Kleve, Germany; LLFG = Centre for Livestock Husbandry and Equipment, Regional Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt (LLFG), Iden, Germany. LVA = State Office for Environment, Agriculture and Geology, Lehr- und Versuchsgut (LVA) Köllitsch, Germany. Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany. Sources: Spiekers and Südekum, 2004; Steingass *et al.*, 2010.

TABLE 15
Amino acid profiles (g/100 g crude protein) of rapeseed meal, soybean meal and wheat

	Rapeseed meal	Soybean meal	Wheat
Lysine	5.6	6.3	2.8
Methionine+Cysteine	4.6	3.0	3.8
Threonine	4.4	4.0	2.9
Tryptophan	1.3	1.3	1.2

Source: Degussa Feed Additives, 1996.

effect, may be reduced by plant breeding to levels close to zero, while indole glucosinolate contributes 2–4 μ moles/g seed (Sørensen, 1990). The high content of fibre and fibre-associated CP contributes to relatively low digestibility for CP and energy in RSM. This is mainly due to the high lignin content of the hulls, which vary considerably (47–517 g/kg) depending on genotype and processing of the seed (Jensen, Olsen and Sørensen, 1990). Table 15 presents average amino acid contents of SBM, RSM and wheat. The lysine content of RSM is slightly less than that of SBM, but threonine and sulphur amino acids (methionine, cysteine) are greater in RSM.

The acceptance of using RSM in pig diets has increased greatly in recent years. This is mainly due to the beneficial price as well as reduced concentration of glucosinolates and improved quality monitoring. Moreover, RSM shows similar values for protein quality compared with SBM, although lysine concentration and digestibilities are lower in RSM. For practical use, this means that other protein supplements or free amino acids are needed to compensate for the loss. In contrast, RSM includes higher concentrations of sulphur amino acids than SBM.

Several trials throughout Germany were performed in order to ascertain the tolerance for the maximum supplementation of RSM in pig diets. In early trials, amounts of 50 g/kg for growing and 100 g/kg RSM for finishing pigs replaced SBM as a protein supplement in the diet. The result was that no differences were observed between groups receiving RSM or SBM. The next trial increased the amount of RSM to 100 g/kg for growing pigs and to 150 g/kg for finishing pigs. Again, no differences in performance and carcass quality were observed when compared with pigs that were fed SBM. It was concluded that diets can contain 100 g/kg RSM in grower diets (40–70 kg live weight) and 150 g/kg RSM in finishing diets (70–120 kg live weight). It is recommended that piglets, which are more sensitive to glucosinolate and high fibre concentrations, can receive up to 50 g/kg RSM in diets, and can tolerate levels of up to 100 g/kg RSM (12–15 kg live weight). However, levels of glucosinolates should not exceed 10 mmol/kg RSM (Weiß and Schöne, 2008; Weber, 2010; Weber *et al.*, 2011).

Other than RSM, rapeseed cake is only produced at smaller oil mills and represents around one tenth of the

total rapeseed feed consumption. The major difference to RSM is that rapeseed cake has a much higher and varying concentration of crude fat (100–160 g/kg vs 20 g/kg in RSM), as well as twice the glucosinolate concentration (6.2–9.4 mmol/kg RSM vs 11.6–17.1 mmol/kg cake). Recommendations for the practical use of rapeseed cake depend mainly on glucosinolate levels. If the acceptable amount is exceeded, animals react with decreased feed intake and performance, and in the worst case an enlargement of the thyroid. Weiß and Schöne (2010) summarized five different trials that were carried out in order to estimate the maximum supplementation of rapeseed cake. It was concluded that fattening pigs may receive between 70 and 100 g/kg rapeseed cake, while sows and piglets may be fed between 50 to 100 g/kg rapeseed cake. The exact amount depends on the glucosinolate level, which should not exceed 1.5 mmol/kg diet. Moreover, crude fat content should be more standardized to be able to use the commodities more easily and reliably.

Rapeseed products are least used in poultry nutrition. For this reason not much research has been conducted, and results vary greatly. Unfortunately, no declaration on glucosinolate levels in the RSM used are reported in most of the literature. Richter *et al.* (1996) noticed a decrease in performance when adding 50 g/kg RSM, while Faghani and Kheiri (2007) observed no differences when RSM was added at a level of 100 g/kg. A few studies with rapeseed cake revealed that it is possible to use approximately 150 g/kg diet without no loss in performance (Peter and Dänicke, 2003). Jeroch, Jankowski and Schöne (2008) reviewed several trials and concluded that broilers, when fed rapeseed cake, tolerate between 3 and 5 mmol/kg glucosinolate. Moreover, it is highly important to add iodine, since glucosinolates act as antagonists. It is suggested that iodine supplementation should be twice general recommendations (GfE, 1999). However, if glucosinolates are present in high concentrations, the negative effects may not be compensated for, even if iodine is supplemented at high levels.

Concluding, it is evident from these data that more widespread use of RSM and rapeseed cake in diets for pigs and poultry requires further reduction in glucosinolate levels.

ENERGY UTILIZATION EFFICIENCY AND SUSTAINABILITY OF CO-PRODUCTS FROM BIOFUEL PRODUCTION IN ANIMAL NUTRITION

The biofuel yield per tonne of rapeseed varies between 250 and 350 kg rapeseed oil, and bio-ethanol yield per tonne of maize or wheat grain is between 300 and 350 kg (Pinkney, 2009). Some losses are caused by CO₂ escape during alcohol fermentation. All other products may be considered as co-products, and may be used in various ways as feedstuff in animal nutrition in wet or dry form, or as fertilizer. Biofuel

co-products can be considered as valuable protein sources for farm animals. Their CP concentration varies between 300 and 400 g/kg DM. Land use scenarios using wheat for biofuel or using wheat and soybean meal to match animal feed value of DDGS have been evaluated by Pinkney (2009). The most effective way to utilize the DGS resulting from biofuel production in large plants is feeding this low DM material (80 g DM/kg) to farm animals. As it is unrealistic to distribute large amounts of DGS in the vicinity of the biofuel plant and due to its short shelf-life, it becomes necessary to dry the material in order to preserve the co-product. Therefore, additional energy expenditures and GHG emissions must be considered in any assessment of ecobalances (carbon footprint, life-cycle assessment) of the co-products or the whole biofuel production chain.

To date, no definite regulations exist in order to classify emissions of the main product and the co-product (Bockisch *et al.*, 2000; Flachowsky *et al.*, 2011). When operating on a causation principle, the producer or the responsible party should be accountable for all emissions. However, drying of DGS is only of interest if the products will be utilized as feedstuffs for animals, and thus emissions associated with processing of co-products are not of interest or necessity for biofuel producing companies.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Even though, much research has already been conducted in the utilization of bio-ethanol and biodiesel co-products for animal nutrition, there are important aspects that need further consideration. Dose-response studies are required for all co-products covered in this chapter, in order to evaluate the exact mode of action as well as the appropriate inclusion level in diets of farm animals. More precisely, this means that methanol must be removed from glycerine as far as technically possible, since separation or purification of glycerine can be fluctuating depending on the plant and the applied process. Rapeseed products fed to pigs and poultry should contain as little glucosinolate as possible. This might be achieved through the breeding process, while the antinutritive impact of the remaining glucosinolates may be compensated for by iodine addition.

Further attention should also be paid to the influence of processing conditions on composition and nutritive value of co-products, depending on raw materials. In particular, rapeseed cake needs further consideration and more reliable data because variations in the processing conditions result in very varying chemical composition, particularly regarding crude fat and CP content. This leads to difficulties in predicting the feeding value of rapeseed cake for all categories of farm animals, and could also affect storage stability. Therefore, the value of rapeseed cake would benefit from a standardization of composition. Similarly,

standardization of processing would be desirable, using constant proportions of raw materials for the production of distillers grain.

Future research should also focus on measuring additional expenditures of the processing of co-products in order to be able to evaluate the carbon footprint and to identify GHG reduction potentials. Factors like harvesting, pressing, drying, conservation and transportation should be accounted for in the same way as animal emissions and manure management, since focusing on single factors does not provide an assessment that reflects the complexity of this subject.

CONCLUSIONS

The results of a number of experiments with lactating dairy cows and fattening bulls suggest that distillers grain as the main protein source could support high productive performance. Trials with grower-finisher pigs suggest that DDGS up to 200 g/kg diet do not influence growth performance and fattening and slaughtering variables. Similarly, laying intensity of hens as well as egg quality and health were not affected by inclusion levels ranging from 150 g/kg to 300 g/kg diet. Trials with broilers suggest that diets that contain more than 100 g/kg DDGS may lower performance. Hence, it is recommended to add non-starch polysaccharide (NSP)-degrading enzymes (e.g. xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS.

Table 16 summarizes current German recommendations for rapeseed products in diets for cattle and pigs. Pigs would particularly benefit from breeding or production progress in further reduction of glucosinolate levels, whereas in cattle, a safer quality assessment of the rapeseed cake is needed.

The chapter reviewed also the use of glycerine as a co-product from biodiesel production, as well as rapeseed products such as rapeseed meal and cake for farm animals. For the benefit of fail-safe usage of glycerine in diets for all farm animals, methanol should be removed as far as technically possible. Glycerine at different purities may help to stabilize the hygienic quality of pelleted compound

TABLE 16
Practical recommendations for daily amounts or dietary concentrations (as-fed basis for dry diets) of rapeseed products for cattle, pigs and poultry

Animal category	Rapeseed meal, solvent-extracted	Rapeseed cake, mechanically extracted
Dairy cow	Maximum 4 kg	1.5–2.0 kg
Beef cattle	Maximum 1.2 kg	1 kg
Fattening pigs	Maximum 100 g/kg	70–100 g/kg
Sows	50–100 g/kg	50–100 g/kg
Piglets	Maximum 50 g/kg	50–100 g/kg
Broiler	50–150 g/kg	50–100 g/kg
Laying hens	0–100 g/kg	0–50 g/kg

Sources: Weiß, 2007; Jeroch, Jankowski and Schöne, 2008.

feeds without compromising physical quality of pellets. Furthermore, glycerine is no direct competitor of propylene glycol, since data on ruminal turnover suggest that glycerine, other than propylene glycol, should replace rapidly fermentable carbohydrates. Mature cattle may consume up to 1 kg glycerine per day, while it may still be necessary to investigate if the sweet taste of glycerine may improve feed intake of diets with inferior palatability.

In conclusion, glycerine can be used as a versatile feed-stuff, in particular for ruminants, but further research is required to explore the full potential of glycerine in dairy cows.

Other rapeseed products for ruminants, such as rapeseed meal, compare well with soybean meal for dairy cows. Recent research on rapeseed meal has shown that it can fully replace soybean meal within dairy cow diets when fed on an approximately isonitrogenous and isocaloric basis, i.e. without considering differences in ruminal degradation or amino acid pattern, or both. Moreover, milk and milk component yields were similar for diets containing soybean meal or rapeseed meal.

Nevertheless, rapeseed cake needs further consideration and more reliable data because variations in the processing conditions result in varying chemical composition, particularly regarding the crude fat and protein content. These circumstances currently lead to difficulties in predicting the feeding value of rapeseed cake for all categories of farm animals, and could also affect storage stability. Therefore, the value of rapeseed cake would benefit from standardization of composition

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Chapter 12

Utilizing co-products of the sweet sorghum-based biofuel industry as livestock feed in decentralized systems

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ABSTRACT

Sweet sorghum-based decentralized crushing and syrup-making units are a major component of sweet sorghum value chains in India. Apart from the main product, syrup, there are several co-products, including grain, bagasse, vinasse, steam, foam and froth. This chapter looks at the state of the art in utilization of these products in livestock feed, as well as exploring emerging opportunities. If the policy framework of the country supports decentralized models, this co-products utilization not only improves economic viability but also has environmental benefits by way of reduced greenhouse gas (GHG) emissions, which are yet to be quantified.

INTRODUCTION TO THE SWEET SORGHUM VALUE CHAIN

Renewable energies are critical contributors to the energy supply portfolio as they contribute to global energy security, reduce dependency on fossil fuels and provide opportunities for reducing emissions of greenhouse gases (GHG), and are expected to play major roles in energy strategies of nations to mitigate adverse global climatic change (Reddy *et al.*, 2008; Srinivasa Rao *et al.*, 2009). The price volatility of global crude oil is more unprecedented and unpredictable than ever before, as seen during the last decade. Hence many policy-makers consider renewable indigenous sources of energy, like biofuels, would be a viable option for energy security. Since biofuels can be produced from diverse crops, each country is adopting a strategy that exploits the comparative advantages it holds with respect to such crops. For example, sugar cane and maize are the main feedstocks for ethanol in Brazil and US respectively, while rapeseed in Europe and palm oil in Malaysia are the main feedstocks for biodiesel. In India, sugar cane, sweet sorghum and tropical sugarbeet are the major bio-ethanol feedstocks, while biodiesel is produced on a limited scale from *Jatropha* (Srinivasa Rao *et al.*, 2010). More than 95 percent of the bio-ethanol in India is produced from molasses, a co-product of the sugar industry, by over 1500 distilleries spread across the country (Aradhya, 2010). As sugarbeet is being grown only on an experimental scale in India the co-products are not available to explore, while *Jatropha* oilcake contains toxins and antinutrient factors such as phorbol

esters, trypsin inhibitors, lectins and phytates, and hence is not suitable for animal feed (Reddy *et al.*, 2008). However, the detoxified *Jatropha* cake, i.e. *Jatropha* meal, can be used as feed. There are currently two models of operation in sweet sorghum value chains, namely a Centralized model and a Decentralized model. This chapter primarily discusses the co-products of sweet sorghum in a decentralized model of the sweet sorghum value chain.

SWEET SORGHUM AS BIO-ETHANOL FEEDSTOCK

Sorghum (*Sorghum bicolor* (L) Moench) is one of the most important food, feed and fodder crops in arid and semi-arid regions of the world. Globally, it was cultivated on about 39.96 million hectares in 2009, with Africa and India accounting for about 80 percent of the global acreage (FAOSTAT data). Although sorghum is best known as a dual-purpose grain and fodder crop, the sweet-stalked sorghums, referred to as sweet sorghums, are similar to the grain sorghums, but possess sweet juice in their stalk tissues, and are traditionally used as livestock fodder due to their ability to form excellent silage; the stalk juice is extracted and fermented and distilled to produce ethanol (Table 1). Thereafter the juice, grain and bagasse (the fibrous residue that remains after juice extraction) can be used to produce food, fodder, ethanol and cogeneration. The ability of sweet sorghum to adapt to drought; to saline and alkaline soils; and to waterlogging has been proven by its wide prevalence in various regions of the world. The

MAIN MESSAGES

- Sweet sorghum is a climate change-ready crop owing to its resource use efficiency and wide adaptability, in addition to apart biotic and abiotic stress tolerance.
- In poor soils with limited inputs, sweet sorghum-based agro-enterprises offer both food for humans and fodder (bagasse) for their livestock, forming a resilient mixed crop-livestock system.
- The sweet sorghum value chain offers immense opportunities to the marginal farmers of the semi-arid tropics as sweet sorghum offers food, feed, fodder and fuel.
- The centralized and decentralized systems complement each other, and benefits percolate down to the associated farming communities.
- The socio-economic, environmental and ecological benefits from sweet sorghum production and processing can be large, and need to be quantified from a systems perspective.
- To benefit from all the above on a large scale in farmers' fields, well structured, sustained, supportive policies and R&D programmes with inclusive market-oriented approaches are required at both national and international levels.

TABLE 1
Favourable traits of sweet sorghum cultivation as biofuel feedstock compared with popular biofuel feedstocks such as sugar cane, maize and sugarbeet

As crop	As ethanol source	As Bagasse	As raw material for industrial products
<ul style="list-style-type: none"> • Short duration (3–4 months) • C₄ dryland crop • Good tolerance of biotic and abiotic constraints • Meets fodder and food needs • Non-invasive species • Low soil N₂O and CO₂ emission • Seed propagated 	<ul style="list-style-type: none"> • Amenable to eco-friendly processing • Less sulphur in ethanol • High octane rating • Automobile friendly (up to 25% of ethanol-petrol mixture without engine modification) 	<ul style="list-style-type: none"> • High biological value • Rich in micronutrients • Use as feed, for power co-generation or bio-compost • Good for silage making 	<ul style="list-style-type: none"> • Cost-effective source of pulp for paper making • Dry ice, acetic acid, fusel oil and methane can be produced from the co-products of fermentation • Butanol, lactic acid, acetic acid and beverages can be manufactured.

Notes: For further details see Srinivasa Rao *et al.*, 2009). N₂O = nitrous oxide; CO₂ = carbon dioxide.

Sources: Reddy *et al.*, 2005; Srinivasa Rao *et al.*, 2009, 2010.

per-day ethanol productivity of sweet sorghum is higher than sugar cane (Srinivasa Rao *et al.*, 2010, 2011), as well as having a shorter growing period (four months) and a low water requirement of 8000 m³/ha (over two crops annually) that is only 25 percent of that required for sugar cane, which has a 12–16-month growing season and needs 36 000 m³ water/ha. It translates to sugar cane needing 900 m³ water for producing 1 tonne of dry matter (DM) while sorghum requires only 200 m³ water, based on productivity of sugar cane at 40 t/ha and sorghum at 20 t/ha.

Sweet sorghum's lower cost of cultivation compared with sugar cane and sugarbeet, and farmer familiarity with cultivation of sorghum, aid in greater adoption of sweet sorghum.

Mixed crop-livestock systems are the dominant form of agricultural production in dryland Africa and Asia. Integrating crops and livestock on the same farm helps small-scale farmers to diversify their sources of income and employment. Livestock act as a storehouse of capital and an insurance against crop production risks, and thus

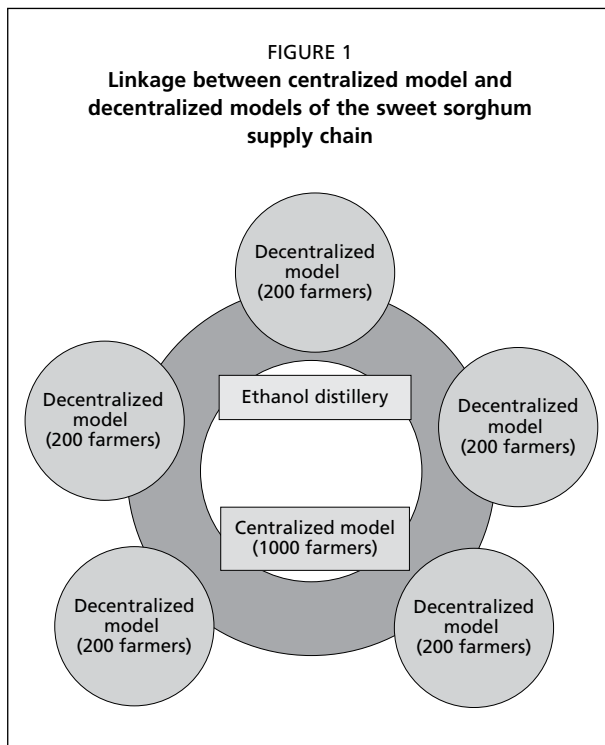
provide a coping mechanism against livelihood shocks as well as a vital source of dietary protein. Development of the livestock sector provides new livelihood opportunities for women, who otherwise often lack access to and control over land-based means of production. For the majority of small-scale farmers, crop residues from dual-purpose crops constitute 40–60 percent of total dry matter intake in their animal feed rations. The rest is made up from other sources.

Sweet sorghum supply chain

Sweet sorghum feedstock supply chains have primarily two models of operation (Figures 1 and 2). These are considered below.

The centralized model

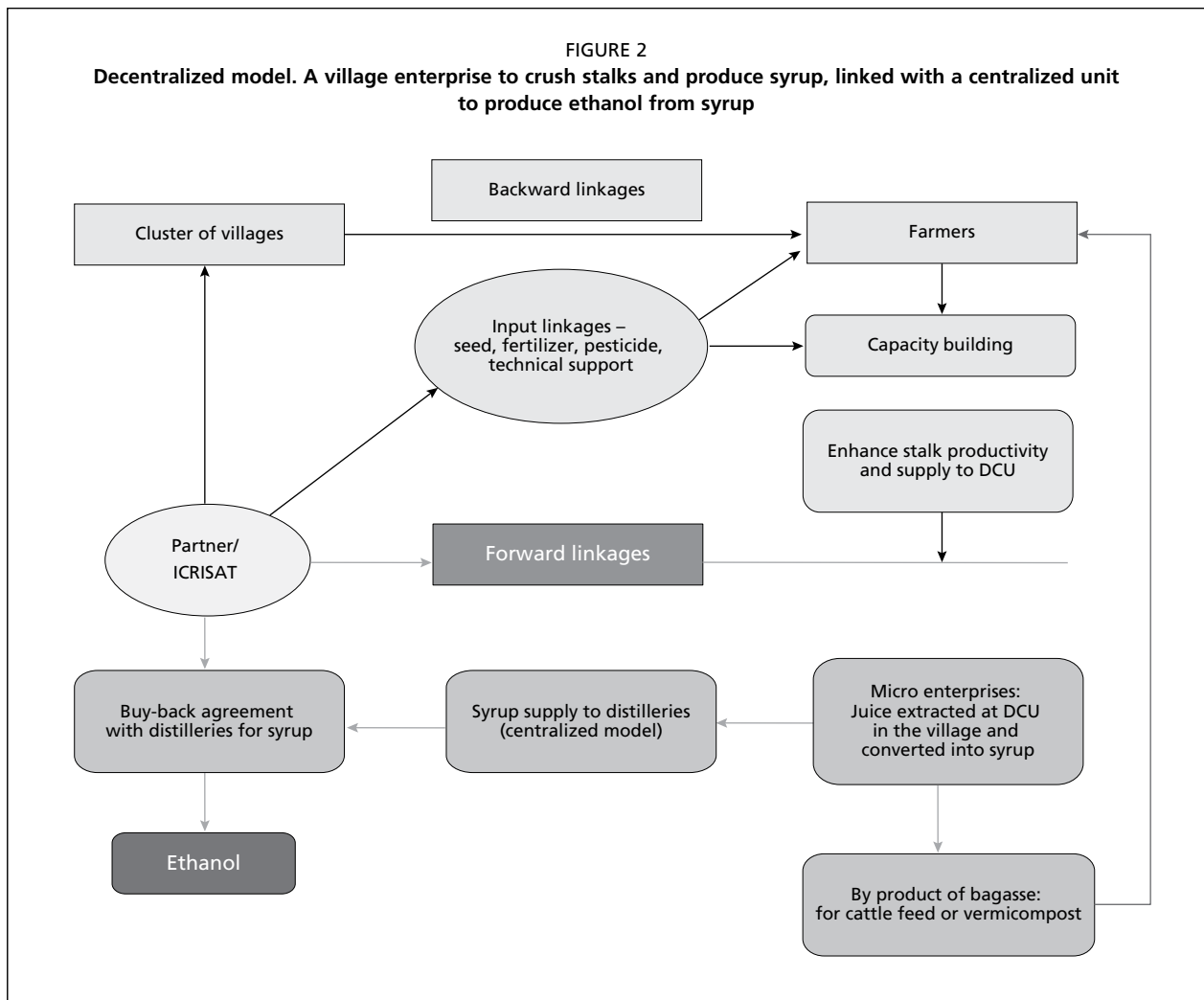
The sweet stalk is directly supplied to the plant from the farmers' fields, and the juice is extracted and fermented to ethanol and allied co-products. Its operational area is generally limited to a 40–50 km radius around the plant owing to high transportation costs involved in bulky raw



material supply. Examples of such centralized plants include Rusni Distilleries Ltd, Sangareddy, Medak District, Andhra Pradesh, India; Tata Chemicals Ltd, Nanded, Maharashtra, India; and ZTE Ltd, Inner Mongolia, China.

The decentralized model

Figure 1 illustrates the overlap of the two models, showing linkages of hundreds of farmers to decentralized crushing units (DCU), while thousands connect to a central distillery. The finer details reflect productivity, capacity utilization and other factors. In simple terms a DCU comprises the crusher and boiling unit, and essentially crushes the stalks to extract juice. The extracted juice is either concentrated to syrup or fermented *in situ* to alcohol. The forward and backward linkages of DCU are illustrated in Figure 2. Sweet sorghum is a seasonal crop that in India can be cultivated in three seasons a year (rainy, post-rainy and summer) to supply raw material for 3 to 4 months annually for ethanol production (Kumar *et al.*, 2010). The grain and sugar yields are best in the rainy and summer seasons, whereas in the post-rainy season the grain yield is high, but with less stalk and sugar yield. A commercial ethanol distillery requires



feedstock year round – for at least 10 months annually – for economical operation. However, in regions with short harvest windows, smaller acreages or with low plantation densities, a typical centralized model with a 30 kilolitres per day (KLPD) processing plant dedicated to sweet sorghum ethanol production could operate only seasonally, requiring a high capital investment that might not be cost effective. In areas with low plantation densities, the transportation costs associated with supplying the plant with sweet sorghum feedstock become prohibitive. Transportation costs are a significant cost factor in all sweet sorghum models studied, with costs ranging from US\$ 34 to US\$ 107 per tonne of fermentable carbohydrates (Bennett and Anex, 2009). Larger plant sizes may not benefit from traditional economies of scale because of the increased transportation costs associated with longer travel distances. Due to these limitations, alternative processing options have been investigated. In view of the need for regular supply of feedstock to the distillery, it is widely believed that DCUs help in sustainability of the supply chain. The juice obtained after crushing the stalks is boiled in pans to produce concentrated syrup (~60 percent Brix) (Photo 1), which is supplied to a distillery for ethanol production (Reddy *et al.*, 2009).

Alternatively, extracted juice can also be fermented *in situ*, resulting in a fermentation mash containing 6–10 percent ethanol. Studies have shown that non-sterile fermentation in the field is possible, with very good ethanol conversion efficiencies, as demonstrated by a research group at the University of Oklahoma, USA (Kundiya *et al.*, 2006). As an alternative to fermentation of the sweet sorghum liquids, several groups have investigated the solid-phase fermentation of sweet sorghum for production of ethanol as it (i) has greater ethanol production per unit volume of the fermenter, (ii) has reduced fermentation capacity requirement, (iii) has no nutrient supplementation requirement, (iv) has lower production costs, (v) leaves smaller volumes of stillage for disposal, and (vi) needs less energy for distillation (Gibbons, Westby and Dobbs, 1986). In these systems, shredded sweet sorghum is injected into a solid-phase fermenter, inoculated with yeast, and mixed during fermentation. Fermenters have been of varied sizes and configurations, including rotary drums and screw augers (Gibbons, Westby and Dobbs, 1986). Solid-phase fermentations typically result in higher ethanol yield than fermentation of the juice alone (78 percent of theoretical ethanol yield in solid state versus 75 percent in juice fermentation) (Bryan, Monroe and Caussanel, 1985), but may have higher capital costs and lower throughput. Other variations to the system have included operating in a semi-continuous rather than batch mode, and application of immobilized yeast in the system, both of which improved system performance.

Potential advantages of small-scale, decentralized ethanol processing are:

- Promotes biodiversity by using more diverse feedstock.
- Enhances food security and food system resilience by ensuring that geographically diverse farms have access to locally-produced renewable fuel for food production.
- Promotes resource cycling by keeping nutritious co-products of ethanol production close to their farm source, where they can be returned to farms for feed or fertilizer.

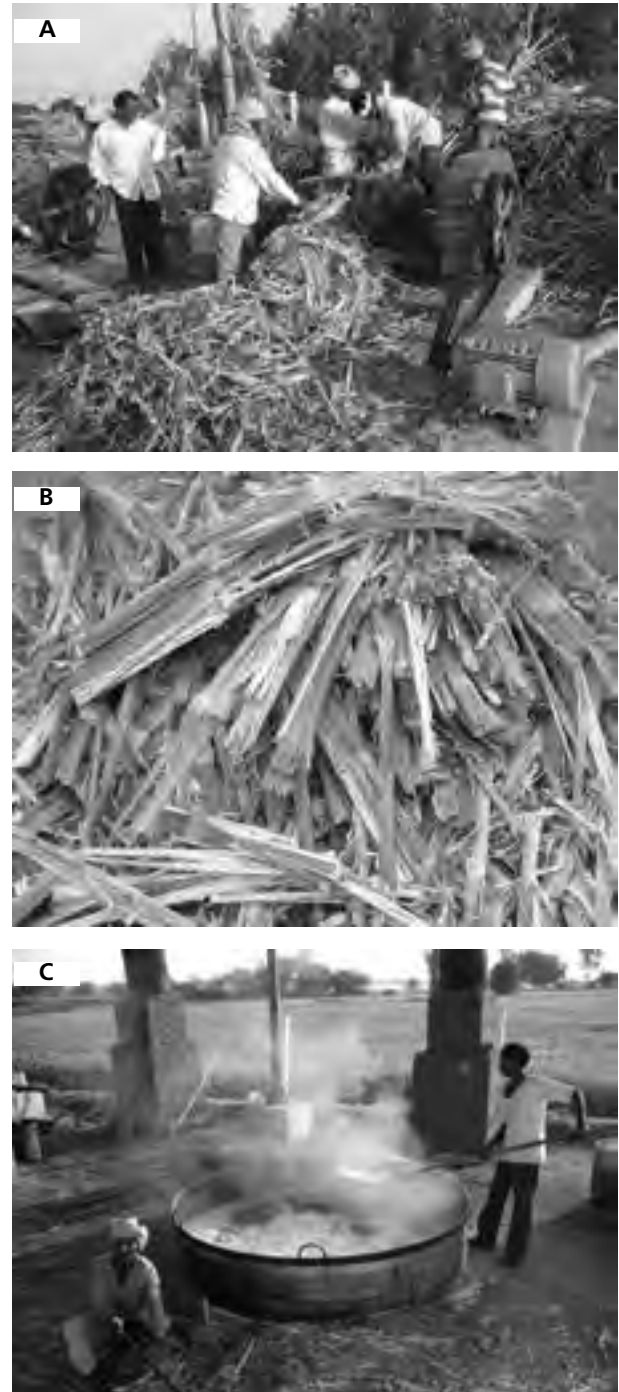


Photo 1
Decentralized sweet sorghum crushing unit. A. Crushing. B. Bagasse. C. Boiling the juice to produce syrup

- Produces feedstock on small farms, which tend to use land more efficiently than large farms.
- Co-products remain with the farmers.
- Reduces farm input needs through promotion of regionally-appropriate, low-input feedstock crops.
- Promotes equitable distribution and greater retention of wealth by rural communities.

CO-PRODUCTS

The processing options discussed above focus on the liquid carbohydrate portion of the sweet sorghum, but do not address the use of grain, the solid bagasse and steam that are generated during the pressing process, or the waste vinasse that is generated during the dewatering process. An ideal system will utilize as many crop components as possible to create a closed-loop system (Worley, Vaughan and Cundiff, 1992).

Grain

Currently the stalk from rainfed sweet sorghum grown in the rainy season is the source of raw material for the decentralized units in India. The grain is considered a co-product here as sweet sorghum is basically grown for production of ethanol by fermenting extracted juice from the sugary stalks. Mould-affected grain can be used as raw material for ethanol production, while mould-free grain can be used for human consumption. The primary product in DCU is syrup, which can be used either in ethanol production or in the food and pharmaceutical industries.

Grain from the rainy season crop is mostly mould-affected due to rains during grain development, maturation and harvest. Grain and stover yield are statistically unrelated in both hybrids and varieties (Blümmel *et al.*, 2009). Stover yield is directly proportional to realizable bagasse yield (Kumar *et al.*, 2010). High grain yields could be associated with above average stover yields. In a recent comprehensive investigation of grain-stover relationships in (non-sweet) sorghum cultivars tested by the Directorate of Sorghum Research (DSR), formerly the National Research Center for Sorghum (NRCS), Hyderabad, India, during the 2002–2006 period, Blümmel and co-workers (2010) observed that grain yields accounted for only 14 percent of the variation in stover yield, i.e. grain and stover yields in sorghum were only weakly positively associated. These findings suggest that grain and stover yield should both be recorded in sorghum improvement, since stover yields cannot be accurately predicted by grain yield measurements. Grain yields do not need to be achieved at the expense of fodder for livestock or feedstock for ethanol production, and vice versa.

Bagasse

The solid bagasse that remains after pressing sweet sorghum has several potential uses. One potential use is

as animal feed, directly after chopping or after ensiling (Linden, Henk and Murphy, 1987). It has also been used as a source of pulp for the paper industry (Belayachi and Delmas, 1997). Another potential use of the bagasse is as a fuel source for the processing plant. With the addition of a solid-fuel boiler, the bagasse can be used to provide process heat to run the plant. With its heating value it is likely to require only 20–30 percent of the available biomass to fuel the plant (Bennett and Anex, 2009). In addition, processes for conversion of lignocellulosic material to ethanol are becoming more economically viable, making sweet sorghum bagasse a possible source of biomass for such a process. Studies have demonstrated that a large portion of the insoluble carbohydrate (cellulose and hemicellulose) from sorghum can be readily converted to ethanol (Sipos *et al.*, 2009).

Foam and froth

Lot of foam and froth is generated during juice boiling. This can be collected separately and used to feed livestock or as organic fertilizer.

Steam

The steam generated during concentration of juice to syrup is a good source of energy, which can be used for several purposes, such as boiling water, which in turn can be used to increase juice extraction, heat treatment of juice before boiling, etc., by installing the necessary equipment to capture the outgoing steam.

Vinasse

Vinasse, also known as stillage, is the liquid co-product after removal of the final products during sugar processing. In a distillation process, vinasse is the liquid remaining after separation of ethanol. In the decentralized model of sorganol production, the dewatering and/or distillation system will produce 10–15 litre of waste vinasse (distillate) for every litre of ethanol produced in the later stages, depending on the initial ethanol concentration of the fermentation broth. The large volume generated and the high organic loading in the waste water make it a major environmental challenge for most commercial applications. Reports of bagasse characterization for sugar cane feedstocks show biochemical oxygen demand (BOD) levels ranging from 25 to 60 g/L, with nitrogen levels from 300 to 2500 mg/L and phosphorus levels from 10 to 300 mg/L. The limited data on sweet sorghum bagasse show comparable results, with BOD = 46 g/L, nitrogen = 800 mg/L and phosphorus = 1990 mg/L (Wilkie, Riedesel and Owens, 2000). Due to its high BOD, disposal into waterways is not an option. One potential option is land application of the vinasse as irrigation water and fertilizer. Several reports suggest that both dilute and concentrated vinasse (from sugar cane)

can be used on agricultural fields (Parnaudeau *et al.*, 2008; De Resende *et al.*, 2005). The vinasse or stillage produced from distillation of sweet sorghum ethanol has been reported to contain 0.2 percent nitrogen, 0.22 percent P₂O₅ and 0.3 percent K₂O. A study conducted in Brazil to determine the long-term effects of disposal of this material onto sugar cane fields found that vinasse applications of 80 m³/ha increased mean yields of both cane and sugar by 12–13 percent (De Resende *et al.*, 2005). A number of other disposal options could be considered, such as anaerobic digestion for production of methane (biogas), on-site combustion for production of energy, or composting to produce bio-fertilizers.

GRAIN UTILIZATION

Rainy season sweet sorghum grain is subject to mould damage if rainfall coincides with grain development, maturation and harvest, which often happens in major sorghum growing regions of India. The moulds have detrimental effects on yield and quality of sorghum grain, including decreasing its nutritive value, and producing mycotoxins and other toxic metabolites. Hence, it is not fit for human consumption, but preferred for alcohol production, and farmers use it as livestock and poultry feed, as the mycotoxins are below permissible threshold levels, and such grain is also inexpensive (Bandyopadhyay *et al.*, 1998; Reddy *et al.*, 2000; Thakur *et al.*, 2006). However, non-mouldy grain from where grain maturation does not coincide with rains and the grain from mould-tolerant sweet sorghum cultivars can be used as food for human consumption by making products like porridge, flat bread (*roti*), *bhakri* (stiff *roti*), flakes, chips, papad, baked products including yeast-leavened breads, cakes, muffins, cookies, biscuits, pasta and health foods. The grain yields among sweet sorghum cultivars vary widely and are cultivar (Table 2) and environment depend-

ent. Hybrids have on average higher grain yield than the original varieties, but all other productivity-related variables were higher in the original varieties. Average grain yields were 10.8 percent (hybrids) and 6.0 percent (varieties) of total biomass yield. This proportionally low partitioning into grain yields probably reflects a sweet sorghum breeding target of high sugar yields in stems. Still, grain yields of up to 2.6 t/ha were recorded in both cultivar types (Table 2) and sweet sorghum grain can contribute significantly to rural food security. Mean juice yield in hybrids amounted to about 47 percent of stem yield, while it was 54 percent for the older varieties. Yields of bagasse plus stripped leaves were on average higher than the juice yields in both hybrids and the varieties, potentially providing 5.8 t/ha (hybrids) and 6.7 t/ha (varieties) of fodder (Table 2).

Grain structure and composition

The sorghum kernel is a naked caryopsis and consists of three main anatomical parts: pericarp (outer layer), endosperm (storage tissue) and germ (embryo), which generally account for 6, 84 and 10 percent of the seed mass, respectively. Sorghum is the only cereal grain known to have starch in the mesocarp layer of the pericarp. The endosperm, composed of the aleurone layer and peripheral corneous and floury areas, is the main storage tissue. The 1000-grain weight of sorghum varieties ranges from 19.0 to 28.5 g (Sehgal, Kawatra and Singh, 2004). Starch is the major grain component in sorghum, followed by protein. Most of the sorghum starch contains 70–80 percent branched amylopectin and 20–30 percent amylose. Waxy or glutinous sorghum varieties contain starch that is 100 percent amylopectin. Sorghum contains high levels of insoluble fibre with low levels of beta glucans. Most of the crude fibre is present in the pericarp and endosperm cell walls. This fibre is composed mainly of cellulose, hemicellulose and small quantities of lignin (Table 3).

TABLE 2

Yields of grain, leaf, stem, stover, juice, bagasse and bagasse plus stripped leaves (B+L) in 34 cultivars of sweet sorghum at Directorate of Sorghum Research (DSR) in 2005

	Mean (and range) in dry matter yields (t/ha)						
	Grain	Leaf	Stem	Stover	Juice	Bagasse	B+L
Hybrids (H)							
Mean	1.6 (10.8%)	1.5 (10.1%)	8.1 (54.7%)	11.7 (79%)	3.8 (25.7%)	4.3 (29%)	5.8 (39%)
Range	0.8–2.6	0.6–2.5	4.7–12.4	7.1–14.9	1.3–7.1	2.6–5.5	3.8–7.9
<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.009
LSD (<i>P</i> <0.005)	0.6	0.5	2.0	2.9	1.8	0.1	2.2
Varieties (V)							
Mean	1.0 (6%)	1.8 (10.8%)	10.7 (64%)	13.9 (83.2%)	5.8 (34.7%)	4.9 (29.3%)	6.7 (40.1%)
Range	0.1–2.6	0.9–2.6	6.9–14.7	8.5–18.8	2.8–8.6	3.2–6.1	4.5–8.1
<i>P</i>	<0.0001	<0.0001	0.03	0.05	0.12	0.02	0.005
LSD (<i>P</i> <0.005)	0.5	0.57	4.2	5.15	–	1.9	2.1
<i>P</i> (H vs V)	0.007	0.07	0.002	0.02	0.002	0.05	0.02

Notes: Stover yield estimates include panicles after grain removal; values in parentheses are proportion of each component in the total biomass. *P* = probability; LSD = least square difference. Source: Blümmel *et al.*, 2009.

TABLE 3
Typical composition of sorghum and sweet sorghum grain

Constituent	Mean	Range	Constituent	Mean	Range
Proximate analyses			Protein fractionation		
Protein (%)	11.6	8.1–16.8	Prolamine (%)	52.7	39.3–72.9
Ether extract (%)	3.4	1.4–6.2	Glutelins (%)	34.4	23.5–45.0
Crude fibre (%)	2.7	0.4–7.3	Albumins (%)	5.7	1.6–9.2
Ash (%)	5.8	1.2–7.1	Globulins (%)	7.1	1.9–10.3
Nitrogen-free extract (%)	79.5	65.3–81.0	Prolamine (%)	52.7	39.3–72.9
Fibre			Essential amino acids (as g/16 g N)		
Dietary insoluble fibre (%)	7.2	6.5–7.9	Lysine	2.1	1.6–2.6
Dietary soluble fibre (%)	1.1	1.0–1.2	Leucine	14.2	10.2–15.4
Acid-detergent fibre (%)	3.3	2.9–3.6	Phenylalanine	5.1	3.8–5.5
			Valine	5.4	0–5.8
			Tryptophan	1	0.7–1.3
			Methionine	1	0.8–2.0
			Threonine	3.3	2.4–3.7
			Histidine	2.1	1.7–2.3
			Isoleucine	4.1	2.9–4.8

Notes: As data from sweet sorghum grain is limited, data are mostly from grain sorghum. All values are expressed on a dry matter basis. Sources: Bach Knudsen and Munck, 1985; Rooney, Kirleis and Murty, 1986; Monti, Di Virgilio and Venturi, 2008.

TABLE 4
Ash and mineral concentrations in the grain of grain sorghum and sweet sorghum

Sorghum type	Ash	N	C	Al	Ca	Cl	Fe	K	Mg	Na	P	S	Si
Grain sorghum	47	13	434	242	1824	6252	141	5587	2451	192	2150	1084	10671
Sweet sorghum	58	14	424	218	2417	5129	159	7125	2895	171	2620	1000	14321

Notes: Ash, N and C are expressed as g/kg DM; the other elements as mg/kg DM. Sources: Jambunathan and Subramanian, 1988; Monti, Di Virgilio and Venturi, 2008.

Utilization as ruminant feed

Both feed and food uses of sweet sorghum grain are compatible; not all grains will have desirable food processing properties, so the poorer quality grain might go into feeds. Obviously, care must be taken to avoid problems with mycotoxins. Sorghum grain is rich in many minerals, including Ca, Mg, P and K (Table 4). Sorghum is a very good feed grain as long as it is properly supplemented for the particular species being fed. Sorghums without a pigmented testa have 95 percent or greater of the feeding value of yellow dent maize for all species of livestock. In India, on average, 250 g grains are consumed per dairy animal per day. Consumption of sorghum grain by dairy cattle is highest in northern India and lowest in southern India. Considering the large population of animals and government policy in support of milk production, the requirement of grains by feed industries will be quite high. Considering the nutritional value of sorghum (Tables 3 and 4) and the probable shortage of grain and roughages, coupled with limitations on other fodder crops cultivation in Asia and sub-Saharan Africa, there is wide scope for more inclusion in feed formulations of sorghum grain harvested from decentralized sweet sorghum production systems.

Utilization as poultry feed

The demand for sorghum for poultry feed largely depends on the price and availability of maize. Inclusion of sorghum at up to 10 percent for layers and 15 percent for broilers is common. However, this rate increases in years of higher maize price. The present non-food share of sorghum grains usage in India is predicted at 77 percent for poultry, 16 percent for dairy, 6 percent for ethanol production and 1 percent for starch production (Dayakarrao *et al.*, 2003). The chemical composition and nutritive value of sweet sorghum grain means it is rich in proteins, starch, fibre, vitamins and minerals. Anti-nutritional factors can be broadly classified as those naturally present in the grains and those developed due to contamination, which modify the nutritive value. Some of them have serious health consequences. Phytic acid, a major phosphorous store in the grain, is present at levels on par with that in maize and is not a problem in diets for chickens. Polyphenols (luteoforol and apiforol) in the seed coat confer bird and mould tolerance (Reddy *et al.*, 2007). However, these compounds reduce digestibility and lead to growth retardation in chickens. Detoxifying methods such as moisturizing with alkali, dilute aqueous ammonia, sodium carbonate solution, formaldehyde, etc., reduce tannins (polyphenols) to tolerable levels in the diet (below 0.26 percent tannins). Aflatoxin contamination is

frequent in mouldy sorghum grain (Waliyar *et al.*, 2008). Published data indicate that sorghum grain can replace up to 60 percent of maize in broiler diets and up to 100 percent in the diet of layers without affecting performance (Reddy and Rao, 2000). However, to be competitive, the sorghum grain market price needs to be about 10 percent lower than that of maize.

Other alternative uses

Sweet sorghum grain can be processed into diverse products to exploit its nutritive value. If the toxin levels are high, it is safe to process sorghum grain to produce ethanol or alcohol and vinegar. Sorghum grain is usually processed by dry milling to make flour for bread. Other processing methods include rolling, steaming, flaking, popping, parching, malting, brewing and fermentation. In rural areas, dehulling (pearling) is practised. These processing techniques, alone or in combination, result in a variety of products and co-products from sorghum grain, such as leavened bread, *injera*, porridge, pasta, grits (semolina), starch, glucose powder, liquid glucose, high fructose syrup, glue, xylitol, spirit, alcohol, beer and non-alcoholic beverages (malta, milo). In 2010, the state government of Maharashtra in India announced a US\$ 0.25 promotional benefit per litre of ethanol produced from mouldy sorghum grains by the distilleries. This is expected to boost rainy season sweet sorghum cultivation, as the stalk will be purchased by the ethanol distillery and the grain by other distilleries and feed manufactures. However, in view of the shortage of human labour, this will be feasible only if mechanical harvesters are available.

Utilization of bagasse

Farmers in the drylands require varieties specifically developed with appropriate combinations of food, feed and fodder traits for use in crop-livestock systems, which will increase farmer income from the sale of grain, feed and fodder. From DCUs the major co-product is bagasse – the fibrous matter that remains after sweet sorghum stalks are crushed to extract their juice. For each 10 t of sweet sorghum crushed, the DCU produces 5 to 6 t of wet bagasse, depending on the genotype, season of crushing, juice extraction efficiency, temperature, etc. The high moisture content of wet bagasse, typically 40 to 50 percent, makes it unsuitable for direct use as a fuel. However, such fresh bagasse is preferred for use as livestock feed. Fodder from crop residues such as stover and straw does not require the allocation of additional land and water because they are a co-product of grain production. This makes crop residues and co-products the single most important – and affordable – fodder resource for small-scale farmers. Thus, any improvement in the nutritive value of crop residues, however small, can have considerable value and impact. Although cereal crop residues generally have low nutri-

tive quality, genetic variation is being exploited to develop dual-purpose types that combine improved fodder quality with acceptable grain production. In many regions of sub-Saharan Africa and Asia the contribution of pastures to livestock feed has declined and been replaced by feed grains, crop residues and other concentrates (Parthasarathy Rao and Birthal, 2008). The problem of finding enough feed for animals raised by small-scale farmers is becoming almost as acute and politically significant as ensuring food security for people. While crop residues, particularly straw, already provide a large component of livestock feed, their nutritive value is often so low that farmers must supplement livestock diets with feed grain and other concentrates.

Bagasse fodder quality and composition

The potential feed value of sweet sorghum bagasse-based livestock feed is described in Table 5 (Blümmel *et al.*, 2009). Nitrogen content was increased in bagasse residue plus stripped leaves (BRSL) compared with whole stover because of the higher leaf content in the BRSL, but all other laboratory fodder quality traits were higher in stover than in BRSL. For example, mean *in vitro* digestibility values for BRSL were around 5 percentile units lower than those of whole stover (Table 5). This reduction in fodder quality seems insignificant considering that highly digestible carbohydrates must have been removed in the extract, which amounted to 47 and 54 percent of stem yields in hybrids and varieties, respectively. This loss of highly digestible carbohydrates was perhaps compensated for by physical changes in the bagasse, facilitating faster and higher microbial colonization and ultimately digestion of residual fibre particles.

The chemical composition and physical properties of sweet sorghum bagasse (Table 6) shows that it has low ash and sulphur content, while being rich in minerals like Ca, Mg, Fe, Na and Zn (Negro *et al.*, 1999).

Bagasse vs forage crops

Fresh bagasse can be sold directly to fodder traders, as shown by an arrangement facilitated in 2009 and 2010 by the International Livestock Research Institute (ILRI) and partners in the National Agricultural Innovation Project (NAIP) decentralized sweet sorghum project set up in Ibrahimbad, Andhra Pradesh, India. After some iterations in fine-tuning bagasse to fodder transactions, an arrangement was implemented in 2010 to sell fresh bagasse leaving the crushing unit to fodder traders from Hyderabad at a rate of 70 paise per kg (US\$ 0.016). The fodder traders chopped the bagasses and transported it by lorry to their customers, 70 km away in Hyderabad. The price of 70 paise per kg fresh bagasse is remarkable given that the whole (i.e. unextracted) sweet sorghum stalks were valued only slightly higher, at 80 paise (US\$ 0.018) per kg, but probably reflects the substantially lower water content of the fresh bagasse.

TABLE 5
Nutritional parameters in hypothetical diets composed of bagasse and leaves of 34 cultivars of sweet sorghum

	Morphological and nutritional composition of bagasse residue and the stripped leaves (BRSL)							ME (MJ/kg)
	Bagasse (%)	Leaf (%)	N%	NDF (%)	ADF (%)	ADL (%)	IVOMD (%)	
Hybrids (H)								
Mean	73.7	26.3	0.73	64.5	41.4	4.9	44.6	6.5
Range	56.1–83.9	16.1–43.9	0.58–1.04	59.2–71.0	36.9–47.5	4.1–6.0	39.3–49.1	5.7–7.3
P	<0.0001	<0.0001	0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD	8.6	8.6	0.22	3.4	2.4	0.44	2.6	0.43
Varieties (V)								
Mean	72	28	0.83	64.6	39.8	4.9	46.6	6.8
Range	60.5–81.9	18.1–39.5	0.73–0.92	60.6–70.9	36.7–45.0	4.3–6.0	42.0–50.4	6.1–7.5
P	0.0005	0.0005	0.75	0.0001	0.0006	<0.0001	0.0002	0.0003
LSD	8.6	8.6		3.8	0.63	0.55	4.04	3.37
P (H vs V)	0.55	0.55	0.0004	0.82	0.11	0.82	0.03	0.02

Notes: N = Nitrogen; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; ADL = acid-detergent lignin; IVOMD = *in vitro* organic matter digestibility; ME = metabolizable energy; P = probability; LSD = least significant difference at $P < 0.005$. Source: Blümmel *et al.*, 2009.

TABLE 6
Chemical and physical properties of sweet sorghum bagasse

Parameter	Value	Parameter	Value
pH (H ₂ O)	6.8	S (%)	<0.01
Electrical conductivity (S/m)	0.027	P ₂ O ₅ (%)	0.08
Organic matter (%)	95.2	K ₂ O (%)	0.20
Total nitrogen (%)	0.5	Na ₂ O (%)	0.08
Ash (%)	4.8	Mg (as MgO, %)	0.08
Ether-soluble fraction (%)	10.9	Ca (as CaO, %)	0.19
Ethanol-toluene extracts (%)	8.7	Cu (ppm)	48
Cellulose (%)	41.7	Zn (ppm)	35
Klason lignin (%)	18.9	Cr (ppm)	29
Elemental analysis		Pb (ppm)	20
C (%)	45.4	Fe (as Fe ₂ O ₃ , %)	0.15
N (%)	0.5	Mn (as MnO, %)	<0.2
H (%)	6.1	Cd (ppm)	<3.0

Source: Negro *et al.*, 1999.

Silage making and quality assessment

For silage preparation, the recommended moisture level is generally 60 percent, and the fodder is chopped for better compaction and anaerobic fermentation, leading to better quality silage. For fresh bagasse leaf residue (BLR), it was observed that the moisture content was 48–52 percent, and experiments were conducted to ensile the fresh material, both whole and chopped, with no further processing (moisture addition or silage additives) to make it as cost effective and practicable as possible. The results showed that ensiling of whole and chopped BLR for 30 days without any additives resulted in good quality silage as assessed by the appearance and smell of the silage. The quality of silage was assessed further by feeding experiments with 4 adult Deccani rams, where the silage was supplemented with 150 g concentrate/animal/day. The trial lasted for 21 days. Intake and nitrogen balance of chopped sweet sorghum BLR was similar to the silage prepared from whole BLR and the intake on a dry matter basis as a percentage of body weight was 2.5 percent (Table 7) (Kumar *et al.*, 2010).

ANIMAL STUDIES WITH SWEET SORGHUM BAGASSE

Nitrogen content, *in vitro* digestibility and metabolizable energy (ME) content of the sweet sorghum bagasse plus stripped leaves-based feed block (BRSLB) were significantly lower than in the commercial sorghum stover-based feed block (CFB), and the BRSLB was significantly superior to normal sorghum stover, but there were no differences in the NDF contents (Table 8). As expected, the laboratory quality indices were lowest for the sorghum stover. An important aspect of the work was to investigate the palatability of feed blocks when sorghum stover was entirely replaced by BRSL. The feeding trials with five murrh bulls (14 day adaptation period and 10 day collection period) showed that there was no (statistical) difference in feed intake between the CFB and the BRSLB (Table 8). For both blocks, the voluntary dry matter feed intake was high at 3.5 (CFB) and 3.7 percent (BRSLB) of animal live weight. Intakes of crop residues by non-lactating livestock are commonly around 2.0 percent or less of live weight (McDonald,

TABLE 7
Performance of sheep fed sweet sorghum bagasse and leaf residue as whole and chopped silage

Sweet sorghum bagasse and leaf residue	Dry matter intake (g/d)	Dry matter intake (as % body weight)	Dry matter digestibility (%)	Organic matter digestibility (%)	Nitrogen balance (g/d)
Chopped	415.4	2.5	59.3 a	60.2 a	5.7
Whole	414.0	2.5	63.1 b	64.3 b	4.8

Notes: a, b = Values followed by different letters in columns denote significant differences ($P < 0.05$). Source: Kumar *et al.*, 2010.

TABLE 8
Comparative feeding results in bulls fed a marketed commercial sorghum stover-based feed block (CFB), an experimental sweet sorghum bagasse/stripped leaves-based feed block (BRSLB) and sorghum stover of the type used in the CFB

Diet	Nitrogen (% DM)	NDF (% DM)	<i>In vitro</i> digestibility (% DM)	ME (MJ/kg)	Intake (kg/day)	Intake (g/day per kg LW)	Weight change (kg/day)
CFB	1.81 a	56.1 a	57.5 a	8.21 a	7.31 a	35 a	0.82 a
BRSLB	1.65 b	56.2 a	54.6 b	7.77 b	7.52 a	37 a	0.73 a
Sorghum stover	0.45 c	70.2 b	50.5 b	7.30 b	2.31 b	13 b	-0.38 b

Notes: NDF = neutral-detergent fibre; DM = dry matter; ME = metabolizable energy; LW = live weight; CFB = commercial sorghum stover-based feed block; BRSLB = experimental sweet sorghum bagasse plus stripped leaves-based feed block; sorghum stover is the type used in the CFB. Different suffixes in columns denote significant differences ($P < 0.05$). Source: Blümmel *et al.*, 2009.

Edwards and Greenhalgh, 1988). In fact, the intake of sorghum stover when fed as sole feed was only 1.3 percent of live weight (Table 8). However, when fed as part of the well-balanced CFB, stover intake was increased. Since sorghum stover was more than 50 percent of the CFB, the intake of sorghum stover was more than 1.75 percent of the live weight in CFB-fed bulls. These findings underline the importance of balanced supplementation in improving the utilization of a basal diet and in optimizing the utilization of crop residues for livestock production. There was no significant difference between the daily liveweight gain of the bulls fed CFB (0.82 kg/day) and the bulls fed BRSLB (0.73 kg/day), which confirms the value of BRSL as a feed block ingredient.

Addition of non-protein nitrogen sources like ammonium sulphate and biuret, either alone or in combination with urea, calcium carbonate or starch sources can also be tried to further improve digestibility, N-content and intake while making silage.

The nutrient digestibility and nutritive value of sweet sorghum bagasse was determined in sheep (deccani rams) and buffalo (murrah bulls) through a digestion-*cum*-metabolism trial using a difference technique. A 7-day adaptation period, 14-day preliminary period and 7-day collection period was used for the trial. The results show that the dry matter intake (as percentage of body weight) with sweet sorghum bagasse was 1.43 in buffaloes and 1.60 in sheep (Table 9). The digestibility (percent) values of proximate nutrients and fibre fractions of sweet sorghum bagasse calculated by different methods in sheep and buffaloes are presented in Table 10. The digestible crude protein (DCP) of sweet sorghum bagasse was 1.0 percent in both sheep and buffaloes, while the total digestible nutrients (TDN) value was 50.7 percent in sheep and 51.8 percent in buffaloes (Kumar *et al.*, 2010).

In another animal experiment, fresh unchopped BLR when supplemented with 500 g cotton cake in milch buffaloes resulted in feed intakes of 22 to 26 kg (fresh matter basis), corresponding to 3.3 percent intake when expressed as a percentage of body weight, indicating that BLR is quite palatable and well accepted by the milch buffaloes (Kumar *et al.*, 2010). The level of milk production was around 3 L/day, and during the one-month feeding period the body condition of the animals also improved, as indicated by the heart girth measurements and the condition of the body coat. After the experiment the animals were fed as per the farmer's usual practice of grazing supplemented with paddy straw and limited rice bran, and it was observed that animals on average lost around

TABLE 9
Effect of supplementing sunflower cake to sweet sorghum bagasse (SSB) on dry matter intake in graded Murrah buffalo bulls and Deccani rams

Parameter	Buffalo	Sheep
Body weight (kg)	344.2 ± 5.99	43.2 ± 1.31
DMI (kg/day)		
Roughage	4.91 ± 0.13	0.69 ± 0.03
Concentrate	0.72 ± 0.00	0.19 ± 0.00
Total	5.63 ± 0.13	0.88 ± 0.03
DMI (g/kg body weight)		
Roughage	61.5 ± 1.21	40.9 ± 1.25
Concentrate	9.0 ± 0.24	11.4 ± 0.25
Total	70.5 ± 1.32	52.3 ± 1.25
DMI (as % body weight.)		
Roughage	1.43 ± 0.03	1.60 ± 0.05
Concentrate	0.21 ± 0.01	0.45 ± 0.01
Total	1.64 ± 0.04	2.04 ± 0.05

Notes: DMI = dry matter intake. Each value is an average of four observations. Source: Kumar *et al.*, 2010

TABLE 10
Nutrient digestibility and nutritive value of sweet sorghum bagasse in graded Murrah buffalo bulls and Deccani rams

Nutrient component	Digestibility (%)	
	Buffalo bulls	Deccani rams
Dry matter	52.47 ± 1.39	50.75 ± 1.84
Organic matter	58.96 ± 0.26	58.82 ± 0.69
Crude protein	40.19 ± 0.83	41.61 ± 0.80
Ether extract	60.97 ± 1.61	58.14 ± 0.31
Crude fibre	51.54 ± 0.40	52.23 ± 0.83
Nitrogen-free extract	58.40 ± 0.84	55.72 ± 1.02
Nutritive value (%)		
DCP	0.98 ± 0.02	1.02 ± 0.02
TDN	51.78 ± 0.43	50.67 ± 0.42

Notes: DCP = digestible crude protein; TDN = total digestible nutrient. Each value is an average of four observations. Source: Kumar *et al.*, 2010.

20 kg within the first 15 days. Farmers appreciated that fresh sweet sorghum bagasse and leaf residue was well accepted by the buffaloes, but pointed out that chopping would have further improved the intake and reduced the refusal of thick stalk pieces. Interestingly, farmers observed that the milk of the fresh BLR fed animals was creamier than those on the previous grass diet due to increased fat content (Kumar *et al.*, 2010).

Other uses

Sweet sorghum bagasse, other than for animal feed, can be used as raw material for a range of purposes, including biofertilizer production, paper making and co-generation. One of the options for bagasse utilization is as organic soil amendment. However, the direct incorporation into the soil of raw wastes such as the bagasse is not usually suitable because they may cause undesirable effects, such as phytotoxicity and soil nitrogen immobilization. It is well known that composting is one of the most suitable ways of transforming wastes into more stable products that are safe and beneficial to plant growth. The finished compost has a low C/N ratio of 13, compared to 90 in the original substrate bagasse, and also has improved levels of macro- and micro-nutrients (Negro *et al.*, 1999).

For the paper industry, cereal straw and sugar cane bagasse are two abundant raw materials in addition to wood from the forest. However, these raw materials are in short supply due to restrictions on cutting trees in the forest, electricity generation from bagasse and residues, and residue use as livestock feed. Hence, sweet sorghum bagasse was assessed for its suitability for paper making (Belayachi and Delmas, 1997). The quality of the pulp obtained from sweet sorghum bagasse is excellent for the paper industry. The pulp exhibits a degree of cohesion higher than 80 percent; a low kappa number, indicating good delignification; a high degree of polymerization; and exceptional physico-mechanical properties, meeting the requirements of the

paper industry, and is expected to be the best alternative to sugar cane bagasse and cereal residues.

Co-generation is the simultaneous production of electricity and process heat from a single dynamic plant. Globally, biomass-based co-generation has been widely applied in forest industries and agro-industries such as sugar factories, rice mills and palm oil factories. The 30 KLPD Tata Chemicals Limited (TCL) plant at Nanded, Maharashtra, India, has a 2 MW per hour power generation capacity using bagasse, thus making it self-sufficient in energy.

Sweet sorghum bagasse, with a bulk density of 70–90 kg/m³ and ash levels of 4–5 percent, is highly suitable for gasification (Rajavanshi and Nimbkar, 2005).

UTILIZATION OF FOAM, VINASSE AND STEAM

Literature is scanty in these areas. The foam, froth and vinasse that is taken out during concentration of juice to syrup is rich in nutrients and can be used in composting of bagasse as well as directly as organic fertilizer. Vinasse needs to be subjected to nutrient analysis. Similarly the steam generated while boiling can be captured and used as a source of heat. This heat can be channelled to warm water when the DCU is aiming for more juice extraction efficiency. Alternatively, it can be used for pre-heating of the juice before boiling.

ECONOMIC IMPORTANCE OF BAGASSE FOR THE SWEET SORGHUM VALUE CHAIN IN THE DECENTRALIZED SYSTEM

The current rate of conversion of a tonne of sweet sorghum stalk to juice is 26.9 percent (269 litres) with 700 kg available as wet bagasse. After drying, about 30 percent (210 kg) of that wet bagasse (700 kg) is available as fuel or as fodder for livestock. In DCUs, about 45 percent of the dry bagasse (95 kg) is utilized as fuel (heating the pans) for converting juice to syrup, and the remaining 55 percent (115 kg) of the bagasse can be used or sold as fodder for livestock. During the early phases of DCU development, bagasse was sold direct to fodder traders with no value addition, and at a low price. However, during subsequent seasons, based on feedback from traders, dried bagasse of sweet sorghum was chopped to realize a higher value. Accordingly, efforts were made toward chopping sweet sorghum bagasse, doubling returns to Rs. 1/kg (US\$ 0.0022) for chopped sweet sorghum bagasse. This value addition through change in physical form of the bagasse increases the overall income from sweet sorghum in the ethanol value chain under the decentralized system. Additionally, sweet sorghum bagasse sold as fodder in the region of sorghum-based crop-livestock systems also helps in meeting the fodder requirements for the growing population of milch animals.

Reduction in cost of syrup production from sale of bagasse

The sale of chopped bagasse as fodder reduces the overall cost of processing syrup for ethanol production. The value realized for 115.5 kg of bagasse that is left over after use as fuel for the pans will be Rs. 115.5 (US\$ 2.6) at current rate of Rs. 1/kg of fodder (costs of chopping not accounted for). Hence, the cost of processing a tonne of stalk, which is currently Rs. 1231 (US\$ 28) (for both raw material and processing), will reduce by Rs. 115.5 ($1231 - 115.5 = 1115.5$) and thus the unit cost of syrup production, which was Rs. 25.65 (US\$ 0.58) will reduce to Rs. 23.23 (US\$ 0.53), a reduction of Rs. 2.40/kg (US\$ 0.05) or 9 percent decline in cost. Since there is further scope for value addition from bagasse sold for fodder (pellets), higher returns can be realized by selling a better product and thus further reducing syrup cost.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The commercial viability of the decentralized model of the sweet sorghum value chain depends on the efficient utilization of co-products in addition to the efficiency of operation and price of the main product, i.e. syrup. The following gaps have been identified based on several years of operation of DCUs in India:

- At present, there is a very limited period of operation of the crushing unit (less than 20–25 days) as the cultivar maturity window is not large. Research should aim at developing sweet sorghum genotypes with adaptability across seasons and months of the year.
- DCUs are being operated only for the rainy season crop (June–September). The post-rainy and summer season crops require an assured irrigation source, thereby increasing the cost of cultivation. Currently there are no suitable sweet sorghum cultivars adapted to post-rainy season conditions. The lower temperatures and shorter day lengths of this season hinder both biomass production and sugar accumulation in the tropical sweet sorghums, which are thermosensitive.
- The majority of the existing sweet sorghum cultivars are not multi-purpose, so do not meet the varying needs of the local agricultural systems. For example, high IVOMD, along with high sugar and biomass yield, are preferable for ensiling to meet livestock feed requirement. In areas where bio-composting is common, biomass with a high C:N ratio is not preferred. Research on hay-type sorghum species suggests that between 1950 and 2000 stem and leaf crude protein decreased and leaf NDF increased due to overemphasis on biomass quantity rather quality (Bolsen *et al.*, 2003).
- Juice extraction efficiency and syrup conversion efficiency are low. A scenario analysis conducted at ICRISSAT showed that improving these even by 5 percent has significant bearing on the economics of the whole value chain.
- As syrup is the main product of a DCU, its quality parameters need to be improved to meet the requirements of diverse end users (such as suitability for use in food, beverage and pharmaceutical industries). Research also needs to focus on improving organoleptic characteristics.
- Commercial dairies are increasingly using the fresh bagasse, after chopping, to feed cattle. Education and training is needed for farmers to raise awareness of the multiple uses of bagasse, such as for feed block making, ensiling or bio-composting.
- Little or no information is available on the utilization of co-products like vinasse, steam, foam and froth. Hence research efforts are needed in using steam for heating or boiling the juice, and in exploring the use of nutrient-rich vinasse, foam and froth as livestock feed and biofertilizers.
- Capacity building of staff at every step – not only syrup production, but also co-product utilization – would go a long way toward improving the operational efficiency and economic viability of DCUs.
- The varied products and co-products of the DCU need to be positioned to exploit locally existing market opportunities, i.e. an inclusive market-oriented development (IMOD) approach, as this brings the DCU closer to the rural farming communities.
- There are no studies on life cycle assessment (LCA) of DCUs with reference to carbon and energy balances. Such assessment studies would help all the stakeholders to understand the real value of this novel system, aside from economic viability analysis.

CONCLUSIONS

The potential uses of co-products from sweet sorghum DCUs for livestock feeding are unequivocally established. Considering the available genetic variability for fodder traits and ensiling parameters of sweet sorghum, the novel DCU system offers unforeseen opportunities, not only for meeting livestock feed demand of poor farmers, but also for offering an environmentally sound agro-enterprise that has tremendous implications for organic recycling related to carbon sequestration, GHG emissions and ecological balance. However, challenges remain pertaining to economic viability and marketability of the products and co-products of DCUs, requiring better linkages of poor and marginal farmers with emerging markets. These challenges must be addressed as a priority if there is to be greater involvement of rural agrarian communities in sweet sorghum cultivation.

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Chapter 13

Utilization of oil palm co-products as feeds for livestock in Malaysia

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ABSTRACT

Several oil palm industry co-products can be utilized as animal feed, notably oil palm fronds (OPF), oil palm trunks (OPT), palm press fibre (PPF), empty fruit bunches (EFB), palm kernel cake (PKC) and palm oil mill effluent (POME). These co-products are obtained either during the harvesting of the fruits, or the extraction and refining of crude palm oil (CPO) or palm kernel oil (PKO). Many of the co-products from the plantation (field residues) and processing mills need further processing before they can be used effectively in livestock diets.

Information on chemical composition, nutritive values, improvement methods and feeding response of ruminants fed oil-palm co-product-based diets are widely documented. Besides livestock feeds, some co-products are also utilized in the manufacturing of industrial products and organic fertilizers. OPF has been successfully utilized as feedstuffs either freshly chopped, as silage, or processed into pellets and cubes. Optimum inclusion level in beef and dairy animals is about 30 percent. Ensiled OPT produced reasonably good liveweight gain (LWG) of about 0.7 kg/day in beef cattle when fed at levels between 30 and 40 percent. PPF has a lower digestibility, which limits its inclusion in ruminant diets to less than 20 percent. PKC is a high-energy source and is a cost-effective ingredient in ration formulations for various livestock species. Beef and dairy production utilizing PKC-based diets are more economical under local dietary and management systems than non-PKC-based diets. High content of fibre and shell can limit use in poultry and aquaculture. With biotechnological treatments, inclusion levels of PKC can be increased to 30 percent for poultry feeding. POME, the residue left from the purification of CPO, can be combined with PKC and OPF to provide a cost-effective and complete ration for feeding ruminant livestock. The use of EFB, the material remaining of fruit bunches after steaming, is very limited and is generally utilized only after irradiation and culture-substrate treatments. The utilization of other locally available oil-palm-based co-products is targeted at increasing dietary energy content and improving nutrient digestibility. These include palm-fatty acid distillates (PFAD) and CPO, which are more suited for supplementing dairy animals, poultry, swine and aquaculture. The use of spent bleaching earth (SBE), another co-product from the oil-palm refineries, is very limited at present. Improvement in feed conversion efficiency (FCE) and maximizing the use of local feedstuffs represents a potential area of application to reduce the high cost of feed in Malaysia, especially in the non-ruminant subsector.

INTRODUCTION

The oil palm industry has become the backbone of Malaysia's economic and social development. It is developing rapidly to meet high global demand for palm oil, oleo-chemicals and biodiesel. In 2008, Malaysia produced about 17.74 million tonne of palm oil from over 4.49 million hectare of planted area. Palm oil and palm kernel oil (PKO) contributed about 30 percent of the total global production of oils and fats in 2008 (Oil World, 2009). The plantation area has increased from 97 000 ha in 1965 to 4.5 million ha in 2008. The planted area in Peninsular Malaysia, Sabah and Sarawak were 2.41, 1.33 and 0.74 million ha, respectively (MPOB, 2009). The private-estate sector occupied the largest area,

amounting to about 60 percent of the total area. The rest of the estates were government and state-schemes (28 percent) and smallholders (12 percent). The government-owned plantations include the Federal Land Development Authority (FELDA), the Federal Land Consolidated Authority (FELCRA), the Rubber Industry Development Authority (RISDA) and the State Economic Development Corporation (SEDC). Of the government-owned plantations, FELDA is the largest owner of oil palm land. As of 2009, there were 252 oil palm mills and 36 refineries in Peninsular Malaysia, 117 oil palm mills and 11 refineries in Sabah, and 41 oil palm mills and 5 refineries in Sarawak. Over the period 1990–2005, the land area under oil palm increased by 6.6 percent per

MAIN MESSAGES

- A large percentage of available palm kernel cake (PKC) should be efficiently used for domestic use as the main energy and protein sources for feeding ruminant and non-ruminant animals.
- Oil palm frond (OPF) is a good fibre source for ruminant feeding, and it is available in Malaysia throughout the year.
- Complete diets based on oil-palm co-products can be produced for various livestock species, including for aquaculture. Recommended levels of PKC feeding are 30–80 percent for growing beef cattle and 20–50 percent for goats, while for lactating dairy cattle it is 20–50 percent. Recommended levels of PKC in feed for poultry and freshwater fish are no more than 10 percent. The optimum level of OPF in feed for ruminant animals is 30 percent.
- Use of various oil-palm co-products as sources of feed for ruminants raised on the plantation itself is to be encouraged and maximized in order to reduce production costs.
- There is a huge potential – currently underestimated – for developing integrated oil palm-based ruminant production in Malaysia.

year, compared with negative growth for rubber, cocoa and coconut areas (MPOA, 2005).

Oil palm, *Elaeis guineensis* Jacq, has an economic life of 20 to 25 years and annually bears 8 to 12 fruit bunches, each weighing between 15 and 25 kg. Each fruit bunch carries 1000 to 3000 fruits, and each palm tree produces about 40 kg of palm oil annually. In palm oil milling, when the fresh fruit bunches (FFB) are processed, the economic end products are crude palm oil (CPO) and palm kernel oil (PKO). In the oil palm industry, the co-products are obtained from two sources, namely from residues in the plantations (field residues) and from palm oil milling. The former produces two major co-products: oil palm trunks (OPT) and oil palm fronds (OPF), while the latter produces empty fruit bunches (EFB), palm kernel cake (PKC), palm oil mill effluent (POME), palm press fibre (PPF), and shell. After processing some of the co-products are suitable for use as animal feed ingredients. The availability of various type of biomass and wastes in the oil palm environment has been intensively reviewed (Zin, 2000). A more recent paper estimated yields of 0.62, 0.04, 0.96 and 0.23 t/ha/year for OPF, PKC, POME and PPF, respectively (Devendra, 2006).

This present paper describes the utilization of the biomass from plantation and milling activities as feeds for livestock. Emphasis is placed on resources with abundant supply and easy to collect and utilize for livestock feeding. Selected products from refining activities that are used as high-energy sources for dairy animals, poultry, swine and aquaculture are also highlighted.

CO-PRODUCTS FROM OIL PALM PLANTATIONS (FIELD RESIDUES)

Oil palm fronds

Availability

Oil palm fronds (OPF) are obtained during harvesting or pruning and felling of palms for replanting. As such, it is available throughout the year. On an annual basis, about 24 fronds are pruned per palm tree, and the weight of fronds

varies considerably with age of the palm, with an average annual pruning of 82.5 kg of fronds per palm (Chan, 1999; Chan, Watson and Kim, 1981). At the time of felling during land clearing for replanting, each crown gives approximately 115 kg of dry fronds. It is estimated that about 30 million tonne of OPF is produced on a dry matter (DM) basis annually during the pruning and replanting operations (Ma, 2000). Traditionally, most OPF is left to rot between the rows of palm trees, mainly for soil conservation, erosion control and ultimately for the long-term benefit of nutrient recycling. However, due to the need to increase the net return per hectare, OPF has been used as resource material for extraction of vitamin E, paper pulp and animal feed. The large quantity of fronds produced by a plantation each year makes this biomass a very promising source of roughage for ruminants.

Nutritive value

OPF comprises three main components: a petiole, rachis and leaflets. About 70 percent of the DM in the OPF is from the petiole, and the rest from leaves and rachis. The leaves contain a higher percentage of crude protein (CP) and ether extract (EE) than the petioles. The DM content of OPF is about 31.0 percent and *in vitro* digestibility of DM of leaves and petioles is uniform throughout the length of the fronds, with a mean value of 35.6 percent (Ishida and Abu Hassan, 1992). OPF also contains between 15 and 26 percent hemicellulose, depending on its age. The moisture contents of chopped fresh OPF, solar-dried chopped OPF, steam-dried ground OPF and OPF pellets were 58.6 percent, 44.6 percent, 12.7 percent and 14.7 percent, respectively, with respective density values of 0.27, 0.08, 0.12 and 0.53 (Oshibe *et al.*, 2001). The chemical composition of OPF in comparison with other oil-palm co-products is shown in Table 1.

Rumen degradability is an appropriate assessment of the nutritive value of a fibrous feed for ruminants because

TABLE 1
Mean chemical composition (percent in dry matter, except for ME) and nutritive value of oil palm frond and other oil palm co-products

Co-products	CP	CF	NDF	ADF	EE	Ash	ME (MJ/kg)
Palm kernel cake (PKC)	17.2	17.1	74.3	52.9	1.5	4.3	11.13
Palm oil mill effluent (POME)	12.5	20.1	63.0	51.8	11.7	19.5	8.37
Palm press fibre (PPF)	5.4	41.2	84.5	69.3	3.5	5.3	4.21
Oil palm fronds (OPF)	4.7	38.5	78.7	55.6	2.1	3.2	5.65
Oil palm trunks (OPT)	2.8	37.6	79.8	52.4	1.1	2.8	5.95
Empty fruit bunches (EFB)	3.7	48.8	81.8	61.6	3.2	–	–

Notes: CP = crude protein; CF = crude fibre; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; EE = ether extract; ME = metabolizable energy. Sources: Wong and Wan Zahari, 1992; Wan Zahari et al., 2000.

TABLE 2
Rumen degradation parameters of whole and different fractions of oil palm frond (OPF) on incubation in nylon bags and using the equation $p = a + b(1 - e^{-ct})$

Incubation (hours)	Petiole	Leaflet	Midrib	OPF
a (g/kg)	21.2	21.7	14.4	18.4
b (g/kg)	24.7	46.1	28.3	38.3
c (% per h)	2.8	1.2	1.5	2.5
(a+b)	45.8	67.8	42.7	56.7

Notes: p = actual degradation at time t; a = intercepts; b = insoluble but potentially degradable component at time t; c = rate of constant of b; (a+b) = total degradability. Source: Islam et al., 1997.

it relates to the availability of nutrients. Table 2 shows the degradation characteristics of different fractions of OPF. A degradability value of 40 percent or more at 48 hours incubation indicates that OPF could be fed directly to ruminants. However, some improvement in terms of nutritive value is needed to increase the degradability level further. The characteristics of rumen degradation, digestibility, voluntary intake and palatability of several types of processed OPF have been reported by Kawamoto, Wan Zahari and Oshio (1999).

Nutritive value improvement

Several processing techniques have been developed to improve the feeding qualities of OPF. These include urea and molasses treatments, preservation as silage, alkali treatment, and steaming under high temperature and high pressure

(Table 3), pelletizing and enzymatic degradation. Urea- and molasses-treated OPF can almost meet the maintenance requirements of ruminants for energy and protein. The optimum level of urea inclusion in the OPF based diet was 30 g/kg ration, and steaming was reported to increase OPF digestibility. Increasing the level of urea in the steamed OPF resulted in reduced dry matter intake (DMI) and dry matter digestibility (DMD). A recent study revealed that microbial fermentation of OPF mixed with rice bran and rice husk through microbial fermentation of Japanese koji (*Aspergillus oryzae*) enhanced the feeding value by improving the CP content, reducing the NDF and improving the DMD of the feed, particularly with *Aspergillus awamori* (Ramli et al., 2010).

Freshly chopped

Freshly chopped OPF has been extensively used by local farmers for feeding to beef and dairy cattle in Malaysia. The growth performance and carcass composition of Brahman-Australian Commercial Cross (ACC) beef cattle fed iso-nitrogenous diets based on a freshly chopped OPF and PKC-based mixture is shown in Table 4. Diet 3 (40% OPF + 60% PKC) was the most economical as indicated by feed cost per weight gain value. Better feed conversion efficiency (FCE) and average daily gain (ADG) were obtained by diet 5 (20% OPF + 80% PKC), but it was not economical in terms of cost. Moreover, there were higher percentages of fat in the carcass. Carcass weight and dressing percentage improved with increasing levels of OPF in the diet.

TABLE 3
Chemical composition of oil palm fronds (OPF), untreated and steam-processed at various pressures (% in DM)

Treatment	NDF	ADF	HC	ADL	NDS	Ash	CP
Untreated	70.9	44.1	26.8	8.5	29.1	4.5	4.3
Fresh, steamed							
10 kg/cm ²	60.7	52.2	8.5	18.9	39.3	4.4	4.3
12.5 kg/cm ²	59.8	49	10.8	15.7	40.2	4.6	4.5
15 kg/cm ²	65.8	51.2	14.6	17.7	34.3	4.7	4.5
Pre-dried, steamed							
10 kg/cm ²	59.8	50.1	9.7	19.9	40.2	4.7	4.2
12.5 kg/cm ²	58.3	48.3	10	18	41.7	4.7	4.3
15 kg/cm ²	56.1	53.3	2.8	20.9	43.9	4.8	4.3

Notes: DM of the untreated and treated materials were almost similar, between 93.2 and 94.0; NDF = neutral-detergent fibre; ADL = acid-detergent fibre; HC = hemicellulose; ADL = acid-detergent lignin; NDS = neutral-detergent solubles (%NDS = 100 - %NDF); CP = crude protein. Source: Bengaly et al., 2000.

TABLE 4
Growth performance and carcass composition of Brahman-Australian Commercial Cross beef cattle fed mixtures with varying ratios of fresh chopped oil palm frond (OPF) and palm kernel cake (PKC)

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
OPF	60%	50%	40%	30%	20%
PKC-based mixture	40%	50%	60%	70%	80%
Number of animals	24	24	24	24	24
Initial LW (kg)	289.8	279	284.4	279	278.9
Final LW (kg)	340.2	327.5	343	343.5	356.9
ADG (kg/day)	0.64	0.61	0.67	0.75	0.85
DMI (kg/head/day)	6.12	6.02	6.5	7.08	7.56
FCR	9.56	9.87	9.7	9.44	8.89
Feed cost	3.09	3.11	3.04	3.45	3.23
Carcass composition					
Dressing %	54	56.3	54.8	57.8	57.2
Meat to bone ratio	2.9	2.57	2.88	3.03	2.85
Meat (% carcass weight)	66.6	57	59.3	55.7	55.6
Bone (% carcass weight)	22.7	21.9	20.9	18.7	19.5
Fat (% carcass weight)	9.6	14.2	14.7	17.2	17.2

Notes: The diets were iso-nitrogenous diet (with about 16.4% CP). The PKC-based mixture contained soybean meal, vitamin-mineral premix and urea. All animals were fed palm fatty acid distillates (PFAD) at 3% of DMI as an energy source. Feed cost is based on Ringgit/kg gain over an 86-day experimental period (US\$ 1 = Ringgit 3.8). CF percentages in diets 1 to 5 were 31.5, 28.6, 25.6, 22.2 and 19.2, respectively. The respective percentage total digestible nitrogen (TDN) values were 58.2, 60.2, 62.3, 65.3 and 67.3. CP = Crude protein; CF = Crude fibre; LW = Live weight; ADG = Average daily gain; DMI = Dry matter intake; FCR = Feed conversion ratio. Source: Mohd. Sukri *et al.*, 1999.

It is evident that the demand for processed OPF began to increase after the ensilation and pelletizing processes were introduced, especially when storage and ease of handling became necessary for commercial farms. However, in some locations, there was no urgent requirement to conserve OPF for silage as fresh OPF is abundantly available throughout the year.

Preservation as silage

Whole OPF can be chopped (to about 2–3 cm in length) and conserved as silage, and can be kept for several years when properly stored. Many trials were carried out to study the effect of additives on silage quality. These include treatment with water, molasses and urea (Table 5). The results indicate that good quality silage could be produced without no additives, provided that OPF was ensiled under anaerobic conditions. Urea addition at the rate of 1–2 percent prevented mould growth, and delayed the initiation of heat production by 28 hours. Inclusion of more than 3 percent of urea reduced the nutritive value of the silage. However, no adverse effect on animals was observed when urea was used at 3 percent (Table 6). Current research shows that *Lactobacillus plantarum*, heterofermentative lactic acid bacteria, is the best isolate for OPF silage, based on its ability to decrease pH faster and attain the lowest pH compared with other isolates (Hussin and Wan Mohtar, 2010).

Processing of pellet and cube

Digestibility studies conducted using mature Kedah-Kelantan (KK) bulls indicated a DMD value of about 45 percent for OPF silage. It was significantly reduced when urea

TABLE 5
Effect of water, molasses and urea addition at ensiling on the fermentation characteristics of oil palm frond silage

Parameter	Treatment			
	Control	Water	Molasses	Urea
pH value	4.02 b	3.93 b	3.93 b	7.38 a
Organic acids (% DM)				
Lactic acid	1.89 bc	2.30 b	3.55 a	1.51 c
Acetic acid	0.89 b	0.65 b	0.78 b	8.99 a
Butyric acid	1.07 b	0.99 b	1.04 b	1.66 a
Percentage spoilage	13.9 a	9.0 a	1.6 a	0.0 b

Notes: Control had no additives. a, b, c = means with different letters in a row differ ($P < 0.05$).

Source: Abu Hassan and Ishida, 1992.

was included at 6 percent of the total diet (Ishida and Abu Hassan, 1992). Further long-term feeding trials were conducted with growing and finishing beef cattle and with lactating cows (Abu Hassan *et al.*, 1993; Ishida *et al.*, 1994). In the trial without urea, the feed required for LWG and lean meat production was reduced with higher inclusion levels of OPF silage (Table 7). This is reflected in reduced feed cost.

The potential of OPF silage as a source of roughage for lactating dairy cows is shown in Table 8. The cows fed 30 percent OPF silage produced more milk than those fed 50 percent OPF silage. There were no adverse effects on the animals, milk yield or flavour, even when the level of OPF silage was increased to 50 percent. In a separate study, the LWG of swamp buffaloes fed 30 percent OPF silage was comparable to those fed 50 percent sago meal (Shamsudin, Mohd. Sukri and Abdullah Sani, 1993). Studies with sheep indicated that OPF silage was better utilized compared with nipa palm (*Nypa fruticans*) frond silage. Additionally,

TABLE 6
Effect of urea level at ensiling on chemical composition, fermentation characteristics, voluntary intake and digestibility of oil palm frond silage

Parameter	Urea level (% in DM)		
	0	3	6
Chemical composition			
Dry matter (%)	30.1 ab	30.7 a	28.6 b
Percentage of dry matter			
Crude protein	6.7 c	11.4 b	17.2
Organic cell contents	20.8 a	20.0 ab	13.0 c
NDF	73.2 b	73.9 b	80.3 a
Fermentation characteristics			
pH value	3.78 a	4.89 b	7.81 c
Total acids (DM percent)	3.68 b	4.76 b	8.96 a
Composition of acids (%)			
Lactic acid	91.0 a	37.4 b	13.0 c
Acetic acid	6.1 c	25.8 b	72.9 a
Propionic acid	0.1 b	3.8 a	0.8 b
Butyric acid	0.9 c	30.9 a	6.7 b
Ammonia (% DM)	0.0 c	0.6 b	1.1 a
Voluntary DM intake (g/day)			
Digestibility (%)	39.9 a	32.1 a	24.0 b
Dry matter	45.3	46.8	35.7
Organic cell contents			
NDF	100	91.7	86.1
TDN (DM%)	29.1	37.5	30.2
	45.5	49.2	37.5

Notes: DM = dry matter; NDF = neutral-detergent fibre; TDN = total digestible nutrient; a, b, c = means with different letters in a row differ ($P < 0.05$). Source: Ishida and Abu Hassan, 1992.

TABLE 7
Effect of oil palm frond levels on growth performance and carcass characteristics of Australian commercial cross bulls

Parameter	Treatment			
	T1	T2	T3	T4
Live weight (kg)				
Initial weight	229.1	226.5	232.9	229.4
Final weight	396.3 a	336.4 ab	333.8 b	357.2 ab
Daily gain (kg/day)	0.75 a	0.62 ab	0.45 c	0.57 bc
Feed intake (kg DM/day)	7.02 a	6.10 ab	5.48 b	5.58 b
Carcass weight (kg)	237.2 a	210.2 ab	189.0 b	195.2 b
Weight of carcass components (kg)				
Meat	127.8	121.5	107	116.7
Fat	76.4 a	58.1 ab	45.8 b	46.0 b
Bone	37.6	33.4	33.2	36.1
% in carcass				
Meat	35.6	58.2	57.2	59.2
Fat	31.6 a	27.6 ab	24.2 b	23.7 b
Bone	16	16.1	17.7	18.4

Notes: T1 = 10% Urea OPF silage + 90% PKC-based concentrate. T2 = 30% Urea OPF silage + 70% PKC-based concentrate. T3 = 50% Urea OPF silage + 50% PKC-based concentrate. T4 = 50% OPF silage only + 50% PKC based concentrate. a, b, c = means with different letters in a row differ ($P < 0.05$). PKC = Palm kernel cake. Source: Ishida *et al.*, 1994.

the provision of molasses was reported to increase the potential degradability of both nipa and oil palm fronds (Abdalla *et al.*, 2001). In this trial, ammonia-N in the rumen liquor of the animals fed OPF supplemented with 0 percent and 30 percent molasses were found to be conducive for

TABLE 8
Effect of feeding oil palm frond silage on performance of Sahiwal-Friesian lactating dairy cows

Parameter	Dietary treatment		
	T1	T2	T3
Number of cows	9	9	9
Body weight (kg)	417	451	450
Ingredient composition of diet (% DM)			
OPF silage	30	50	-
Fodder	-	-	50
Concentrates	70	50	50
Feed intake and milk production			
DM intake (kg/day)	6.46 b	5.86 c	8.28 a
Yield of 4% FCM (kg/day)	6.93	5.73	6.48
4% FCM to ME intake ratio (kg/MJ)	0.109 a	0.088 b	0.096 b

Notes: T1 = 30% OPF silage diet. T2 = 50% OPF silage diet. T3 = 50% fodder diet. FCM = fat corrected milk. ME = metabolizable energy. Concentrates contained 24.0% CP and 11.3 MJ/kg of ME. a, b, c = Means with different suffixes differ ($P < 0.05$). Source: Abu Hassan *et al.*, 1993.

optimum rumen environment, with values of 141.5 mg/litre and 142.9 mg/litre, respectively. These values were, however, lower than suggested levels of 200–250 mg/litre for ruminants (Preston and Leng, 1997).

Feeding beef cattle

The effects of varying levels of OPF pellet on intake and growth performance of local beef cattle has been reported by Oshibe *et al.* (2000). The trial was conducted to evaluate the effect of OPF-based diets varying in CP content on intake and growth performance of growing Charolais × KK crossbred cattle. The animals were fed iso-caloric pelleted diets (containing about 9.13 MJ/kg DM) based on ground OPF at a 30 percent inclusion level. Over the 172-day feeding period, the LWGs achieved were 0.50, 0.52, 0.30 and 0.44 kg/day, respectively, when the animals were fed 10, 12, 14 and 15 percent CP (Table 9). The respective mean DMDs of the diets were 55.7, 68.6, 56.8 and 52.7. The LWGs obtained were comparable to those raised on 30 percent roughage and 70 percent concentrate. Provision of 12 percent CP improved DMD by about 23 percent compared with those fed 10 percent CP. Further addition of protein increased neither intake nor DMD, as shown in the groups fed higher CP levels. What contributed to the differences was not clear, as energy contents among the diets were very similar. It is unlikely that this is due to small differences in CF content as the values from the four diets only varied between 20.5 and 23.3 percent. Levels of EE for all of the diets were below 5 percent, and hence unlikely to cause any significant impairment in CF digestibility for pelleted diets based on OPF. Body scoring of cattle fed 30 percent OPF-based diets was from medium to good. Meat quality was excellent, with less deposition of fat in the carcasses. Irrespective of protein levels, the ranges for carcass weight and mesenteric fat were from 130.9 to

TABLE 9
Intake and growth performance of beef cattle raised on OPF pellet based diet

Treatment	Mean DMI (kg/day)	DM digestibility (%)	Initial LW (kg)	Final LW (kg)	LWG (kg)	Mid-abdomen (cm)
10% CP	6.40	55.7	242.5	328.5	0.50	181–214
12% CP	5.94	68.6	234.8	324.0	0.52	172–226
14% CP	5.88	56.8	231.5	283.4	0.30	182–192
15% CP	5.94	52.7	236.6	312.6	0.44	171–212

Notes: DMI = dry matter intake; LW = live weight; LWG = live weight gain; CP = crude protein.
Source: Wan Zahari *et al.*, 2000, 2002.

215.3 kg and 5.0 to 6.6 kg, respectively. The meat to bone ratio ranged from 0.7:1 to 3:1.

Distended rumen was reported in beef heifers fed pellets made from ground OPF at a 30 percent inclusion level (Wan Zahari *et al.*, 2002). This is associated with the rapid rate of passage of finely ground materials from the pellet, which is unfavourable for optimum rumen fermentation. Faster passage of feed through the rumen is known to depress DMD. Hence, rumen retention time should be reduced to stimulate better digestibility. Longer particle size (>15 mm) should be considered for making complete diets based on OPF. One option to make OPF cube, a process that does not require grinding (Hayakawa and Ariff, 2000). Small particle size of the diet is also known to depress the population of protozoa in the rumen, but what particle size is best for the protozoa to stimulate optimum fermentation is another issue. A high protozoan population density could also increase requirements for supplementary protein. Additionally, reducing the protozoan population in the rumen generally increases animal productivity on low-protein diets. Moreover, an optimal ratio of nitrogen to sulphur is vital for efficient ruminal microbial growth for diets based on fibrous materials like OPF. Contrary to what has been thought, distension of the rumen was not associated with bolus formation, which has been found in growing sheep raised on OPF silage and urea molasses mineral blocks (Wan Zahari, unpublished). Irrespective of the treatments, there seemed to be large variations between animals for the weight of the rumen, intestine and other organs (Table 10). There were also no abnormalities with regard to the structural and physical appearances of organs and other body tissues. The meat and organs were safe for consumption and of superior quality due to less deposition of body fat (Wan Zahari *et al.*, 2000, 2002). The average concentration of lead (Pb) residues in OPF feed was lower than the concentration specified for the maximum residual limit level (3000 ppb) (Faridah *et al.*, 2002).

The LWG of Brahman × KK male cattle fed diets containing 70 percent OPF + 30 percent cassava fodder was significantly less than for those fed 70 percent OPF + 30 percent concentrate or 70 percent OPF + 15 percent cassava fodder + 15 percent grain concentrates (Tung *et al.*, 2001). The

TABLE 10
Body composition of beef cattle raised on oil palm fronds based diet

Parameter	Bulls	Heifers
Live weight before slaughter (kg)	274.0–407.0	186.0–238.0
Carcass weight (hot) (kg)	130.9–215.3	98.7–136.4
Rumen weight (empty, kg)	7.5–10.4	4.2–5.8
Intestinal weight (full, kg)	10.85–13.30	8.0–11.0
Intestinal weight (empty)	6.0–9.0	3.8–7.0
Liver (kg)	2.15–4.40	1.92–3.96
Spleen (kg)	0.758–1.172	0.71–1.82
Kidney (kg)	0.508–0.714	0.175–0.304
Mesenteric fat (kg)	5.00–6.60	2.60–5.50
Fat in carcass (kg)	3.50–10.10	1.52–4.47
Sirloin (kg)	1.36–3.40	0.74–2.00
Loin (kg)	2.60–9.30	2.52–4.10
Meat:Bone ratio	2.70–3.10	2.42–3.10

Notes: The animals were Kedah-Kelantan × Charolais crosses. SOURCE: Wan Zahari *et al.*, 2000, 2002.

values for DMI (kg/head/day), N retention (% of N intake) and LWG (g/head/day) for the respective treatments were 4.01, 4.78 and 4.66; 15.49, 19.04 and 17.93; and 277.8, 412.7 and 373.0 respectively.

Feeding dairy cattle

Research and development on OPF feeding for dairy cattle reflects the intensive system of rearing that is suitable for Malaysia, considering the high cost of pasture land. Several experiments have been conducted that were aimed at developing feeding programmes based on OPF pellets or OPF cubes.

A study was conducted to evaluate ground OPF-based diets as a complete ration for lactating Sahiwal-Friesians dairy cows. The lactation performance and LW change of the animals fed 30 percent OPF pellet ration is shown in Table 11. Milk yields of cows used in this experiment varied from 11.1 to 20.3 L/day for the duration of the trial. The highest recorded 28-day milk yield period was 609 litres, equivalent to an average daily yield of 21.75 litres. The overall milk fat was 3.5 percent, and daily supplementation with 100 g long hay was insufficient to increase the fat content to the level of 4.6–4.8 as obtained when feeding concentrate-grass mixture or dairy cattle pellets (Abu Bakar *et al.*, 2001).

TABLE 11
Effects of oil palm frond (OPF)-based pellets on milk yield and milk composition

Ration	Milk yield (L/28 days)	Milk fat (%)	Milk protein (%)	Weight change (kg)
30% OPF pellets	366	3.5	3.5	22.5
30% OPF pellets + LG	375	3.5	3.5	16.5

Notes: LG = Unchopped guinea grass hay given at 100 g/cow/day as long fibre supplement. Four Sahiwal-Friesian cows per group, assigned to a treatment sequence in a 4x4 Latin square design involving four 28-day measurement periods following a 2-week adjustment period. Daily ration fed to each cow was limited to 14 kg/day. Source: Abu Bakar *et al.*, 2001.

In a separate study, Sahiwal-Friesian heifers fed molasses-treated OPF were observed to consume 30 percent more ($P < 0.05$) total feed DM compared with untreated-OPF (Abu Bakar *et al.*, 2000). The improvement in intake could be attributed to improvement in the palatability and digestibility of nutrients. There was no obvious advantage of brine treatment (salty water containing 39.12 percent sodium chloride by weight, commonly used for food preservation) in stimulating intake of OPF pellets. LWGs of the animals fed molasses-treated OPF were comparable to those fed brine-treated OPF, with values of 0.69 kg/day and 0.68 kg/day, respectively. In comparison, Sahiwal-Friesian heifers fed maize stover silage and guinea grass produced gains of 0.43 and 0.47 kg/day, respectively (Abu Bakar, Aminah and Mansor, 1990). In addition, Friesian heifers fed complete rations based on 70 percent sugarcane bagasse as roughage recorded mean LWG between 0.38 and 0.56 kg/day, depending on quality of the energy-protein sources used (Van Horn *et al.*, 1980). Dried grated coconut meal (containing 64 g CP/kg; 359 g CF/kg; 24 g EE/kg; and 10.8 MJ ME/kg) and PKC are equally good as supplemental feed with OPF pellets for growing Sahiwal-Friesian heifers diets, provided that the protein content is enriched (Abu Bakar *et al.*, 1999).

Oil palm trunks

Availability

Oil palm trunk (OPT) is only available after oil palms are felled for replanting at an age of about 25–30 years (Mohamad *et al.*, 1986). The main economic criteria for felling are the height of palms exceeding 13 m, and annual yield of bunches falling below 10–12 t/ha. The biomass consists mainly of vascular bundles and parenchyma tissues. The parenchyma recovery is about 38 percent (Oshio *et al.*, 1991).

Nutritive value

The nutritive value of OPT is similar to PPF. It contains about 3 percent CP. The vascular bundles contain less lignin than the parenchyma tissues and in digestibility studies with sheep the parenchyma tissue had higher DM and organic matter values.

Processing and livestock feeding

OPT can be collected and processed into chips (about 2–3 cm) and preserved in the form of silage. Vertical or bunker concrete silo are recommended. OPT silage can be utilized for feeding after 21 days in the silo. OPT silage results in excellent fermentation due to low pH (3.2) and good production of lactic acid. Without any treatment, the DM digestibility of OPT is comparable to rice straw. Feeding trials with ACC beef cattle showed that OPT silage produced better FCE than rice straw, with good rate of growth and eating quality (Oshio *et al.*, 1991). The DMD of OPF silage without urea was 45 percent, compared with 44.2 percent and 35.8 percent when urea was added at 3 percent and 6 percent, respectively. Insecticide residues were not detected in the OPT samples (Ong and Abu Hassan, 1991).

The parenchyma is an excellent source of roughage for beef cattle in feedlots. The biomass was readily consumed by the animals, even at 50 percent level. It can be integrated with OPT fibre processing where the fibre can be used for production of pulp, paper and composite panels. The nutritive value of the material can be further enhanced by physical, chemical or biological treatment. OPT-based ration can be formulated for feeding large ruminant animals and the maximum level of inclusion is suggested to be 30 percent.

CO-PRODUCTS FROM OIL PALM MILLING

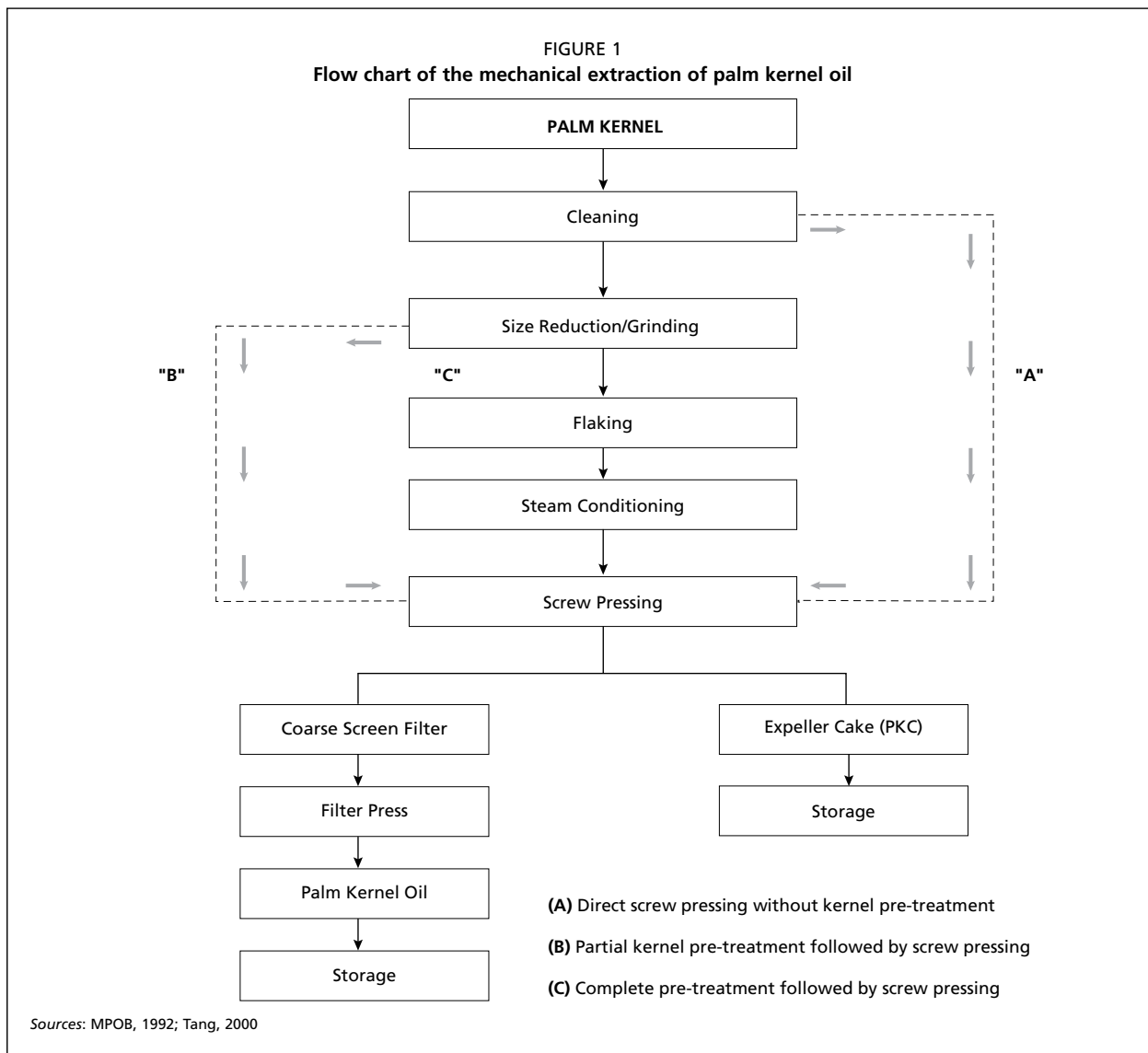
Palm kernel cake

Availability

Palm kernel cake (PKC) is an important feed for livestock in Malaysia. It is produced after the extraction of PKO from the kernels of the oil palm fruits. PKC is also known as palm kernel meal (PKM), or palm kernel expeller (PKE) (Figure 1). Two types of oil extraction process are employed, either screw press (expeller) or solvent extraction. The oil milling industry differentiates PKC as the solvent extraction type, while PKE is the screw-pressed type. PKE is subject to heat damage during screw pressing. More than 99 percent – over 2 million tonne – of the meal produced is PKE, of which 95 percent is exported, mainly to the European Union. In this paper, the term PKC is used as it is accepted widely in Malaysia and other countries.

Nutritive value

In general, the solvent extracted PKC has a lower oil content, ranging from 1.2 to 5.0 percent, while the expeller pressed PKC has 4.5 to 17.3 percent (Tang, 2000). Generally, PKC can be classified as an energy-feed (Table 12) and its chemical composition is somewhat similar to copra meal, rice bran or corn gluten feed. The ME values for ruminants and poultry are 10.5–11.5 MJ/kg and 5.9–7.0 MJ/kg, respectively (Yeong, 1985). The ME for swine is generally higher than for poultry, with the values between



10.0 and 10.5 MJ/kg. The CP content is considered to be more than sufficient to meet the requirement of most ruminants. PKC has a good amino acid profile (Table 13), with availability between 62 and 87 percent (Yeong, Mukherjee and Hutagalung, 1981). Limiting amino acids are lysine, methionine and tryptophan. The protein quality of the MPOB-Q-PKC, recently introduced by the MPOB is superior to the existing PKC (Atil, 2009). This product is obtained after pre-processing the palm nuts to remove completely the shell and the fibrous testa of the kernels. However, this product is still under development. PKC also contains high residual fat (about 10 percent), carotene and vitamin E (about 0.3 IU/kg), which can act as a natural antioxidant. Table 14 shows the fatty acid content in PKC. Its low content of unsaturated fatty acids also reduces rancidity problems.

PKC is high in minerals, with P and Ca contents of 0.48 to 0.71 percent and 0.21 to 0.34 percent, respectively (Table 15). The Ca:P ratio is very low (about 0.36:1) and

TABLE 12
Chemical composition and nutritive value of palm kernel cake

Parameter	
Dry matter (DM as %)	88.0–94.5
Chemical composition (% in DM)	
Crude Protein (CP)	14.5–19.6
Crude Fibre (CF)	13.0–20.0
Ether extract (EE)	2.0–8.0
Ash	2.0–10.0
Nitrogen-free Extract (NFE)	46.7–75.8
Neutral-detergent Fibre (NDF)	66.8–78.9
Oil content (%) +	4.5–17.3
Shell and dirt	3.6–21.4
Metabolizable energy (MJ ME/kg)	
Ruminants	10.5–11.5
Poultry	6.5–7.5
Swine	10.0–10.5

Notes: Oil content values adapted from from Siew, 1989.

TABLE 13
The amino acid profile of palm kernel cake

Amino acid	Composition (%)
Alanine	0.92
Arginine	2.18
Aspartic acid	1.55
Cystine	0.2
Glycine	0.82
Glutamic acid	3.15
Histidine	0.29
Isolucine	0.62
Leucine	1.11
Lysine	0.59
Methionine	0.3
Phenylalanine	0.73
Proline	0.62
Serine	0.69
Threonine	0.55
Tyrosine	0.38
Valine	0.93
Tryptophan	0.17

Notes: The concentration values are based on total protein content in palm kernel cake of 16.01%. Source: Yeong, 1983.

TABLE 14
The fatty acid content of palm kernel cake

Fatty acids	g/100 g oil
C6:0	0.2
C8:0	3
C10:0	4
C12:0	48
C14:0	16
C16:0	8
C18:0	3
C18:1	15.4
C18:2	2.4
C20:0	0.1

Source: MPOPC, 1995.

TABLE 15
The mineral content of palm kernel cake

Element	Level
Calcium (Ca) (%)	0.21–0.34
Phosphorus (P) (%)	0.48–0.71
Magnesium (Mg) (%)	0.16–0.33
Potassium (K) (%)	0.76–0.93
Sulphur (S) (%)	0.19–0.23
Copper (Cu) (ppm)	20.5–28.9
Zinc (Zn) (ppm)	40.5–50.0
Iron (Fe) (ppm)	835–6130
Manganese (Mn) (ppm)	132–340
Molybdenum (Mo) (ppm)	0.70–0.79
Selenium (Se) (ppm)	0.23–0.30

Source: Alimon, 2004.

diets based on PKC need to be supplemented with Ca to meet animal requirements. The level of Mg, K, S, Zn, Fe, Mn, Mo and Se are within acceptable ranges. However, Cu content in PKC (21–29 ppm) is higher than

TABLE 16
The digestibility coefficients of nutrients in palm kernel cake

Nutrient	Sheep	Cattle
Dry matter	0.70	0.76
Crude protein	–	0.78
Ether extract	0.91	0.84
Ash	–	0.67
Neutral-detergent fibre	0.52	0.76
Acid-detergent fibre	0.53	0.73

Source: Wong and Wan Zahari, 1997.

required by ruminants. More than 75 percent of PKC is cell wall component, which consist of 58 percent mannan, 12 percent cellulose and 4 percent xylan (Mohd. Jaafar and Jarvis, 1992). Table 16 shows the average digestibility coefficients of nutrients in PKC, based on studies with sheep and cattle. The digestibility values for ADF and NDF are much higher in cattle than in sheep, suggesting that sheep are less efficient than cattle in digesting fibre. The digestibility of NDF in forage hays are also higher in cattle than in sheep (Reid *et al.*, 1990). Earlier studies suggested that differences in the concentrations of urea and sulphur in blood, and lower excretion of N, P and Ca by the cattle, could have increased microbial activity in the rumen and digestion of fibre (Playne, 1978)..

PKC is normally free from aflatoxin, and therefore very safe for livestock feeding. It is also free from any chemicals, heavy metals, pesticides and dioxins. High DM content inherent in the PKC discourages growth of micro-organisms and mould, and it can therefore be stored for periods of up to three months without much problem.

Livestock feeding.

Feeding beef cattle and swamp buffaloes

PKC is widely used as the main ingredient in rations for feedlot cattle and buffaloes. In Malaysia, feedlot cattle are normally fed diets containing up to 80 percent PKC, with LWG of 0.6–0.8 kg/day for local KK cattle and 1.0–1.2 kg/day for crossbred cattle (Wan Zahari *et al.*, 2000). Diets containing almost 100 percent PKC have been fed to feedlot cattle with no negative effects, provided that the supply of Ca and vitamins (in particular A and E) are sufficient to meet requirements. Studies have shown that supplementing traditional rations of beef cattle with 30–50 percent PKC increased LWG (Wan Zahari and Alimon, 2004). It is common practice in Malaysia to produce complete feed based on PKC, either in the form of pellets, cubes or as total mixed ration (TMR) (Wan Zahari, Wong and Hussain, 2009). Apart from PKC, other common ingredients that are included in TMR include rice bran, brewers grain, palm oil mill effluent (POME), tapioca waste, urea, salt and minerals (Wan Zahari *et al.*, 2003). An example of the formulation for beef cattle feeding is PKC (80%) + grass/hay (17.5%) +

limestone (1.5%) + mineral premix (1.0%). A low cost fattening programme for beef cattle can be developed based on PKC and PPF, with LWG between 0.60 and 0.75 kg/day (Wan Zahari *et al.*, 2000)

Owing to its small particle size, the level of PKC in beef cattle diets should not be more than 85 percent to avoid occurrence of metabolic problems such as acidosis and kidney stones. Grass or hay or other long-fibre sources should be included at at least 10 to 15 percent in the total ration. Addition of grasses or other forages will reduce the rate of passage of PKC in the gastro-intestinal tract of the animals, thus increasing retention and digestibility of nutrients (Oshibe *et al.*, 2001; Wan Zahari *et al.*, 2002). Moreover, when feeding PKC at high levels, attention should be given to Ca supplementation (Wan Zahari and Alimon, 2004). Limestone (calcium carbonate) is the most appropriate Ca supplement as it is cheap and easily available. It is important to ensure that the ratio of Ca to P in the rations is within the range of 1:1 to 3:1 in order to preclude skeletal deformities and mineral imbalances. Sodium chloride and vitamin A should be supplemented at the appropriate levels to meet requirements. Feeding PKC at 100 percent inclusion level may cause wet faeces and digestive disorders, and is contrary to principles of proper ruminant nutrition.

Feeding dairy cattle

In dairy cattle rations, PKC is used as a source of energy and protein at an inclusion level of 30–50 percent. PKC-based pellet is a common feed supplement for dairy cattle in Malaysia and it is usually fed together with grass and other concentrates (Abu Hassan, 2005; Abu Bakar *et al.*, 2000). The grass to concentrate ratios fed are around 50–70 percent:30–50 percent (Abu Hassan *et al.*, 1996). In the Malaysian environment, daily milk yields of 10–12 L/head can be achieved, and, with good formulation, higher yields can be expected (Wan Zahari *et al.*, 2000). Other common ingredients in rations for dairy cattle are rice bran, brewers grain, palm oil sludge (POS) or POME, soybean waste, bakery waste, salt and minerals (Abu Bakar *et al.*, 2001). In some areas, grass and other forages high in protein are given *ad libitum*. An example of a dairy cattle formulation is PKC (50%) + molasses (5%) + grass/hay (42%) + limestone (1.5%) + mineral premix (1%) + common salt (0.5%) (Alimon, 2004). Most of the PKC exported to the European Union is used in dairy cattle rations, but the level of inclusion is known to be limited to 15 percent.

Feeding sheep and goats

Recommended maximum inclusion level of PKC in sheep rations is 30 percent. Long-term feeding of PKC at high inclusion level (>80 percent) can cause Cu toxicity in sheep, as sheep are known to be very susceptible to Cu poisoning (Hair Bejo *et al.*, 1995; Al-Kirshi, 2004). Some sheep breeds

(especially crossbreds) accumulate Cu in the liver, causing liver damage. Addition of 100 ppm of zinc sulphate or 5.2 mg/kg ammonium molybdate together with 440 mg/kg sodium sulphate in the rations can overcome the Cu toxicity problem (Hair-Bejo *et al.*, 1995). Cu toxicity does not appear in cattle, buffaloes, goats and other animals, but long-term feeding of PKC can result in high levels of Cu concentrations in the liver. An example of a formulation for goats is PKC (50%) + grass/hay (30%) + rice bran (10%) + soybean meal (9%) + mineral premix (1%) (Wan Zahari and Alimon, 2003).

Feeding poultry

Owing to its high fibre content, non-starch polysaccharides and shell content, the use of PKC in poultry rations is very limited, with wide variation in the optimum inclusion level. The main difficulty is the origin and variation in the oil and shell content of the PKC used. Broiler chicken can tolerate up to 20 percent PKC in their diets without affecting growth performance and FCE (Yeong, 1987; Abu Hassan and Yeong, 1999). In layer rations, PKC can be included up to 25 percent without any deleterious effects on egg production and quality (Yeong, 1987; Radim *et al.*, 2000). However, inclusion of PKC at levels greater than 20 percent was reported to reduce egg production and egg quality (Yeong *et al.*, 1981), although in another study reduced egg production was only observed at levels exceeding 40 percent (Onwudike, 1988).

Muscovy ducks can be fed PKE at the 30 percent level without any deleterious effects on performance (Mustafa *et al.*, 2001). Low-shell PKC with higher energy and CP content is important to maximize utilization in poultry. However, high inclusion levels of PKC require supplementation with high levels of fat, making the rations economically uncompetitive in comparison with conventional maize-soya-based diets.

Current research focuses on enhancing the nutrient content of PKC for poultry. Topics include enzyme treatment and solid-state fermentation of the PKC. Enzymic depolymerization of PKC releases digestible sugars that will be fully absorbed and metabolized by poultry. Supplementation with specific enzymes can improve nutrient digestibility and has worked efficiently to break down mannans in PKC (Noraini *et al.*, 2002; Saenphoom *et al.*, 2010). Broilers can be fed diets containing 30 percent fermented PKC without any adverse effect on performance (Noraini *et al.*, 2008). Fermentation with *Aspergillus niger* was reported to increase the true metabolizable energy of PKC from 5.5 MJ ME/kg to 8.1 MJ ME/kg. *Aspergillus niger* up to generation F₆ can be used as inoculum for fermentation of PKC (Abdul Rahman *et al.*, 2010). Chemical treatment using sodium hydroxide and formaldehyde have also been investigated, but with variable results. Further research is required to

enhance the nutrient content of PKC for poultry (Wong *et al.*, 2009).

Feeding swine

PKC is also suitable for swine at an inclusion level ranging from 20 to 25 percent for growers and finishers. In some areas in Peninsular Malaysia, PKC is used at lower levels (between 5 and 10 percent). An example of a formulation for feeding swine is PKC (20) + maize (65.5%) + soybean meal (9.5%) + fish meal (3.0%) + dicalcium phosphate (1.5%) + mineral premix (0.2%) + common salt (0.3%) (Wan Zahari and Alimon, 2003). In Nigeria, PKC is fed to swine at levels ranging from 15 to 40 percent without no negative effects on performance (Codjo *et al.*, 1995).

Feeding in aquaculture

The availability of PKC in many tropical countries where aquaculture is practised has generated much interest in its potential use in fish diets. Early studies indicated that PKC can be tolerated up to 30 percent in catfish (*Clarias gariepinus*) and 20 percent in tilapia (*Oreochromis niloticus*) rations with no deleterious effects on growth and performance (Sukkasame, 2000). An example of a formulation for African catfish is PKC (30%) + fish meal (20%) + cassava flour (15%) + soybean meal (31%) + sago (1%) + mineral and vitamin (2%) + vegetable oil (1%). PKC pre-treated with commercial feed enzymes resulted in better growth and FCE than with raw PKC. The fermentation of PKC with *Trichoderma koningii*, a cellulolytic fungus, increased the CP content in PKC from 17 percent to 32 percent (Ng *et al.*, 2002). At a 40 percent feeding level of PKC, the rate of growth was reduced and this was not rectified with the addition of 1.2 percent dietary L-methionine (Ng, 2006). It is suggested that 30 percent is the maximum inclusion level for enzyme-treated PKC in tilapia diets. More R&D is needed to optimize the use of feed enzymes in PKC-based diets in order to reduce the cost of using imported maize as an energy source.

Table 17 shows the recommended levels of PKC in the feeds for beef cattle, dairy cattle, sheep, goats, poultry, swine and freshwater fish.

TABLE 17
Recommended levels of palm kernel cake in livestock feeds

Species	Recommended level (%)
Beef Cattle	30–80
Dairy Cattle	20–50
Sheep and Goats	20–50
Poultry – broiler	<10
Poultry – layer	<10
Swine	<20
Freshwater fish	<10

Source: Wan Zahari and Alimon, 2003.

Palm oil mill effluent and palm oil sludge

Palm oil mill effluent (POME) is a general description for the discharge from palm oil extraction in the mill. This is the residue left from the purification of the crude palm oil (CPO) and includes various liquids, dirt, residual oil and suspended solids, mainly cellulosic material from the mesocarp of the fruits. When fresh, it is in the form of a thick, brownish-yellow, colloidal slurry comprising about 95 percent water with an average pH of about 4.7 and biological oxygen demand of 25 000 mg/L (Ngan, 2000). Some mills may use decantation to complement the clarifier in order to reduce the volume of effluent by 10 to 20 percent. By using the decanter-drier system, a lighter co-product is recovered in the form of decanter solid. In order to avoid confusion, the term POME should be restricted to only the raw untreated effluent. The decanter solid is obtained when most of the solids in the effluent is removed before the waste water is discharged into the pond. The effect of different chemical treatments on the settling ability of POME has been reported (Hassan *et al.*, 2001).

Availability

The average production of POME is 670 kg for every tonne of FFB processed. In 1997, Malaysia produced about 32 million tonne of POME from 290 mills.

Nutritive value

The material is characterized by high content of ether extract (11.7%), ash (19.5%) and medium CP content (12.5%) (Table 1). Wide variability in ash content and CP digestibility in POME results in widely different feeding values (Gurmit Singh, 1994). The content of CF, cellulose, NDF and gross energy (GE) are 20.1 percent, 20 percent, 63 percent and 8.37 MJ/kg, respectively.

POME is non-toxic as no chemical is added during the oil extraction process. It is rich in minerals and therefore suitable to be used as an organic fertilizer in crop cultivation. The average concentrations of Ca, P, K and Mg are 0.8, 0.3, 2.5 and 0.7 percent, respectively (Gurmit Singh, 1994). Ammonia N, B, Fe, Mn, Cu and Zn are 35, 7.6, 46.5, 2.0, 0.89 and 2.3 mg/litre, respectively (Ma and Ong, 1985).

Feeding ruminants

Feeding raw POME to growing sheep at levels ranging from 10–60 percent of the diet showed that the 10 percent level of inclusion gave the highest digestibility (Devendra and Muthurajah, 1976). However, an assessment of feeding value using sheep indicated that up to 40 percent POME can be used either alone in molasses-urea-based diets or when combined in equal proportions with PPF. Retardation in rate of growth and skeletal mineralization have been observed when POME was fed at the 100 percent level in dairy cattle. In this case, supplementation with protein, energy and

minerals is necessary. The combination of POME and sago meal (40% POME + 45% sago meal) has successfully been used for feeding local sheep, with daily liveweight gains of 59.1–64.0 g in the males and 50.5–54.3 g in the females. Field trials with cattle on estates have shown improved LWG. Satisfactory gains of between 0.18–0.43 kg/day for buffaloes and 0.47–0.78 kg/day for cattle were obtained with POME, PPF and PKC-based diets (Dalzell, 1977).

Feeding non-ruminants

Most of the studies in poultry utilized the solid portion of POME, which was dehydrated mechanically in the raw or in fermented form, or in mixtures with other feed materials. Dehydrated POME was used to replace part of the protein and energy sources in poultry diets. LWG and FCE of birds were significantly lower when the POME level in the diet exceeded 15 percent. Supplementation of the diet with lysine and methionine did not reverse the situation. Meat to bone ratios were 3.1:1 to 3.4:1, whereas diets with 20 and 25 percent POME gave ratios of 2.6:1 to 2.8:1.

In a layer trial, the optimum dietary level of inclusion was 10 percent (Yeong, 1983). The average percent egg production, total egg mass and feed:gain ratio were 76.4 percent, 8.9 kg and 2.77:1, respectively, as compared with 77.9 percent, 9.2 kg and 2.52:1, respectively, for the maize-soybean control diet. Inferior results were apparent in those birds fed diets with more than 10 percent POME. The optimum POME levels in diets were 15 percent for broilers and 10 percent for layers. The levels have also been confirmed with studies with pigs. Local and Pekin ducks were able to utilize 10 percent POME efficiently without exhibiting any adverse effect on growth and FCE (Yeong, 1983).

There are several commercial feeds derived from POME, specifically developed to have a high protein content. Examples are Censor (Centrifugal solid recovery), Prolima and Central solids (Centriplus). Prolima was used in poultry diets as a protein source to replace soybean meal. This product contained 2.42 Mcal ME/kg, 43.3 percent CP, 7.6 percent CF, 12 percent EE and with an amino acid profile comparable to groundnut meal. The optimum level of Prolima inclusion in diets was 30 percent. At this level, the birds showed feed intake, LWG, FCE and carcass quality comparable to those fed with the maize-soybean control diet. The optimum level of Prolima inclusion in layer diets was 20 percent (Yeong *et al.*, 1980). The digestibility of lysine and methionine were 8.3 and 22.1 percent, respectively, for POME and 80.0 and 76.1 percent, respectively, for Prolima. POME has very low amino acid digestibility. Incorporating 14 percent of Centriplus solids in the diets of growing pigs resulted in a reduction in LWG, increased feed intake and poor FCE compared with pigs fed the control diet.

Two types of Censor meals, prepared by using cassava-PKC as absorbents or cassava-PKC-grass meal as absorb-

ents for palm oil effluent, were used to replace maize at feeding levels of 25–100 percent for laying hens. Birds fed with both types of Censor meals showed adverse effects on egg production and feed efficiency. When Censor meals replaced 50 percent maize, the LWG and FCE were comparable to the control diet. Substitution of maize by 50 percent Censor in pigs increased feed intake without affecting LWG. No significant differences in carcass traits were found. Both Prolima and Centriplus were not commercialized due to high cost of production.

In a separate study, four types of processed oil palm slurry (OPS), using rice bran as an absorbent, were tested on the performance of broiler chicks. The dietary treatment did not have significant impact on feed intake, LWG or FCE. Carcass yields were similar and mortality was unaffected by the dietary treatments (Atuahene, Donkoh and Ntim, 2000). Improving the quality of POME in terms of uniformity and nutrient availability can help to upgrade its status as a feed ingredient for the poultry industry. A recent study revealed that through submergence fermentation and using selected yeast cultures, the CP value increased from 11.2 percent to 14.1 percent, with the highest digestible amino acid being phenylalanine (digestibility coefficient 0.705) and the highest percentage of digestibility improvement was for lysine (20.3 percent) (Jame'ah *et al.*, 2010).

Empty fruit bunches

Ripe fruit bunches are harvested at intervals of 10–14 days throughout the economic life of the palm. Each oil palm bunch usually weighs about 15–25 kg and, depending upon the age of the palm and variety, there is about 24 percent oil in the bunch. Empty fruit bunches (EFB) are the remains of the fruit bunches after the fruits have been stripped and sterilized, following the steaming process at the oil palm mill. It is in the form of stalks with empty spikelets, and is commonly used as a mulching material during the early stages of planting in the plantation, or as raw material for fibreboard.

Availability

The average production of fresh EFB is about 4.42 t/ha/year, which is equivalent to 1.55 t/ha/year of dried EFB (Chan, Watson and Kim, 1981). Burning of EFB is now prohibited by regulation to prevent air pollution.

Nutritive value

EFB contains about 50 percent CF, 3.5 percent lipid, 3.6 percent CP, 81.8 percent NDF and 61.6 percent ADF.

Processing and livestock feeding

Although large quantities of EFB are produced yearly, very limited research has been done on its use as feed for livestock. Early studies on the treatments of EFB by irradiation

and substrate culture have met with limited success. EFB fermented by inoculating *Pleurotus sajor-caju* was found to be palatable to beef cattle (Mat Rasol *et al.*, 1993). At present, EFB is widely used as pulp for making paper, bunch ash after incineration, mulch and recycling of nutrients for oil palms, wood composite products and fibreboard. Intensive R&D is required to improve its value for feeding if EFB is to be utilized as a major ingredient in livestock rations. EFB is also used as a substrate for cellulose enzyme production by solid-state bioconversion.

Palm press fibre

Availability

Palm press fibre (PPF) is a fibrous co-product of crude oil extraction of the mesocarp. More than 12.2 million tonne of PPF is produced annually in Malaysia, at a rate of 2.70 t/ha.

Nutritive value

PPF has 5.4 percent CP, 41.2 percent CF and 26 percent lignin (Table 1).

Processing and livestock feeding

Due to its poor nutritive value, PPF is commonly used as fuel to generate heat for boilers, for making pulp and paper, roof tiles and fibreboard. Being highly lignified and fibrous, it is not commonly used as feed for livestock, and when fed to cattle its intake by the animal is low because of the poor digestibility (24–30 percent).

Based on balance trials on sheep, optimum DMD of PPF was obtained when it was fed at 30 percent level of inclusion. Several treatments have been applied to PPF to improve its digestibility and palatability. Alkali treatments using sodium hydroxide and calcium hydroxide have been used, but had little effect in enhancing the digestibility of PPF. Steaming at 15 kg/cm² for 10 minutes improved the organic matter digestibility (OMD) of untreated PPF from 15 percent to 42 percent. Higher OMD levels were achieved by explosive depressurization at 30 kg/cm² for 1 minute (OMD reaching 51.6 percent). Other researchers found no benefit from sodium hydroxide treatment and steaming in improving the digestibility of PPF.

Formulated feedlot rations containing 30 percent PPF fed to LID × Red Dane male calves produced an average LWG of 117 kg per animal during the 251-day feeding. Rations containing 50 percent PPF and 30 percent PKC for dairy cattle provided the cheapest source of energy compared with cattle pellets based on starch equivalent.

The widespread use of PPF is still constrained by its low digestibility and the potential problem of rumen impaction. Farmers operating in the vicinity of oil palm mills can utilize PPF, either fresh or ensiled, to some extent for feeding cattle, and thus reduce cost of feeding. However, it is advocated that the feeding level should be maintained at less than

30 percent. Further research on chemical and physical treatments are necessary to improve its utilization in livestock.

Crude palm oil

Availability

Crude palm oil (CPO) is extracted from the mesocarp of the fruit of the oil palm tree (Figure 2). The mesocarp comprises about 70–80 percent by weight of the fruit, and about 45–50 percent of this mesocarp is oil. Two co-products produced during the refining of CPO are palm fatty acid distillates (PFAD) and spent bleaching earth (SBE).

Nutritive value

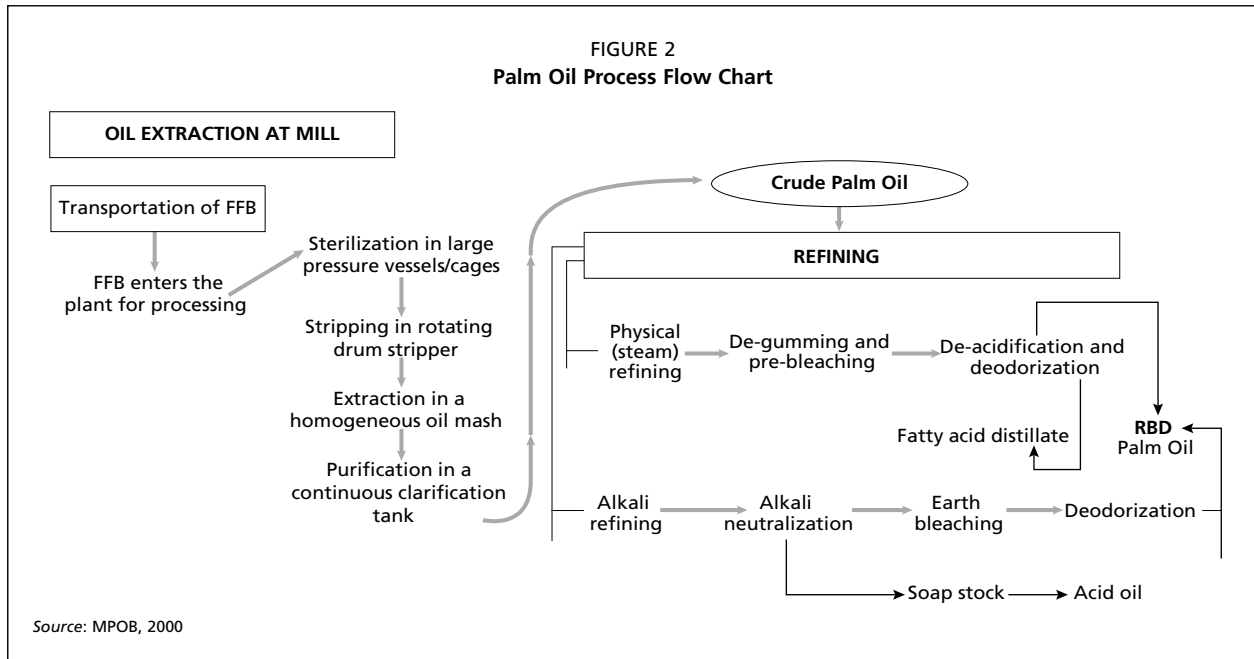
Like all natural fats and oils, CPO comprises mainly mono-, di- and triglycerides. There are free fatty acids, moisture, dirt (about 0.25 percent) and minor components of non-oil fatty matter, collectively referred to as unsaponifiable matter. CPO has a deep orange-red colour due to the high content of carotenoids, and is a rich source of vitamin E (300–600 ppm), consisting of tocopherols and tocotrienols. The content of palmitic acid (C16:0, saturated) and oleic acid (C18:1, unsaturated) are quite high (about 37.0 percent and 47.0 percent, respectively). The B-carotene content is 54 g/100 ml of oil, and maximum fatty acid content is 5 percent. The pro-vitamin A activity is about 640 IU/g. CPO does not contain n-3 highly unsaturated fatty acids, which are required by marine species. The GE value is about 8500 Kcal/kg, equivalent to about 34 MJ/kg.

Livestock feeding

Palm oil is traditionally used at about 3 percent level in diets for pigs and poultry as a source of vitamins A and D, as well as to reduce dustiness of the diets. Higher levels of dietary palm oil of up to 10 percent have also been used successfully in diets for growing and finishing pigs in Malaysia. The percentage of lean cuts and backfat thickness increased with increasing levels of palm oil. In lactating cattle, supplementation with 2–8 percent of CPO increased both milk yield and milk fat content. The digestibility of CPO determined in balance trials with sheep gave a value of 85.4 percent. Information on the use of palm oil products in fish diets is currently limited to a few species only (Ng, 2010). About 90 percent of fish oil in the diets of catfish, *Hemibagrus bongan* (Popta 1904) (*syn. Mystus nemurus* (Valenciennes 1840)), could be replaced by CPO without affecting growth, FCE or body composition (Ng *et al.*, 2002). In another study, African catfish, *Clarias gariepinus*, was observed to show better growth when fed semi-purified diets containing 10 percent palm oil as the sole dietary lipid (Ng *et al.*, 2004).

Palm fatty acid distillate (PFAD)

Palm fatty acid distillate (PFAD) is a co-product from refining of CPO at very high temperature (240–260 °C) under



reduced pressure (2–6 mm Hg). Normally, the refinery mixes all the distillates, irrespective of whether from refining of CPO, crude palm olein or crude palm stearin (Figure 2). The final product is generally called PFAD. It is a light-brown solid at room temperature, melting to a brown liquid on heating.

Nutritive value

PFAD is composed of free fatty acids (81.7%), glycerides (14.4%), squalene (0.8%), vitamin E (0.5%), sterols (0.4%) and other substances (2.2%) (Ab Gapor, 2010). It is used in the animal feed, oleo-chemical and soap industries. Vitamin E, squalene and phytosterols are valuable constituents that can be extracted from PFAD and are of potential value for the nutraceutical and cosmetic industries.

Livestock feeding

Most of today's market for by-pass fats consumption is for dairy cow feed. High producing cows, especially in early lactation, are typically in negative energy balance. The loss in appetite and the effect on live weight caused by insufficient dietary nutrient intake to meet the demands of milk output subjects the high yielding cow to considerable weight loss over the first 60–80 days of lactation, and this can have substantial effects on subsequent performance. Consequently, the cow mobilizes body reserves such as body fat to meet the energy demand. Fats in their crude form have only limited application in ruminant feeds because they become hydrolyzed in the rumen into free fatty acids, which may cause many problems. The major problem is the tendency to reduce the rate and level of fibre digestion in the rumen. The maximum efficiency of milk production is achieved when fat contributes between 16 percent and 18 percent of the dietary ME intake.

There are several protected fats based on PFAD or calcium soaps that are marketed worldwide under various trade names. Most of the products are in the form of hydrogenated triglyceride with energy content of about 9000 Kcal/kg and a digestibility above 90 percent. The products can be absorbed in the small intestine and have a very low stearic acid (C-18:0) content of between 1 and 5 percent. Improved PFAD specifically derived from palm oil increased milk production and the total SNF of lactating cows (Farah Nurshahida *et al.*, 2008). The digestibility of fatty acids in hydrogenated distillate was lower than for Ca salts of fatty acids, but intake and production responses were similar or greater for diets containing hydrogenated distillate (Elliott, Drackley and Weigel, 1996). Calcium soaps of PFAD were satisfactorily stable till pH 5.5 in the rumen (Sukhija and Palmquist, 1990). Increasing dietary intake of Ca salts of PFAD resulted in increase ratio of C_{18:1}:C_{18:0} in Holstein cows, but not in Jersey cows (Beaulieu and Palmquist, 1995). The use of PFAD is a practical and cost-effective way to produce high-energy diets without causing side effects due to increased lipids (Ng *et al.*, 2004).

Spent bleaching earth

In refining the CPO and PKO, Bleaching Earth is used to remove colour, phospholipids, oxidized products, metals and residual gums from the oil, impurities that can cause the oil to have an unattractive colour and taste. The residue is termed Spent Bleaching Earth (SBE). It absorbs approximately 0.5 percent by weight of the oil in the process. The SBE generated annually by Malaysian palm oil refineries is estimated to be approximately 120 000 tonne. Disposal of SBE by incineration, inclusion in animal feeds, as land fill or in concrete manufacturing is generally practised (Kheang *et al.*, 2006).

Nutritive value

The free fatty acid content of SBE ranges from 14 to 31 percent, with an unsaturated to saturated ratio of 46.5:53.5 (Lai, 1987). Apart from the original bleaching earth, the SBE also contains residual water, inorganic acids, organic acids, silicates and active carbon used in the refining process. The content of the output varies greatly, depending on the type of bleaching agents used and the method applied. Two main methods are chemical and physical refining. Chemical refining uses alkali to neutralize the free fatty acids, which are then removed as soap. Physical refining subjects the oil to steam distillation under high temperature and vacuum. Table 18 outlines the nutritive value of the SBE collected from a CPO refinery in Selangor, Malaysia (Wan Zahari, Mohd. Sukri and Wong, 2004). Ash content is excessively high, while the protein content is low (CP <6 percent). The heavy metal contents are within normal ranges and therefore SBE is considered safe for livestock consumption.

Livestock feeding

There is no published report on the utilization of SBE for ruminant livestock, even though the material is known to be used by small-scale farmers in certain areas in Peninsular Malaysia. Supplementation of protein is required if SBE is to be used as a main ingredient for ruminants. The high level of residual oil in SBE could be exploited for dairy feeding. Reflecting its high Ca content, SBE is suitable for combining with PKC in order to achieve a better Ca:P ratio. More studies need to be carried out to evaluate the effect of SBE on animal performance, especially on broiler and layer poultry. These should include studies to determine ME values and optimum inclusion levels. SBE can be further fortified or enriched with addition of certain nutrients or compounds to increase the feeding value. Apart from blending into animal feed, SBE can also be used as binder in feed processing, especially in diets with high fibre content, such as OPF-based diets. The free-flowing characteristic of SBE is very well suited for feed processing purposes and it can be pneumatically conveyed via a vacuum line.

MAXIMIZING LIVESTOCK PRODUCTION IN AN OIL PALM ENVIRONMENT

Of the land area under oil palm, only 2.1 percent is currently used for integration with ruminants, emphasizing the enormous potential for expanding this system (Devendra, 2011). The concept of integrating ruminants with tree crops is not new and has been practised with varying degrees of success. Grazing the undergrowth and providing supplementary feeding with feeds such as PKC and POME is economically feasible. The basic model for integrated systems involving cattle and oil palm has been intensively reviewed (Devendra, 2006; Devendra, 2007). Based on this

TABLE 18
Nutritive value and elemental composition of spent bleaching earth

Parameter	Value
Energy value	
Gross Energy (MJ/kg)	10.9
Proximate analysis (% DM)	
Ash (%)	57.9
CF (%)	1.0
CP (%)	0.44
EE (%)	14.6
ADF (%)	34.6
Heavy metal	
Cd (ppb)	44.35
Co (ppb)	4277
Cr (ppb)	43310
Mo (ppb)	Trace
Ni (ppb)	12229
Pb (ppb)	2001
Se (ppb)	Trace
Hg (mg/kg)	–
Major elements	
Ca (%)	6.28
K (%)	0.72
Mg (%)	2.44
Na (%)	0.70
P (ppm)	57.07
S (%)	0.42
Trace elements	
B (ppm)	2143
Cu (ppm)	8.61
Fe (ppm)	13.42
Mn (ppm)	217.50
Zn (ppm)	25.86

Source: Wan Zahari, Mohd. Sukri and Wong, 2004.

model, theoretical calculations for a 500 000 ha oil palm plantation gives the following results:

- Carrying capacity utilizing native herbage alone at 4 kg DM/head/day = 214 286 head.
- Carrying capacity utilizing native herbage plus co-product feeds at 4 kg DM/day = 736 581 head (an increase of 245% over grazing alone).
- Using a 50 percent dressing percentage and a liveweight at slaughter of 420 kg, the quantity of beef produced using oil palm co-product feeds is 154 682 tonne.
- Annual gross revenue based on US\$ 1260/t live weight = US\$ 194.9 million.
- Rate of return on investment is from 8.1 percent for indigenous cattle to 16.3 percent from exotic cattle.

Lack of feeder cattle is one of the limiting factors in beef production in Malaysia. This is mainly associated with the high cost of rearing, as most of the feeder cattle are imported. It is estimated that about 1.8 million head of breeding females could be produced were the available feeds from 4.0 million hectare of oil palm to be effectively used, contributing about 0.5 million feeders per year. Based on these figures and assuming a 30 percent

concentrate + 70 percent roughage ration is used for feeding, the total requirement for concentrate would be about 1.6 million tonne per year. About 78 percent of locally available PKC would be needed annually for beef production were it to be used in this way as the concentrate in the above feeding regimen, with OPF utilized as the main roughage source.

CONCLUSIONS

The rapid expansion of the palm oil industry in Malaysia has generated large quantities of wastes from the field and palm oil mill. Most of the wastes and residues are basically cellulosic and organic biomass with high nutrient content. Most of the resources can be used as feeds for livestock.

At the plantation site, potential feedstuffs include OPF and OPT, while co-products from the milling and refining activities include EFB, PPF, PKC, POME and SBE. The availability of these resources provides potential for more practical and cost-effective feeding systems, as feeding values and outcomes from the previous and current R&D activities are known. Significant development in the processing of these feedstuffs, either as an ingredient for total mixed rations or as complete and balanced feeds, would encourage further growth in the local goat, sheep, beef and dairy industry. Intensive rearing of beef cattle on oil palm plantations also offers tremendous potential for beef production in view of the availability of OPF, PKC, POME and SBE for use as feedstuffs. With changes in livestock production systems towards semi-intensive and fully intensive systems, the demand for feed is growing in Malaysia. Growth of the local livestock sector aims to meet the self-sufficiency level for beef and milk over the next decade, and this creates further demand for feed. It is also evident that these fibre sources are in high demand in markets in Japan, South Korea, Taiwan and the Middle East, in addition to the Malaysian domestic market. Promotion and marketing of the agro-industrial co-products from the oil palm industry should be intensified to further expand their use and commercial potential. CPO, PFAD and other specialty fats, though not usually categorized as oil palm co-products *per se*, have great potential to be utilized as energy sources for dairy animals, poultry, swine and in aquaculture. The utilization of oil palm co-products thus aims to convert the large plantation biomass not only into animal feed, but also into other commercially viable value-added products.

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Chapter 14

Use of palm kernel cakes (*Elaeis guineensis* and *Orbignya phalerata*), co-products of the biofuel industry, in collared peccary (*Pecari tajacu*) feeds

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ABSTRACT

The oil palm (*Elaeis guineensis*) and the babassu (*Orbignya phalerata*) are palms of commercial interest in tropical countries and are found in the Brazilian Amazon. The oil from these palms has diverse uses, such as food, production of charcoal, soap and, most recently, biodiesel. The remainder of the plant, which is the bulk, is not normally commercialized, making it an ideal alternative source of low-cost energy for animal feed. The systems for breeding wild animals in captivity for commercialization and sustainability have an important role in conservation, because these species of game animals are under constant environmental pressure. For the collared peccary (*Pecari tajacu*) production system, the major part of the cost is feed. If alternative sources of low-cost animal feed could be used in the animal's diet, the production of the collared peccary could provide a new source of income for rural Brazilian producers. The use of co-products of oil palm and babassu has been found to be positive both for performance and for carcass characteristics of those animals bred in captivity. The replacement of 40 percent and 15 percent of the energy components of the traditional collared peccary diet with babassu and oil palm, respectively, showed the best improvement in the productive performance, demonstrating that they could reduce feeding costs while maintaining good animal development.

INTRODUCTION

Palms are plants typical to the tropics, and some are sufficiently prolific to be relevant to the subsistence of indigenous and traditional peoples (Clement, Lleras Peres and Van Leeuwen, 2005), providing an important contribution to the economies of several tropical countries (Lopes *et al.*, 2008). The oil palm and babassu are examples of species of commercial interest.

In recent years, production of the oil palm has expanded greatly on a large scale in many tropical countries (e.g. Brazil, Colombia, Ecuador, Indonesia, Malaysia and Thailand). The oil palm belongs to the monocotyledonous class, order Palmales, family Arecaceae and genus *Elaeis*. There are two species of commercial interest: *E. guineensis* Jacq, of African origin, known as oil palm, and *E. oleifera* Cortés, known as American oil palm or Caiaué. The palm of African origin is the principal species planted commercially,

using varieties of the Tenera type. The American species is used in improvement programmes to obtain interspecific hybrids (*E. oleifera* × *E. guineensis*) especially for plantations in regions subject to fatal yellowing disorder. The ideal climatic conditions for its cultivation are: annual rainfall of more than 2000 mm that is well distributed, without a defined dry season, and a minimum of 100 mm per month; an average maximum temperature between 29 and 33 °C, with a minimum temperature between 22 and 24 °C; a daily insolation period of between 5 and 7 hours, and daily radiation of 15 MJ/m² (Corley and Tinker, 2003).

The oil palm, a perennial plant with continuous production throughout the year, has an economically productive life of around 25 years. This species is the most productive oleaginous palm and can produce from 6 to 10 tonne of oil per hectare per year. The oil palm produces at least 3 to 8 times more oil than most other oleaginous seeds. The oil

MAIN MESSAGES

- Babassu cake substitution for maize as an energy source up to a level of 40 percent improved productive performance of collared peccaries, and good results were obtained with respect to dressing percentage of collared peccaries slaughtered at the terminal phase.
- Oil-palm cake can be used to replace 15 percent of the energy components of the traditional collared peccaries diets at the terminal phase.
- Babassu and oil-palm cakes could reduce feeding costs while maintaining good animal development.

palm produces its fruit in clusters, varying in size from 10 to 40 kg per cluster. The individual fruit consists of an exterior layer (exocarp), pulp (mesocarp), endocarp and seed. The primary products produced from the fruit of the oil palm are oil and cake. The palm oil is extracted from the pulp of the fruit (mesocarp), and the palm kernel oil from the seed (endosperm). The ratio between the quantities produced by these types of oils is approximately 9:1 (palm oil:palm kernel oil). The cake results from the process of extracting oil from the seed and contains 17–19 percent protein and acceptable bromatological characteristics, particularly in ruminant diets due to its high proportion of fibre, and is rich in arginine and glutamic acid. The average composition of palm kernel cake is 48 percent carbohydrate, 19 percent protein, 13 percent fibre, 5 percent palm kernel oil, 11 percent water and 4 percent ash (Hartley, 1988). Oil palm oil production exceeds 35 million tonne per year, with marked growth in the last two decades, and has become the most produced and commercialized vegetable oil in the world (USDA, 2006; FEDEPALMA, no date; Oil World, 2008).

Biofuel demand might greatly exceed that for edible use, and the interchangeability of the major oils, for edible and biofuel uses, means that this demand will drive oil palm expansion, whether or not palm oil is actually used for biodiesel (Corley, 2009).

Although the oil palm plantations are, in some situations, world-challenged by presenting some environmental risks (e.g. Friends of the Earth, 2005; Rosenthal, 2007; Fitzherbert *et al.*, 2008; Koh and Wilcove, 2008; Butler and Laurence, 2009), these risks can be considerably reduced through sustainable development practices, with proper management (Basiron, 2007; Corley, 2009; Boyfield, 2010; Nelson *et al.*, 2010).

The palm oil industry could supply sufficient vegetable oil to meet the growing food requirements for the global population in 2050, and there is sufficient land available for necessary expansion without the need for deforestation (Corley, 2009). Due to the fact that Malaysia does not have physical space to increase its plantation area (Thoenes, 2006), it is necessary to increase cultivation of oil palm elsewhere. Various countries could emerge as major producers of palm oil (East and West Africa, other Asian countries, and Central and South America). Brazil, in spite of currently having little

market penetration in terms of global production of palm oil, has a great potential for expansion and has recently expanded production in this sector. To control expansion of oil palm plantations in the Brazilian Amazon and minimize possible negative environmental impacts, the Brazilian government has requested the implementation of agri-ecological zoning for the culture. This zoning is a technico-scientific basis for achieving sustainability by defining lands suitable for oil palm culture (Ramalho Filho and Motta, 2010). The focus area, set in the Amazonian biome (5 million km²), refers to areas already deforested, with the exception of strictly protected areas (state and national parks, and indigenous reserves). The areas already deforested and considered suitable for the cultivation of oil palm total 30 million ha (300 000 km²), being some 5.9 percent of the Brazilian legally-defined Amazon (Ramalho Filho *et al.*, 2010).

The babassu (*Orbignya phalerata* Mart.) is a palmeous plant of the Arecaceae family, found in abundance in the Brazilian Amazon region, especially in the States of Maranhão, Tocantins, Pará and Piauí, and possesses a high energy potential. Maranhão State has around 65 percent of the national occurrence of the palm, which represents 30 percent of the State surface (Ferreira, 1999). Babassu is a native of the transition zone between the savannah and open forests of the southern Amazon, and is in areas anthropogenically altered (Clement, Lleras Peres and Van Leeuwen, 2005), often appearing in spontaneous homogeneous groupings. This species covers extensive regions in Brazil, Bolivia and Suriname (Zylbersztajn *et al.*, 2000).

The babassu produces drupe type fruits with oleaginous and edible seeds from which the oil is extracted in sufficient quantities for local needs. Fundamental aspects for the exploitation of the babassu are the harvesting and the gathering system. There are no commercial plantations of these palms in the world, and the fruits are collected from natural forests by native populations. It is a natural resource whose economic importance has been recognized. Its exploitation is characterized by the collection of fruits from natural stands of native vegetation with no additional management action.

Natural babassu density in the forest varies from 1 to 4000 plants per hectare, with an average of 1111 plants per hectare (Ferreira, 1999), but not all these plants can be utilized. Each adult plant produces approximately

2000 fruits per year (Lorenzi *et al.*, 1996). Each fruit can weigh between 40 and 400 g dry weight (Revilla, 2002). Each 17.6 kg of fruit provides 2.6 kg of epicarp, 3.5 kg of mesocarp, 10.4 kg of endocarp and 1.1 kg of kernels (Wisniewski and Melo, 1981).

The seed is the principal product extracted from the fruit, and represents the greatest commercial and industrial value. One fruit contains from 3 to 5 seeds, which are extracted manually by traditional cottier families, being the most important source of income for the landless population in the interior regions where babassu is found. In the state of Maranhão, seed extraction involves more than 300 000 families, especially women (called "breakers").

The food products from the babassu and oil palm production could significantly contribute to food security in the Amazon forest region, and currently provide a large variety of foods and an adequate health standard for the population (Alencar *et al.*, 2007). These palms could be used for numerous purposes, such as the production of starch, charcoal, soap, margarine, oil tar, alcohol, palmetto and, more recently, biodiesel. Nevertheless, the remainder of the plant, which constitutes the bulk of the plant, is not normally commercialized, and could be considered as an alternative source of low-cost energy for animal feed.

USE OF BABASSU (*ORBIGNYA PHALERATA*) IN THE FEED OF COLLARED PECCARIES RAISED IN CAPTIVITY

Very few studies have been carried out regarding sustainable production systems for native wild animals maintained in captivity for commercial purposes. These systems may play an important role in conservation because these species are under constant human pressure due to subsistence and commercial hunting, fragmentation of the habitat and deforestation.

In the Amazon region, subsistence hunting of game animals provides a significant proportion of the protein component of the diet of rural families (Robinson and Bodmer, 1999; Peres, 2000, 2001). In certain regions, the trade in bushmeat and other co-products of game animals is a great

source of income (Bodmer, 2000; Baia Junior, Guimarães and Le Pendu, 2010.).

The collared peccary (*Pecari tajacu*) is a wild species which is frequently hunted. Its diet in its natural environment is basically fruit, leaves and roots, and in captivity can easily adapt to different types of feed, including grain, fruits, potherbs, roots and fodder, and accepts porcine commercial feed (Albuquerque and Hühn, 2001; Albuquerque *et al.*, 2004)

The collared peccary belongs to the Suiformes suborder and the Tayassuidae family. The animals belonging to his family possess a stomach subdivided into compartments, and some authors suggest that its digestive physiology could be similar to that of ruminants. Due to its low requirements for protein and its high digestive performance, these animals are able to adapt to green foods such as fodder (Comizzoli *et al.*, 1997; Cavalcante Filho *et al.*, 1998; Mendes, 2008), and the wild collared peccary resort to this type of diet when there is a scarcity of fruits.

Captive breeding of collared peccary has been proposed by Nogueira-Filho (1999), Albuquerque *et al.* (2004) and Garcia *et al.* (2005). This could be a new source of income for rural Brazilian producers, supported by supplementing the animal's diet with alternative sources of low-cost feed.

Albuquerque (2006) studied the use of babassu cake as an alternative energy source in the captive collared peccary's diet. In the experiment, babassu cake substituted maize at varying levels in feed formulated for animals in the termination phase, and animal performance was evaluated using daily weight gain and daily feed consumption. After the experimental phase the animals were slaughtered to analyse the carcasses.

Table 1 shows the chemical characteristics of the experimental feed, and Table 2 shows the average composition of the ingredients used in the experiment. The experimental feed was based on maize and soy bran, replaced with varying levels of babassu cake.

At the end of the experimental phase, when the experimental animals reached slaughter weight (average of 16.25 kg and 7 months old), they were weighed. After this, the animals were fasted for 24 hours, re-weighed and

TABLE 1
Average chemical characteristics of the ingredients of the experimental feed

Ingredient	DM	MM	P	CF	CP	Ca	EE	NDF	ADF	Sodium
Soy bran ⁽¹⁾	88.1	6.6	0.6	5.9	45.5	0.3	1.4	14.1	7.8	0.1
Maize ⁽¹⁾	87.1	1.3	0.2	2.0	8.6	<0.1	3.5	11.4	3.4	<0.1
Babassu (cake) ⁽²⁾	90.2	4.6	0.7	26.0	17.3	0.1	3.1	–	–	–
Soy oil	99.3	–	–	–	–	–	99.0	–	–	–
Dicalcium phosphate	–	–	18.5	–	–	24.8	–	–	–	–
Calcitic lime	–	–	<0.1	–	–	31.9	–	–	–	–
Salt	–	–	–	–	–	–	–	–	–	39.7
Lysine-HCl	–	–	–	–	79.1	–	–	–	–	–

Notes: DM = dry matter; MM = mineral material; P = phosphorus; CF = crude fibre; CP = crude protein, Ca = Calcium; EE = ether extract; NDF = neutral-detergent fibre; ADF = acid-detergent fibre.

Sources: (1) Rostagno *et al.*, 2000. (2) Embrapa, 1991.

TABLE 2
Average composition of experimental feeds

Ingredient	Inclusion levels of babassu cake in the feed			
	TA	TB	TC	TD
Babassu (cake)	0.0	15.7	31.3	47.0
Maize	78.3	62.7	47.0	31.3
Soy bran	14.6	14.6	14.6	14.6
Soy oil	1.0	3.5	3.5	3.5
Dicalcium phosphate	1.25	1.25	1.25	1.25
Calcitic lime	0.78	0.78	0.78	0.78
Salt	0.40	0.40	0.40	0.40
Lysine-HCl	0.15	0.00	0.00	0.00
Vitamin supplement ⁽¹⁾	0.40	0.40	0.40	0.40
Mineral supplement ⁽²⁾	0.10	0.10	0.10	0.10
Inert	3.00	0.65	0.65	0.65
Total	100.00	100.00	100.00	100.00
Calculated Values				
Digestible energy (Kcal)	3304	3304	3304	3304
Gross protein	13.5	13.5	13.5	13.5
Calcium	1.19	1.19	1.19	1.19
Total phosphorus	0.78	0.78	0.78	0.78
Available phosphorus	0.53	0.53	0.53	0.53
Lysine	0.49	0.49	0.49	0.49
Methionine+cystine	0.41	0.41	0.41	0.41
Threonine	0.41	0.41	0.41	0.41
Tryptophan	0.11	0.11	0.11	0.11
Sodium	0.19	0.19	0.19	0.19

Notes: TA = Control feed based on maize and soy bran; TB = Feed containing 20% babassu cake and 80% maize; TC = Feed containing 40% babassu cake and 60% maize; TD = Feed containing 60% babassu cake and 40% maize.

(1) Vitamin supplementation per kg of feed: vitamin A = 625 000 IU; vitamin D3 = 125 000 IU; vitamin E = 3375 IU; folic acid + 875 mg; biotin = 27.56 mg; choline chloride = 2475 mg; niacin = 4000 mg; pantothenic acid = 2000 mg; thiamine = 175 mg; riboflavin = 550 mg; pyridoxine = 175 mg; vitamin B₁₂ = 2800 mg; antioxidant = 200 mg.

(2) Mineral supplementation per kg of feed: Iron = 22 000 mg; copper = 5000 mg; zinc = 18 750 mg; manganese = 12 500 mg; iodine = 238 mg; selenium = 56.3 mg; cobalt = 116 mg.

Values calculated in accordance with the nutritional demands in basal feed for swine of low genetic potential.

Source: Rostagno *et al.*, 2000.

TABLE 3
Average daily weight gain (DWG) and daily feed intake (DFI) of the collared peccary in the terminal phase

Parameter	Inclusion levels of babassu cake in the feed (%)				SE
	0	20	40	60	
DWG (g)	32.7	38.0	44.7	37.0	4.6
DFI (g)	355.5	359.1	356.1	362.2	11.4

Notes: SE = Standard error. Source: Albuquerque, 2006.

then sent to the abattoir. The characteristics of the animal carcasses included in this study were dressing percentage, corporal composition, carcass measurements, organs and glands, and commercial cuts. Table 3 shows the daily weight gain and daily feed intake in the terminal phase.

In this experiment, no significant ($P > 0.05$) relationships were observed between the levels of babassu cake and DWG and DFI. The DWG at the 40 percent babassu cake inclusion level showed an increase of 36.74 percent

compared with the basal diet. No significant effects were observed in DFI.

Evaluation of the carcass

Tables 4, 5, 6 and 7 show the variables studied in the carcass evaluation of the experimental collared peccaries. The levels of babassu cake did not affect the variables of live weight, fasting weight, hot carcass, cold carcass, length, hide, hind and front feet, as shown in Table 4.

Albuquerque (1993) evaluated the carcasses of male, female and castrated male capybaras (*Hydrochoerus hydrochaeris*) slaughtered after the terminal phase, and found no significant differences ($P > 0.05$) in carcass components among different experimental groups.

Silva *et al.* (2002) studied the effects on the animal carcass of different levels of CP in the diet of collared peccaries slaughtered after the terminal phase, but they found no significant differences. The carcass length was between 55.25 and 57.63 cm, and was greater than reported by Albuquerque (2006). The authors did not report the age of the animals studied, but it is thought that they were older, due to the differences in body length.

Albuquerque (2006) observed no significant differences ($P > 0.05$) in hot or cold dressing percentages related to varying levels of babassu cake (Table 5), and ribs, gammon, shoulder blades and percentage of gammon in relation to the cold, left half-carcass (Table 6). There was an increase over basal feed of 7.1 percent for ribs, 8.9 percent for gammon, 6.4 percent for shoulder blades, and 21.6 percent for percentage of gammon relative to the cold, left half-

TABLE 4
Measurements of the carcass components of slaughtered collared peccaries after the terminal phase

Parameter	Levels of babassu cake in the feed (%)				SE
	0	20	40	60	
Live weight (g)	16533	15633	16600	16233	834.8
Fasting weight (g)	16467	15700	16400	16000	746.8
Hot carcass (g)	9233	8267	9500	9500	407.5
Cold carcass (g)	9141	8184	9405	9405	403.4
Carcass length (cm)	23	21	21	21	0.8
Blood (g)	148	212	217	204	27.0
Hide (g)	2088	1892	1998	1980	103.5
Hind feet (g)	123	122	130	117	4.7
Front feet (g)	122	122	120	120	2.7

Notes: SE = Standard error. Source: Albuquerque, 2006.

TABLE 5
Averages of the dressing percentage of slaughtered collared peccaries after the termination phase

Parameter	Levels of babassu cake in the feed (%)				SE
	0	20	40	60	
HDP (%)	56.1	53.2	57.8	59.4	2.62
CDP (%)	55.5	52.6	57.2	58.8	2.59

Notes: SE = Standard error; HDP = Hot dressing percentage; CDP = Cold dressing percentage. Source: Albuquerque, 2006.

TABLE 6
Average features of the commercial cuts removed from the cold, left half-carcass of the collared peccaries slaughtered after the termination phase

Parameter	Levels of babassu cake in the feed (%)				SE
	0	20	40	60	
Ribs (g)	1320	1147	1147	1413	186.6
Gammon (g)	1428	1420	1468	1555	80.2
Shoulder blade (g)	967	953	943	1028	67.7
% Gammon ⁽¹⁾	30.6	32.4	35.3	37.2	3.4

Notes: SE = Standard error. (1) % of gammon in relation to the left side cold half carcass. Source: Albuquerque, 2006.

TABLE 7
Average percentages of organs and glands in relation to the carcass of the collared peccaries slaughtered after the terminal phase

Parameter	Inclusion levels of babassu cake in the feed (%)				SE
	0	20	40	60	
Stomach (%)	5.0	4.7	5.2	4.0	0.65
Heart (%)	0.7	0.8	0.7	0.6	0.08
Lung (%)	1.3	1.8	1.5	1.2	0.11
Liver (%)	2.1	2.7	2.5	2.2	0.19
Spleen (%)	1.1	0.8	0.7	0.5	0.24
Kidneys (%)	0.5	0.6	0.6	0.6	0.07
Intestines (%)	5.9	8.2	7.3	6.5	0.93
Total (%)	16.6	19.7	18.3	15.3	1.73

Notes: SE = Standard error. Source: Albuquerque, 2006.

carcass. In the diet with an inclusion level of 40 percent babassu cake, the increase was 2.8 percent for gammon and 15.4 percent for percentage of gammon in relation to the cold, left half-carcass.

Silva *et al.* (2002) studied the effect of different inclusion levels of CP in the feed on carcass and meat of collared peccaries slaughtered after the terminal phase, and found no significant differences ($P > 0.05$) for the carcass parameters studied. Similar to observations of Albuquerque (2006), the average dressing percentage was between 56.88 and 59.47 percent. The percentage of gammon in relation to the carcass was between 35.0 and 38.2 percent, showing slightly higher values than reported in Albuquerque (2006).

Some bovine data for dressing percentage were poorer when compared with that of collared peccaries reported by Albuquerque (2006), such as the data found by Schwarz *et al.* (1993), who found average dressing percentages of between 57.7 and 58.4 percent, and Holzer *et al.*, (1999), who reported an average dressing percentage between 55.4 and 57.4 percent. The inclusion of different levels of babassu cake showed no significant differences ($P > 0.05$) in the values for organs and glands (Table 7).

Meat properties and fatty acids profile in the collared peccary gammon

Albuquerque *et al.* (2009) studied the organoleptic properties (cooking losses, shearing force, pH and water

holding capacity) of gammon from 12 collared peccaries, and the fatty acid (FA) profile of the oil extracted from the meat. No significant differences ($P > 0.05$) were observed in meat properties, and unsaturated FA (mono- and polyunsaturates) were more frequent than saturated fatty acids in the collared peccary gammon meat. When comparing the meat from collared peccaries, bovines, ovines and swine, the collared peccary had more unsaturated FA (mono- and polyunsaturates) than saturated FA. The FA polyunsaturates are responsible for a reduction in cholesterol blood levels (Monteiro, Mondini and Costa, 2000), suggesting that the meat from the collared peccary is a healthy source of animal protein (Albuquerque *et al.*, 2009).

PALM KERNEL CAKE (*ELAEIS GUINEENSIS*) USE IN THE FEED OF COLLARED PECCARIES RAISED IN CAPTIVITY

The use of oil palm cake in the diet has been studied in various animal species: fish – *Pieractus mesopotamicus* and *Oreochromis niloticus* (Oliveira *et al.*, 1997, 2008; Pascoal, Miranda and Silva-Filho, 2006.); chicken (Onwudike, 1986, 1988; Farias-Filho *et al.*, 2006); and in swine (Rhule, 1996; Gómez, Benavides and Diaz, 2007.).

Embrapa Amazônia Oriental, in partnership with the Universidade Federal do Pará, embarked on a research project (PROFAMA, 2008) that evaluated the performance of collared peccaries bred in captivity on diets of oil palm kernel cake as an alternative feed source. Animal performances (daily weight gain and daily feed intake), the characteristics of the carcass and the non-carcass components were observed, and the bacterial microbiota in the gastro-intestinal tract of these animals was studied.

Forty male animals were used, aged between 8 and 10 months, in their final growth phase and weighing an average of 13.20 kg. During the experiment, the animals received varying levels of oil palm cake (T1 = 0% cake; T2 = 7.5% cake; T3 = 15% cake; and T4 = 22.5% cake). The proximate analysis of the feed is shown in Table 8, and the nutritional analysis in Table 9.

At the end of each experimental phase, the animals were slaughtered to evaluate the effects of the feed utilized on the carcass and non-carcass characteristics (gammon and carcass dressing percentage, head, hide, blood, feet, carcass length, organs and glands, and commercial cuts) and live weight and fasting weight.

The results observed in the feed with the inclusion of oil palm cake demonstrated that its use in the diet of the collared peccary in an intensive breeding system could be a regional low-cost nutritional component.

Rhule (1996) studied the effect of breed on the growth of swine with varying levels of oil palm cake in the feed, and observed more weight gain in swine than in collared

TABLE 8
Chemical characteristics (percentage basis) of the experimental feed

Ingredient	DM	MM	P	CF	CP	Ca	EE	NDF	ADF	Na
Soy bran ⁽¹⁾	88.1	6.6	0.6	5.92	45.54	0.3	1.4	14.1	7.8	0.1
Maize ⁽¹⁾	87.1	1.3	0.2	1.95	8.57	<0.1	3.5	11.4	3.4	<0.1
Oil palm (cake) ⁽²⁾	94.9	3.1	–	–	15.70	–	–	83.2	80.3	–
Wheat bran ⁽³⁾	88.0	5.6	1	9.52	50.63	0.2	3.5	44.3	13.5	<0.1
Meat/bone flour ⁽³⁾	93.4	25.0	5.0	1.61	59.9	8.6	12.4	–	–	–
Dicalcium phosphate	–	–	18.5	–	–	24.8	–	–	–	–
Calcitic lime	–	–	<0.1	–	–	31.7	–	–	–	–
Salt	–	–	–	–	–	–	–	–	–	39.7

Notes: DM = dry matter; MM = mineral material; P = phosphorus; CF = crude fibre; CP = crude protein, Ca = Calcium; EE = ether extract; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; Na = sodium.

Sources: (1) Rostagno *et al.*, 2000. (2) Unpublished data from Animal Nutrition Laboratory, CENA-USP. (3) Valdares Filho *et al.*, 2006.

TABLE 9
Composition of experimental feeds (on a percentage basis)

Ingredient	Oil palm cake inclusion level			
	0% (control)	7.5%	15%	22.5%
Maize grain	60.2	60.2	60.2	60.2
Palm oil cake	0.0	7.5	15.0	22.5
Wheat bran	31.0	23.5	16.0	8.5
Soy bran 45%	2.5	2.5	2.5	2.5
Meat and bone flour 55%	5.0	5.0	5.0	5.0
Lime	0.5	0.5	0.5	0.5
Vitamin supplement	0.4	0.4	0.4	0.4
Common salt	0.3	0.3	0.3	0.3
Mineral supplement	0.1	0.1	0.1	0.1
Total	100	100	100	100
Calculated values				
SDE (Mcal/kg)	3.05	3.06	3.07	3.07
Crude protein (%)	14.2	14.1	14.0	13.8
NDF (%)	5.7	10.9	16.1	21.3
ADF (%)	19.8	23.0	26.2	29.4
Ca (%)	0.68	0.67	0.66	0.65
Na (%)	0.18	0.18	0.18	0.18
Available P (%)	0.35	0.33	0.31	0.29
Total P (%)	0.66	0.59	0.52	0.45
Total lysine (%)	0.55	0.51	0.46	0.41
Total Methionine+cystine (%)	0.48	0.44	0.40	0.36
Total methionine (%)	0.22	0.21	0.19	0.17
Total threonine (%)	0.48	0.44	0.41	0.37
Total tryptophan (%)	0.13	0.11	0.10	0.08
Fat (%)	3.68	3.43	3.186	2.92

Notes: Values are calculated in accordance with the nutritional demands in basal feed for swine of low genetic potential (Rostagno *et al.*, 2000). SDE = Swine Digestible Energy; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; Ca = calcium; Na = sodium. Vitamin supplementation was (per kg of feed): vitamin A = 625 000 IU; vitamin D₃ = 125 000 IU; vitamin E = 3375 IU; folic acid = 875 mg; biotin = 27.56 mg; choline chloride = 2475 mg; niacin = 4000 mg; pantothenic acid = 2000 mg; thiamine = 175 mg; riboflavin = 550 mg; pyridoxine = 175 mg; vitamin B₁₂ = 2800 mg; antioxidant = 200 mg. Mineral supplementation was (per kg of feed): Iron = 22 000 mg; copper = 5000 mg; zinc = 18 750 mg; manganese = 12 500 mg; iodine = 238 mg; selenium = 56.3 mg and cobalt = 116 mg.

Source: Rostagno *et al.*, 2000.

peccaries. The differences observed in the weight gain between the collared peccary and the swine may be related to the physiological metabolism of each species, and genetic improvements.

TABLE 10
Daily weight gain (DWG) and daily feed intake (DFI) of collared peccaries fed with varying inclusion levels of oil palm cake

	Oil palm cake inclusion level			
	0%	7.5%	15%	22%
DWG (g)	38	54	70	28
DFI (g)	452	429	425	455

Source: Projeto PROFAMA 109/2008 FAPESPA/SEDECT/UFGPA/Embrapa.

TABLE 11
Characteristics of the carcass and non-carcass components of collared peccaries fed with varying levels of oil palm cake

Parameter	Oil palm cake inclusion percentage			
	0%	7.5%	15%	22.5%
Live weight (kg)	15.05	14.25	16.00	15.3
Fasting weight (kg)	14.45	12.75	15.00	14.65
Dressing percentage (%)	58.4	58.4	56.8	60.4
Gammon dressing percentage (%)	29.7	32.1	31.2	29.8
Carcass length (cm)	56.3	57.0	57.5	61.8
Head (kg)	1.37	1.24	1.45	1.35
Hide (kg)	1.79	1.55	1.83	1.73
Organs and glands (kg)	1.65	1.43	1.36	1.35
Front and hind feet (g)	141	134	138	168
Blood (g)	197	206	205	200
Gammon (kg)	1.35	1.37	1.32	1.46
Ribs (kg)	1.17	1.12	1.34	1.31
Shoulder blade (g)	740	692	813	832

Source: Projeto PROFAMA 109/2008 FAPESPA/SEDECT/UFGPA/Embrapa.

The characteristics of the carcass and of the non-carcass components in collared peccaries fed with varying levels of oil palm cake are shown in Table 11. Dressing percentage variations from 56.8 to 60.4 percent were observed. These values are close to those of Silva *et al.* (2002), who observed dressing percentages from 56.9 percent to 59.5 percent when evaluating different levels of diet crude protein, and slightly higher than those reported by Albuquerque (2006), who tested increasing levels of babassu cake (20, 40 and 60 percent) giving dressing percentages of 53.2, 57.8 and 59.4 percent, respectively.

In the captive white-lipped peccary fed with fodder and feed (13 percent of crude protein and 2800 kcal/kg), the average dressing value was 53.8 percent, slightly below that observed in collared peccaries (Ramos *et al.*, 2009), probably related to the different nutritional composition in the diet offered. This fact can be verified in domesticated swine breeds fed with different diets containing oil palm cake and which present distinct dressing percentages (Rhule, 1996; Gómez, Benavides, Diaz, 2007; Oluwafemi and Akpodiete, 2010).

In javelinas (*Sus scrofa*) fed with sugar cane, vegetables and commercial swine feed, dressing percentages were observed similar to those of domestic swine fed with diets containing oil palm cake (Marchiori, 2001), suggesting that this diet supports good animal performance.

The dressing percentages of collared peccaries are similar or better than other free-ranging artiodactyl wild animals, such as: *Lama glama* (Pérez *et al.*, 2000), *Lama guanicoe* (Gonzalez *et al.*, 2004), *Aepyceros melampus* (Hoffman, 2000), *Tragelaphus strepsiceros* (Hoffman *et al.*, 2009), and *Damaliscus dorcas philipsi* (Hoffman, Smith and Muller, 2008).

The gammon dressing percentage (29.7 to 32.1 percent) observed in the collared peccary (Table 11) was close to the values observed by Silva *et al.* (2002) (36.1 percent) and Albuquerque (2006) in the same species. These observations suggest that the inclusion of oil palm cake in the diet does not appear to prejudice collared peccary performance.

The weight of the shoulder blade was similar to that encountered by Albuquerque (2006) feeding varying levels of babassu cake in the diet of the collared peccary (953.3 g with 20 percent; 943.3 g with 40 percent; and 1028.3 g with a level less than 60 percent). These results were higher than those in the capybara, which did not exceed 800 g (Albuquerque, 1993).

The weight of the ribs was lower than that observed by Albuquerque in the same species and similar to those observed in capybara (Albuquerque, 1993).

Study of the bacterial microbiota from the gastro-intestinal tract

The project PROFAMA (2008) evaluated the bacterial population in the gastro-intestinal tract of collared peccaries and studied the adaptation of the bacterial populations with respect to different feed treatments. Microbiological evaluations were carried out on different components of the gastro-intestinal tract of 26 slaughtered collared peccaries.

In the 27 bacterial microbiota isolated, only Gram-negative bacteria were observed, including *Escherichia coli* (85.2 percent), *Shigella* spp. (7.4 percent), *Salmonella* spp. (3.7 percent) and *Klebsiella oxytoca* (3.7 percent). These results are similar to those reported in literature based on

TABLE 12
Gram-positive and Gram-negative bacteria (percentage) isolated post-slaughter from the gastro-intestinal tract of 26 collared peccaries

Bacterial species	Pre-stomach	Stomach	Intestine
<i>Corynebacterium</i> spp.	10	6.2	9.0
<i>Escherichia coli</i>	40	71.4	58.8
<i>Klebsiella oxytoca</i>	20	9.5	11
<i>Klebsiella pneumoniae</i>	10	0	0
<i>Micrococcus</i> spp.	90	56.2	63.6
<i>Salmonella</i> spp.	10	4.7	5.8
<i>Serratia</i> spp.	0	0	11.7
<i>Shigella</i> spp.	10	0	0
<i>Staphylococcus</i> spp.	0	25	18.8
<i>Streptococcus</i> spp.	0	12.5	9.0
<i>Yersinia enterocolitica</i>	10	14.2	11.7

Source: Projeto PROFAMA 109/2008 FAPESPA/SEDECT/UFPA/Embrapa

isolations of faecal micro-organisms from both domestic and wild animals (Adesiyun *et al.*, 1998; Melville *et al.*, 2004; Marinho, Meireles and Souza, 2004; Oliveira *et al.*, 2009).

Eighty-five isolated bacterial microbiota were obtained, including 20 samples (23.5 percent) from the pre-stomach, 37 samples (43.5 percent) from the stomach, and 28 samples (32.9 percent) from the intestine.

Some of the genera and bacterial species identified are similar to those reported in swine (Jensen, 2001). Of these, *Lactobacillus* spp., *Streptococcus* spp., *Clostridium* spp., *Eubacterium* spp., *Fusobacterium* spp., *Bacterioides* spp. and *Peptostreptococcus* spp. are those most frequently isolated.

Some bacteria, namely *Clostridium perfringens*, *Salmonella* spp., *E. coli*, *Klebsiella* spp., *Campylobacter* spp. and *Pseudomonas aeruginosa* are etiologic agents responsible for enteritis in various animal species, including humans. Despite finding these highly pathogenic micro-organisms, the experimental animals did not present symptoms suggestive of gastro-enteritis.

Irrespective of the treatments the animals received, the results demonstrate that this does not affect the presence or frequency of the bacteria isolated from the gastro-intestinal tract of the collared peccary in captivity, with the majority of isolations having *E. coli* as part of the normal microbiota. It has become necessary to institute strict feed handling procedures to maintain the integrity of the gastrointestinal system in order to prevent diseases and to reinforce food safety measures.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

In addition to the collared peccary, it is important to develop further studies on the captive management of other non-domestic neo-tropical animals of commercial interest, such as white-lipped peccary (*Tayassu pecari*), capybaras

(*Hydrochoerus hydrochaeris*), paca (*Cuniculus paca*), agouti (*Dasyprocta* spp.), broad-snouted caiman (*Cayman latirostris*), yacare caiman (*Caiman yacare*) and greater rhea (*Rhea americana*).

In order to make intensive neo-tropical animal production systems viable for those wild species that may be of economic importance, and for their sustainability and conservation, it will be necessary to study alternative feed resources, such as those already studied with the domestic species. This should be done with feed resources deriving from the agro-processing co-products of cassava, fruits and oil palms. To this could be added sugar cane forage, as suggested by Archimede and Garcia (2010), as this could provide a sustainable feed supply.

CONCLUSIONS

- Babassu cake substitution for maize as an energy source up to a level of 40 percent was a success in feed for collared peccaries in the terminal phase.
- Babassu cake, used to replace up to 40 percent of maize, obtained good results with respect to dressing percentage and commercial cuts of collared peccaries slaughtered at the terminal phase.
- Oil palm cake can be used to replace wheat bran as an energy source in feed for collared peccaries at the terminal phase.
- Oil palm cake used to replace wheat bran gave satisfactory results with respect to dressing percentage and commercial cuts of collared peccaries slaughtered at the terminal phase.

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Chapter 15

Sustainable and competitive use as livestock feed of some co-products, by-products and effluents generated in the bio-ethanol industry

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ABSTRACT

A combination of factors, including rapid increase in fossil fuels prices, climate change effects and especially the need to provide rural jobs, is catalysing a growing interest in biofuel production. Biofuel processing operations need to meet technical, social and environmental sustainability parameters. Technical aspects are usually met, considering the vast array of options already available. The social aspects are more easily met when farmer groups are included as full participants in operations. Satisfying the environmental sustainability parameters is more difficult, as high volumes of effluent are generated as by-products. These by-products can be converted into co-products for use in animal feeding programmes. The Rural Social Biorefineries (RUSBI) approach, as presented in this chapter, for the production and local use of biofuels includes value-added management of co-products and residues generated. In this approach, the organic content of the effluents is flocculated and agglomerated through the use of a biopolymer-based technology, and the flocculated biomass is used to prepare nutritional supplements for ruminants. The use of these supplements in feeding experiments with ruminants has allowed net weight gains in calves and steers of 350–550 g/day, with better economic efficiency than feeding programmes based on commercial nutritional supplements. Transforming biofuel effluents into nutritional supplements for animal feeding is a sound approach to reducing or eliminating contamination of soils and waters, reducing the high costs involved in the management of the high volumes of effluents generated, and generally improving the overall energy and economic efficiency of the biofuel processing operation.

INTRODUCTION

In recent years, the problems associated with the increasing production and use of fossil fuels (such as national security, pollution and global warming) have prompted discussion about the real contribution of biofuels in reducing greenhouse emissions, and how to minimize the impacts caused by the eventual change of land use into food supply and socioeconomic development of rural communities (Walter and Leal, 2010). Today it is considered that the sustainability of biofuels depends on the fulfilment of prerequisites in three dimensions: economic, environmental and social. Bioethanol production in developing countries will have to prioritize the social dimension to ensure aggregate income and social inclusion of the rural communities involved. The growing global demand for biofuels may create new economic opportunities in rural areas, associated with the production, use and marketing of biofuels. Rural communities can also derive income from the processing of by-products

and co-products of biofuels, such as high-protein livestock feeds and fertilizers (UNDESA, 2007).

Despite the wide variety of raw materials available for production of first generation ethanol, more than 90 percent of current world ethanol production is made from maize and sugar cane. However, there is increasing interest in the use of unconventional raw materials that have good levels of sugar or starch, good agronomic productivity, tolerance to low soil fertility, pest and disease resistance and resistance to environmental stress conditions, such as cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas*), sweet sorghum (*Sorghum bicolor* Moench), Jerusalem artichoke (*Helianthus tuberosus* L.), arrowroot (*Maranta arundinacea* L.), biri (*Canna edulis*), yam bean (*Pachyrhizus tuberosus*), yam (*Discorea* spp.), taro (*Colocasia esculenta*) and taioba or tannia (*Xanthosoma sagittifolium*) (Patino et al., 2009). These crops are produced on small farms and therefore their use in ethanol production schemes needs

MAIN MESSAGES

- Satisfying the environmental sustainability parameters in biofuel processing operations is a challenge, as high volumes of effluent are generated as co-products.
- The Rural Social Biorefineries (RUSBI) approach for the production and local use of biofuels includes value-added management of co-products and residues generated.
- The organic content of the effluents is flocculated and agglomerated through the use of a biopolymer-based technology, and the flocculated biomass is used to prepare nutritional supplements for ruminants.
- The use of these supplements in feeding experiments with ruminants has allowed net weight gains in calves and steers of 350–550 g/day, with better economic efficiency than feeding programmes based on commercial nutritional supplements.
- Transforming biofuel effluents into nutritional supplements for animal feeding is a sound approach to reducing or eliminating contamination of soils and waters, reducing the high costs involved in the management of the high volumes of effluents generated, and generally improving the overall energy and economic efficiency of the biofuel processing operation.

to include the implementation of associative structures of production, public policies to add value to the product and appropriate management of co-products, by-products and effluents to minimize environmental impact.

The Latin American and Caribbean Consortium to Support Research and Development of Cassava (CLAYUCA), the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil, and collaborators have been working over the past five years on the development of a technology platform known as the Rural Social Bio-refineries (RUSBI), to promote local production and use of ethanol (ETOH) (96% v/v) using cassava, sweet potato and sweet sorghum as raw material. The ethanol can be used as fuel for farm machinery and implements (tractors, irrigation pumps, power generators, clean-cook stoves, etc.) or sold in public or private niche markets at higher prices (social ethanol, pharmaceutical companies, green plastic industries, etc.). In this approach, wastes and effluents are converted into products that can be used to develop nutritional supplements for animal feed.

The RUSBI approach is not a technical package designed for biofuel production in large-scale commercial enterprises. On the contrary, RUSBI is an approach for small-scale production and local uses of biofuel, as a strategy to promote agricultural and economic development of those billions of farmers around the world living in marginal areas and facing a lack of resources, especially energy. RUSBI is meant to address the needs of these people and become an alternative model for promoting more inclusive, equitable bio-energy development efforts. The production of the biofuel is not considered the final product, as is the case of the commercial, large-scale operations. In the RUSBI approach, the biofuel becomes an intermediate objective that allows farmers groups to have access to energy, and to use this new energy-security status for implementing other agro-industrial transformation processes, adding value to their agricultural products and creating new employment and income oppor-

tunities. These combine to help reduce levels of poverty and to improve standards of living. The RUSBI approach focuses on developing an alternative approach for biofuels production that overcomes the social inequalities that characterize the modern, large-scale, commercial biofuel operations that are booming around the world, characterized by the limited participation of the farmers in the distribution of the benefits, acting merely as providers of raw material for the distilleries (Ospina, Gallego and García, 2009).

The RUSBI approach for biofuel production

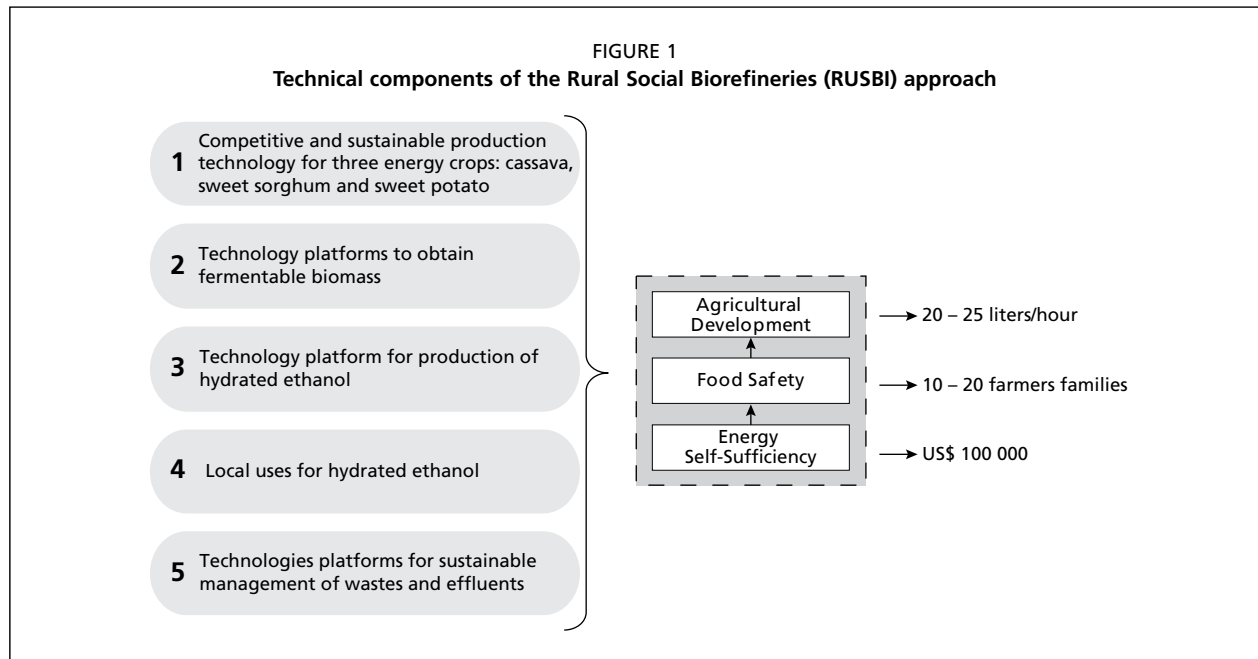
The RUSBI approach for the production of biofuels has five technical components that integrate modern concepts of agricultural management, process engineering and effluent management (Figure 1).

The end objective of the RUSBI approach is to promote agricultural development, food safety and energy self-sufficiency within small-scale farmer groups and rural communities, living in isolated, marginal areas. The scale of the rural social biorefinery is small to facilitate the participation of poor farmer groups: the capacity of the ethanol distillery is 20 to 25 litre/hour, so groups of 10 to 20 farming families could produce enough cassava, sweet potato or sweet sorghum to run the plant, with a total investment cost for a rural community of around US\$ 100 000.

The various elements in a RUSBI are presented in Photo 1. They comprise:

- a drying plant and a refining unit to produce cassava and sweet potato flour, and a milling section to produce sweet sorghum juice,
- a pilot plant to produce ethanol (96 percent), with a capacity to produce 20 L/hour, and
- a plant for treating the effluents.

The biorefinery equipment also includes a stationary engine to generate bio-electricity, and a cooking stove. Both use the ETOH as fuel.



a) Cassava or sweet potato flour milling and refining plant



b) Ethanol processing plant



c) Effluents treatment plant

Photo 1

Equipment included in a typical Rural Social Biorefinery (RUSBI)

The process for production of the ETOH in the RUSBI approach is shown in Figure 2. When the feedstock is cassava, biofuel can be obtained from cassava flour or from cassava roots. When cassava flour is used, the roots are first processed into refined flour, which is then converted into a slurry or liquid biomass by adding water.

Incubation conditions (pH and temperature) are adjusted to prepare for the hydrolysis and fermentation steps. The operating conditions include: pH of fermentation medium 4.5, adjusted with hydrochloric acid (30 percent m/m); substrate concentration 30 percent; fermentation time 72 hours; with 0.23 percent urea, 0.5 percent enzyme and 0.33 percent yeast. The fermented must is then passed through the distillation columns to obtain the ETOH, with the vinasse as the co-product.

When fresh cassava roots are used, the roots are grated into a pulp with very fine particle size to facilitate the hydrolysis and fermentation stages. In this method, less water is required for the process but the fermented biomass requires a filtering step to reduce the high fibre content

prior to the distillation step. One important difference in the process when cassava flour is used instead of fresh cassava roots is that two co-products are generated during the milling and refining processes, which can be used in animal feed, helping to offset the additional cost of processing the cassava roots into flour.

Hydrolysis is one of the most important phases of the process, converting starches into fermentable sugars, which are then metabolized by yeast during fermentation, producing bio-ethanol. The enzymatic hydrolysis or saccharification is catalysed by enzymes whose function is to break down large starch molecules to produce units of glucose. For hydrolysis of starches, two methods can be used: liquefaction, saccharification and conventional fermentation (LSF); or simultaneous hydrolysis and fermentation (SHF).

The LSF method consists of the starch being first liquefied, then converted into glucose (saccharification), and finally fermented using a yeast (*Saccharomyces cerevisiae*). The thermostable enzymes used in the liquefaction and saccharification steps are, respectively, alpha-amylase and

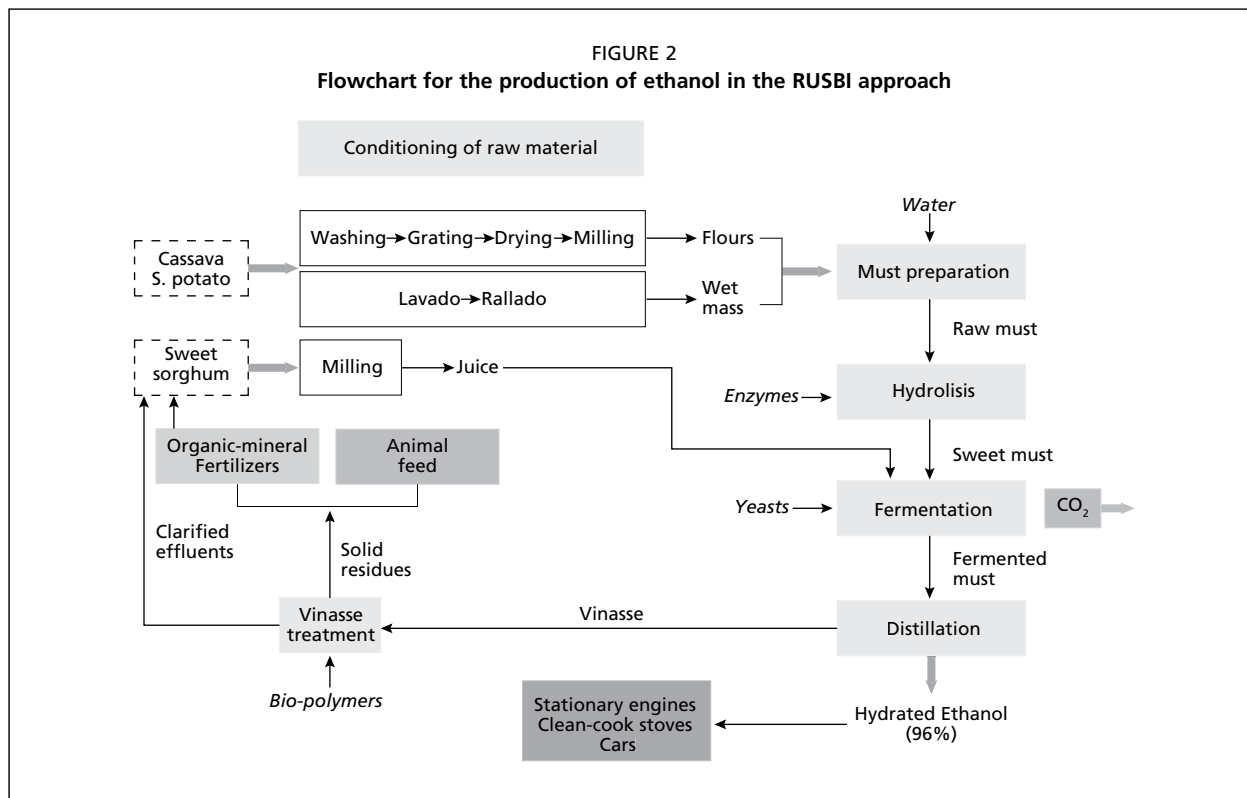


TABLE 1
Operating conditions in the early stages of hydrolysis and fermentation of the conventional process (LSF) for biofuel production

Parameter	Hydrolysis		Fermentation
	Liquefaction	Saccharification	
T (°C)	82–86	65–70	32
pH	5.7–6.0	4.3	4.5

Source: Genencor International web site (www.genencor.com)

gluco-amylase. Operating conditions conventionally used for this method are given in Table 1.

In the SHF method, a mixture of enzymes is used to carry out the saccharification of starch without the liquefaction stage. In this method, special enzymes are used (StarGen™), that are able to perform the hydrolysis stage at room temperature, and allow the combination of the saccharification and fermentation stages in one single step, because they work under the same conditions of temperature and pH as the yeast (*Saccharomyces cerevisiae*). The operating conditions for this method are given in Table 2.

The RUSBI approach to producing bio-ethanol is based on the SHF method, seeking to reduce processing time, power consumption and installation costs, since it does not need installation of a heat exchanger. After the SHF step is finished, a fermented mash is obtained. To separate the ethanol from this mash, a distillation stage is required, in which the ethanol evaporates at 78 °C. The ethanol

TABLE 2
Operating conditions of the simultaneous hydrolysis, fermentation and saccharification process (SHF) for biofuel production

Parameter	Hydrolysis (Liquefaction + Saccharification)	Fermentation
	T (°C)	
pH	4.0–4.5	4.5

Source: Genencor International Web site (www.genencor.com)

vapours are captured and condensed, yielding ETOH and leaving the vinasse (Ospina *et al.*, in press).

BIO-ETHANOL PRODUCTION TRIALS WITH THE RUSBI APPROACH

The work conducted by CLAYUCA with the RUSBI model for production of biofuel has focused on the optimization of the enzymatic hydrolysis of starch in cassava (Cajamarca, 2009), and on estimation of the efficiency in the production of bio-ethanol from cassava flour, by calculating the mass and energy balances in the process (Martínez, 2009). Some of the tests conducted with cassava flour and cassava roots for the production of ETOH using the SHF method at room temperature are presented in Table 3.

Management of the vinasse co-product resulting from the bio-ethanol production process

The operation of a biofuel production process, such as ETOH from cassava, generates a large quantity of effluent

TABLE 3
Production of ethanol from cassava roots and cassava flour in a CLAYUCA RUSBI pilot plant

	Cassava flour	Fresh cassava roots
Raw material		
Cassava refined flour (kg)	120	300
Enzymes (Stargen) (kg)	0.600	0.380
Yeast (Ethanol red) (kg)	0.400	0.500
Urea (kg)	0.300	0.300
Water (kg)	400	450
Products generated		
Ethanol 96% v/v (Litre)	44.7	48
Vinasse (Litre)	630	801
Quantitative analysis		
Yield (L ethanol/t cassava flour)	372.5	
Yield (L ethanol/t cassava root)		160
Yield (L ethanol/ha) ⁽¹⁾	2660	4000
Ethanol production efficiency ⁽²⁾ (%)	61	89
Ratio of vinasse to ethanol (v/v)	14.1	16.7

Notes: (1) Average cassava yield = 25 t/ha. (2) Production/theoretical conversion.

as a by-product of the process. This effluent, known as vinasse, is produced in large volumes and needs to be managed properly in view of its potential environmental effects and energy costs parameters. The vinasse has the form of a dark organic liquid, with very low pH (3.5 to 4.3), and is the result of the fermentation of carbohydrates (sugar cane and sweet sorghum juices, cassava and sweet potato slurry) and subsequent distillation of the fermented mash. The vinasse contains a high percentage of organic matter (organic acids and dead yeast), minerals (mainly potassium, calcium, magnesium and sulphur) and non-fermentable constituents of the raw material (Patino *et al.*, 2007).

On average, for every litre of ethanol obtained, between 10 and 15 litre of liquid effluent are generated, depending on the feedstock used, the time of harvest, the grinding process, the fermentation and distillation technology, the soil type and fertility level, and other parameters (Mutton, Rosetto and Mutton, 2010.). In a CLAYUCA biorefinery, the ethanol production from cassava had a ratio of vinasse to ethanol equivalent to 14:1 (Del Ré *et al.*, 2010).

Vinasse has historically been used as a fertilizer, with Brazil being the pioneer in the development of fertirrigation systems using sugar cane vinasse. The use of the vinasse has improved sugar cane productivity in Brazil (Penatti, 2007) through chemical (Leal *et al.*, 1983), biological (Matiazzo and da Gloria, 1985) and soil physical benefits (Gloria and Orlando Filho, 1983), as well as reduced fertilization costs. However, the excessive and continuous application of vinasse in agricultural soils can create serious problems in terms of cane quality (Silva, Pozzi de Castro and Magro, 1976) and water source contamination (Gloeden *et al.*, 1990).

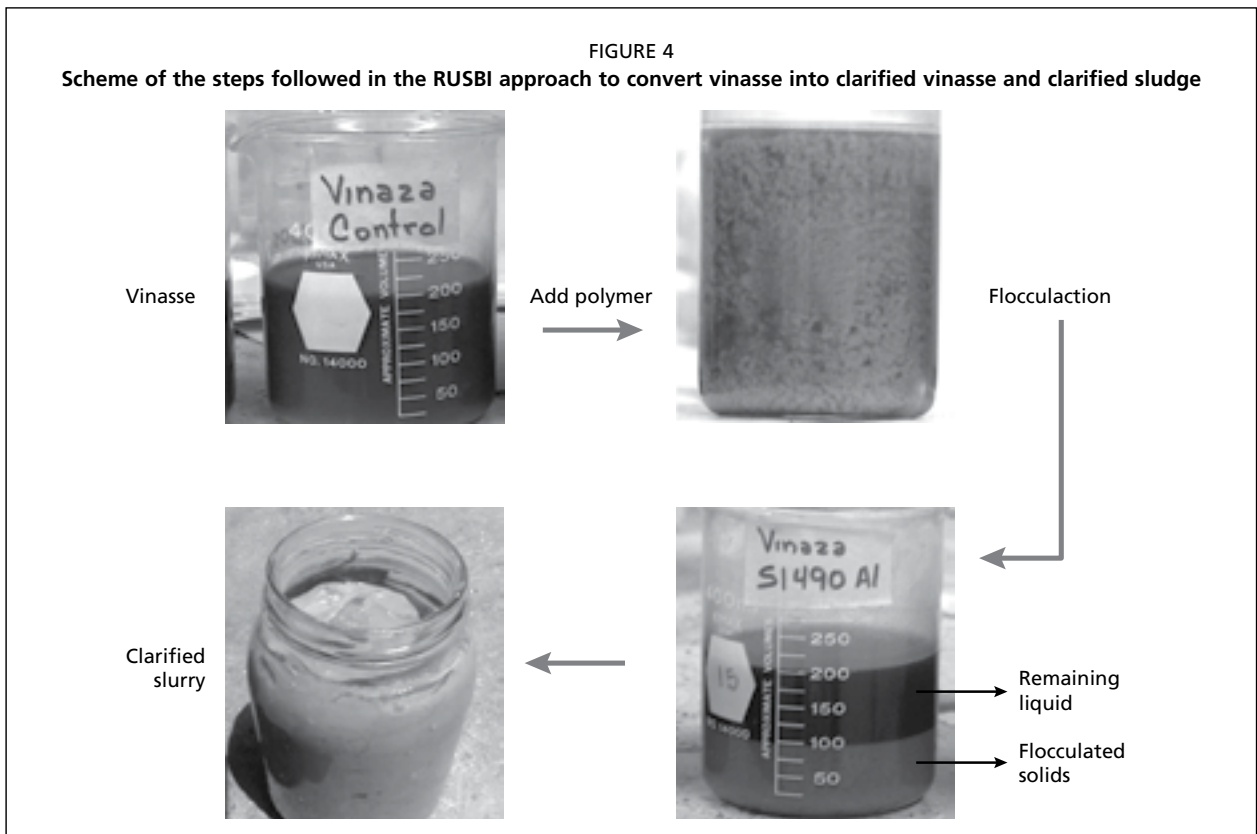
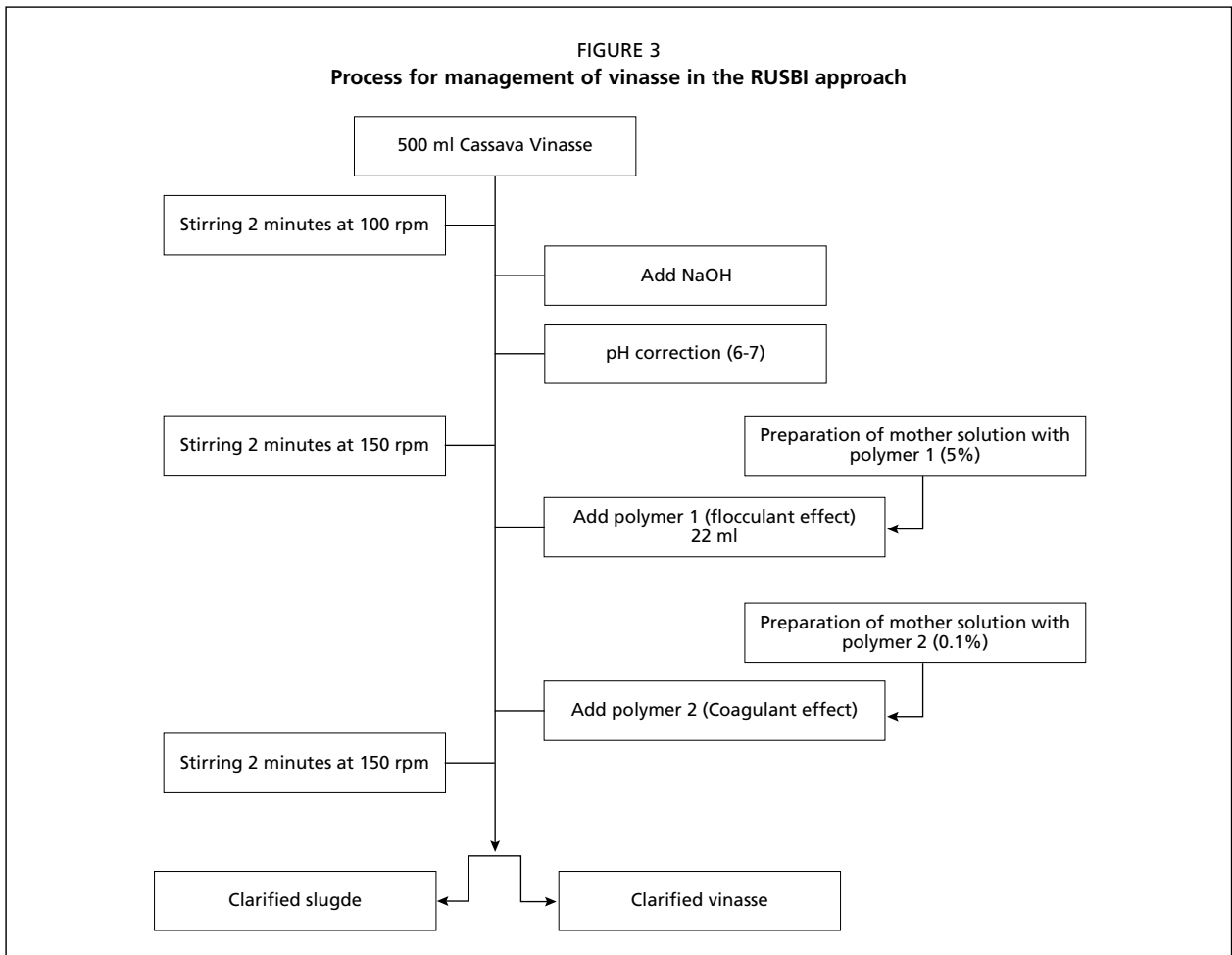
The use of vinasse in fertirrigation takes two general forms. The first is to use the vinasse directly after leaving it to cool, as the temperature at which the product leaves the distillation process is above 70 °C. After cooling, and with the addition of minerals (N, P), the vinasse is used to directly irrigate the fields.

The second method is to reduce the water content to facilitate its incorporation or mixing with other raw materials. This can be accomplished by physical processes, such as sedimentation in ponds (this can take between 48 and 72 hours). Alternatively, chemicals can be added, such as polymers that accelerate the process of flocculation and coagulation, precipitating the solids in the vinasse more rapidly. The polymers are diluted to 1 part per thousand and added to the vinasse, which results in a rapid clarification response (Orts *et al.*, 2007).

In addition to use as fertilizer, the vinasse can also be concentrated by evaporation or drying and used in the preparation of animal feed products (Albers, 2007) or in the production of fertilizers (Barbosa *et al.*, 2006). However, this alternative has limitations due to the high energy cost of the concentration process. An alternative use of the vinasse is the production of biogas (methane) through anaerobic fermentation by methanogenic bacteria, which also reduces the environmental impact of the vinasse by reducing its biological oxygen demand (BOD) and chemical oxygen demand (COD) (Peres, 2007; Zhang *et al.*, 2010). The vinasse has also been used for single-cell protein production in aerobic fermentation systems (Murakami *et al.*, 1993; Diaz, Maria Gualtieri and Semprun, 2003; Cazetta and Celligoi, 2006). Another alternative for the management of the vinasse is the production of compost for use as fertilizer. This latest technology, despite its potential as an environmentally friendly process, requires high investments in area, capital and time for its operation.

TRANSFORMATION OF CO-PRODUCTS, BY-PRODUCTS AND EFFLUENTS INTO NUTRITIONAL SUPPLEMENTS FOR ANIMAL FEEDING

In bio-ethanol production with the RUSBI approach, vinasse treatment is done using electrically charged chemicals known as biopolymers, which are made from starch and have been used to ensure the slow release of minerals contained in fertilizers, to reduce erosion, to increase the penetration of water in the soil and to produce fertilizer-coated seeds. When the biopolymers are introduced into solutions that have basic pH and with high loads of ionic solids, flocculation and coagulation of the organic load occurs. Once the organic matter contained in the vinasse is flocculated, coagulated and removed, the clarified water can be used for other purposes in the biorefinery (irrigation, washing, cleaning, etc.). Figures 3 and 4 present the



flocculation and coagulation processes, which result in two products, vinasse and clarified sludge. Table 4 shows the bromatological composition of four types of vinasse. Table 5 presents the minerals and nutrient content of pure vinasse, clarified vinasse and clarified sludge, from sugar cane biofuel processing.

CLAYUCA-CIAT, in partnership with Soil Net (Soil Net LLC, Polymers Solutions, a private company in the United States) and Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS; a Brazilian University), has developed new potential solutions and alternatives for sustainable, competitive management of the effluents generated in biofuel distilleries. One of these alternatives is protein and energy supplementation for ruminants by mixing the vinasse with cassava products (roots and foliage). The nutritional supplements developed with vinasse have been oriented principally to feed ruminants. The composition and characteristics of products can be adjusted to suit the age and type of animal to be fed.

The organic matter contained in the flocculated sludge is mixed with other products and co-products obtained

during the process, such as cassava and sweet potato leaves and stems, and sweet sorghum bagasse. Other components that are included are urea, minerals and additives. The formulation of the nutritional supplement is scientifically designed with the help of a computer program to obtain a final product that is competitive, nutritionally balanced and highly efficient in the feeding of ruminants. Photo 2 presents the different steps required to prepare the nutritional supplement.

Organic matter removed from vinasse, together with products and co-products from biofuel processing (cassava and sweet potato leaf and stalks; sweet sorghum and sugar cane bagasse) and other ingredients such as urea, minerals and additives, are combined to provide a balanced protein, mineral and energy supplement for ruminants (Patino *et al.*, 2007; Martin, 2009). The supplements can be presented in different forms, depending on the animal feeding programme: multinutritional blocks, pellets or meal (Photo 3).

In the preparation of the multinutritional blocks, the ingredients (bagasse, molasses, vinasse, urea, sodium

TABLE 4
Chemical composition and *in vitro* dry matter digestibility (IVDMD) of four types of vinasse (all values are percentages except for trace minerals)

Nutriment/Parameter	Vinasse source			
	Cassava	Sugar cane	Sweet potato	Sweet sorghum
Dry matter	8.5	13.0	2.6	3.4
Organic matter	93.5	–	92.8	90.8
Crude protein	11.6	2.0	12.5	7.2
Starch	0.7	–	–	–
Ether extract	4.9	0.4	22.3	0.8
Crude fibre	60.4	–	27.0	–
IVDMD	64.7	–	–	–
Ash	5.2	32.3	7.2	9.2
Total Digestible Nutrients	–	–	74.5	77.8
P	1.42	0.45	0.39	–
Ca	5.38	1.04	0.50	–
K	1.49	2.08	1.9	–
Mg	0.40	0.24	0.63	–
S	0.48	0.30	0.18	–
Na	0.34	–	0.31	–
Zn (ppm)	40	–	44	–
B (ppm)	16	–	10	–
Mn (ppm)	104.5	–	58	–
Fe (ppm)	3305	86	584	–
Cu (ppm)	14	1	17	–
Al (ppm)	3121	–	–	–

Sources: CLAYUCA, 2008

TABLE 5
Nutritional content of the products obtained in clarification of sugar cane vinasse

Product	P total	K total	Ca total (%)	Mg total	S	Fe	Cu	Na	Zn	Crude protein (%)	OM
Sugar cane vinasse	2.97	10.24	0.88	1.14	1.23	986	6.0	3.066	54.0	7.0	56.8
Clarified sugar cane vinasse	0.00	1.06	0.48	0.12	0.14	32.0	0.0	366.0	3.0	0.8	6.8
Sugar cane clarified sludge	2.75	2.99	14.26	0.20	9.30	525	47.0	467.0	19.0	5.2	27.5

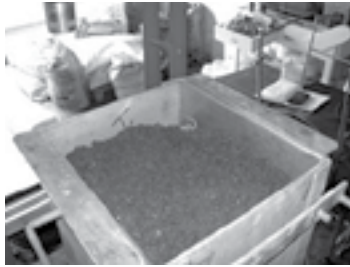
Note: OM = organic matter. Source: CLAYUCA, 2007.



Step 1. Raw material preparation



Step 2. Mixing ingredients



Step 3. Pressing



Step 4. Drying 8-15 days

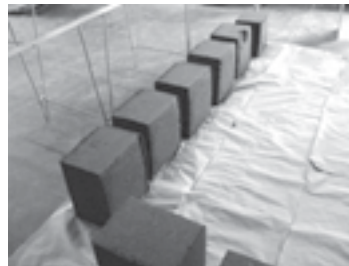


Photo 2

Outline of the steps followed to prepare the nutritional supplements in the RUSBI approach

bentonite and minerals) are first weighed and mixed in a horizontal mixer. The order of introduction of ingredients is defined to avoid losses of molasses by adhesion to the walls of the mixer and to enhance chemical reactions and heat generation, thus ensuring that the mix is in a form suitable for the block compaction operation. First the bagasse, minerals and sodium bentonite are mixed. Then a solution of urea, diluted in the vinasse and molasses, is incorporated in the mix. Finally, the calcium oxide is incorporated. The mixture is agitated for 15 minutes until homogenous. The blocks are formed by pressing 18 kg of the mixture in a steel mould under compaction pressure of 2000 kg/cm² for 5 minutes. Finally, the blocks are removed from the mould and placed in a shaded area to dry for one week. For transportation and commercialization, the blocks are packed in cardboard boxes.

In the preparation of supplements specifically for cattle feeding, co-products from sugar cane-based ethanol can be included at between 50 and 80 percent. Tables 6 and 7 present the components and nutritional composition of two products for ruminants: a multinutritional block and a mineral salt block, made with co-products from sugar cane-based biofuel processing, using the RUSBI process. Table 8 presents the bromatological composition of the two

TABLE 6

General characteristics of a block nutritional supplement

Raw material	Inclusion level (%)	Nutritional composition (%)	
Pre-digest bagasse	25.10	Crude protein	24.0
Vinasse sludge	36.82	NPN (max.)	3.9
Fly ash	4.32	TDN	33.0
Molasses	9.89	Ca	2.21
Other ingredients	23.87	P	1.00
Total	100.00	S	0.36

Notes: Other ingredients comprise urea, NaCl, flowers of sulphur, dicalcium phosphate, calcium oxide, sodium bentonite, micromineral premix. NPN = Non-protein nitrogen; TDN = Total digestible nutrients. Source: CLAYUCA, 2009

nutritional supplements (energy and protein), prepared as blocks with salt.

As indicated in Table 8, the two nutritional supplements have TDN equivalence, but they differ in the percentage of protein. Both the blocks and the meals are recommended for use in situations in which the available pastures have protein contents below 6 percent and where the TDN:CP ratio exceeds 8. Also, the energy supplements are recommended with better quality pastures, with protein content over 8 percent. All the nutritional supplements were formulated to obtain a balance of 10 to 11 percent between con-

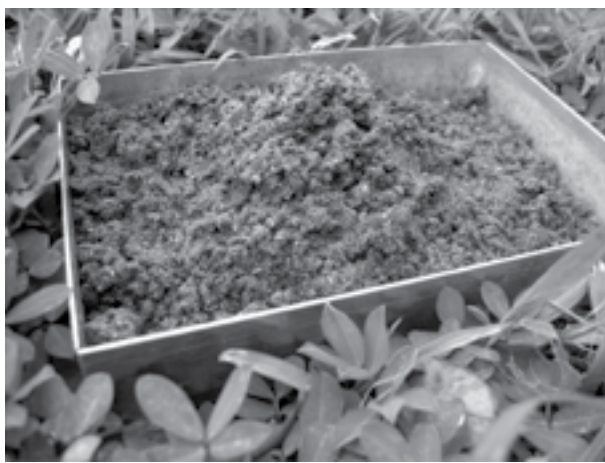


Photo 3
Different forms of nutritional supplement and standard packaging

TABLE 7
General characteristics of a nutritional supplement in meal form

Raw material	Inclusion level (%)	Nutritional composition (%)	
Pre-digest bagasse	24.45	Crude protein	24.0
Clarifications sludge	35.86	NPN (max.)	0.9
Filter cake	4.63	TDN	34.0
Molasses B	9.90	Ca	2.21
Other Ingredients	25.16	P	1.00
Total	100.00	S	0.36

Notes: Other ingredients comprise NaCl, flowers of sulphur, dicalcium phosphate, sodium bentonite, urea, mineral premix. NPN = Non-protein nitrogen; TDN = Total digestible nutrients.

Source: CLAYUCA, 2009

sumption of degradable protein in the rumen and energy consumption (TDN), in animals grazing pastures of low to medium quality. At least 25 percent of the total nitrogen in the nutritional supplement is from true protein and the rest is non-protein nitrogen.

The multinutritional blocks based on co-products and effluents from ethanol production are very attractive in the market because they have good palatability and good levels of protein and energy (i.e. TDN) (Loaiza, 2008; Torres, 2010). The microbiological quality of the nutritional supple-

TABLE 8
Bromatological composition of two nutritional supplements (energy and protein), elaborated as blocks and meals, using vinasse and other products and co-products from sugar cane-based biofuel processing

Nutrients	Protein supplement		Energy supplement	
	Block	Meal	Block	Meal
Dry matter (%)	78.0	93.4	79.0	94.2
Organic matter (%)	67.6	59.4	67.7	65.0
Crude protein (%)	33.1	39.5	9.6	17.2
Fat	0.8	2.2	1.3	1.6
Total Digestible Nutrients (TDN)	65.5	64.3	69.9	65.5

Source: Ruminants Nutrition Laboratory-LANUR. UFRGS, 2007. Pers. Comm.

ments developed by CLAYUCA was measured under differing storage conditions to check if they were meeting the quality standards and guidelines established by Colombian legislation (implemented through Instituto Colombiano Agropecuario – ICA). The products were stored according to the conditions recommended in the local standards for Good Manufacturing Practices for Food (BPFA), which require that after 40 days the nutritional supplements must retain their intrinsic characteristics and have good microbiological status.

TABLE 9
Microbiological analysis of multinutritional blocks made with co-products and effluents from sugar cane-based ethanol production

Analysis	Nutritional block	ICA Specification
Count of mesophile aerobic micro-organisms (CFU/ml)	1×10^6	Up to 1 000 000 CFU/g
Count of fungi (CFU/mL)	<10	Up to 100 000 CFU/g
Count of yeast (CFU/mL)	1×10^4	Up to 100 000 CFU/g
MPN of faecal coliforms per mL	Not detected	Absence
Salmonella in 25 g	Not detected	Negative in 25 g

Notes: CFU = Colony Forming Units; MPN = most probable number. Source: ICA Microbiological Laboratory. Pers. Comm.



Photo 4

Cattle eagerly consuming nutritional blocks made with co-products and effluents from sugar cane-based ethanol processing

Table 9 shows the results of the microbiological analysis of multinutritional blocks made with co-products and effluents from sugar cane-based ethanol production. The microbiological count indicated absence of *Salmonella* and faecal coliforms over 40 days, as well as a constant low fungal count (<10) (Loaiza, 2008; Torres, 2010). Palatability tests of the nutritional supplements were also conducted by CLAYUCA, with positive responses from the animals in terms of consumption rates for the blocks and liveweight gain. These nutritional supplements are very attractive for the animals due to their high palatability (Torres, 2010), and also for their high levels of dry matter digestibility (Loaiza, 2008).

Another feature of the block preparation process for nutritional blocks is an increase in crude protein content as levels of vinasse increase in the formulation. This change is due to the presence of yeast residues in the vinasse, which enrich the nutritional value of the product (Loaiza, 2008). These positive features make the nutritional blocks a very attractive product, with great market potential in the animal feed sector. Photo 4 shows the acceptance of the product by the animals.

BIO-ECONOMIC ANIMAL FEEDING TRIALS WITH THE NUTRITIONAL SUPPLEMENTS

The quality and efficacy of nutritional supplements made with co-products and effluents from sugar cane-based and cassava-based ethanol processing has been tested in bio-economic animal feeding trials.

In a commercial test with calves with initial weight of less than 200 kg, a nutritional supplement was fed for 90

days to complement a *Pennisetum purpureum* grass basal diet. Average weight gains per animal per day of 0.602 kg were obtained, almost double the average weight gains (0.316 kg/animal/day) obtained by the animals before starting the supplementation. The short duration of this feeding trial does not allow firm conclusions to be made. The main objective of this experiment was to make a first evaluation regarding the acceptance of the block by the animals, and to have an initial estimate of the consumption potential. The animals in the study consumed the block from the first day of exposure, without any rejection related to the smell or taste of the product. The nutritional block retained its structure during the whole supplementation period.

Another trial aimed at assessing the consumption and weight gain of heifers on pasture, supplemented with protein supplements prepared from cassava root and leaf flour, and vinasse from sugar cane-based biofuel production (Gil *et al.*, 2007). The study included 20 replacement Holstein heifers, with an average initial weight of 168 kg, divided into two groups of 10 animals each. One group was used for evaluating the protein supplement based on cassava and vinasse, and the other to assess the use of a commercial supplement. The experiment lasted for 120 days (September to December). Four grazing plots planted with an African star grass (*Cynodon nlemfluensis*), with an average area of 5518.5 m² for each plot, were used in the rotation of the animals (each group used two pastures). The forage dry matter on offer was on average 2320.5 kg DM for each grazing plot, equivalent to 4204.9 kg DM/ha. The trial was conducted at a site near Palmira, Valle, Colombia.

Animals were distributed randomly into two groups: the first group received 1.5 kg/day/animal of a commercial concentrate (18 percent protein and 67 percent TDN), and the second group received 1.0 kg/day/animal of supplement based on cassava and vinasse (21 percent protein and 56 percent TDN). The group receiving the vinasse-based supplement was given a period of 10 days to accustomize to the product. Weighing was conducted every 21 days and supplement consumption assessed, taking into account the daily supply of supplement. The commercial supplement and the supplement based on cassava were weighed in the morning. In the afternoon, the feeders were reviewed to collect and weigh the wastes or leftovers. In both cases, the consumption of supplements was complete. The assessment of the weight gains indicated that those animals that consumed the supplement of cassava and vinasse had better performance than the animals given the commercial product. Weight gains were on average 0.48 kg/day whereas the commercial concentrate gave weight gains averaging 0.36 kg/day ($P < 0.05$). The slightly higher weight gain obtained by the animals consuming the cassava-based supplement could be explained by the higher protein content of the cassava-based supplement and the better ratio of nutrients (rumen degradable protein vs TDN).

Another trial was carried out in the Cauca River Valley, classified by Köppen as tropical climate. The experimental area consisted of 17 paddocks divided with electric fences, each approximately 0.25 ha, planted with an African star grass (*Cynodon plectostachyus*). Each paddock had an automatic water supply and a feeder for the nutritional supplement. Rotational paddock grazing was used, with about 2 days of occupation and 17 days of rest. The pastures were fertilized with 80 kg P_2O_5 /ha/yr and 50 kg N/ha/yr. During the dry season, the pastures were uncompacted and irrigated. A total of 71 steers of undefined breed, aged approximately 24 months, and with an initial average live weight of 234 kg, were used. The treatments evaluated consisted of a conventional mineral supplement and a protein-mineral block supplement (Table 10).

Statistical analysis of the data obtained indicated that daily weight gains of animals consuming the nutritional blocks was 21 percent higher than the weight gains among the animals consuming the mineral supplement ($P < 0.05$) (Table 11). The weight gains obtained indicate the potential of the nutritional supplements for use in animal feeding. The economic efficiency parameter was also positive. The average daily weight gain of animals consuming multi-nutritional blocks was 94 g/day higher than in the animals supplemented with mineral blocks to 6 percent. This improved efficiency represented a 17 percent increment on gross margin (US\$ 0.69 vs US\$ 0.59), making it an attractive option for cattle producers (Table 12). The

TABLE 10
Composition of nutritional supplements offered to cattle

Nutrient	Conventional mineral blocks (6%) ⁽¹⁾	Multi-nutritional blocks
Crude protein (%)		24
Non-protein nitrogen (NPN) (% max.)		3.85
Total Digestible Nutrient (TDN) (g/kg)		330
Sodium chloride (%)	38.52	19.62
Phosphorus (g/kg)	60.0	10.04
Calcium (g/kg)	120.0	22.12
Magnesium (g/kg)	0.5	1.91
Sulphur (g/kg)	6.0	3.60
Copper (ppm)	2500	82
Zinc (ppm)	8000	247
Iodine (ppm)	150	5.96
Cobalt (ppm)	40	0.82
Selenium	100	0.82

Notes: Mineral block analysis data from www.somexnutricion.com. Source: CLAYUCA, 2009

TABLE 11
Initial live weight, final live weight and average daily weight gain of grazing animals with nutritional supplementation

Variables	Treatments	
	Multinutritional block	Mineral block 6%
Initial weight (kg)	231.5	235.4
Final weight (kg)	273.2	269.8
Weight gain (g/day)	541 a	447 b

Notes: a, b = different suffixes indicate a significant difference based on the Tukey Test ($P < 0.05$). Source: CLAYUCA, 2009

TABLE 12
Weight gains and economic benefits from grazing animals with nutritional supplementation

Parameter	Multi-nutritional block	Mineral block 6%
Average initial weight (kg)	231.0	235.0
Average final weight (kg)	273.2	269.8
Average weight gain (kg)	42.20	34.80
Duration of trial (days)	78	78
Average daily weight gain (kg/day)	0.541	0.446
Price live kg (US\$)	1.47	1.47
Price average daily weight gain (US\$)	0.80	0.66
Supplement consumed (kg/day)	0.177	0.071
Nutritional supplement consumed (kg)	1046.6	1565.02
Costs of nutritional supplement (US\$)	0.10	0.06
Gross margin (US\$)	0.69	0.59

Source: CLAYUCA, 2009

objective of this experiment was to validate the option of developing a nutritional supplement that could give the animal not only minerals, but also protein and some energy. The question that this experiment was trying to answer was "Is it possible to have a complete nutritional supplement (minerals, energy, protein) that was competitive relative to the mineral supplements available in the market?"

ECONOMIC VIABILITY OF THE USE OF NUTRITIONAL SUPPLEMENTS IN ANIMAL FEEDING

The economic viability of the use of nutritional supplements for animal feeding based on the by-products and co-products from sugar cane and cassava biofuel operations will depend on the cost of producing the nutritional supplements and their price competitiveness in relation to the price of similar products available in the commercial market. Table 13 presents the complete cost of producing a nutritional supplement (block) using the RUSBI approach.

In the Colombian cattle sector, the use of nutritional supplements is common, although the percentage of cattle growers that uses them is still limited. In some cases, the transportation costs to the areas with large cattle opera-

tions increases the final costs of the nutritional supplements. The products commercially available are presented in the form of blocks, with a weight of 25 kg each, usually including molasses and urea. As of August 2011, the cost of a multinutritional block was 28 000 Colombian pesos (US\$ 15.55). The unit cost of nutritional block is US\$ 0.622/kg from the RUSBI process, while commercial blocks are 52 percent more expensive. This large margin implies tremendous market potential for these nutritional supplements in the animal feed sector.

The technical and economic feasibility of using by-products and co-products coming from a sugar cane- or cassava-based biofuel operation to produce supplements for animal feeding has been demonstrated. It is possible to use the nutritional supplements in animal feeding programmes, with good results in terms of both biological and economic

TABLE 13
Production costs for a nutritional block, based on producing 100 nutritional blocks of 15 kg each

Parameter	Unit	Quantity	Unit cost (US\$)	Total cost (US\$)
Variable costs				
Bagasse	Kilogram	289.5	0.13	37.64
Fly ash	Kilogram	300	0.016	4.80
Clarified sludge	Kilogram	375	0.05	18.75
Molasses	Kilogram	150	0.19	28.50
By-products total cost		1114.5		89.69
Inputs				
Urea	Kilogram	60	0.49	29.40
Mineral salt	Kilogram	185.25	0.72	133.38
Sulphur	Kilogram	3	2.10	6.30
Polymer	Kilogram	2.25	7.30	16.43
Calcium oxide	Kilogram	135	0.55	74.25
Inputs total cost		385.5		259.76
Total costs of raw material		1.500.0		349.45
Other costs				
Electric power	kwh	13.78	0.11	1.52
Water	m ³	6	0.75	4.50
Qualified operator 1	Hour	4.78	2.13	10.18
Qualified operator 2	Hour	4.78	4.27	20.41
Total other costs				36.61
Selling cost				
Packaging	1 cardboard box	100	0.06	6.00
Transport	US\$/block	100	0.05	5.00
Total cost of sales				11.00
Total variable costs				397.05
Fixed costs				
Administration (5% of production cost)				18.81
Unforeseen (5% of production cost)				18.81
Maintenance (5% of production cost)				18.81
Total fixed costs				56.43
Total production cost per 100 blocks of 15 kg = 453.48.				
Total production cost per kilogram of nutritional block = 0.302				

Source: CLAYUCA, 2009

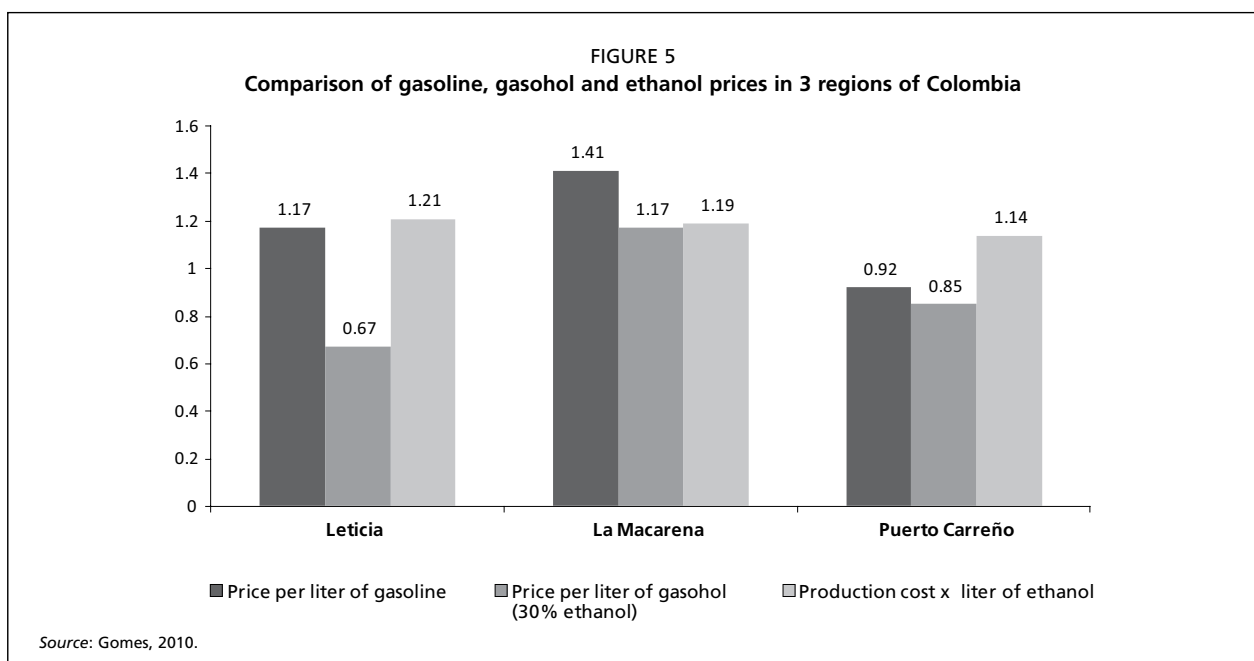
efficiency. It is also feasible to establish market linkages with the animal production sector and to position the nutritional supplements based on their competitive production price in comparison with commercially available products. However, the work conducted by CLAYUCA and collaborating agencies, institutions and private sector companies has focused on a strategy designed to promote biofuel production and use by small-scale communities and farmer groups, i.e. the RUSBI approach. In this sense, the initial beneficiaries of the technology developed for the preparation and use of the nutritional supplements will be the commercial groups that are already operating the bio-ethanol distilleries, with large volumes of effluents that need to be managed with economic and environmental efficiency. The small-scale rural communities, cooperatives and farmer groups that the RUSBI approach is targeting will not be able to compete with the large-scale biofuels distilleries and sugar cane operations. The objective of the RUSBI approach is not to enter this market. What RUSBI aims to achieve is to add value to the biofuels that can be produced by small-scale farmers, promoting local use, for their own consumption, or for commercialization in local markets, supported by the government (social ethanol) or by private-sector initiatives. The sustainable, competitive management of the effluents becomes a plus component of this approach, with potential to help farmers improve the feeding systems for their animals and increase incomes.

For facilitating access by target farmers to the potential benefits of these technologies, the rural social biorefineries have to be promoted and established in the rural areas, and this process may still require some time, considering the initial investment required (around US\$ 100 000 for a 300 L/day distillery). CLAYUCA has been working on

generating the data required to convince and sensitize national and local governments, rural development agencies and the donor community, regarding the importance of supporting strategies aimed at promoting production and local uses of biofuel by poor farmers, located in remote villages, and lacking access to any source of energy. A study was conducted (Gomes, 2010) to evaluate the technical and economic feasibility of the implementation of a rural social biorefinery (500 L/day) in three rural areas of Colombia (Puerto Carreño, La Macarena and Leticia), with problems of high energy costs as a consequence of their total dependence on fossil fuel. The study concluded that the implementation of the rural social biorefinery project is viable in one region (La Macarena), in which all the gasoline consumed has to be brought in from other regions, at very high cost. In contrast, in other regions, due to their proximity to other countries (Venezuela and Brazil) that guarantees a steady supply of gasoline at lower prices, the bio-ethanol produced in the rural social biorefinery would not be competitive (Figure 5).

Another study, conducted in Brazil (Rosado, 2009), evaluated the economic feasibility of establishing a small-scale biorefinery, with a specific focus on small rural properties. The viability of the operation of the distillery was analysed for both a cooperative system and an association type of organization. The operation of the biorefinery as part of a productive model within a large rural property was also simulated. The analysis considered two raw material options: sugar cane plus sweet sorghum, and sweet potato plus sweet sorghum.

The economic analysis was carried out through a cash flow simulation for a period of ten years, including the taxation element as appropriate for each case. Different levels



of funding of the project were also tested, with differentiated parameters for small and large properties. Parameters estimated included the Net Present Value (NPV) and the Internal Rate of Return (IRR). The biorefinery as a cooperative model was found the best option, with or without external financing, as compared with the associative model, mainly due to a lower tax regime for the cooperatives.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The use of co-products, by-products and effluents from bio-ethanol production as nutritional supplements in animal feed has been receiving increased attention in recent years, and although information about the technology options for treatment and use of these effluents is available, there are still some areas where more information, knowledge and research is required.

The huge volume of effluents generated in the biofuels processing operation is a major challenge. There is an urgent need to develop processing technologies that could reduce considerably the large volumes of effluents generated. In large-scale operations, with high capital investments, this problem could be reduced to a large extent by evaporation of the effluents.

In operations at smaller scale, with poor farmer groups and rural communities, this option is more difficult to implement because they generally lack the resources to invest in processes that demand high capital and energy costs, and a long time scale. Composting is one example of these options. Substantial capital investments are required, large areas need to be allocated, and a good composting process usually requires from 70 to 90 days. Therefore, it is very important to work on developing technologies that help to reduce the amount of water used in the production of the biofuel and, consequently, the volume of vinasse that is generated.

An area that needs to be strengthened, one that could help to improve the overall efficiency of the biofuel production process, is the conversion of vinasse into biogas, through an anaerobic fermentation process. The biogas generated could then be used in the distillery, helping to reduce energy costs. The residue could be used as fertilizer. Finding and developing new bacterial strains that could perform under the hard conditions and characteristics of the vinasse would be a major breakthrough for this process.

Another area, in which there is still a large gap in knowledge and information, is in the identification and validation of products that can act as flocculants and agglomerants of the organic load present in the vinasse. Up to now, the most common products in use are the biopolymers. CLAYUCA, UFRGS and SoilNet have had very good results using biopolymers. This has been the basis for the technologies developed for the formulation of the nutritional supplements described in this chapter. Although the cost of the

biopolymers is low (only 1.5 grams is required to prepare 1 kilogram of nutritional supplement, and only 2.4 percent of the costs of producing 1 ton of nutritional supplements is due to the biopolymers), the primary constraint is that the biopolymers are usually produced by multinational companies, and there could be some difficulties in importing and distributing them, especially if they are intended for use by small-scale, resource-poor farmer groups. Thus, there is a need to develop alternative products that would function in the same manner as the biopolymers, but that could be produced and distributed locally and thus be more easily purchased by small farmer groups.

Finally, there is a need to develop and refine technology protocols for the production of products with greater value-added, with good economic potential for use in the animal feed market. The use of biofuel co-products in the production of single-cell protein is one example of an emerging technology. CLAYUCA and UFRGS have already obtained promising results in pilot activities in which sugar cane-based vinasse has been used as a substrate to grow yeast (*Candida utilis*), with acceptable performance parameters. The biomass harvested from this process is the basis for an excellent yeast cream with high protein percentages, that could have multiple uses in animal feeding and industry. This is an exciting field that will probably grow very rapidly in the coming years.

CONCLUSIONS

The effluents and different products and co-products generated during the biofuel production process have very good potential as nutritional supplements in animal feed, especially for cattle. Co-product use in this way is an activity that helps to improve the overall economic efficiency of the biofuel production process and has positive impacts on the environment. Different technology options exist and their application to biofuel production enterprises is very easy, especially in large-scale, commercial operations with enough economic resources available for implementation.

Scenarios of biofuel production and use with small-scale farmer groups and rural communities, in which the RUSBI approach is applied, have been presented in this chapter. The technologies that are currently available for the management of the effluents through transforming them into nutritional supplements for animal feed (such as flocculation with biopolymers) need to take into account the specific context of the target groups, which usually have limited financial resources for investing, and with low educational levels so learning to handle and assimilate sophisticated processes and technologies takes time. The technologies offered have to be simple, efficient and sustainable.

The transformation of the effluents from biofuel processing into nutritional supplements for use in animal feed, especially cattle, could be a very important strategy

to promote social inclusion and more active participation of the farmers in the distribution of the benefits obtained in the biofuels value chain, helping them to improve the feeding systems of their animals, and to gain more control over their natural resources through a more sustainable management of the wastes and residues generated in the biofuel processing operation.

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Chapter 16

Scope for utilizing sugar cane bagasse as livestock feed – an Asian perspective

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ABSTRACT

Sugar cane is one of the important commercial crops grown in tropical regions, including Asia, and is emerging as major feedstock for bio-ethanol production. Bagasse, the fibrous residue after extraction of juice from sugar cane, is an important co-product, generated in large quantities and with the potential to be used as a roughage source for ruminants. Currently, a major part of bagasse is used as a source of fuel in the sugar and jaggery production process. It is also used as raw material in board or paper manufacture. Use of bagasse for livestock feeding is very limited due to poor nutritive value and palatability. The low nutritive value of bagasse is mainly due to its high lignin content and low protein, energy and mineral content. Considerable research has been carried out to improve utilization of bagasse in various production systems, and for productive functions in different livestock species. Because of its low nutrient density and fibrous nature, bagasse cannot be used as sole feed to fulfil animal nutrient requirements, or even for maintenance, and has to be supplemented with other, high quality feeds. There is a need for economic analysis of the use of processed bagasse as feed as comparative price advantage of feed use vs non-feed use would be a decisive factor. Cost of conventional cereal straw used for feeding ruminants (paddy, wheat or sorghum) will also influence use of bagasse for livestock feeding. Policy decisions, such as subsidizing biofuels and tax concessions for sugar mills generating power, are other factors that can have a major negative impact on the usage of bagasse as a feed resource.

INTRODUCTION

Use of various agricultural commodities as raw materials for biofuels has a major impact on the usage patterns, leading to changes in crop acreage and cropping patterns. This, at times, could lead to fuel-food and fuel-feed conflicts, affecting local food and feed security. Depleting fossil fuel reserves, environmental concerns and long-term sustainability are factors that favour the promotion of bio-fuel production. Aggressive promotion of biofuels through policy interventions would lead to increases in the area of crops that serve as biofuel feedstocks and disturb the balance between food and feed and other crops. Sugar cane is a crop with multiple utility. Besides sugar production, it is one of the important feedstocks for ethanol production. Efficient utilization of biofuel co-products can mitigate the impact of food-feed conflicts and add value to the biofuel value chain. This crop is a major feed and fodder resource in sugar cane growing areas through its co-products and integrates well with dairy production (Rangnekar, 1986). Sugar cane bagasse is another co-product available in large quantities and, in view of a fodder deficit situation in countries like India, there is need to consider ways of optimizing its use as feed. The present status of and prospects for use of

sugar cane bagasse as livestock feed in the Asian context is briefly reviewed in this chapter.

SUGAR CANE PRODUCTION AND CO-PRODUCTS

Sugar cane production trends over the last two decades (1990–2009) globally have shown that the area under sugar cane has expanded by 34 percent and production of sugar cane has increased by 53 percent. Brazil is the largest producer of sugar cane and India ranks next to Brazil in both area and production. Globally, Brazil has 33 percent of the area and 37 percent of production of sugar cane, while India has 21 percent of the area and accounts for 20 percent of global production. The Asian region has recorded an increase of 3 million hectare under cultivation and an increase in production of around 195 million tonne during the same period (1990–2009). The share of area and production of sugar cane in Asian region in the global context has remained in the range of 40 to 45 percent during the same period (FAOSTAT data). Within the Asian region, based on data for 2007–2009, India is the largest producer, accounting for about 50 percent of the region's output, followed by China (18%), Thailand (10%), Pakistan (9%), Indonesia (4%) and the Philippines (4%) (Table 1).

MAIN MESSAGES

- Bagasse is the fibrous co-product of the sugar processing industry, a major part of which is used as a fuel source in the sugar processing industry itself.
- The surplus bagasse available from sugar mills has the potential to be used as a roughage source in ruminants, with the major limitation on bagasse use being its low nutritive value, due to high fibre and low content of protein, energy and minerals.
- Using appropriate interventions – supplementation with limiting nutrients, treatment of bagasse, and a combination of the two approaches – will facilitate inclusion of bagasse up to 40 to 60% of the total diet to support various productive functions (milk, meat, maintenance and reproduction) in ruminants.
- Feed use versus non-feed use of bagasse would be dictated by relative economic advantage, and current usage and policies are in favour of non-feed uses.

TABLE 1
Production trends in major sugar cane producing countries of Asia (million tonne)

Country	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
India	299	296	297	287	234	237	281	356	348	285
China	69	78	92	92	91	88	93	114	125	116
Thailand	54	50	60	74	65	50	48	64	74	67
Pakistan	46	44	48	52	54	47	45	55	64	50
Indonesia	24	25	26	25	27	29	29	25	26	27
Philippines	24	22	21	24	26	23	24	22	27	23
Asia	554	552	585	596	537	514	562	680	703	606
World	1257	1261	1329	1372	1333	1313	1415	1611	1729	1661

Source: FAOSTAT data.

TABLE 2
Composition of sugar cane and co-products (on a percentage dry matter basis)

	CP	EE	CF	Total ash	NDF	ADF	Lignin	Reference
Whole sugar cane	6.0	2.1	30.6	4.7	49.6	32.5	8.4	Dhage <i>et al.</i> , 2009.
Bagasse	2.7	–	–	2.5	84.2	51.0	11.2	Krishnamoorthy, Singh and Kailas, 2005.
Bagasse	3.7	1.1	44.2	5.0	92.3	81.5	25.7	Nagalakshmi and Reddy, 2010.
Sugar cane tops	5.9	1.7	33.5	8.5	65.3	40.4	4.8	Naseeven, 1988.

Notes: CP = crude protein; EE = ether extract; CF = crude fibre; NDF = neutral-detergent fibre; ADF = acid-detergent fibre.

The major co-products of sugar cane are sugar cane tops, bagasse, molasses and filter mud, of which the first three are used as feed resources for livestock. The composition of the sugar cane and its co-products are shown in Table 2. Although no specific data are available regarding the usage pattern of these co-products, in most of the countries in South Asia, sugar cane tops are used as the main fodder for ruminants during the sugar cane harvesting season due to shortage of roughages. Even though molasses is a preferred feed resource, and there is a huge demand from the livestock sector, its availability for feeding livestock has always been a constraint, due to high demand for other industrial uses, chiefly for distilleries and export. FAO, through an expert consultation in 1986 reviewed available information related to use of sugar cane and co-products of the sugar industry for feeding livestock in sugar cane growing countries (FAO, 1988). The publication also provides information on alternative uses of sugar cane

and co-products of sugar industry (see Paturau, 1988, and Alexander, 1988).

In the Indian context, the potential uses of sugar cane co-products include use in the production of paper and boards, moulded products, rayon-grade pulp, electric power, biogas, ethanol, furfural, food additives, animal feeds, soil amendments and fertilizers (Yadav and Solomon, 2006). This chapter reviews work done on sugar cane bagasse as livestock feed in the Asian context, and, wherever relevant, includes work carried out elsewhere.

Sugar cane bagasse

Sugar cane bagasse is produced in large quantities at the crushing units, either small-scale units at village level or large-scale sugar factories, and is used as fuel for heating boilers or generating steam. Sugar factories with efficient boilers and cane juice processing machinery have surplus bagasse. Part of the surplus bagasse is sold as fuel and

TABLE 3
Sugar cane bagasse production in Asian countries (million tonne)

Country	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
India	46.6	56.7	66	64.9	70.1	58	72.5	79.4	81.8	91.7	84.6
China	24.3	23.4	22.5	20.9	28.4	34.1	33.3	29.8	30.7	40.9	47.0
Thailand	13.3	15.6	16.6	12.1	15.7	18.4	17.4	18.7	16.1	23.3	19.5
Pakistan	11.4	12.0	6.6	8.8	10.8	13.2	14.5	9.2	10.6	14.2	16.3

Source: United Nations Statistics Division

to the board and paper industry. The feeding of bagasse to livestock is very limited. It has, however, been successfully used as drought feed. Average bagasse production is around 30 percent of the cane crushed and it is mainly used as fuel in the sugar factories (Rangnekar, 1986). Bagasse production in major sugar cane producing countries in the Asian region is presented in Table 3. Shortage of feed resources and high cost of conventional feeds have necessitated exploring alternate feed resources, like bagasse, which are available in plenty at affordable prices.

Of late, considerable research is exploring the possibility of utilizing bagasse as raw material for second-generation biofuels and alternative uses. At the same time, research on bagasse for utilization as a feed resource is unfortunately declining. This is evident from the number of publications appearing on the subjects "Bagasse" and "Bagasse and feeding" in the Commonwealth Agricultural Bureaux International (CABI) Animal Production Database over the last four decades. The number of publications on various uses of bagasse increased from 350 in 1972–1991 to 397 in 1992–2010, indicating continuing interest in bagasse, while publications related to bagasse and livestock feeding fell from 167 to 115 in the same periods.

Bagasse contains more than 60 percent of its dry matter in the form of cellulose and hemicellulose, and its degradability in the rumen is very poor. High levels of lignin, low levels of soluble carbohydrates and the relative absence of both fermentable nitrogen and by-pass protein result in low nutritive values for crop residues (Preston and Leng, 1984; Hamad and El-Saied, 1982; Sundstol, 1988). Sugar cane bagasse contains around 50 percent cellulose, 27.9 percent hemicellulose, 9.8 percent lignin and 11.3 percent cell contents (Kewalramani *et al.*, 1988). Pith, a co-product of bagasse obtained from bagasse-based paper mills, is nutritionally better than the bagasse as it is devoid of lignified rind and so has better digestibility. The components of bagasse are in their natural, resistant conformation, and hence susceptibility to enzymatic hydrolysis is extremely limited (Rivers, 1988).

There are basically three approaches to improving the nutritive value of bagasse: pre-treatment, supplementation and a combination. Most of the studies use the combined approach. As the ruminal degradability of bagasse is very low, some form of pre-treatment may be essential to enable the rumen microbes to digest the complex carbohydrates

present and thus improve its degradability. Furthermore, as bagasse is low in energy, protein and minerals, it has to be supplemented to ensure optimum rumen fermentation, so as to fulfil the role of a basal diet. Studies conducted by researchers using different approaches, and their findings, are reviewed briefly. Although these approaches are discussed under separate headings, many of these involve a combination of approaches. In addition to the above approaches, Preston (1980) proposed fractionation of different components of sugar cane to ensure its optimal utilization, and a brief description of the approach is also given.

Fractionation of sugar cane

On observing the extremely low fermentability of sugar cane fibre in the rumen and the negative effect this has on voluntary intake of the overall diet, Preston (1980) developed a method for fractionating the juice and the residual fibre-sugar in the pressed stalk, so they can be treated as separate entities. The justification for this system is that the juice comprises soluble carbohydrates (sucrose, glucose and fructose) and is completely digestible by both ruminant and non-ruminant livestock and is thus a viable alternative for the starch in cereal grains. The sugar cane tops, and even the bagasse, may still contain appreciable amounts of sugars in the residual juice, and have a potential digestibility ranging between 50 and 60 percent. If adequately supplemented with fermentable nitrogen (urea or ammonia), these could have a nutritive value similar to Elephant grass. It can also be fed to small ruminants, which are able to select the sugar-rich pith, leaving the lignified rind as a source of fuel (Preston, 1988).

Pre-treatment of bagasse

It is well recognized that pre-treatment of the plant material is required to improve the nutritive value of lignocellulosic materials for livestock (Helmling *et al.*, 1989). The pre-treatment could be physical, chemical, biological or combinations thereof, which would result in significant changes in the structural characteristics of the lignocellulosic matrix, resulting in better contact of microbial enzymes with fibre for improved digestion (Rolz *et al.*, 1987). Of the various treatments, steam and alkali treatment have been most widely used by different researchers to improve the utilization of bagasse. Pre-treatment must meet the following requirements: (1) improve enzymatic hydrolysis, (2) avoid

degradation or loss of carbohydrate, (3) avoid formation of products inhibitory to the subsequent hydrolysis and fermentation processes, (4) improve palatability, and (5) be cost-effective (Ye and Cheng, 2002).

Steam treatment

Major reasons for using steam as pre-treatment for improving the nutritive value of bagasse is the ready availability of steam at sugar plants, which could be easily used with minimum investment and, as it does not involve use of any chemicals, it is likely to be safe. The steam pressure treatment completely modifies the hemicellulose fraction of raw bagasse, changing it into more soluble components, but does not affect the lignocellulose components (Wong *et al.*, 1974; Pate, 1982; Kling *et al.*, 1987). Replacement of maize silage with equal proportions of cubed hay and bagasse (steamed and pelleted with wood chips) resulted in similar energy intake, milk yield and protein content, but lowered milk fat and total solids in a bagasse-fed group of milch animals (Sekiguchi *et al.*, 1981). Steam treatment of bagasse was found to improve its digestibility and acceptability to animals due to changes in colour, smell and palatability (Rangnekar *et al.*, 1982, 1986). Rumen dry matter degradability in Zebu cattle determined by the nylon bag technique for untreated, steam ammoniated (NH_3 ; 3%) and steamed bagasse was found to be 17, 20 and 31 percent against 35 percent in the control that contained cotton wool (de la Cruz, 1990). Heat treatment in the presence of water (solvolysis) or aqueous orthophosphoric acid at 2.9 percent w/w (phosphorolysis) was also used to increase the nutritional value of sugar cane bagasse for cattle feeding (Fontana, Ramos and Deschamps, 1995).

Steaming of fresh bagasse at a pressure of 15 kg/cm² for 10 minutes and fed at 50 percent of dietary dry matter in wethers resulted in improved digestibility and was found equivalent to wild grass. The estimated total digestible nutrients (TDN) value of steam-treated bagasse was 48.7 percent (Tanabe and Kume, 2004). Ammonia pressurization at 1 g/g of bagasse in a reactor in liquid phase for 5 minutes at 50 percent bagasse moisture resulted in maximum solubilization of lignocellulosic contents, leading to enhanced value of bagasse as feed substrate in animals (Pernalet *et al.*, 2008). Steam-treated bagasse pith could replace 30 percent of the concentrate component of the diet (15 percent of total diet) without any negative effect on physiological and productivity parameters (body weight changes in ewes and lambs, milk composition, blood parameters) in pre- and post-lambing Lorie ewes, over a period of 120 days (Ebrahimi *et al.*, 2009).

Alkali or acid treatment

Alkali or acid treatment of lignocellulosic material has been quite widely used by different workers to improve the nutri-

tive value of fibrous feed stuff. Ensiling of green sorghum alone or with 20 percent wheat straw and poultry litter, or 20 percent bagasse with poultry litter, resulted in comparable dry matter, protein and fat digestibility between the animal groups fed three types of ensiled diets. The digestible crude protein (DCP) and TDN of sorghum silage, wheat straw and bagasse-added groups were 2.0, 60.1; 4.3, 45.3; and 6.1, 50.3 percent, respectively (Parthasarathy and Pradhan, 1982). Tudor and Inkerman (1986) reported an increase in organic matter digestibility *in vitro* from 28 to 63 percent in sugar cane bagasse with increasing concentrations of NaOH. Supplementation of black liquor, an effluent containing NaOH (10.5 g/litre) from the paper industry to bago-molasses and ensiling for 90 days (bagasse:molasses mixed in a 10:1 ratio and DM adjusted to 70%) resulted in higher digestibility of lignocellulosic materials in male buffalo calves (Prasad and Prasad, 1986). Nour and El-Tourky (1987) reported that treatment of bagasse or sugar cane pith with 5 percent NaOH and supplemented with cottonseed cake resulted in improvement in the intake and digestibility of nutrients, and better nutritive value of diets in Rahmany rams, compared with those fed untreated bagasse. Further, the productive performance of animals fed a pith-containing diet was better than bagasse-containing diets.

The response in Holstein bulls fed corn brewers grain-bagasse silage with alfalfa pellets versus concentrate with alfalfa pellets resulted in comparable growth rates, nutrient digestibility and carcass traits with economic advantage in bagasse-fed groups. However, there were differences in the blood parameters (Su and Yan, 1998a). The use of distillers corn brewers grain-bagasse silage with alfalfa pellets versus concentrate with alfalfa pellets in crossbred goats resulted in similar weight gains, nutrient digestibility, blood parameters and carcass traits, with economic advantage in the bagasse-fed group (Su and Yan, 1998b). The feed intake, digestibility of nutrients, carcass characteristics and blood parameters did not differ between the groups of yellow cattle fed either distillers rice grain with bagasse silage and concentrate or Pongola grass silage and concentrate, with the feed cost per kg of liveweight gain was more economic in the bagasse-based silage-fed group (Su and Yan, 2000). Odai *et al.* (2002) reported that bagasse silage can be kept for at least 90 days and then used as a source of roughage for dairy cattle during the dry season. A combination of 25 percent each of bagasse and rice straw and 50 percent brewer's grains can be used for fattening beef cattle. Yong and Zhou (2002) reported that treatment of bagasse with 5 or 7 percent hydrogen peroxide, urea + $\text{Ca}(\text{OH})_2$, or Urea + NaOH increased the degradation rate and fibre degradation index, while the treatment with urea alone could not achieve the same effect. Calcium hydroxide treatment at 8 percent of bagasse dry matter decreased the contents of NDF, ADF

and lignin by 23, 5 and 7 percent, respectively, while the *in vitro* digestibilities of DM and neutral-detergent fibre (NDF) increased by up to 60 percent. It was concluded that calcium hydroxide treatment can enhance the fermentation of sugar cane bagasse by rumen micro-organisms, and is most effective at 5.1–6.5 percent of dry bagasse (Guo and Meng, 2006). Nasuer, Chaudhry and Khan (2006), reported that urea treatment of bagasse should always include a source of urease to enhance the utilization of the crude protein content of the treated bagasse.

Studies in lactating buffaloes using four different roughage sources: (1) maize silage; (2) a mixture of sugar beet silage and sugar cane bagasse; (3) a mixture of sugar beet silage and wheat straw; and (4) a mixture of sugar beet silage, sugar cane bagasse and wheat straw, resulted in comparable milk yield, fat and solids-not-fat content. The cost of feeding for the group fed a mixture of sugar beet silage and sugar cane bagasse was found to be significantly lower than the other treatments on a 4 percent fat-corrected milk basis (Ebrahim, Reza and Hassan, 2008).

Biological treatment

While many studies have been conducted on the physical (steam) and chemical (acid or alkali) treatments of bagasse, there is little literature available on the biological treatment of bagasse, using lignolytic fungi through solid state fermentation. Bagasse is considered to be an ideal substrate for applications of microbial fermentations for the production of value-added products because of its rich organic content (Zadrazil and Puniya, 1996). Solid state fermentation with *Pleurotus sajur-caju* for 30 days in a chain of flasks resulted in significant improvement in digestibility of bagasse, from 45 to 63 percent (Puniya *et al.*, 1996). Biological treatment of bagasse with *Lentinula edodes*, a white rot fungus, for 12 weeks improved the *in vitro* organic matter digestibility from 45.6 to 68.6 percent (Okano *et al.*, 2006). Microbial fermentation of bagasse for 21 days, using chicken dropping (10 percent) improved its digestibility to the extent that it could be utilized as an alternative livestock feed (Anakalo, Abdul and Anakalo, 2009).

Pre-treatment of fibrous crop residues has been most widely studied and documented approach for improving the nutritive value, while physical, chemical and biological approaches, or a combination, have improved the digestibility of bagasse and pith. Treated bagasse or pith in most of the reports had a positive effect on the digestibility and production response in different species. Treated bagasse can be used to replace the conventional feed resources, augmenting other locally available feed resources, and can also be used to cut down feeding costs as bagasse is usually cheaper than other feed resources. Up-scaling of treatments to commercial scale, and the cost efficiency of these approaches, are the major factors

in determining the practical application of the treatment approaches in utilizing bagasse. Studies on these aspects are virtually non-existent.

Supplementation of bagasse

Sugar cane bagasse can only provide a basal diet and it has to be supplemented with other, high quality feed resources to maintain and promote desired levels of production (milk, meat, draught, reproduction). The nature and quantity of supplements would be determined by a number of factors; of these, the level of production, nature of the supplement, cost of the supplement and produce value are important parameters. Of all the supplements, urea and molasses have been tried most extensively due to ready availability of molasses at sugar plants and low cost of urea. Kaushal, Kochar and Chopra (1972) observed that the soluble carbohydrate contents of factory bagasse did not supply sufficient energy for proper utilization of the urea in Sahiwal calves. Increasing levels (5 to 40% of diet) of alkali-treated bagasse (treated with 4 or 6% urea) together with molasses, resulted in decreased dry matter digestibility in sheep, with 4 percent alkali treatment found to be a better level. Furthermore, between sheep and goats fed 20 and 40 percent bagasse, goats were found to be able to digest significantly more fibre than sheep (Devendra, 1979)

Enriched bagasse with urea (2%) and molasses (20%) with or without alkali (4%) fed *ad libitum* to crossbred bulls with limited concentrate resulted in similar feed intake and digestibility of dry matter, protein and fat (Vaidya, Reddy and Mohan, 1981). Alkali treatment of bagacillo (the short fibre of sugar cane bagasse) at 6 percent NaOH with 20 percent moisture in the finished product, when fed with molasses resulted in higher weight gain, fibre digestibility and increased nitrogen retention than in the untreated bagacillo fed lambs. The superior performance of the lambs on the treated bagasse diet was attributed to its higher palatability (Chicco *et al.*, 1983). Crossbred bulls fed sugar cane bagasse-based complete feeds consisting of 5 kg of green maize and molasses (1–2 kg/day) over a period of 5 months resulted in satisfactory semen production and sperm concentration (Bhosrekar *et al.*, 1988). Use of pith as a “Molasses urea and pith” mixture in cattle diets up to 30 percent of the concentrate, replacing coconut cake totally, resulted in comparable quality and palatability of feed, body weight gain and feed efficiency. It was concluded that pith used as a “Molasses urea pith” mixture can substitute for coconut meal as a protein source in the concentrate for beef cattle (Wardhani *et al.*, 1985). Based on the series of experiments conducted in Taiwan over a period of 10 years, Wang (1986) concluded that feed cost can be reduced by utilizing sugar co-products such as cane top, bagasse, bagasse pith, molasses and processed sugar co-products

Huang *et al.* (1993) reported that bulls fed a diet containing 34 percent sugar cane bagasse, together with concentrates, wherein soybean oil soap stock partially replaced cane molasses, over a period of 97 days could result in daily weight gains of around 1 kg. Sugar cane bagasse supplemented with 15 percent molasses and urea or poultry manure was as good as grass hay in crossbred goats fed 1 kg concentrate daily in supporting milk production and body weights over a period of 90 days (Sanchez and Garcia, 1994). Bagasse and sawdust-based poultry litter can replace up to 30 percent nitrogen in conventional concentrate mixture given with wheat straw to maintain adult crossbred cattle and Murrah buffaloes (Parthasarathy and Pradhan, 1994).

Reddy, Reddy and Nagalakshmi (2001) reported that sugar cane bagasse can be used as a sole roughage source at 40 percent of the diet containing 60 percent concentrate and converted into total mixed rations in pelleted as well as in mash form. As a total mixed ration diet, the digestibility was significantly improved compared with conventional diets containing 40 percent bagasse. Haque and Rahman (2002) reported that bagasse supplemented with 2 percent urea vs a group fed urea-molasses-straw resulted in lower feed intake and significantly lower digestibility, but had no significant effect on daily weight gains in indigenous bulls.

Supplementation of yeast in pelleted sugar cane bagasse feed in fattening sheep significantly improved the average daily gain (ADG) without affecting the dry matter intake (DMI), blood profile or carcass characteristics (Monjeghtapeh and Kafilzadeh, 2008). An economic analysis conducted by Cabello, Torres and Almazan (2008) to compare the economic viability for milk production of a diet based on bagasse, revealed that the net value of bagasse was in the range of US\$ 20–30/tonne, being lower than the net value of bagasse for electricity generation at sugar mills. Similarly, the calculations revealed that blackstrap molasses gives negative revenue when used for fattening cattle in comparison with its export price for ethanol production.

The success of supplementation strategies are mainly dependent on the volume and price structure of the supplements to support a given level of production, besides the quality of the basal roughage. Low nutrient density and digestibility of bagasse necessitates a reasonably good level of concentrate supplements to support various productive functions in livestock. Besides the supplement need, the form of feed, e.g. total mixed ration in the form of feed blocks or complete feed mash, can improve the nutrient utilization, as evident from some of the above studies. Furthermore, using locally available supplements, such as sugar cane tops or molasses, could make the feeding economic and promote the use of bagasse.

Further studies on responses in different categories of livestock fed untreated or processed bagasse are summarized in Table 4.

From the findings reported by different workers (Table 4) on the responses recorded from livestock fed treated and untreated bagasse, certain generalizations can be made. First, bagasse is a low quality roughage and it cannot be fed as sole diet to ruminants and must be supplemented with nitrogen, energy and minerals to sustain the animals. Second, the proportion of bagasse and supplements are dictated by production levels. In low and medium producers, bagasse can be fed up to 40–60% of the diet, provided the concentrate supplement is balanced properly to fulfil the animals requirements. Finally, a balanced bagasse-based diet can probably reduce the cost of feeding for milk and meat production, particularly when straw prices are high.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The bulky and fibrous nature of bagasse makes it a poor roughage source and most of the times it has to be used locally. Its efficient use is directly linked to quality, cost and local availability of other feed supplements. Keeping in view the availability of feed resources and the production levels of animals in a particular area, there is a need to develop region-specific feeding regimens for different productive functions, integrating the sugar cane co-products (sugar cane tops, bagasse and molasses) with locally-available resources, for optimizing livestock production.

Furthermore, in view of the ongoing research activities on second-generation biofuels, where the use of complex carbohydrates trapped in crop residues are used as sources of ethanol production through appropriate pre-treatments, one can only hope that such studies may provide a lead to newer approaches for effective delignification of bagasse to improve its feeding value. The current need is for economic analysis and feasibility studies of the options for using sugar cane bagasse (treatments, supplementation, complete feed, etc.) for feeding livestock vs biofuel and non-feed uses. This should be undertaken through pilot projects, through field size operations and not laboratory-scale experiments, under various circumstances, to better understand the feasibility of various approaches (processing, supplementation) to using bagasse for livestock feeding.

CONCLUSIONS

Considerable information is already available on the nutritive value of bagasse and the different approaches that have been adopted to improve its nutritional quality. Thus, the use of bagasse with different supplements for various productive functions in several species has been well documented. In general, the treated bagasse can be safely used up to 30–40 percent in ruminant diets to support a medium

TABLE 4
Summary of reported responses in different categories of livestock fed untreated or processed bagasse

Treatment	Species and response	Remarks	Reference
Untreated bagasse/pith			
Bagasse with molasses mixture at 10, 20 and 30% replacing maize	Pigs (local and exotic breeds)	ADG, DMI and FCR were significantly lower in pigs given 30% mixture.	Reddy <i>et al.</i> , 1985.
Untreated bagasse (UB)	Bulls fed complete feed blocks containing 40% wheat straw or UB	Rumen fermentation in sugar cane bagasse fed diet was comparable to wheat straw based diet	Hozhabri and Singhal, 2006.
Complete feeds (i) 30% Untreated bagasse + 70% unconventional concentrates (ii) 30% wheat straw + 70% concentrate	Crossbred calves fed for 40 weeks. DMI and ADG were comparable in both groups	Complete feed with 30% sugar cane bagasse and non-conventional feeds was economical	Pandya <i>et al.</i> , 2009.
Complete feeds (i) 40% wheat straw + 60% concentrate (ii) 40% untreated bagasse + 60% concentrate	Crossbred calves fed for 4 months	DMI, ADG and FCR were comparable between the groups, with bagasse diets being economical	Fardin and Singhal, 2009.
Steam treated bagasse and pith			
Steam pressure treated bagasse	Milch cows fed for 28 days, replacing 18–32% in complete diets	Greatly depressed DMI, milk yield and milk fat content	Horn <i>et al.</i> , 1984.
Steaming of bagasse at 170–195 °C for 60 minutes. + NaOH addition @ 5% on DM basis	Improved the DM digestibility from 27–30% to 52% in sheep Further improved the digestibility to 65–66% in sheep	Palatability was impaired	Ali, 1991.
Steam treated bagasse supplemented with legumes, urea molasses, rice bran and poultry litter	Crossbred bulls fed for 141 days	Steam-treated bagasse was well consumed and ADG varied from 0.57 to 0.75 kg	Héctor, 1990.
Steam treatment of bagasse followed by anhydrous ammonia treatment (3% by weight) for 15 days, plus supplements.	Crossbred bulls fed for 169 days	ADG in bulls fed steam treated bagasse (0.64 to 0.54 g) was significantly higher than the steam-ammonia treated bagasse (0.30 g)	Héctor, 1990.
Steam treated pith (STP)	Arabi lambs fed for 70 days. STP constituted 0, 11, 22 and 33% of diet and replaced barley at 0, 25, 50 and 75%, respectively	DMI and ADG did not differ. FCR was significantly lower at 33% of STP. STP at 11% level had the best economic efficiency	Ensiyeh, Najafgholi and Hamideh, 2009.
Chemical treatments			
(i) Complete diets containing 40% alkali treated (2% NaOH) bagasse (ATB) (ii) Complete diets containing 40% untreated bagasse (UB) (iii) Control: pasture + concentrates @ 360 g/liter milk	Lactating cows fed for 300 days Feed consumption- (i) 16.5 kg, (ii) 14.2 kg and (iii) 6 kg concentrate + pasture. Milk production kg – (i) 17.2 (ii) 12.5 and (iii) 16.5	UB resulted in significant drop in milk production. ATB was comparable to controls in milk production but it resulted in significant drop in milk fat and total solids %	Randel <i>et al.</i> , 1972.
(i) urea-molasses enriched sugar cane bagasse (ii) (i) with alkali treatment (iii) 4 kg green + paddy straw	Crossbred heifers fed for 61 days All three roughages were fed ad libitum with 2 kg concentrate	ADG g (i) 158 (ii) 55 & (iii) 356 Urea-molasses enriched bagasse, without or with alkali treatment was not suitable as the only source of roughage.	Reddy, Mohan and Das, 1981.
Untreated and alkali-treated (5% NaOH solution) bagasse	Awassi lambs 25, 40 and 50% untreated and alkali-treated bagasse	Treatment had no significant effect on ADG and FCR NaOH treatment appeared mainly to increase its palatability leading to higher ADG	Al-Tawash and Alwash, 1983.
Steam and alkali treated bagasse (ATB)	Dohne Merino lambs fed to appetite for 56 days. ATB improved ADG and FCR at 19 and 40% inclusion levels.	Steam treatment improved performance at lower inclusion level, while at higher levels it had a negative effect.	Jacobs and van Niekerk, 1985.
(i) Spray drying of fresh bagasse with NaOH solution (30%) containing 5% NaOH of dry fibre (TAB). (ii) Supplementation of (i) with molasses (20: 40 w:w) and urea (1.5 to 2.0%) (iii) supplementation of (ii) with cotton seed	Increased IVDMD from 30 to 55% Maintained weaner cattle Daily gain up to 0.7 kg in growing cattle	TAB can be stored up to 6 months without problem. No health problems associated with the feeding TAB-based diets provided the concentration of NaOH does not exceed 5% on dry fibre.	Tudor and Inkerman, 1989.
Raw bagasse pith (RBP) and urea ammoniated bagasse pith (UABP) (4% urea, 40% moisture and 21 days treatment)	Crossbred bulls fed for 28 days Complete feeds having 50 : 50 roughage and concentrate	RBP was inferior to wheat straw and UABP was superior to wheat straw based diets	Singh <i>et al.</i> , 2004.

TABLE 4 (Cont'd)

Treatment	Species and response	Remarks	Reference
Complete feed pellets containing 50% concentrate and (i) Urea-ammoniated bagasse (UAB) 50%, or (ii) Tree leaves 50%	Goat kids fed for 90 days. ADG – (i) 68.7 g and (ii) 44.1 g FCR – (i) 8.6 and (ii) 10.8	UAB Improved performance, rumen fermentation and blood biochemical characteristics	Dhore <i>et al.</i> , 2006.
Complete feed containing 60% concentrate, 20% wheat straw and 20% Urea ammoniated bagasse –UAB	Crossbred bulls fed for 30 days	No adverse affects	Tiwari, Garg and Singh, 2006.
Concentrate 500 g/day + <i>ad libitum</i> 1% urea treated: (i) wheat straw (control) (ii) sugar cane tops (T1) (iii) bagasse (T2)	Said rams were fed for 90 days and used for breeding. ADG, testicular size, scrotal circumference and semen characteristics increased significantly in T1 and T2.	Pregnancy rates in groups (i), (ii) and (iii) were 74.1, 86.7 and 81.5%, respectively, suggesting that urea-treated sugar cane tops and bagasse was better than wheat straw.	Megahed and Etman, 2006.
Urea fortified bagasse pith + sugar cane bagasse with 15% molasses	Holstein lactating cows fed for 75 days, replacing 0, 40, 50, 60 or 70% of alfalfa	Milk yields in 0, 40, 50, 60 and 70% replacement were 15.3, 14.5, 14.4, 14.1 and 13.4 kg milk/day. Feed cost at 60% replacement was most economical.	Ahmad, 2009.
Biological treatment			
Acid/grinding/enzymatic hydrolysis followed by culturing of <i>Geotrichum candidum</i> or <i>Oidium lactis</i> of bagacillo(1)	Fish. Feeding the granulated product replacing 60% of concentrate	Improved the WG and FCR in grass carp	Yu, 1990.
Solid state fermentation using ligninolytic white-rot fungus, <i>Lentinus edodes</i>	Sheep Degradation of lignin was 34.4%,	Increase of 34.3% digestibility in sheep and the material was free from toxins	Pham and Ramirez, 1996.
Basal diet of Bermuda hay supplemented with <i>ad libitum</i> (i) Fermented bagasse feed - Solid state fermentation of bagasse with wheat bran (w/w) in 1:3 using <i>Aspergillus sojae</i> (ii) Lucerne hay cubes	Crossbred bucks fed for 196 days WG, DMI, FCR, DCP and TDN intakes were comparable to groups fed Lucerne hay. Sensory attributes of meat were superior in bagasse-fed group	Fermented bagasse feed could be an alternative to Lucerne hay cube and thereby reduce the feeding cost	Ramli <i>et al.</i> , 2005.
Combination of approaches			
(i) Untreated bagasse + GNC	Bull calves fed for 51 days	(i) DMI: 1.9 kg, ADG: 124 g/day and DM digestibility: 53.8%	Joshi <i>et al.</i> , 1984.
(ii) Steam-treated bagasse (7 kg/cm ²) + GNC		(ii) DMI: 4.2 kg, ADG: 385 g/day and DM digestibility-60.0%	
(iii) Alkali-treated bagasse (4% NaOH – 1 litre/kg) + GNC		(iii) DMI: 2.3 kg, ADG: 182g/day and DM digestibility: 62.8%	

Notes: (1) Bagacillo is the waste from paper manufacture using sugar cane bagasse. ADG = Average daily gain; DMI = Dry matter intake; FCR = Feed conversion ratio; UB = Untreated bagasse; UAB = Urea-ammoniated bagasse; STP = Steam-treated pith; ATB = Alkali-treated bagasse; WG = Weight gain; DCP = Digestible crude protein; TDN = Total digestible nutrients; GNC = Groundnut cake; RBP = Raw bagasse pith; UABP = Urea-ammoniated bagasse pith; TAB = Treated alkaline bagasse.

level of production. With better quality supplements or processing, the level of bagasse in the diet of low producers could be increased, even up to 60%. Steam treatment, alkali treatment and supplementation with urea, molasses and locally available concentrate sources have been quite effective in improving the utilization of bagasse as ruminant feed. Bagasse as such is not harmful, but steam treatment in the presence of certain chemicals, especially alkali at higher levels, can induce certain changes that may prove harmful to animals. So one has to be careful in combining steam treatment of bagasse with other chemical treatments. However, most of the bagasse generated at sugar processing units at present continues to be primarily used for fuel purposes, and the practice of feeding bagasse to livestock is very limited and at times only seasonal. The ongoing "livestock revolution" of greater demand for livestock products, resulting in greater demand for feed resources, and thus increasing the cost of feed resources,

both roughages and concentrates, are some of the factors that could have a positive impact on the use of bagasse for livestock feed. National policies favouring energy security, leading to greater emphasis on biofuels, and providing tax incentives and subsidies to the energy sector could favour the diversion of this potential feed resource, namely bagasse, to non-feed uses.

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Chapter 17

Camelina sativa in poultry diets: opportunities and challenges

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ABSTRACT

Feed represents over 65 percent of the cost for poultry production. Fast-growing and high-producing poultry are fed high calorie and high protein maize-soybean-based diets. Considering the high demand for maize and other oil crops for biofuel production, finding alternative sources of energy could reduce production costs. *Camelina sativa* is an oilseed crop of the Brassica family that is emerging as an important biofuel crop. Nutrient composition of camelina meal indicates that the meal has 36–40 percent crude protein, 11–12 percent fat, and 4600 Kcal/kg gross energy. The fat in camelina meal is rich in α -linolenic acid (~30 percent), the parent fatty acid of the health-promoting omega-3 family, and γ -tocopherol, an antioxidant vitamin. In addition, camelina contains other bio-active compounds such as flavonoids and phenolic products. Therefore, incorporating camelina in poultry diets will: (1) provide energy and protein to the birds, (2) provide health-promoting omega-3 fatty acids and tocopherol-rich-foods to humans, (3) improve the antioxidant activity and lipid stability of poultry products, and (4) increase the market value of the crop. Feeding trials aimed at evaluating the optimum amounts of camelina meal in feed for meat-type broilers and egg laying hens were conducted. Special emphasis was given to omega-3 fatty acid and tocopherol incorporation in meat and eggs, and thus developing value-added functional poultry foods. The results obtained were: (1) camelina meal could be incorporated into broiler and layer rations at 10 percent without affecting bird performance and meat or egg quality; (2) feeding camelina meal led to over 3-fold increase in omega-3 fatty acids in chicken meat and 8-fold increase in eggs; (3) incorporation of 10 percent camelina meal led to 2.5- to 3.2-fold reduction in the omega-6:omega-3 ratio in meat and eggs; and (4) inclusion of camelina meal at 5 and 10 percent led to significant reduction in lipid oxidation products and an enhancement in γ -tocopherol and antioxidant activity in the dark meat. Investigating factors that can enhance the feeding value as well as the health-promoting and antioxidant properties of camelina will provide greater potential for developing camelina-based functional feeds and value-added wholesome poultry foods for human consumption.

INTRODUCTION

Camelina sativa or false flax (“gold of pleasure”) is an oilseed crop of the Brassica (Cruciferae) family. The crop can be grown on marginal farmland, with relatively low inputs and no irrigation. Although camelina has been cultivated in Europe for over 2000 years for oil and livestock fodder, the crop has gained increased popularity recently as a biofuel source due to its oil content (Putnam *et al.*, 1993). Camelina is not a food crop; however, co-product (i.e. meal) obtained after oil extraction from the seed is valuable as animal feed (Pilgeram *et al.*, 2007). To use camelina meal as a potential animal feed requires information on its chemical composition, nutritive value, digestibility and product quality aspects. In this context, studies on using camelina in the diet of beef heifers (Moriel *et al.*, 2011), dairy cows (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau *et al.*, 2011) and ewes (Szumacher-Strabel *et al.*, 2011) have been reported. In the current chapter, opportunities

and challenges associated with using camelina meal in the diets of meat-type broilers and egg-laying birds are discussed.

CAMELINA SATIVA MEAL: CHEMICAL COMPOSITION AND NUTRITIONAL VALUE

Camelina sativa is an oilseed producing plant. Camelina seeds contain over 38.9 percent fat, 30 percent α -linolenic acid (18:3 n-3) (an omega-3 fatty acid), and 25.8 percent crude protein. Due to the high oil content, omega-3 fatty acid and crude protein, finding alternative use of camelina meal (a co-product obtained from camelina seed after oil extraction) in animal diets will increase the market value of the crop. The nutrient profile of camelina meal is shown in Table 1. Cold-pressed camelina meal contains 35–40 percent crude protein, 6–12 percent fat, 6–7 percent ash, and 41 percent neutral-detergent fibre. The gross energy is 4600–4800 kcal/kg. The oil content, fatty acid composi-

MAIN MESSAGES

- Camelina meal is rich in protein, fat and essential n-3 and n-6 fatty acids, and could be incorporated into poultry rations as a source of energy, protein and essential n-3 and n-6 fatty acids.
- Feeding camelina meal up to 10 percent of the diet did not affect growth performance and feed consumption, nor meat and egg quality.
- Feeding 10 percent camelina meal led to increases in health-promoting omega-3 fatty acids of over 3-fold in chicken meat and 8-fold in eggs.
- Camelina meal at 10 percent led to significant reduction in lipid oxidation products and an improvement in antioxidant activity in the dark meat.
- Consuming two large eggs from hens fed 10 percent camelina meal could provide over 300 mg omega-3 fatty acids to the average human diet.

tion and other nutrient profiles of the meal can vary due to cultivar, season, processing method and other agronomic factors (e.g. soil type).

The fatty acid composition of the camelina meal has received considerable attention due to its high content of essential fatty acids. The meal is rich in omega-3 and omega-6 essential fatty acids (also known as n-3 and n-6 fatty acids). The omega-6:omega-3 fatty acid ratio is 0.90 to 0.70. α -Linolenic acid (18:3 n-3) is the major omega-3 fatty acid, constituting over 29 percent, with linoleic acid (18:2 n-6) constituting up to 23 percent. Oleic acid is the major mono-unsaturated fatty acid, followed by eicosenoic acid (20:1). Other mono-unsaturated fatty acids include palmitoleic (16:1) and erucic acids (22:1, <2 percent). Altogether, total mono-unsaturated fatty acid constitutes over 32 percent. Saturated fatty acids in the meal include palmitic acid (16:0, 9 percent) and stearic acid (18:0, 2.5 percent). Fat-soluble vitamin (tocopherols) content of the meal is over 200 μ g/g. The protein of camelina meal contains several essential amino acids, such as threonine, glycine, methionine, valine, isoleucine, leucine, lysine and phenylalanine. Lysine and methionine are usually the first-limiting acids in poultry nutrition, which makes camelina meal a potential source of protein for poultry. Among the minerals in camelina meal, potassium is the major mineral, followed by sulphur, phosphorus, magnesium and calcium. Camelina meal also contains other phenolic compounds and glucosinolates. The high protein, energy, omega-3 and omega-6 fatty acid and essential amino acid content of camelina meal makes it a potentially suitable source of plant protein, essential fatty acids and amino acids for use in poultry rations.

FEEDING CAMELINA MEAL TO POULTRY

Feed represents the major cost for food animal production. Therefore, development of non-traditional, low-cost feed sources may reduce production costs. High producing and fast growing poultry (egg layers and meat-type) are fed

high calorie (2800–3200 kcal/kg/day) and high protein diets (16–22 percent crude protein). In the United States, poultry diets are based on maize and soybean meal. Considering the high demand for maize and other oil crops for biofuel production, there has been great interest in finding alternative sources of energy and protein to reduce poultry production costs. In this respect, camelina meal has attracted attention from nutritionists due to its gross energy and crude protein content. Recently, studies were conducted with meat-type broiler chickens, egg layers or turkeys on feeding camelina meal, and results are summarized in Table 2. These studies were aimed at (1) finding the optimum levels that can be incorporated into the ration without affecting production performances, (2) assessing product quality, and (3) testing the efficacy of camelina meal in enriching the meat or eggs with omega-3 fatty acids.

Effect on production performance of feeding *Camelina sativa* meal to broiler chickens

The effect of feeding camelina meal to broiler birds led to conflicting results in growth performance (Table 2). Aziza, Quezada and Cherian (2010a) fed 2.5, 5 and 10 percent camelina meal to broiler birds and these authors reported no difference in 42-day body weight gain, carcass weight or feed efficiency (gain:weight) when compared with maize-soybean-based control diet-fed birds. However, Ryhanen *et al.* (2007) and Pekel *et al.* (2009) reported that feeding 10 percent camelina meal or expeller cake to broiler chickens impaired the growth between 15 and 37 days of age, decreased feed intake during the starter phase, impaired feed efficiency and reduced the final body weight by 7–10 percent compared with the control group. Studies on feeding camelina meal to turkey hens by Frame, and Petersen (2007) reported no significant differences in final weight, weight gain or feed conversion when 10 percent camelina meal was included in the diet from 9 weeks through to 13.5 weeks of age. The discrepancies in growth performance of birds fed diets containing camelina meal

TABLE 1
The nutrient profile of camelina meal

Parameter	Value
Gross energy	4755 kcal/kg
Crude protein	36.2 %
Crude fibre	8.4 %
Ash	6.5 %
Neutral-detergent fibre	41.8 %
α -Tocopherol	5.2 μ g/g
γ -Tocopherol	201.7 μ g/g
Phenolics	4006 μ g/g
Flavonoids	21.2 mg/g
Glucosinolates	21.4 μ mol/g
Minerals	ppm
P	10 214
K	13 204
Ca	2 703
Mg	4 696
S	9 122
Na	15.4
Fe	151
Mn	25.1
Zn	61.1
Cu	9.18
Al	5.37
Amino Acid	%
Aspartic acid	2.84
Threonine	1.34
Serine	1.36
Glutamic acid	5.50
Glycine	1.82
Alanine	1.60
Valine	1.89
Isoleucine	1.38
Leucine	2.32
Tyrosine	0.82
Phenylalanine	1.47
Lysine	1.77
Histidine	0.84
Arginine	2.16
Tryptophan	0.43
Methionine	0.92
Cystine	0.95
Fatty Acid	%
Palmitic (16:0)	8.29
Palmitoleic (16:1)	0.25
Stearic (18:0)	2.38
Oleic acid (18:1)	20.33
Linoleic (18:2n-6)	23.87
α -Linolenic (18:3n-3)	29.48
Eicosenoic (20:1)	10.67
Eicosadienoic (20:2n-6)	1.52
Eicosatrienoic (20:3n-6)	1.05
Erucic (22:1)	1.75

Notes: Values are indicative, subject to variation due to differences in batch, cultivar, soil type or processing method used. Amino acid values are expressed as g per 100 g sample (as-is basis). Fatty acid values are reported as percent of fatty acid methyl esters.

Sources: Adapted from Aziza, Quezada and Cherian, 2010a, b; Cherian, Campbell and Parker, 2009.

could be due to the availability of nutrients in the meal. Camelina belongs to the Brassica family, which is high in non-starch polysaccharides and glucosinolates that can affect feed consumption and growth performance of broiler chickens (Budin, Breene and Putnam, 1995). In addition, phenolic compounds such as phenolic acids and tannins that are present in the Brassica family, soil type, bird age and meal preparation methods can affect digestibility, leading to discrepancies in reported results. Pekel *et al.* (2009) reported that addition of copper (150 mg/kg) enhanced feed consumption and body weight of birds fed camelina meal. The beneficial effect of Cu may be due to its ability to alleviate the negative effects of glucosinolates present in the meal. Although glucosinolates themselves show no toxic effects on animals, the breakdown products of glucosinolates can form toxins by the endogenous plant enzyme myrosinase or can influence gut microflora, affecting growth and feed efficiency (Schuster and Friedt, 1998). These factors should be taken into consideration when evaluating results from feeding camelina meal to broiler birds.

Effect on production performance of feeding *Camelina sativa* meal to egg laying hens

Oil seeds and oilseed meals are incorporated into laying hen rations as a source of energy, crude protein and essential omega-3 fatty acids. In this respect, much work on feeding flax seed to laying birds for omega-3 egg production has been well documented (Cherian, 2008). Typically, oil seeds or their meals are restricted to less than 10 percent of the rations (Bean and Leeson, 2003). Lipid quantity and type of fatty acids in oil seeds in the laying hen diet can significantly affect the content of fatty acids, fat soluble vitamins and pigments in the egg yolk. The alteration of egg lipid nutrient profile is due to the fact that chickens are monogastric (single stomach) animals and that there is a high turnover of lipids in laying hens, causing egg lipid to mirror dietary fats. This has led to the successful production and marketing of omega-3 fatty acid- and vitamin-modified specialty eggs worldwide (Cherian, 2009). Considering the high content of α -linolenic acid in camelina meal, studies were conducted to test the efficacy of the meal in enriching eggs with omega-3 fatty acids. Feeding trials conducted in our laboratory showed that inclusion of camelina meal at over 10 percent of the ration can affect egg production, feed consumption and egg yolk weight. When the meal was included at 5, 10 and 15 percent of the ration, it was observed that hen-day egg production ([total number of eggs produced/total number of hens \times number of days on test diet] \times 100), was lowest for the 15 percent inclusion level (Cherian, Campbell and Parker, 2009) (Table 2). Yolk weight as a percentage of egg weight was lower for the 10 and 15 percent inclusion levels. However, decrease in

TABLE 2
Summary of studies investigating the effect of camelina meal in poultry

References	Bird type	Salient findings
Acamovic <i>et al.</i> , 1999.	Broiler	Nutritional evaluation of the meal done in broiler birds by precision method (tube feeding) reported reduced digestibility coefficient for nitrogen and dry matter.
Aziza, Quezada and Cherian, 2010a.	Broiler	Feeding camelina meal at 10% led to over 2.5-fold increases in omega-3 fatty acid of white and dark meat. No difference in final body weight at 10% inclusion.
Aziza, Quezada and Cherian, 2010b.	Broiler	Feeding 10% camelina meal led to: (1) a >1.5-fold increase in γ -tocopherols in the thigh meat; (2) increase in thigh meat antioxidant activity; and (3) reductions in thiobarbituric acid reactive substances in the meat during storage and cooking.
Cherian, Campbell and Parker, 2009.	Layer	Feeding camelina meal at 10% led to over 8-fold increase in omega-3 egg fatty acids. Camelina meal at low levels (5 and 10%) did not lead to any changes in egg production or egg quality. Addition of camelina at higher levels (15%) led to reductions in egg production, yolk fat and yolk size. No effect on egg weight.
Frame and Petersen, 2007.	Turkey	Camelina meal could be included in turkey diets up to 5%.
Pekel <i>et al.</i> , 2009.	Broiler	10% camelina meal reduced body weight when fed during the first 3 weeks of life.
Pilgeram <i>et al.</i> , 2007.	Layer	Fed 15% camelina meal containing diets to layer birds. No adverse effect on bird health or egg production. Over 140 mg of α -linolenic acid reported in eggs.
Rokka <i>et al.</i> , 2002.	Layer	No effect of feeding camelina seed oil on the sensory attributes of chicken eggs.
Ryhanen <i>et al.</i> , 2007.	Broiler	Inclusion of 5 or 10% <i>Camelina sativa</i> expeller cake reduced feed intake and feed conversion ratio during starter phase. Feeding the meal had no effect on meat sensory quality aspects.

TABLE 3
The systematic and trivial names of different omega-6 and omega-3 fatty acids in white meat and egg and their shorthand notation and concentrations

Systematic name	Common name	Shorthand notation	Content (%)	
			White Meat	Egg
Omega-6 Fatty Acids				
all-cis-9,12-octadecadienoic	Linoleic acid	18:2 n-6	17.5	9.3
all-cis-6,9,12-octadecatrienoic	γ -Linolenic acid	18:3 n-6	0.7	0.1
all-cis-8,11,14-eicosatrienoic	Dihomo- γ -linolenic acid	20:3 n-6	0.9	0.1
all-cis-5,8,11,14-eicosatetraenoic	Arachidonic acid	20:4 n-6	3.2	1.5
all-cis-7,10,13,16-docosatetraenoic	Adrenic acid	22:4 n-6	0.7	0.1
all-cis-7,10,13,16,19-docosapentaenoic	Docosapentaenoic acid	22:5 n-6	0.3	0.2
Omega-3 Fatty Acids				
all-cis-9,12,15-octadecatrienoic	α -Linolenic acid	18:3 n-3	1.1	0.2
all-cis-5,8,11,14,17-eicosapentaenoic	Eicosapentaenoic acid	20:5 n-3	0.2	0.0
all-cis-7,10,13,16,19-docosapentaenoic	Docosapentaenoic acid	22:5 n-3	0.6	0.1
all-cis-4,7,10,13,16,19-docosahexaenoic	Docosahexaenoic acid	22:6 n-3	0.7	0.6

Notes: Values are reported as a percentage of fatty acid methyl esters and are subject to variation reflecting bird diet, age or strain.

yolk weight was not associated with egg weight. No difference was found in egg weight, albumen weight, albumen height, shell weight or shell thickness due to camelina meal feeding at any level. Minimal effects were noted to yolk colour due to feeding camelina meal to laying hens.

Effect on meat and egg lipid composition of feeding *Camelina sativa* meal

Birds have a high capacity for lipid biosynthesis and most of the accumulated fat is of dietary origin. Lipids constitute over 30 percent in egg and 10 percent in broiler carcass. Fatty acids are the major components of egg and meat lipids. Among the different fatty acids present in animal products, omega-3 fatty acids have received considerable attention in the past decade due to their several health-promoting effects (Barceló-Coblijn and Murphy, 2009; Palmquist, 2009). Some of the common omega-6 and

omega-3 fatty acids present in chicken meat and egg, their systematic and trivial names and concentrations are shown in Table 3. It should be noted that the concentrations of fatty acids are highly dependant on the dietary lipid source.

Effect on changes in meat fatty acid composition of feeding *Camelina sativa* meal

The use of feeds containing omega-3 fatty acids in poultry diets provides a straightforward and well-adapted, successful approach to fortifying poultry food lipids with health promoting omega-3 fatty acids (Cherian, 2002, 2008). In this respect camelina meal has attained interest due to its high (>29 percent) α -linolenic acid content (Table 1). One of the major goals of feeding camelina meal to broiler birds is to test its efficacy in enriching meat with α -linolenic acid and other long-chain omega-3 fatty acids. Studies in our laboratory investigated changes in white (breast) and dark (thigh) meat lipid charac-

TABLE 4
Omega-3 fatty acid content and Omega-6:Omega-3 ratio in white and dark meat from birds fed diets containing 10 percent camelina meal

Diet	α -Linolenic (18:3) (mg/100 g)	Total long chain omega-3 (mg/100 g)	Total omega-3 (mg/100 g)	Omega-6: Omega-3
Camelina Meal				
Dark Meat	0.56	0.32	0.88	7.54
White Meat	0.26	0.19	0.45	6.84
Control Diet				
Dark Meat	0.22	0.07	0.29	21.45
White Meat	0.05	0.09	0.14	17.28

Notes: Adapted from Aziza, Quezada and Cherian, 2010a. Control is maize-soybean meal basal diet.

teristics of birds fed different levels of camelina meal. In birds fed diets containing 10 percent camelina meal, 2- and 3-fold or greater increases in α -linolenic acid were observed. In addition to α -linolenic acid, other 20- and 22-carbon omega-3 fatty acids, such as eicosapentaenoic (20:5 n-3), docosapentaenoic (22:5 n-3) and docosahexaenoic (22:6 n-3) acids, were also enhanced upon feeding camelina meal. The total omega-3 fatty acids (>18C) in the dark and white meat was 2- to 2.5-fold greater than from birds fed a maize-soybean-based control diet (Aziza, Quezada and Cherian, 2010a). The incorporation of 10 percent camelina meal led to 2.5- to 2.8-fold reduction in the omega-6:omega-3 ratio. The total omega-3 fatty acid content and omega-6:omega-3 ratio in the dark and white meat from birds fed 10 percent camelina meal are shown in Table 4.

Thus, consuming a 100 g portion of dark or white meat from birds fed 10 percent camelina meal could provide 0.88 and 0.45 mg/100 g of omega-3 fatty acids when compared with 0.29 and 0.14 mg/100g from birds fed a maize-soybean-based diet. Traditionally, flaxseed has been the major terrestrial source of omega-3 fatty acid used in animal feeding for omega-3 fatty acid enrichment purposes (Gonzalez and Leeson, 2001; Cherian, 2008). Feeding broilers diets with 10 percent flaxseed could provide more than 300 to 500 mg of n-3 fatty acids in 100-g servings of dark meat (Cherian, 2008).

Effect on egg total fat and fatty acids of feeding *Camelina sativa* meal

Feeding high levels of camelina meal (>10 percent) led to over 6 percent reduction in egg total fat content (Cherian, Campbell and Parker, 2009). Feeding 10 percent camelina meal led to an 8-fold increase in total omega-3 fatty acids, a 10-fold increase in α -linolenic acid and an 8.5-fold increase in docosahexaenoic acid compared to control birds fed a maize-soybean diet (Cherian, Campbell and Parker, 2009). The total omega-3 fatty acid content in eggs was 0.3 vs 2.69 for eggs from birds fed a control or 10 percent camelina meal diet. The omega-6:omega-3 fatty acid ratio was 14.8 vs 4.6 for eggs from birds fed the control diet and 10 percent camelina meal. Thus, consuming two large eggs

from hens fed camelina meal could provide over 300 mg omega-3 fatty acids to the human diet.

Effect on meat and egg oxidative stability and quality aspects of feeding *Camelina sativa* meal

The oxidative stability of food lipids is inversely related to the degree of unsaturation or number of double bonds present in the carbon chain. Introduction of a double bond in the carbon chain introduces a kink in the molecule and changes the biochemical reactivity of the fatty acid, ultimately affecting food lipid quality. Thus, highly unsaturated structures in the food lipid matrix are less stable because unsaturated fatty acids will favour the abstraction of a hydrogen atom and will initiate the oxidation process. In addition, factors such as total lipid content, types and amount of iron present, reducing compounds (e.g. ascorbic acid), concentration of natural antioxidants (e.g. carnosine, anserine and tocopherol), antioxidant enzymes (catalase, superoxide dismutase) and others (oxygen, heating, cooking, salt, temperature, storage) can also affect oxidative stability and meat quality aspects (Min and Ahn, 2005). Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde which is a reactive aldehyde and forms lipid oxidation products. Malondialdehyde and other "thiobarbituric reactive substances" condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative that can be assayed spectrophotometrically and is commonly used to measure lipid oxidation products in food lipids.

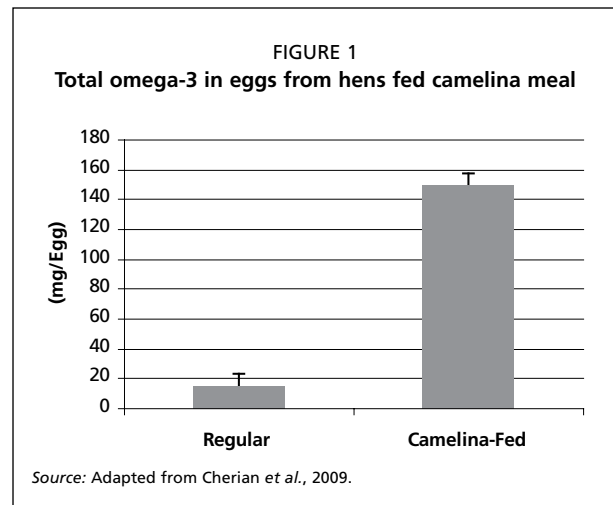
Limited studies have been reported on meat and egg oxidative stability and quality upon including camelina meal in broiler diets. In a recent study, when camelina meal was incorporated at either 5 or 10 percent in broiler diets, 49 and 36 percent reductions in thiobarbituric reactive substances were observed during short-term (2 day) and long-term (90 day) storage, respectively, in the dark meat (Aziza, Quezada and Cherian, 2010b). Similarly, upon cooking, thiobarbituric reactive substances were reduced over 48 percent in dark meat from birds fed a 10 percent camelina meal diet compared with birds fed the control diet (Aziza, Quezada and Cherian, 2010b). The improvement

in meat stability may be due to the tocopherols and other flavonoids supplied through the diet. This is justified by the 1.7-fold increase in γ -tocopherols and antioxidant activity in the thigh meat of camelina meal-fed birds (Aziza, Quezada and Cherian, 2010b). While assessing sensory qualities of meat from birds fed camelina meal, Ryhanen *et al.* (2007) reported that inclusion of meal had no adverse effect on meat taste, juiciness or tenderness. Therefore, inclusion of camelina meal rich in bio-active compounds may prove to be beneficial for providing omega-3 fatty acids while reducing oxidative stress associated with omega-3 polyunsaturated fatty acid enrichment. However, the observed beneficial effect of thiobarbituric reactive substances noted in meat was not observed in eggs from hens fed >10 percent camelina meal. Very few studies have reported sensory aspects of eggs from hens fed camelina. Rokka *et al.* (2002) fed camelina seed oil to hens and these researchers reported that inclusion of camelina oil had no effect on the sensory attributes of chicken eggs.

Using *Camelina sativa* to increase human supply of functional nutrients

Major advancement has been made in the past two decades in our understanding of the mechanisms whereby diet can influence health. As a result, functional nutrients have been introduced as a new concept for nutrients that provide health benefits beyond basic nutrition. The health-promoting effects of such nutrients have led to the development of “functional foods” or “nutraceuticals”. Increased awareness of such “functional foods” has led consumer to seeking these nutrients from food or supplements. Among the different nutrients, several animal food lipid components (e.g. omega-3 fatty acids, fat-soluble vitamins and pigments, conjugated linoleic acid, antioxidants, phospholipids) have been widely researched. As animal food lipids contribute a major portion of fat in the western diet, much work has been done to enrich poultry food lipid portions (egg, white and dark meat) with n-3 fatty acids (Rymer and Givens, 2005; Cherian, 2009). Table 4 and Figure 1 show the omega-3 content of meat and eggs from hens fed camelina meal.

Consuming 1 egg could provide over 140 mg of omega-3 fatty acids and 100 g of thigh meat could provide 0.9 mg of omega-3 fatty acids. In addition, the meal could also enrich food products with tocopherols and other phenolic compounds (Aziza, Quezada and Cherian, 2010b). Feeding flax to broiler birds is associated with negative effects on performance (Ajuyah *et al.*, 1991; Gonzalez and Leeson, 2001). Flax is also approximately twice the price of wheat and maize. Therefore, for reducing feeding costs while increasing omega-3 fatty acids and other functional nutrients in animal food lipids without affecting bird growth, use of biofuel-based co-products should be investigated.



DEVELOPING *CAMELINA SATIVA* AS A FUNCTIONAL FEED: CHALLENGES

The nutritional value of feedstuffs is largely determined by their content of available nutrients. To use camelina meal effectively in poultry feed, further information is needed on its metabolizable energy, and protein and amino acid digestibility and availability in different age groups and strains of meat- or egg-type birds. Accurate determination of dietary amino acid digestibility and availability is essential for balancing feed for optimum growth as well as limiting N excretion to the environment. The effects of processing or use of enzymes in enhancing nutrient digestibility need to be investigated. Such research may provide answers to the reduction in growth observed in young birds fed camelina meal. In addition, effects of the meal on product organoleptic quality during storage and cooking, along with long-term effects on bird health aspects, need to be further investigated. Camelina varieties typically have low levels of glucosinolates (20–24 $\mu\text{mol/kg}$) and erucic acid (22:1) (<2 percent) compared with other mustard species. Investigating factors that can enhance the nutritive value as well as the health-promoting effects of camelina will provide greater potential for developing camelina-based functional feeds and value-added poultry foods for human consumption. Such results may also lead to gaining approval for unrestricted use of camelina meal as a feed ingredient.

CONCLUSIONS

Consumer demand for animal protein has made poultry production one of the fastest growing livestock industries around the world. Livestock feeds are potentially the highest value applications for camelina meal. Being a new co-product, farmers and researchers have not established optimal use of camelina meal and many questions remain about how to best use it effectively. Efforts to minimize nutrient variation among cultivars and processing methods may help to produce a stable feed product, and will help

in comparing results obtained from different locations and laboratories. Feed represents over 65 percent of the cost of poultry production. Using co-products from biofuel production, such as camelina meal, can reduce feed cost while promoting environmental equilibrium and sustainability. Studies conducted on feeding camelina meal to broilers and egg laying hens show that the meal can be included in broiler and layer diets up to 10 percent without compromising bird performance, while potentially increasing the omega-3 fatty acid content 3-fold in the meat and 8-fold in eggs. In addition, dietary camelina meal at 10 percent led to significant reduction in lipid oxidation products and an improvement in γ -tocopherol content and antioxidant activity in the dark meat. The increase in omega-3 fatty acids and tocopherols of eggs and meat will be beneficial to human nutrition as poultry products are the major source of animal protein around the world. The research results obtained will increase the market value of the crop because meal is the by-product of oil extraction and accounts for 70 to 80 percent of the oilseed harvest. Therefore, finding the optimum level of camelina meal in poultry diets without affecting bird performance, health, product quality and sensory characteristics will reduce food production cost while achieving greater independence of food supply.

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Chapter 18

Utilization of lipid co-products of the biofuel industry in livestock feed

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ABSTRACT

Biofuels, which are new and renewable alternative fuels of biological-origin, have been receiving more attention globally due to energy needs and environmental consciousness. The biofuels industry owes its feasibility not only to high petroleum prices and governmental support, but also to the added-value co-products suitable for use as animal feed and supplements.

The objective of this review chapter is to collate and describe the lipid co-products derived from the biofuel industry and to further discuss their potential role as feeds or supplements in the diet of ruminants, their effects on animal performance and public health, and possible risks.

INTRODUCTION TO BIOFUELS

The oil crisis and the constant search for environmentally friendly, acceptable and relatively safe alternatives for fossil fuels have encouraged mass production of biofuels in a number of countries. The growing concern over greenhouse gas (GHG) emissions, as well as fluctuations in gasoline and diesel fuel prices, has prompted several governments to encourage the introduction of renewable biofuels into the market. Promotional measures, such as economic and local regulations, subsidies, tax exemptions and penalization of all fuel not including the required amount of biofuel fixed by law, have been implemented in order to foster the development of the biofuel industries (Behzadi and Farid, 2007; Bloch *et al.*, 2008; Colibar, Korodi and Popovici, 2010).

In addition to the reduction of hazardous pollutants emitted from the transportation sector, increased rural development and national energy security are other important outcomes that benefit the communities. The increment in intellectual and profitable management of unutilized energy sources that potentially exist in urban and agricultural waste fractions, to produce the so called second-generation biofuels, is another advantage influenced by the economic incentives (Najafi *et al.*, 2009; Willson, Wiesman and Brenner, 2010).

Currently, the two main types of first-generation biofuels produced and commercialized around the world are biodiesel and bio-ethanol, both derived from plant parts and generally recognized as clean sources. Biodiesel is obtained by the esterification of vegetable oils to alkyl mono-esters that can be used directly as combustible fuels (Van Gerpen, 2005). The most common crops used for this purpose are feedstocks rich in oil content, like rapeseed

(canola oil), sunflower and soybean. Bio-ethanol is obtained from the fermentation of hydrocarbons, such as those concentrated in cereal grains, sugar cane and sugar beet.

In recent years the expansion of the biofuel industry was in part responsible for a sharp rise in the prices of grains and oilseeds destined for livestock. Fortunately, the solution for that threat to the farm feed sector was found in the utilization of co-products derived from the manufacturing process of biofuels as low cost feed alternatives (Willson, Wiesman and Brenner, 2010).

SOAPSTOCK

The increased need for intestinal absorption of unsaturated fatty acids (FA) in cattle is driven mainly by nutritional guidelines that promote reduced intake of saturated fatty acids by humans and the observed enhanced animal performance (Jenkins and Bridges, 2007). Rumen-protected fat sources provide essential and non-essential fatty acids that otherwise would have been transformed by micro-organisms in the rumen to yield other end products. For example, dietary unsaturated fatty acids are processed by an array of bacterial enzymes and form trans- fatty acid intermediates and stearic acid (Wallace, 2002). Hence, although essential linoleic and α -linolenic acids comprise the majority of fatty acids consumed by cattle, stearic acid comprises most of the fatty acids leaving the rumen and reaching body tissues. This results in a loss of specific essential and non-essential fatty acids that are provided in the diet not only in order to supply the nutritional requirements of man but also to achieve certain benefits to animals. Even though some fatty acids can be synthesized from stearic acid in ruminant body tissue, the economic loss is great also due to unnecessar-

MAIN MESSAGES

- Lipid co-products from the bio-ethanol and biodiesel processing industries can be excellent sources of nutrients for ruminants.
- With the growth of biofuel production from various feedstocks, livestock producers will have many nutrient-dense co-product feed resources readily available at economical prices.
- As more novel extraction and refining technologies are developed, better quality co-products destined for livestock feed will be achieved.
- Potential risks should be taken into consideration, so adequate risk assessments should be conducted in order to avoid adverse effects in animals and to safeguard public health.

ily wasted metabolizable energy. Moreover, fatty acids are known to inhibit fibre digestion in the rumen, whereas the pre-formed calcium soaps of FA have little or no such effects (Enjalbert *et al.*, 1997).

Soapstocks, from a nutritional point of view, are saponified fatty acids formed when free fatty acids and divalent cations (usually calcium) are combined. They were originally developed as a form of rumen-inert fat to avoid ruminal fermentation and are commercially available today for enhancing the tissue supply of unsaturated fatty acids in cattle (Palmquist, 1994; Brown, 2006).

COMPOSITION

The refining process for biodiesel generates a distillate rich in free fatty acids and other lipid components (Haas, 2005). It comprises an aqueous phase and an oily phase (also termed acid oil). The acid oil consists of acylglycerols, phosphoacylglycerols, free fatty acids (FFA), triacylglycerides (TG), di-acylglycerides, mono-acylglycerides, pigments and other lipophilic materials (Haas *et al.*, 2003; Wang *et al.*, 2007). When the distillate is reacted with calcium oxide, calcium salts of the fatty acids present in the distillate are formed and separate further from the unsaponifiable matter.

Fatty acid content of soapstock is a reflection of the parent oil composition (Table 1). The glycerides that will probably be detected have their origin in the partial hydrolysis of the remained TGs, during refining of the biodiesel (Dumont, Suresh and Narin, 2007). For instance, the major TG in canola oil is triolein; hence, a relatively high concen-

tration of mono-olein and di-olein glycerides will be present in the soapstock (Durant *et al.*, 2006).

Effect on ruminants

Palm fatty acids distillate reacted with calcium oxide to develop a rumen-protected fat (commercialized as Megalac®) (Gardner and Rudden, 2004) was proven to be effective in the protection of fatty acids against ruminal biohydrogenation (Scollan *et al.*, 2001; Palmquist, 1994). It was also found to significantly increase the digestibility of feed neutral-detergent fibre (NDF) compared with unprotected fatty acids (Palmquist, 1994). Fatty acids from palm oil were the source of choice due to the reliability and consistency of the fatty acid profile, in addition to their stability at the average and optimal rumen pH (Gardner and Rudden, 2004). (Tables 2 and 3)

Later, calcium salts of fatty acids from other sources of vegetable oils (such as rapeseed and soybean oils) were developed and their effectiveness was investigated. For example, after studying the response of dairy cows to Ca salts of fatty acids, it has been observed that rapeseed oil fatty acids were not as inert as palm oil Ca salts in the rumen (Ferlay, Chilliard and Doreau, 1992.). Thus, it was concluded that saponification of polyunsaturated FAs was probably not an efficient way to protect them against ruminal biohydrogenation and to increase their secretion in the milk (Ferlay *et al.*, 1993). At the same time, calcium salts of fatty acids from rapeseed distillate (commercialized as Energol) were observed to augment the oleic, linoleic,

TABLE 1
Fatty acid content of different soapstock sources according to Dumont, Suresh and Narine, 2007

Fatty acids	Concentration (g/kg) reported on dry basis			
	Cottonseed	Maize grain	Groundnut seed	Canola seed
Myristic acid	2.4	0.31	6.6	Not detected
Palmitic acid	93.1	8.62	86.2	46.3
Palmitoleic acid	1.8	Trace	Trace	1.5
Stearic acid	9.6	5.07	4.58	Not detected
Oleic acid	60.7	93.6	11.6	155.7
Linoleic acid	165	178	77.5	33.1
α -linolenic acid	Not detected	Not detected	Not detected	0.024
Arachidic acid	0.77	0.76	Traces	1.2

TABLE 2
Chemical composition of MEGALAC®

Component	Concentration (g/kg)
Oil (Werner-Schmidt)	84
Protein	0
Fibre	0
Ash	12
Calcium	9
Moisture	5

Source: Gardner and Rudden, 2004.

TABLE 3
Fatty acid profile of MEGALAC®

Fatty Acid	g/100 g of total fatty acids
C14:0	1.5
C16:0	48.0
C18:0	5.0
C18:1	36.0
C18:2	9.0

Source: Gardner and Rudden, 2004.

α -linolenic and stearic acid content in the milk of dairy cows and reduce that of palmitic acid (Komprda *et al.*, 2005).

In general, a reduction in milk fat total saturated FAs (including palmitic acid, which imposes a negative effect on cardiovascular disease risk to dairy cows, was observed (Givens *et al.*, 2009). The concentrations of cis-mono-unsaturated FAs were enhanced probably due to their escaping rumen biohydrogenation.

Regarding the ability to manipulate the fatty acids profile in meat, evidence suggests that feeding linseed soapstock to finishing steers raised the total amount of omega-3 fatty acids in the longissimus muscle (Quinn *et al.*, 2008). Brandt and Anderson (1990) reported the same when they compared supplementing finishing steers with tallow or soybean soapstock as fat sources. In another study, although the FA composition of the muscle tissue was not altered, subcutaneous adipose concentrations of 9-cis,12-cis-linoleic acid (LA) and liver tissue concentrations of (all-cis)-5,8,11,14,17-eicosapentaenoic acid (EPA) were the highest in lambs fed 4 percent calcium salts of palmitic and oleic acid (Seabrook, Peel and Engle, 2011).

PHYTONUTRIENTS

Extensive research with palm biodiesel (palm oil methyl esters) revealed that phytochemicals such as carotenes (pro-vitamin A, lycopene, phytoene), tocopherols and tocotrienols, sterols, squalene, a mixture of phospholipids (better known as lecithin), polyphenols and co-enzyme Q₁₀, remain intact in the biodiesel after the trans-esterification reaction (Wattanapenpaiboon and Wahlqvist, 2003). Data about palm phytonutrients concentrations and percentage of the components in crude palm oil are presented in Table 4.

TABLE 4
Palm phytonutrient concentrations and percentage of the components in crude palm oil

Palm phytonutrient	Concentration (mg/kg)
Vitamin E	600–1000
Carotenoids	500–700
Phytosterols	300–620
Squalene	250–540
Phospholipids	20–100
Co-enzyme Q ₁₀	10–80
Polyphenolics	40–70

Components in palm oil	g/kg
Tryglycerides	>900
Dyglycerides	20–70
Monoglycerides	<10
Free fatty acids	30–50
Phytonutrients	10

TABLE 5
Concentration of selected phytonutrients in crude palm oil (CPO) and palm phytonutrients concentrate (PPC)

Component	Concentration (mg/kg)	
	CPO	PPC
Carotenes	530	23710
Vitamin E	1020	14640
Sterols	910	15780
Squalene	44	320

Source: Chandrasekaram *et al.*, 2009.

TABLE 6
Concentration of selected phytonutrients in unsaponified crude palm oil (Unsap CPO) and unsaponified palm phytonutrients concentrate (Unsap PPC)

Component	Concentration (mg/kg)	
	Unsap CPO	Unsap PPC
Carotenes	19570	209880
Vitamin E	39290	78660
Sterols	32430	30500
Squalene	2770	8110

Source: Chandrasekaram *et al.*, 2009.

These high value phytochemicals are recovered with the aid of several processes incorporating short-path distillation, supercritical fluid technology, saponification, crystallation and solvent treatment (Choo *et al.*, 2006, 2009). The presence of phytonutrients in the palm phytochemicals concentrate (also termed palm fatty acid distillate – PFAD) obtained after the distillation of the palm biodiesel was found to be ten times higher than that in crude palm oil (Chandrasekaram *et al.*, 2009) (Tables 5 and 6). A saponification process is necessary to further separate the unsaponifiable matter (the phytonutrients) from the small amount of free FAs and esters remaining in the PFAD, to reach a purity of more than 90 percent (Lin and Yoo, 2009).

Rapeseed, soybean and sunflower are also a good sources of phytochemicals, especially tocopherols and

sterols (Ooma and Mazza, 1999; Buczenko, De Oliveira and Von Meien, 2003). Extraction of these valuable compounds from the co-products (for example fatty acid distillate) obtained during the biodiesel production will therefore be interesting for their trade as value-added co-products of this industry.

Dried distillers grain (DDGS), a co-product from the bio-ethanol industry (comprising principally bran, protein and germ fractions of the grain used in the fermentation process, together with remnants of yeast cells), also contains significant amounts of phytonutrients. The main constituents are tocopherols and phytosterols. The oil extracted from DDGS may be further processed to yield a distillate rich in these various bio-actives (Winkler *et al.*, 2007; Winkler-Moser and Vaughn, 2009; Leguizamon *et al.*, 2009; Winkler-Moser and Breyer, 2011).

The above-mentioned bio-active components are much appreciated for application as standard reference materials, functional food, nutraceuticals and cosmeceuticals for human well-being. With novel technologies for the production of biodiesel and the consequent increased quantities of the relevant co-products in the future, a greater proportion of them can be shifted for use as feed additives and vitamins destined for livestock, at more appealing prices.

EFFECT ON RUMINANTS

Vitamin E

'Vitamin E' is the generic name for a group of eight natural compounds: α -, β -, γ - and δ -tocopherol and α -, β -, γ - and δ -tocotrienol, (Figure 1) which differ in the location of methyl groups on their chromanol ring structure (Williams *et al.*, 1993; Röhrle *et al.*, 2011). The principal and most investigated vitamin E form, with antioxidant and immune functions, is α -tocopherol. However, important or more effective, or both, functions of non- α -tocopherol like γ -tocopherol and tocotrienols are being revealed (McDowell *et al.*, 2007).

Concentrations and distribution of tocopherols significantly depend on kind of oil analysed (Tables 7 and 8), but α - and γ - tocopherols are usually dominant (Ooma and Mazza, 1999).

Vitamin E is essential for body functions such as growth, reproduction, prevention of various diseases (white muscle disease in young ruminants, foetal death and resorption, retinal degeneration) and protection of the integrity of tissues (McDowell *et al.*, 1996; Rooke, Robinson and Arthur, 2004). Supplementation of domestic animals with vitamin E has potentiated their antibody responses to a variety of pathogens and their adaptability to stressful situations (Finch and Turner, 1996; Rajesh *et al.*, 2008; Cusack *et al.*, 2009).

In addition, feeding levels of vitamin E that are considerably higher than NRC requirements is required to improve animal product quality (Liu, Lanari and Schaefer, 1995) such as extending beef colour stability and minimizing off-flavors

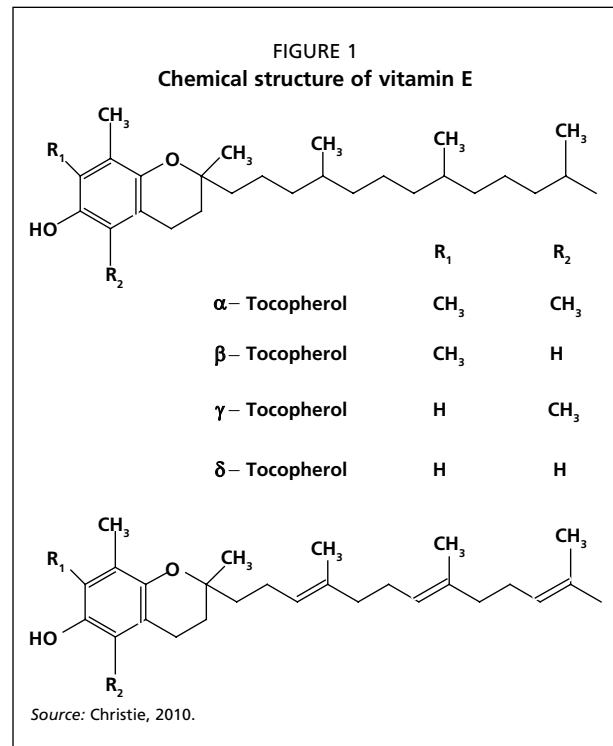


TABLE 7

Tocopherol contents (mg/kg) in selected vegetable oils (possible raw materials for biodiesel production)

Oil	α -Tocopherol	β -Tocopherol	γ -Tocopherol	δ -Tocopherol
Rapeseed	268	–	426	–
Canola	272	0.1	423	–
Soybean	116	34	737	275
Maize	134	18	412	39

Source: Ooma and Mazza, 1999.

TABLE 8

Tocopherol content (mg/kg) in plant oils

Oil	Total tocopherol content (mg/kg)
Grapeseed	121 ± 6
Linseed	367 ± 8
Olive	177 ± 3
Groundnut	226 ± 4
Sunflower	535 ± 8

Note: Values are mean + SD. Source: Gryszyńska-Swigło *et al.*, 2007.

in milk due to lipid oxidation. Higher levels of vitamin E in the ruminant diet increases α -tocopherol concentrations in the tissues and, owing to its antioxidant properties, it protects not only membranal lipids but also myoglobin from oxidation. This results in delayed onset of discoloration in fresh, ground and frozen beef, and in suppression of lipid rancidity (Liu, Lanari and Schaefer, 1995).

Carotenes

Carotenes belong to the carotenoids family, a group of natural pigments that encompasses more than 600 molecules synthesized by higher plants and algae. These compounds

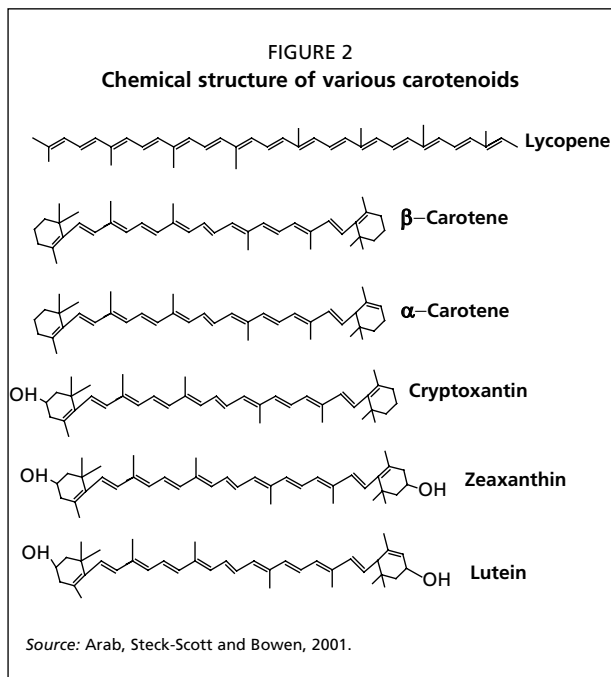


TABLE 9
Various types and composition of carotenes in palm oil

Carotene type	Part in general carotenes (g/100 g)
Phytoene	1.27
Cis- β -carotene	0.68
Phytofluene	0.06
β -carotene	56.02
α -carotene	35.16
Cis- α -carotene	2.49
ζ -carotene	0.69
γ -carotene	0.33
δ -carotene	0.83
Neurosporene	0.29
β -zeacarotene	0.74
α -zeacarotene	0.23
Lycopene	1.30

Source: Puah *et al.*, 2005.

are characterized by a linear poly-isoprene structure with conjugated double bonds either *per se* (as in lycopene, $C_{40}H_{56}$) (Figure 2) or as derived by cyclization of the two extremities, with oxidation (as in xanthophylls such as lutein and zeaxanthin, $C_{40}H_{56}O_2$) or without oxidation (carotenes, $C_{40}H_{56}$) (Calderon *et al.*, 2006; Noziere *et al.*, 2006).

Concentration of carotenes in crude palm oil is approximately 640–700 ppm (Choo, 1994) and 0.25–3.6 ppm in virgin olive oil (Tanouti *et al.*, 2011).

Non-oxidized carotenes are known as general components of the carotenes fraction (Table 9).

The β -carotene content of forages is reduced by sun-curing, ensiling and storage, and is quite variable. Hence, green pasture is the most abundant natural source of carotenes for ruminants (Miller, 1968; Kalac and McDonald, 1981).

Ruminants depend entirely on feed as their source of carotenoids, not being able to synthesize them *de novo*, but metabolize or convert them into other carotenoids.

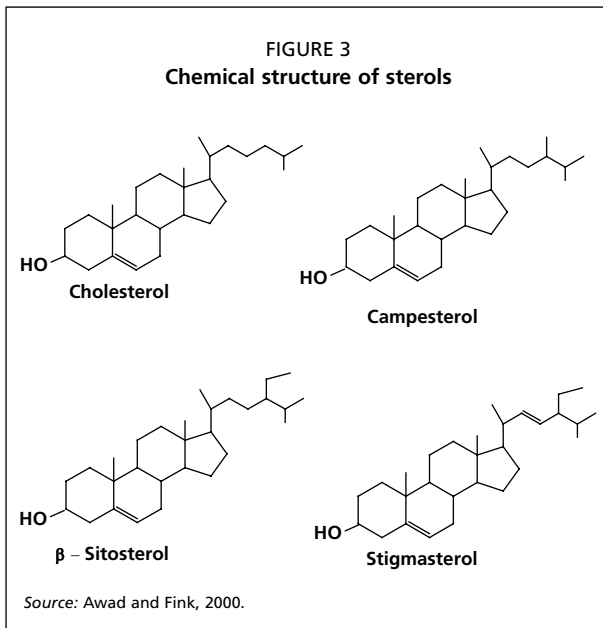
In sheep and goats, absorbed β -carotene is assumed to be almost entirely transformed into retinol (vitamin A) in the enterocytes. In contrast, in cattle, not all absorbed β -carotene is transformed into retinol and thus β -carotene is the main carotenoid present in their plasma, stored in tissues and secreted in milk fat (Mora *et al.*, 1999; Cardinault *et al.*, 2006; Lucas *et al.*, 2008).

A deficiency in retinol may cause xerophthalmia (a night blindness disease) and reduce reproductive efficiency in dairy cows, through impaired ovarian function and increased incidence of abortion (Wang *et al.*, 1987; Haliloglu *et al.*, 2002). Apart from having pro-vitamin A properties, β -carotene *per se* also plays an important role as antioxidant. Some positive effects of β -carotene on mammary gland health, rumen function, milk yield and immunity have been reported (Hino, Andoha and Ohgi, 1993; De Ondarza and Engstorm, 2009a, b).

Certain changes in the organoleptic characteristics of meat and milk from ruminants fed on diets rich in β -carotene were reported (Ellis *et al.*, 2007). Some of them are most desired from the point of view of public health, consumer acceptability or preference on the one hand, and animal producers and food manufacturers on the other. The augmented levels of β -carotene and vitamin A in milk as a consequence of supplying them in the ruminant diet, could be beneficial for the production of functional foods (i.e. butter, margarine) (Ellis *et al.*, 2007). Additionally, their abundance in meat and milk can supply the nutritional requirements recommended for humans (Simmons, Green and Bransby, 1996; De Ondarza, Wilson and Engstrom, 2009). It should be noted, though, that high levels of β -carotene and vitamin A were found to adversely affect the fatty acid profile in intermuscular fat tissue and marbling deposition (Siebert *et al.*, 2000, 2006; Pyatt and Berger, 2005; Dikeman, 2007).

Phytosterols

Plant sterols and stanols (their reduced form), also called phytosterols and phytostanols, are natural constituents of plants and are part of the triterpene family (Moreau, Whitaker and Hicks, 2002). They are non-nutritive compounds whose chemical structure resembles that of cholesterol, a predominant sterol in animals (Figure 3). Phytosterol content ranges from 0.14 percent in olive oil to 1.6 percent in maize oil (Gul and Amar, 2006). In plants they are responsible for the regulation of the fluidity and permeability of cell membranes, serve as substrates for the synthesis of numerous secondary plant metabolites, and act as biogenic precursors of plant growth hormones and hormonal precursors.



The best dietary sources of phytosterols are unrefined plant oils, seeds, nuts and legumes; in certain plants, such as *Amaranthus* spp. or *Butyrospermum parkii* (shea butter tree), it can reach more than 10 percent. The predominant forms being β -sitosterol, campesterol and stigmasterol, followed by brassicasterol, avenasterols and ergosterol (the latter is a known precursor of vitamin D₃, that is also formed in fungi) (Tapiero, Townsend and Tew, 2003; Milovanovic, Banjac and Vucelic Radovic, 2009).

Studies with animals and humans show that phytosterols reduce the absorption of cholesterol, thus lowering its serum level and leading to a reduction in the risk of cardiovascular diseases (Kamal-Eldin and Moazzami, 2009; Weingartner, Bohm and Laufs, 2009). In addition, they are considered to have anti-inflammatory, anti-bacterial, anti-ulcerative and anti-tumor properties (Awad and Fink, 2000).

Phytosterols supplied as immuno-modulators (commercialized as Inmunicin Maymoim, a product consisting primarily of β -sitosterol) in the diet of pigs during the nursery and finishing periods have been shown to fortify the immune system (decrease mortality and percentage of culls) and improve average daily gain and feed efficiency (Fraile *et al.*, 2009). Hence, it will be interesting to conduct trials aiming to prove the same utility in ruminants.

Polyphenols

Polyphenols are secondary metabolites of plants, known to be involved in defence mechanisms and the survival of the plant in its environment (Manach *et al.*, 2004). These compounds possess characteristic aromatic rings (single, as in simple phenols, to several, as in flavonoides and condensed tannins) (Figure 4) attached to a hydroxyl group, which confers on the molecule part of its diverse biological activities (Singh, Bhat and Singh, 2003).

Polyphenols are present in a variety of plants utilized as important components of both human and animal diets. Polyphenols in vegetable oils are a complex mixture of compounds that include derivatives of hydroxybenzoic and hydroxycinnamic acids, as well as oleuropeins, coumarins, flavonoids and lignins (Kozłowska *et al.*, 1990; Valavanidis *et al.*, 2004).

Polyphenols are usually soluble in basic media and alcohols, but they can present in plant oils at low concentrations. Concentration of polyphenols in virgin olive oil may be from 63 mg/kg to 406.5 mg/kg (Tanouti *et al.*, 2011). As a rule, they are dissolved in the dispersed water phase. This phase is stable due to presence in oils of such substances like lecithin and other phospholipids.

The presence of polyphenols in the diet of ruminants improves the efficiency of protein degradability and digestibility (except when the level of tannins is not monitored correctly and reaches high levels), thus ameliorating feed conversion. It also reduces the concentration of urea excreted in cattle manure (Reed, 1995; Frutos *et al.*, 2004). Additionally, polyphenols augment ruminant performance by inhibiting bloat and reducing the incidence of subclinical helminth infections (O'Connell and Fox, 2001).

As they possess potent antioxidant activity, their deposition in animal tissues and secretion in milk is mostly desired, because it protects the lipid components in meat and milk products as well as providing dietary antioxidants for human consumption. In this manner, functional-healthy products are achieved (Weisburger *et al.*, 2002; Priolo and Vasta, 2007; Moñino *et al.*, 2008; Cuchillo Hilario *et al.*, 2010; Jordan *et al.*, 2010).

The prohibition on use of growth-promoting antibiotics in animal feeds (EU, 2003) and the constantly increasing demand for organically produced milk and meat, have prompted livestock producers to look for more acceptable alternatives (Wallace, 2004). In addition, some phenolic extracts have been demonstrated to inhibit hyper-ammonia-producing bacteria in the rumen and exert beneficial effects on rumen fermentation (Flythe and Kagan, 2010). They have also been shown to inhibit certain pathogens, hence their potential role as natural and less hazardous replacements for antibiotics (Wells, Berry and Varel, 2005).

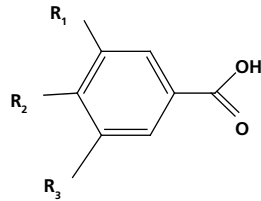
Lecithin

Lecithin is primarily a natural mixture of phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidic acid (PA) (Figure 5), and which contains minor quantities of other water-soluble or hydratable components (glycolipids and oligosaccharides) (Pickard, 2005).

Soybean is the predominant vegetable source of lecithin due to its availability, and the lecithin has outstanding func-

FIGURE 4
Chemical structure of polyphenols

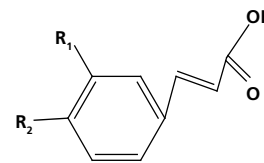
HYDROXYBENZOIC ACID



Protocatechuic acid $R_1=R_2=(-OH)$; $R_3=(-H)$

Gallic acid $R_1=R_2=R_3=(-OH)$

HYDROXYCINNAMIC ACID



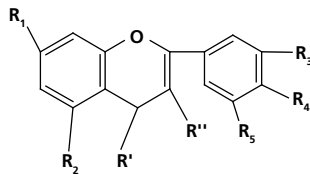
Coumaric acid

$R_1=(-OH)$; $R_2=(-H)$

Ferulic acid $R_1=(-O-CH_3)$;

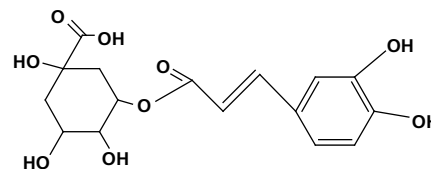
$R_2=(-OH)$

FLAVONOIDS



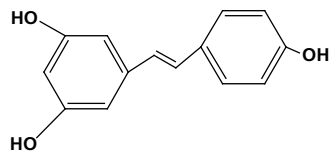
If $R'=(=O)$; $R''=(-H)$

If $R'=(-H)$; $R''=(-OH)$



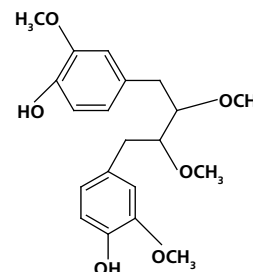
Chlorogenic acid

STILBENES



Resveratrol

LIGNANS



Secoisolariciresinol

tional characteristics, mainly as a surfactant and emulsifier (Wilson, 2003). However, lecithin products from seeds of rape, sunflower, glandless cotton and maize are also potential commercial sources. Seed of glanded cotton contains more phospholipids than any other oilseed (with the exception of soybean), but has the disadvantage that gossypol (a toxic compound normally present in the cotton seed) tends to bind to the phospholipids during the solvent extraction process (Pickard, 2005).

Information about the chemical structure of lecithins from different oils are presented in Tables 10 and 11.

Lecithins are used in animal feed recipes as dust suppressors, economic emulsifiers (e.g. stabilization of milk replacers for feeding calves) and essential FA sources (Van

TABLE 10
Fatty acid composition of vegetable lecithins (g/100 g)

Fatty acid	Soybean	Sunflower seed	Rapeseed
16:0	16	11	7
18:0	4	4	1
18:1	17	18	56
18:2	55	63	25
18:3	7	0	6
Others fatty acids	1	4	5

Source: Van Nieuwenhuyzen and Tomas, 2008.

Nieuwenhuyzen and Tomas, 2008). Feeding soy lecithin to ruminants was found to favourably change the FA profile in longissimus muscle and subcutaneous adipose tissue in lambs (Lough *et al.*, 1992). It also increased FAs and

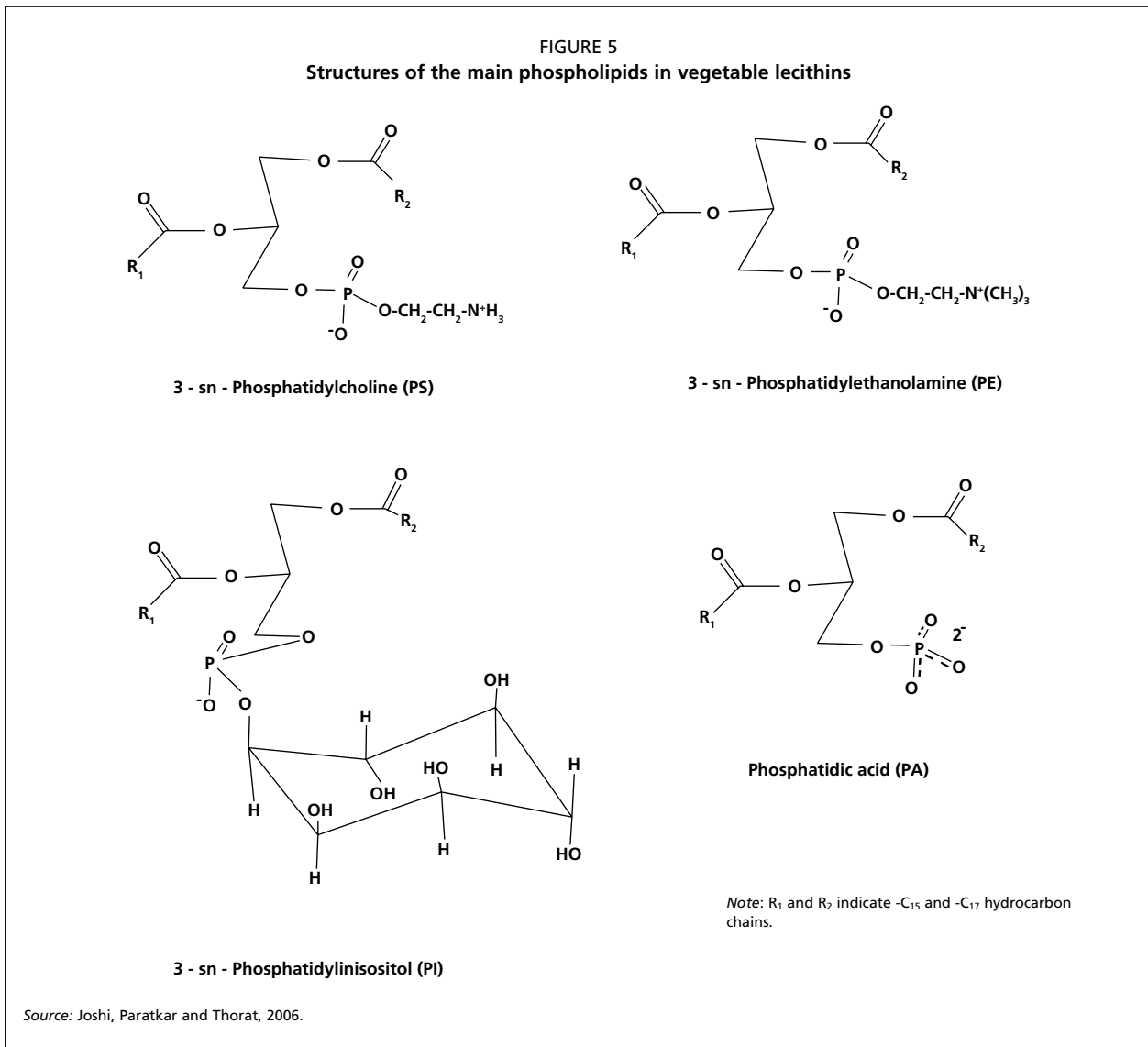


TABLE 11
Phospholipid composition of liquid vegetable lecithins (g/100 g of lecithins fraction) by ³¹P-NMR

Phospholipid	Soybean	Sunflower seed	Rapeseed
PC	15	16	17
PE	11	8	9
PI	10	14	10
PA	4	3	4
Other phospholipids	7	6	6

Notes: ³¹P-NMR indicates analysis of P isotope-marked lecithins using nuclear magnetic resonance techniques. For PC, PE, PI and PA see Figure 5. Source: Van Nieuwenhuyzen and Tomas, 2008.

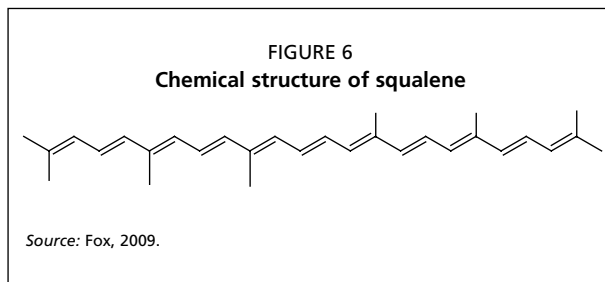
protein digestion in the hindgut both *in vitro* and *in vivo* (Jenkins and Fotouhi, 1990; Wettstein, Machmuller and Kreuzer, 2000; Hristov, Neill and McAllister, 2003; Pivoda *et al.*, 2010). Both methane production and ammonia concentration in the rumen were significantly reduced, implying that efficiency of feed conversion was increased.

Squalene

Squalene – an isoprenoid compound with 6 isoprene units (triterpene) – is an intermediate metabolite in the synthesis of cholesterol and phytosterols (Figure 6). This unsaturated, thermally unstable and light-sensitive hydrocarbon appears in high concentrations (50–90 percent by weight) in the liver oils of certain species of deepsea sharks (Bakes and Nichols, 1995; Wetherbee and Nichols, 2000). It is also present in lower concentrations in foods such as avocado, aubergine, poultry and tuna, as well as in some common edible oils such as olive, palm, groundnut, and rapeseed (Catchpole and von Kamp, 1997; Catchpole, Von Kamp and Grey, 1997; Chua *et al.*, 2007).

Concentration of squalene in olive oil can be from 136 to 708 ppm (Kiritsakis, 1990).

Squalene has been demonstrated to be effective in decreasing total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels. It is also used extensively as a strong antioxidant in the food and cosmetic industries



(Fan *et al.*, 2010). Dietary supplementation with squalene enhanced the reproductivity of boars and improved semen count and quality in meat-type male chicken (Zhang *et al.*, 2008; Li *et al.*, 2010). Therefore, the administration of squalene with other vitamins and feed additives is expected to strengthen the immune system and to improve livestock productivity.

A surprising revelation regarding the accumulation of squalene in the intermuscular fat in reindeers (northernmost freely ranging ruminants in Scandinavia) fed pellets that contained squalene, was made by Sampels, Pickova and Wiklund (2005). The levels of squalene found in the reindeer meat (0.5–1 percent) were above the recommended values for common human dietary fats and oils (0.002–0.3 percent squalene in total fat) (Sampels, Pickova and Wiklund, 2005). This discovery may encourage research regarding the deposition of squalene in the tissues of ruminants and its secretion in milk, in order to promote the creation of functional foods.

POTENTIAL RISKS FROM FRACTIONS CONTAINING SUCH PHYTOCHEMICALS

Deodourizer distillates, by-products of the refinement of vegetable oils, are a known repository for hazardous substances such as dioxins, furans, PCBs (polychlorinated biphenyls) and pesticides. They have been banned from direct use in animal feeds in the United States, due to the elevated levels of these contaminants that may accumulate in livestock tissues (biomagnification) (Halbert and Archer, 2007).

Therefore, although the new biodiesel production plants aim to minimize the presence of harmful impurities by utilizing novel advanced technologies, this crucial issue must be supervised by the corresponding authorities (Brambilla and De Filippis, 2005). It is advised that a thorough examination of the biodiesel lipid co-products should be carried out in order to assess possible presence of other possible toxic compound that can harm the health of both animals and humans (EU, 2003).

CONCLUSIONS

When used correctly and with prudence, the lipid co-products from the biofuel industries could offer significant benefits to agriculturalists, animal producers and consumers of functional-healthy products. However, adequate risk

assessments should be conducted in order to avoid adverse effects in animals and on public health.

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Chapter 19

Potential and constraints in utilizing co-products of the non-edible oils-based biodiesel industry – an overview

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ABSTRACT

The biofuel industry is undergoing exponential growth, fuelled by the high demand for renewable sources of energy and advancing technology. With the increasing production of biofuels, the volume of co-products has, in parallel, grown dramatically. During the last few years, many non-edible oil feedstocks were suggested that resulted in new co-products to supplement those resulting from conventional feedstocks and that are accepted by the livestock feed industry. These earlier co-products are also used in applications ranging from soil fertilizers to pharmaceuticals, which is not the case with the emerging co-products from non-edible oil feedstocks, many of which contain either toxic or antinutritional compounds. Sustainability of the biofuel industry hinges on the use of feedstocks that are not competitive with human and animal nutrition and that are produced from plants that grow in poor and marginal soils. Another important criterion that ensures sustainability is the use of the resulting co-products as value-added products. Since the biofuel-derived cakes and meals constitute a rich source of crude protein, ranging from 11 percent (*Mesua ferrea*) to 58 percent (*Crambe abyssinica*), these have the potential to be used as animal feeds. In this chapter, current knowledge on the potential and constraints of using oil cake or meals from the emerging biodiesel industry based on non-edible oil for livestock feed is examined. This information will assist in enlarging the feed resource base by identifying promising novel feed resources and in identifying potential detoxification treatments where necessary.

INTRODUCTION

The worldwide production of renewable fuel is expected to grow quickly and its share in global energy production is expected to increase. Biodiesel production, which started on a small scale in the early 1990s, quadrupled between 2000 and 2005 (Brown, 2009). Conversion of vegetable oils into biodiesel has undergone several new developments (Meher, Sagar and Naik, 2006). This has resulted in some of the feedstocks traditionally used as animal feed, e.g. soybean and rapeseed, becoming feedstocks for the biofuel industry. Europe, the leader in biodiesel production processed from vegetable oils, is largely dependent on these two crops to sustain production. Biofuel production, like any agriculture-based industry, will absorb agricultural products, but will also result in co-products, including protein-rich oilcakes and meals, which can be used as animal feed.

Unlike other agro-industrial activities, biofuel production should not compete for oil and other natural resources needed for human food production. A convenient way to avoid competition with food production is to promote the use of plant species with products that are non-edible

and that can grow on poor soil and under harsh climatic conditions. Based on this concept, biodiesel production from non-edible oils presents a promising option. However, concerns have been raised about the sustainability of using non-edible oils for this purpose as the resulting co-products are often toxic if fed directly to livestock. This would limit complementarity among the sectors of agriculture, the biofuel industry and the animal feed industry. The toxic co-products obtained during biodiesel production can also pose risks to the environment.

The toxicity of non-edible oil feedstock originates from the plant secondary metabolites they contain. These secondary metabolites are present in plants for their protection, including acting as antioxidants, thus enabling the plants to grow in harsh environments. However, their antinutritional and toxic factors result in the resultant oil and co-products being non-edible.

The multiple and widespread use of biofuel co-products from edible oil resources, including the use of cakes and meals for livestock feed, is well documented. Literature is scarce and isolated on the use of biofuel co-products from

MAIN MESSAGES

- Many potential feedstocks have shown promising results in feeding trials after detoxification. Studies on oil cakes and meals of *Ricinus communis*, *Crambe abyssinica*, *Azadirachta indica* and *Pongamia pinnata* show possibilities for feeding to farm animals after subjecting them to appropriate detoxification treatments.
- Further studies are needed to fill the gaps in knowledge for the possible detoxification and further use of *Hevea brasiliensis*, *Thevetia peruviana*, *Mesua ferrea*, *Calophyllum inophyllum* and *Croton tiglium*.
- Scaling up of promising detoxification processes is needed. Implementation of positive results can be successful only if large quantities of the derived meals can be treated and used for animal feeding.
- The development, use and scaling up of the detoxification processes should be accompanied by socio-economic analysis.
- Preparation of high-value protein isolates and peptides for use in livestock feeds could be an alternative approach to use of otherwise non-edible cakes and meals – an area that so far has received little attention.

non-edible oils as animal feed. Also, data on nutritional value, intake, digestibility and toxicity are scattered and not systematically collated. This presents a challenge in estimating the potential of these products for animal feed. The present chapter synthesizes information on the nutritional composition of the co-products, their toxicity and the attempts made to enhance by detoxification their utilization as animal feed.

PROMISING NON-EDIBLE OIL PLANT SPECIES

Many plant species are known for their oil seeds, but exploitation of the oil cakes from non-edible oil species originates from the fact that many of these plants are non-ubiquitous in distribution, their production is seasonal and their co-products are usually non-edible for livestock (Sivaramakrishnan and Gangadharan, 2009). The list of such non-edible oil seed plants is long, but this chapter only considers nine promising oleaginous species, whose oil is potentially suitable for use as biodiesel (Azam, Waris and Nahar, 2005) and their co-products are reported to be toxic when used as animal feed. *Jatropha curcas*, another promising non-edible oil plant that is being extensively promoted, is not discussed here. The utilization of *Jatropha* seed meal, cake and protein isolate is presented elsewhere in this publication.

Castor (*Ricinus communis* L.)

Commonly known as castor, *Ricinus communis* is a wild plant growing in large quantities in most tropical and sub-tropical countries. The plant requires air temperatures ranging between 20 and 26 °C, with low relative humidity. However, its extreme toxicity limits its cultivation in many countries. Castor is grown mainly for its oil. On average, castor seeds contain 46 to 55 percent oil by weight (Ogunniyi, 2006). The oil is used in production of viscous lubricants, important oleochemicals, surface coatings, soaps, cosmetics and pharmaceuticals (Ghandi, Cherian and Mulky, 1994).

Rubber (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.)

Para rubber is a perennial tree, indigenous to South America. It has been cultivated as an industrial plantation crop since its introduction to Southeast Asia (Abdullah and Salimon, 2009). The important contribution of para rubber trees is the latex used for natural rubber production (Ravindran and Ravindran, 1988). Rubber tree seed yields are between 100 and 150 kg/ha (Stosic and Kaykay, 1981). The seed has a high content (43 percent) of semi-drying oil, which can be used in the paint industry (Lauw Tjin Giok *et al.*, 1967).

Although the seeds contain cyanogenic glycosides, they are locally used as an ingredient in human nutrition after appropriate treatment (soaking and cooking). This procedure reduces the cyanide content from 330 mg to 8.9 mg/100 g in seeds (Lauw Tjin Giok *et al.*, 1967).

Crambe (*Crambe abyssinica* Hochst)

Crambe is an oil plant of the cruciferous family, a native to the Mediterranean region that adapts well to the cold weather of much of Europe (Falasca *et al.*, 2010). A low water requirement, a short crop cycle of about 90 days, hardiness and the possibility of using it as a catch-crop between main cropping seasons has attracted attention for its use as a feedstock for biodiesel production (Macagnan, Chaves and Café-Filho, 2010). Yields vary widely: from 1125–1622 kg/ha in Russia to 450–2522 kg/ha in the United States (Falasca *et al.*, 2010). The thousand-seed weight is about 6–10 g, with the hull representing 25 to 30 percent of the total weight (Carlson and Tookey, 1983).

Thevetia (*Thevetia peruviana* K.Schum.)

Thevetia is a native of tropical America, but has been naturalized in tropical regions worldwide. It is grown as an ornamental shrub, generally as hedges, despite the high oil (61 percent) and protein (37 percent) content of the seed

(Ibiyemi *et al.*, 2002). Seeds, leaves, fruits and roots are used in traditional medicine as a purgative, as an emetic and for intermittent fever treatment (Gata-Gonçaves *et al.*, 2003). However, latterly it has been regarded as a potential source of biologically active compounds, including insecticides (Reed, Freedman and Ladd, 1982), rodenticides (Oji *et al.*, 1994; Oji and Okafor, 2000), fungicides (Gata-Gonçaves *et al.*, 2003) and bactericides (Saxena and Jain, 1990; Obasi and Igboechi, 1991).

Polanga (*Calophyllum inophyllum* L.)

Commonly called Alexandrian laurel, *Calophyllum inophyllum* is a tree found mainly in the tropics. It grows on rocky and sandy seashores, requires moderate temperatures and an annual rainfall ranging from 750 to 5000 mm. It is planted up to 1200 m altitude (Louppe, Oteng-Amoako and Brink, 2008). A mature tree may yield 50 kg of dry fruits (45 percent kernel). According to Ajayi *et al.* (2008), the seed contains 49.2 percent oil, an oil that has long been used for lighting in India and across the Pacific. The purified oil is used in cosmetics and also to treat glandular swellings in the neck and jaws (Louppe, Oteng-Amoako and Brink, 2008).

Nahar (*Mesua ferrea* L.)

Mesua ferrea is an evergreen tree growing naturally in the sub-canopy of moist tropical and subtropical forests in India. It grows at 100 to 1000 m altitude, but does not coppice well (Khan *et al.*, 1999).

It is used as firewood. The tree yields a timber used for heavy construction. The flowers are used in dyeing. In traditional medicine, the flowers are used to terminate pregnancy. The kernels and the seed oil are used for dressing wounds (Orwa *et al.*, 2009).

Neem (*Azadirachta indica* A.Juss.)

Commonly known as neem, *Azadirachta indica* is one of the most important native trees of India. It grows also in South and South East Asia and other tropical regions. Neem survives at annual average temperatures ranging between 21 and 32 °C with an annual rainfall between 120 and 1120 mm. It is usually found on plains and low-lying hilly areas, and altitudes between 700 and 800 m. Its resistance to drought and its ability to grow in poor soils leads to its incorporation in forestation programmes (Yakkundi, 1997; Uko *et al.*, 2006). All the tree parts (roots, trunk, bark, leaves and fruits) have been used in industry and folk medicine. Neem oil is considered as non-edible because it is rich in sulphur compounds (acyclic di-, tri- and tetra-sulphides with di-n-propyl disulphide being the major component). These sulphur compounds and limonoids (tetranortriterpenoids) give the oil seed cake a bitter taste (Yakkundi, 1997).

Karanj (*Pongamia pinnata* (L.) Pierre)

Karanj, as it is commonly called, is native to the Asian sub-continent, is found naturally along coasts and riverbanks as it is tolerant of water-logging, and both saline and alkaline soils. It can withstand harsh climates and is suitable for degraded lands (Wani and Sreedevi, 2011). The seeds yield non-edible karanj oil, which has medicinal properties (Wani and Sreedevi, 2011).

Croton (*Croton tiglium* L.)

Croton is native to tropical Asia. It grows in subtropical humid to tropical dry conditions up to an altitude of 1500 m, with an annual rainfall from 700 to 4300 mm, temperatures from 21.0 to 27.5 °C and a soil pH from 4.5 to 7.5 (Duke, 1983).

Croton oil is a very strong laxative and is highly toxic when used as such. The oil has also been used in preparations as a counterirritant on the skin (Alexander *et al.*, 2008a), although other studies resulted in the oil being deemed unsafe for either use due to its carcinogenic activity (Hecker, 1968) and the presence of phorbol esters, known for their tumour promoting activity (Goel *et al.*, 2007).

CHEMICAL COMPOSITION OF CO-PRODUCTS OF THE NON-EDIBLE OIL-BASED BIODIESEL INDUSTRY

Oil cakes or oil meals are solid residues obtained after oil extraction from the seeds. Their composition varies depending on plant species, growing conditions and extraction methods used (Kolesárová *et al.*, 2011). The oil cake is the co-product obtained after oil extraction by mechanical pressing and usually contains residual oil. However, in order to maximize oil extraction, the cake can be exhaustively extracted by organic solvents. The resulting co-product, called oil meal, is low in residual oil but it contains more crude protein (CP) than oil cake. One common feature of oil cakes and meals is their high protein content (Ramachandran *et al.*, 2007).

The chemical compositions of the different non-edible oil cakes and meals are summarized in Table 1. Traditionally oil cakes and meals from edible plants are used in livestock feeding because of their high protein content. Non-edible oil cakes and meals are also rich in protein. CP content is highest for crambe meal and lowest for mesua meal.

Castor meal contains 27 percent CP and its fibre content is higher than the other non-edible cakes and meals. Castor meal is deficient in the essential amino acids methionine, lysine and tryptophan (Table 2). Rubber seed meal contains 22 percent CP, but its ash content is lower than many other oil cakes and meals. The amino acid composition of rubber seed meal shows a well balanced profile with high levels of glutamic acid, aspartic acid and leucine. Lysine and sulphur amino acids are deficient (Oyewusi, Akintayo and Olaofe,

TABLE 1
Nutritional and antinutritional components (g/100 g DM) of different oil cakes and meals

	Co-product	Ether extract	Crude protein	Crude fibre	Minerals	Toxic compounds										Reference						
						Ricin (thermolabile protein), ricinine (alkaloid), CB-1A (stable allergen)	Cyanogenic glycosides (linamarin and lotaustralin), phytohaemagglutinin (antifertility factor)	Epi-progoitrin (thioglucoiside)	Cardiac glycosides (thevetin A, thevetoside, gluco-peruvoside and acetylated monoside)	Calaustralin (phenylcoumarin derivative)	Unknown apolar toxic factor(s)	Azadirachtin (tetranoortriterpenoid antifeedant), isoprenoids and nimbidin (sulphurous compound)	Karanjinin (furanoflavonoid), antinutritional factors (phytates, tannins and protease inhibitors and glabrin)	Met	Phe		Pro	Ser	Thr	Trp	Tyr	Val
<i>Ricinus communis</i>	Meal	0.3	27.1	41.1	7.5																	Ghandi, Cherian and Mulky, 1994.
<i>Hevea brasiliensis</i>	Meal	15.8	21.9	ND	2.3																	Oyewusi, Akintayo and Olaofe, 2007.
<i>Crambe abyssinica</i>	Meal	0.9	46 – 58	6.7	8.6																	Carlson and Tooke, 1983.
<i>Thevetia peruviana</i>	Meal	0.5	42.8 – 47.5	5.20	ND																	Atteh, Ibiyemi and Ojo, 1995; Usman <i>et al.</i> , 2009.
<i>Calophyllum inophyllum</i>	Cake	ND	24	ND	ND																	Mohapatra and Samal, 2002.
<i>Mesua ferrea</i> Linn	Meal	0.96–14.0	11.3–15.7	4.5–9.2	5.3 – 5.4																	Baruah, Kalita and Saikia, 1997; Konwar, Ahmed and Medhi, 1999.
<i>Azadirachta indica</i>	Cake	3.6–9.1	45.0–49.4	5.5–8.6	7.6 – 9.5																	Rao, 1987.
<i>Pongamia pinnata</i>	Cake	14.4	24.2	3.9	5.2																	Natanam, Kadirvel and Viswanathan, 1989.

Notes: ND = not determined.

TABLE 2
Amino acid composition of different oil cakes and meals (g/16 g N)

	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Lys	Leu	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Reference
<i>Ricinus communis</i>	4.1	7.9	9.1	1.4	18.3	4.2	1.6	4.7	2.8	6.1	1.6	3.7	3.6	5.4	3.3	0.3	2.3	5.5	Mottola <i>et al.</i> , 1968; Vilhjalmsson and Fisher, 1971.
<i>Hevea brasiliensis</i>	2.4	5.1	8.0	1.5	11.2	4.0	2.4	3.5	5.0	7.2	1.5	4.9	1.8	3.0	2.3		3.4	3.8	Oyewusi, Akintayo and Olaofe, 2007.
<i>Crambe abyssinica</i>	4.0	6.5	6.8	2.7	15.6	5.0	2.4	3.9	5.3	6.3	1.7	3.7	5.9	3.8	3.8	1.5		5.0	Carlson and Tooke, 1983; Lazzeri <i>et al.</i> , 1994.
<i>Thevetia peruviana</i>	10.1	10.5	44.6	2.2	31.9	9.0	3.5	4.0	9.1	9.2	1.4	5.8	8.5	8.8	4.5		3.9	8.5	Atteh, Ibiyemi and Ojo, 1995; Usman <i>et al.</i> , 2009.
<i>Calophyllum inophyllum</i>		9.2		1.3			1.2	2.3	3.3	4.9	0.6	3.5		2.3	0.9			3.1	Venkatesan and Rege, 1973.
<i>Azadirachta indica</i>	3.7	8.5	10.6	0.3	24.2	4.3	1.9	2.6	3.3	6.5	1.1	3.8	3.9	4.9	3.2	1.2	2.1	3.6	Rao, 1987.
<i>Pongamia pinnata</i>	4.1	6.2	13.3	0.1	19.1	4.7	2.8	4.2	8.4	10.1	0.4	6.6	5.1	4.5	2.7		2.0	5.4	Vinay and Sindhu Kanya, 2008.

2007). In addition to a significant amount of CP in crambe meal, protein efficiency tests showed that its protein is of good nutritional quality, with a well balanced amino acid profile (Ghandi, Cherian and Mulky, 1994).

Thevetia meal has a protein content comparable to that of soybean meal (Atteh, Ibiyemi and Ojo, 1995). Thevetia meal protein is rich in lysine but deficient in methionine, cysteine and isoleucine.

Calophyllum meal has medium protein content (24 percent). The amino acid composition shows very low methionine, appreciably lower than that of ricinus and crambe meals (Venkatesan and Rege, 1973). Mesua meal has the lowest protein content among the species discussed here, with only 11 to 16 percent CP (Baruah, Kalita and Saikia, 1997). Azadirachta cake is similar in composition to crambe cake. Unlike crambe, azadirachta protein is limited in sulphur-containing amino acids, although it is rich in lysine (Rao, 1987). Meal from pongamia is a good source of protein, rich in lysine, leucine, tyrosine and phenylalanine, and in sulphur-containing amino acids (Vinay and Sindhu Kanya, 2008).

TOXICITY OF NON-EDIBLE CAKES AND MEALS

Non-edible oil cakes and meals are characterized by the presence of anti-nutritional and toxic factors (Table 1) which preclude the utilization of these co-products directly as animal feed (Sivaramkrishnan and Gangadharan, 2009).

Castor cake is poisonous and allergenic to animals because of the presence of three antinutritional compounds: ricin (a heat labile toxic protein), ricinine (a toxic alkaloid) and a stable allergen known as CB-1A (Gardner *et al.*, 1960; Ogunniyi, 2006; Gowda *et al.*, 2009). For details on the detoxification of castor meal and its utilization in animal diets, see Anandan and Sampath (this volume).

Rubber cake is toxic because of the presence of linamarin and lotaustrolin, cyanogenic glycosides which after enzymic hydrolysis by linamarinase (an endogenous glucosidase) liberate HCN (Ukpebor *et al.*, 2007). Raw rubber meal is suspected of containing an unknown anti-fertility factor and phytohaemagglutinin. Feeding raw rubber meal caused a decline in semen volume and sperm count when fed up to 20 percent of the diet for white leghorn cockerels (Ravindran, Rajaguru and De Silva, 1987). It caused a depression in plasma protein and albumin when fed at more than 10 percent in the diet of growing swine (Babatunde, Pond and Peo, 1990).

Crambe meal contains epi-progoitrin, a thioglucoside, which undergoes a hydrolysis reaction sequence, initiated by the thioglucosidase enzyme system, leading to any of four major products: two diastereomeric (2S)-l-cyano-2-hydroxy-3,4-epithiobutanes and (S)-1-cyano-2-hydroxy-3-butene (Daxenbichler, Van Etten and Wolff 1968); and 5-vinyloxazolidine-2-thione (goitrin), which suppresses thyroidal iodine

uptake and causes thyroid hyperplasia and hypertrophy (Gould and Gumbmann, 1980). Thus feeding raw crambe meal with intact glucosinolates and active thioglucosidase can reduce palatability and cause growth inhibition and pathological changes in body organs (Carlson and Tookey, 1983).

The most important active constituents of thevetia responsible for exerting cardiotoxic effects are the cardiac glycosides (Langford and Boor, 1996), among which are thevetin A, thevebioside, gluco-peruvoside, acetylated monoside and other cerebrosides (Bisset and Bogor, 1962). Raw thevetia cake was extremely toxic when fed up to 15 percent in the starter and finishing diets for broilers (Atteh, Ibiyemi and Ojo, 1995).

Mesua cake is toxic when oil extraction is not complete, due to the presence of unknown deleterious substances in the residual oil (Konwar, Ahmad and Medhi, 1999).

One of the toxic compounds of calophyllum cake has been identified as calaustrolin (Dash *et al.*, 1990).

Toxicity of azadirachta cake is caused by the presence of azadirachtin, tetranortriterpenoid (an antifeedant), isoprenoids and nimbidin, a sulphurous compound (Yakkundi, 1997; Usman *et al.*, 2005; Saxena *et al.*, 2010). Uko *et al.* (2006) incorporated up to 30 percent raw full fat azadirachta kernels into cockerel chick diets. Feed intake and body weight gain were depressed independently of the inclusion level, and starting from 15 percent in the diet, anaemia and leucocytosis occurred. Defatted azadirachta meal included up to 10 percent in the diet of in-lay Japanese quails reduced feed efficiency (but intake, egg production and quality were not affected) and caused adverse effects in liver and kidney tissues with long-term feeding (Elangovan *et al.*, 2000).

Pongamia cake contains anti-nutritional factors such as phytates, tannins, protease inhibitors, glabrin and a fat-soluble constituent karanjinin (a furano-flavonoid) (Vinay and Sindhu Kanya, 2008). When fed untreated to chicks, karanj expeller cake depressed weight gain when included at 10 percent of the diet, and elicited 100 percent mortality at 40 percent inclusion rate (Natanam, Kadirvel and Ravi, 1989). At 10 percent, untreated karanj cake and meal fed to 18-week-old white leghorn pullets decreased feed efficiency, egg production and quality (Natanam, Kadirvel and Viswanathan, 1989). Long-term feeding at 20 and 24 percent cake or meal in lamb concentrate had deleterious effects on lamb performance, especially spermatogenesis (Singh *et al.*, 2006).

Croton meal, in addition to containing carcinogenic phorbol esters, contains a toxic glycoprotein belonging to the type II group of ribosome inactivating proteins, crotonin, similar to ricin (Stirpe *et al.*, 1976). Crotonin showed LD₅₀ of 20 mg/kg body weight when administered intraperitoneal in mice (Alexander *et al.*, 2008a). Non-toxic lectin, with effects on agglutination and haemolytic abilities

of erythrocytes in sheep and rabbits, was isolated from croton seeds (Banerjee and Sen, 1981).

POSSIBILITY OF FEEDING SOME UNTREATED NON-EDIBLE CAKES AND MEALS FROM SEEDS THAT GIVE NON-EDIBLE OILS

Feeding non-edible cakes and meals is not recommended before the appropriate treatment. However, feeding trials with untreated non-edible cakes and meals have been carried out based on two principles: (1) feeding up to, but not beyond, the threshold level of toxicity; and (2) the apolar toxic compounds get extracted with the oil, making the oil non-edible and the residual meal edible.

Untreated mesua cake feeding is reported to be possible when the oil extraction is complete, because the toxic compounds are soluble in the oil (Konwar, Ahmad and Medhi, 1999). Raw mesua meal containing 14 percent residual oil could be included up to 15 percent in the starter diet of chicks without any effect on body weight, but at higher levels feed efficiency was reduced (Baruah, Kalita and Saikia, 1997). When included in the diet of white leghorn layers at 15 percent of the ration, egg production and weight were significantly depressed (Baruah, Kalita and Saikia, 1997).

Calaustralin, a phenylcoumarin derivative present in *Calophyllum inophyllum* (Bhushan, Rangaswani and Seshadri, 1975), is not polar and thus can be extracted with the oil. De-fatted calophyllum cake can be fed at up to 15 percent in the diet of chicks, but with slight growth depression (Dash *et al.*, 1990). Mohapatra and Samal (2002) reported that an amino acid deficiency was the cause of the decline in weight gain of laying hens when offered calophyllum cake at 37 percent of their diet.

POSSIBILITY OF FEEDING SOME TREATED NON-EDIBLE CAKES AND MEALS FROM SEEDS THAT GIVE EDIBLE OILS

There are some non-edible meals and cakes that originate from seeds whose oils are edible. Examples are: *Balanites aegyptica*, *Terminalia bellirica*, *Putranjiva roxburghii*, *Perilla frutescens*, *Madhuca indica* and *Moringa oleifera*. *Camelina sativa*, which belongs to this group, is not discussed here. The utilization of its meal and cake in animal feeding is discussed in Chapter 17 of this document.

Balanites aegyptica cake is regarded as unsuitable for livestock feeding because it contains steroidal sapogenins (Chapagain and Wiesman, 2007). Sapogenin content was reduced from 3.2 g/100 g protein in the cake to 2.4 g/100 g in the fine fraction (Mohamed, Wolf and Spiess, 2000). Protein extraction by wet sieving using methanol reduced sapogenin to 1.7 g/100 g protein in the protein extract (Mohamed, Wolf and Spiess, 2000). Either fraction, obtained from air classification or wet sieving, has lower *in vitro* protein digestibility (82.0 and 86.4 percent,

respectively) compared with the balanites cake (93.7 percent), due probably to their (fractions) enrichment in phytic acid (Mohamed, Wolf and Spiess, 2000).

Due to the high content of total phenols and tannins (Alexander *et al.*, 2008b), *Terminalia bellirica* seeds are used traditionally for tanning purposes (Rukmini and Rao, 1986). Terminalia meal contains unidentified heat stable antinutritional factors that result in lower feed intake and death in rats, mice and chicks (Rukmini and Rao, 1986).

Putranjiva roxburghii kernels contain phenyl, isopropyl and sec-butyl iso-thiocyanates of glucosides (Puntambekar, 1950). Chaudhary *et al.* (2008) isolated a trypsin inhibitor from the putranjiva seeds, active over a broad range of pH (2–12) and temperature (20–80 °C). Raghavendra *et al.* (2010) found that the methanol extracts of the seeds, which contain phenols, alkaloids, steroids, flavonoids and glycosides, showed cytotoxicity with an LC₅₀ of 427.7 µg/ml in the brine shrimp lethality assay.

Although perilla seed oil is edible, perilla seed may be a source of a food allergen. Two cases were reported and studied by Jeong *et al.* (2006), where perilla seed caused anaphylaxis in two patients.

Mahua cake contains sapoglucosides that are bitter and toxic to livestock (Varma and Singh, 1979). Because of the harvesting time (at peak rainfall), the occurrence of aflatoxins constitute an additional problem when feeding mahua cake (Sidhu, Chandra and Behl, 2009). Mahua meal can be fed raw, up to 22 percent of the concentrate to rams, without any differences in slaughter weight and carcass characteristics (Kesava Rao *et al.*, 1998). Feeding mahua meal up to 15 percent in broiler chick rations induced lower feed intake, lower body weight gain and poor feed conversion ratio (Kumar, Vaishnava and Sajjan, 2000). Hot water and isopropanol (60 to 80 percent) treatment resulted in reducing the saponins content by 74 and 90 percent, respectively (Varma and Singh, 1979).

Moringa seeds contain glucosinolates that yield 4-(α -L-rhamnosyloxy)-benzyl isothiocyanate after crushing (Makkar and Becker, 1997; Bosch, 2004). The glucosinolates present can be removed by water treatment (Makkar and Becker, 1997). However, the seeds also contain cationic peptides that have antibiotic properties and at high levels could decrease productivity (Ben Salem and Makkar, 2009).

A summary of feeding trials with these non-edible cakes and meals, either raw at low inclusion rates or after appropriate treatment, is reported in Table 3.

DETOXIFICATION METHODS

The risk of toxicity can be less serious with decreasing contents of the toxic compounds and anti-nutritional factors following appropriate treatments. Methods of detoxification can be classified into chemical, physical, biochemical and a combination of these processes.

TABLE 3
Effects on animal performance of feeding non-edible cakes and meals (from seeds that give edible oil) after different detoxification treatments

	Toxic compound	Detoxification methods and animal response	Reference
<i>Balanites aegyptica</i>	Steroidal sapogenins, diosgenin as the aglycon.	Up to 20% in the sheep diet. No significant difference in feed intake, liveweight gain and carcass analysis with the control group fed cotton-seed meal. A distinct black mucous membrane of the rumen was observed. At up to 12.5%, the diet fed to laying hens induced diarrhoea and retarded growth and led to cessation of egg laying.	El Khidir <i>et al.</i> , 1983.
<i>Terminalia bellirica</i>	Heat stable factors	Fed up to 10% either raw or cooked to rats, mice and chicks. Feed intake was 1 g/animal/day. In two weeks, all the animals receiving raw as well as cooked kernel meal died.	Rukmini and Rao, 1986.
<i>Perilla frutescens</i>	Not reported	Raw meal up to 28% in rat diet when fed for 4 weeks did not affect significantly the feed intake but because of its deficiency in valine, body weight gain was less. De-hulled and cooked meal fed up to 28% in diet resulted in comparable body weight gain in rats fed casein.	Longvah and Deosthale, 1998.
<i>Madhuca indica</i>	Sapogluosides	Fed treated cake (first with 2.5% ferrous sulphate, then cooked or treated with 2.3% HCHO) up to 22% of the concentrate in rams' diet did not affect the slaughter weight, carcass characteristics or meat quality attributes. Washed meal (repeated cold water washing) replaced up to 100% of groundnut cake in buffalo diet. No significant difference in feed intake, nutrient digestibility or milk yield and composition.	Kesava Rao <i>et al.</i> , 1998. Tiwari and Patle, 1983.
<i>Moringa oleifera</i>	Glucosinolate, cationic peptides (anti-fermentative)	Raw cake could be fed up to 6 g daily to growing lambs. At 4 g inclusion per day of raw cake the growth rate of the lambs improved.	Ben Salem and Makkar, 2009.

Chemical treatments

Chemical treatments include additives, alkaline and acidic treatments and solvent extraction. Although chemical treatment can reduce substantially the content of toxic compounds, the resulting meal or the protein extract has lower protein and amino acid content. Sodium hydroxide treatment reduced up to 98 percent the allergen content in castor meal (Gardner *et al.*, 1960) and reduced the toxicity of pongamia meal by converting karanjinin to less toxic intermediates (Panda, Sastry and Mandal, 2008).

Ammoniation of crambe meal resulted in the disappearance of glucosinolates (Kirk, Mustakas and Griffin, 1966), but this treatment decreased lysine levels (Liu, Steg and Hindle, 1993) and formed undesirable cyanobutane and other aglucon products in the meal which were still toxic (Carlson and Tookey, 1983). Ammoniation of azadirachta cake was found to result in a detoxified product suitable for animal feeding (Nagalakshmi *et al.*, 1999).

Hydrochloric acid treatment (soaking the meal in 2 percent HCl for 1 hour at room temperature, bringing up the pH to iso-electric point by diluted alkali and washing the residue) of pongamia meal resulted in the removal of up to 54 percent of the tannins, up to 72 percent of the phytates and up to 74 percent of trypsin inhibitor activity. This had the corollary of reduction of the protein content from 33 percent in the raw meal to 23 percent in the treated meal, but without affecting available lysine (3.6 percent to 3.5 percent) (Vinay and Sindhu Kanya, 2008).

Other chemical additives have also been used for inactivation of the toxic compounds. Calcium hydroxide was less effective than HCl for the detoxification of pongamia meal.

Although 0.5 percent Ca(OH)₂ reduced the content of tannins and phytates substantially, it also led to a significant decrease in nutritive value of proteins and destruction of lysine, with the production of toxic constituents such as lysino-alanine (Vinay and Sindhu Kanya, 2008). Sodium carbonate left 1.7 percent epi-progoitrin in crambe meal, thus reducing its content by 82 percent (Mustakas *et al.*, 1976), while only 0.6 percent remained in ferrous sulphate-treated meal (Kirk *et al.*, 1971).

Solvent extractions are used depending on the polarity of the toxic compounds. Water washing is one of the successful methods of detoxification carried out on crambe meal (Baker *et al.*, 1977), azadirachta meal (Agrawal, Garg and Nath, 1987) and pongamia meal (Vinay and Sindhu Kanya, 2008), despite the loss of water-soluble nutrients. Water washing of crambe meal after inactivation of thioglucosidase resulted in 20 to 25 percent DM loss, but the resulting meal contained 50 percent CP, a balanced amino acid composition and 0.6 percent residual epi-progoitrin (Baker *et al.*, 1977). Rubber meal soaked in water (1:3) for 24 hours resulted in a substantial reduction in HCN content after one month of storage (from 120 to 2.6 mg/kg) (Narahari and Kothandaraman, 1983). Acetone extraction of crambe meal resulted in total removal of thioglucosides and epi-progoitrin from the meal, with good residual biological value protein (Van Etten *et al.*, 1969). Alcohol extraction of thevetia meal by a mixture of ethanol+methanol (80:20) resulted in 98 percent reduction in the glycoside content, with 18 percent DM loss and 25 percent CP increase (Oluwaniyi, Ibiyemi and Usman, 2007). Extraction of azadirachta meal with 80 percent methanol resulted in

TABLE 4
Effects on animal performance of feeding non-edible cakes or meals after different detoxification treatments

	Treatment	Animal response	Reference
<i>Ricinus communis</i>	Roasted meal	Adverse effect when fed up to 10% to ducks for four weeks.	Okoye <i>et al.</i> , 1987.
	Two-stage cooked dehulled meal (100 °C for 50 minutes)	Up to 10% in the diet of six week-old broiler birds for optimum performance.	Ani and Okorie, 2009.
	Meal supplemented with Ca(OH) ₂ at 4 to 6%	Up to 10% and 15% in the diet of sheep and beef cattle, respectively, without adverse effects on feed intake or daily body weight gain.	De Oliveira <i>et al.</i> , 2010. Diniz <i>et al.</i> , 2010.
	Meal mixed with <i>Shorea robusta</i> seed meal (1:1), followed by treatment with ammonia and heat.	The mixture was fed at 20% of the diet to rats. The rats survived and had weight gain and feed intake comparable to the control group (15% casein).	Ghandi, Cherian and Mulky, 1994.
<i>Hevea brasiliensis</i>	Meal prepared from soaked-cooked-dried seeds	Fed up to 60% in the diet of rats (approximately 20% of protein in the diet). No evidence of toxicity. Feed intake, protein efficiency and growth rate comparable to casein-fed group at the same incorporation level.	Lauw Tjin Giok <i>et al.</i> , 1967.
<i>Crambe abyssinica</i>	Meal extracted with aqueous acetone	Replaced 20% of casein in the rat diet. Normal growth and equivalent protein efficiency compared with the casein.	Van Etten <i>et al.</i> , 1969.
	Meal supplemented with Na ₂ CO ₃	When fed at dietary levels of 20 to 30% to chicks, there was a growth limitation (70–80% of the control). Some adverse side effect on organs.	Carlson and Tookey, 1983.
	Water-washed meal	Up to 10% in broiler chicken diet but with a decrease in feed intake.	Kloss <i>et al.</i> , 1994.
	Heat-Carbonate-treated meal	Up to 70% in beef cattle diet. Lower feed intake and daily weight gains, without significant differences in feed efficiency. Increased palatability with dehulling. Dehulled crambe meal so prepared, can replace up to two-thirds of soybean meal in the supplement. Up to 30% in the pig diet. Crambe meal so treated has higher energy digestibility but lower protein utilization than rapeseed meal.	Lambert <i>et al.</i> , 1970. Liu <i>et al.</i> , 1995.
<i>Thevetia peruviana</i>	Alkali and acid treatment of the cake	Reduced feed intake and weight gain at up to 15% in the chick diet. Alkaline and acid hydrolysis was not efficient.	Usman <i>et al.</i> , 2009.
	Protein concentrate from the cake	Fed up to 30% to replace soybean in the chick diet with satisfactory performance of 90% of the animals and 10% mortality.	Odetokun, Akindumila and Ibukun, 1999.
	Autoclaved cake	Up to 10% in rabbit diet, no mortality, reduced feed intake, diarrhoea and rough and dry coat observed. Autoclaved cake could not support productive growth.	Taiwo, Afolabi and Adegbuyi, 2004.
<i>Calophyllum inophyllum</i>	Protein extract from the meal	Diets with 25 and 50% were able to support normal growth in rats when adequately supplemented with deficient amino acids.	Venkatesan and Rege, 1973.
<i>Azadirachta indica</i>	Water-washed cake	Up to 45% of concentrate fed to growing calves with no adverse effects on intake, digestibility and weight gain.	Nath, Rajagopal and Garg, 1983.
		Fed up to 40% of concentrate to buffalo calves. Led to higher weight gain, higher nitrogen balance and reduced urinary N. No significant difference in intake and CP digestibility.	Agrawal, Garg and Nath, 1987.
		Could be fed up to 25% of concentrate to growing goats without significant difference in feed intake, body weight gain and feed conversion efficiency.	Verma, Sastry and Agrawal, 1995.
	Organic solvent-extracted cake	Ethanol-hexane extracted cake fed up to 84% of the diet to rats without toxic effect. Protein use efficiency was comparable to the conventional cake.	Rao, 1987.
	Urea-ammoniated meal	Methanol extracted cake fed to rats at a rate of 25% in diet was promising in terms of feed efficiency and weight gain.	James, Ameh and Agbaji, 2009.
		Replaced groundnut meal totally (22.5% of concentrate) in the diet of goats, without significant effect on feed intake and body gain weight.	Anandan <i>et al.</i> , 1999.
		Lower digestibility parameters in lambs when included at 33% of concentrate. Similar average daily weight gain but enlarged kidneys observed.	Musalía <i>et al.</i> , 2000.
Up to 10% inclusion in the diet could support the overall productive performance of white leghorn, without any obvious adverse effect.		Verma, Gowda and Elangovan, 1998.	
<i>Pongamia pinnata</i>	Water-washed cake	Fed at 13.5% of the concentrate to lambs, it did not affect the feed intake, body weight gain and nutrient digestibility.	Soren and Sastry, 2009.
	Toxin-bonded cake	Fed at 13.5% of the concentrate to lambs; it decreased feed intake and body weight gain.	Soren and Sastry, 2009.
	Alkali-treated meal	NaOH-treated meal could replace up to 12.5% of the soybean meal in the starter diet of the broiler chicken without significant difference in body weight gain and feed efficiency.	Panda, Sastry and Mandal, 2008
	2% hydrochloric acid-treated meal	At 30% in the rat diet, no deleterious effects.	Mandal, Ghosh Majumdar and Maity, 1985.

a spent meal free from the antinutritional factors (Saxena *et al.*, 2010).

Protein extraction is another procedure to obtain pure protein isolates for use as animal feed and additives. The method consists of protein solubilization in alkaline solution followed by protein precipitation by acid at the iso-electric pH (Saetae and Suntornsuk, 2011). Usman *et al.* (2005) isolated proteins from azadirachta meal using 0.5 M NaCl at pH 7.5.

Physical treatments

Physical treatments lead to denaturation of the active toxic compounds and include thermal treatments (autoclaving, moist heat and microwave) (Liu, Steg and Hindle, 1993). Dry heating was effective in the de-allergenization of alkali-treated ricinus meal at 205 °C for 95 minutes (Gardner *et al.*, 1960). Cooking ricinus meal for 10 minutes destroyed its ricin content (Barnes, Baldwin and Braasch, 2009). Steam cooking of crambe meal reduced its content of epi-progoitrin by 30 percent, but increased the toxic nitrile content and decreased the level of available lysine (Liu, Steg and Hindle, 1993).

Biochemical treatments

Biochemical detoxification treatments are based on enzymic and fermentative reactions. Trypsin (6 percent by weight of the meal) digestion of ricinus meal resulted in a complete de-allergenization of the treated meal (Gardner *et al.*, 1960). The binding properties of tannins were used by Ghandi, Cherian and Mulky (1994). In their work, the toxic factor of the castor cake was neutralized by the tannins present in sal (*Shorea robusta*) seed meal.

Fermenting rubber cake and meal with the mycelium of the edible mushroom *Pleurotus tuberregium* for 96 hours at room temperature resulted in a decrease of its total cyanogens content from 500 to 5 ppm for the cake and from 300 to 4 ppm for the meal. The treatment resulted additionally in an increase in CP content from 29 to 39 percent (Ukpebor *et al.*, 2007).

A study realized by Hundsdoerfer *et al.* (2005) indicated that the larvae of *Hyles euphorbiae* could metabolize synthetic phorbol esters (12-tetradecanoyl-phorbol-13-acetate) either injected or fed. Phorbol esters occur in different species of the Euphorbiaceae, e.g. croton and jatropa.

EFFECTS OF FEEDING TREATED NON-EDIBLE CAKES OR MEALS ON ANIMAL RESPONSE AND PERFORMANCE

The usefulness of treated non-edible oil cakes and meals as animal feed depends on the efficiency and economic viability of detoxification methods and the possibility of using the treated co-products. The results of the feeding trials conducted on laboratory and farm animals after appropri-

ate detoxification treatments of the non-edible cakes and meals are summarized in Table 4. Some of the detoxification attempts have shown promising results, and, at low levels of their inclusion in diets, many can be adopted without adverse effects on animal welfare and performance. Although many treatments are promising, the challenge lies in developing cost-effective and simple processes that can be adopted by farmers.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Studies on the possible use of co-products of the biofuel industry based on non-edible oils for animal feeding show that a lot still needs to be done. Main knowledge gaps are:

- The nature of some toxic compounds in these co-products is unknown. The current methodologies for analysing many of the toxic and antinutritional factors need improvement. For developing an effective detoxification process, it is necessary to define the chemical nature of the toxic compound(s) and their mode of action. This information is not available for many of the co-products.
- There is a need to further improve the detoxification processes for *Thevetia peruviana*, *Hevea brasiliensis*, *Calophyllum inopylum*, *Mesua ferrea* and *Croton tiglium*. Studies on the utilization of the detoxified material by various farm animal species should also be conducted.
- Scaling up is needed for promising detoxification processes. The implementation of positive results can be successful only if large amounts of the co-products can be treated and used for animal feeding.
- The development, use and scaling up of the detoxification processes should be accompanied by socio-economic analysis.
- Preparation of high-value protein isolates and peptides for use in livestock feeds could be an alternative approach for use of otherwise non-edible cakes and meals, an approach that so far has received little attention. Processing for preparation of protein isolates and peptides could eliminate the toxic and antinutritional factors. Future work is warranted on this topic.

CONCLUSIONS

To make the biofuel industry more profitable and sustainable, use of the co-products-derived cakes and meals, generally rich in protein is of utmost importance. Detoxification has been successful for some of these products:

- *Ricinus communis* meal cooked at 100 °C for 50 minutes could be considered for addition at up to 15 percent in chick diets. The addition of lime at 4 percent was also promising when fed at up to 10 and 15 percent in the diet of sheep and beef cattle, respectively.
- *Hevea brasiliensis* meal soaked in water and left to ferment, or meal obtained from originally soaked seeds,

contains less HCN (reduced from 120 to 2.6 mg/kg DM after one month of storage). However feeding trials on farm animals need to be conducted to confirm the safety of feeding.

- Heat-carbonate-treated dehulled-meal from *Crambe abyssinica* has been shown to have acceptable palatability and can replace up to two-thirds of soybean meal in the supplement for beef cattle.
- Water washing, methanol extraction, urea and alkali treatments of *Azadirachta indica* meal gave promising results when fed to farm animals. Water-washed neem cake could be fed at up to 45 percent of concentrate for calves.
- Water-washed *Pongamia pinnata* meal can be incorporated at up to 13.5 percent of the concentrate in lamb diet. Alkali treatment was also effective.

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Chapter 20

Status of biofuels in India and scope of utilizing castor (*Ricinus communis*) cake – a biofuel co-product – as livestock feed

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ABSTRACT

Biofuel policy in India is unique in that it has been clearly spelt out that feedstock must be based on non-food sources, thus avoiding a possible food vs fuel conflict. Further, the policy views biofuels as a potential means to stimulate rural development and generate employment opportunities by using vast areas of land that are otherwise unfit for agriculture. Although the policy has the ambitious target of achieving 20 percent biofuel blending by 2017, currently less than 5 percent blending of petrol has been achieved. Based on current production levels, it is unlikely that India will fulfil the set targets. Major reasons include slow progress in establishing the area under jatropha (*Jatropha curcas*) cultivation; low productivity and poor market infrastructure for jatropha; land availability constrained by sugar cane expansion; a plateau in productivity of sugar cane; the price structure for biofuels; and import policy. Among the various co-products of biofuel, castor (*Ricinus communis*) cake is one of the potential resources that could be used for feeding livestock. Although castor cake has high protein, its use in livestock feeding is restricted due to the presence of toxic factors and it is currently being used as organic fertilizer, leading to under-utilization of a precious resource. Substantial research has been carried out to identify the nature of the toxins, their toxicity, susceptibility to various treatments and production response of different livestock to feeding processed cake. In spite of all the efforts, castor cake has not found a place as a feed resource and continues to be used as organic fertilizer, leading to its under-utilization. All the major castor producing countries – Brazil, China and India – have large livestock populations and big demand for protein supplements, so an appropriate detoxification technology to make use of castor cake could add great value to the castor, to the benefit of livestock producers and processing industries.

INTRODUCTION

India is one of the fastest growing economies in the world and energy is a critical input for socio-economic development. Fossil fuels will continue to play a dominant role in fulfilling the energy needs of India in the next few decades. Provisional estimates have indicated that domestic crude fossil fuel oil is able to meet only about 25–30 percent of demand, while the rest is met from imported crude. Biofuels are globally considered sustainable and an eco-friendly source of energy, and these also enhance national energy security and decrease dependence on imported fossil fuels. The growing interest in and demand for biofuels have resulted in diversion of grains, oilseeds, land and water resources to biofuels which otherwise could have potentially contributed to food and feed resources. For a large country like India, with a billion-plus human population and limited land mass, the role of biofuels has to go beyond the objective of achieving energy security and sustainability, towards addressing food and feed security.

Choice of feedstock for biofuel production and efficient utilization of biofuel co-products can to a great extent address these issues. In the light of the above, an attempt has been made here to review the present status of biofuels in India, and the available technologies for utilizing castor cake – a potential biofuel co-product – as livestock feed.

STATUS OF BIOFUELS IN INDIA

India is one of the largest users of hydrocarbons and it is imperative that the country has a biofuel policy in place to address the issues of the economy (import expenditure), environment and energy security. The Government of India is seriously looking for use of alternative fuels to meet energy demand in a technically efficient, economically viable and environmentally sustainable manner. There are many concerns and challenges to be overcome if biofuels are to contribute positively to an improved environment as well as to agricultural and rural development (FAO, 2008). The 'National Policy on Biofuels' of India, released in 2009,

MAIN MESSAGES

- Biofuel policy in India is based on non-food feedstock to minimize food-fuel conflict, and aims at utilizing the vast wastelands otherwise unfit for agriculture to stimulate rural development and employment.
- Castor cake – a protein rich, castor oil industry co-product – continues to be used as organic fertilizer, leading to under-utilization of a precious protein resource.
- The toxic principles in castor cake limit the direct use of castor cake as livestock feed, and research efforts to evolve suitable detoxification procedures have yielded variable results.
- Research conducted so far has shown that processed castor cake can certainly be incorporated at low levels in ruminant feeds, and with better processing methods higher levels of incorporation are possible.
- A concerted effort by the castor processing industry, researchers, feed industry and livestock farmers could lead to evolving efficient and commercially viable technologies for utilizing castor meal as livestock feed.
- Utilizing castor meal as livestock feed will have great relevance for countries like India, China and Brazil, which are not only the largest producers of castor cake but have large livestock populations and high demand for protein supplements.

foresees biofuels as a potential means to stimulate rural development and generate employment opportunities, as well as aspiring to reap environmental and economic benefits arising out of their large-scale use. The Policy aims at mainstreaming biofuels by setting an indicative target for their blending up to 20 percent with petrol and diesel in the transport sector by 2017 (GOI, 2009). It is categorically mentioned in the Policy that the programme is to be carried out based solely on the non-food feedstocks that are raised on degraded or wastelands not otherwise suitable for agriculture, thus avoiding a possible conflict between food and fuel security. Bio-ethanol produced from sugar cane molasses and biodiesel produced from non-edible oilseed crops like jatropha (*Jatropha curcas*) and pongamia (*Millettia pinnata*) are currently being promoted for commercial use.

The biofuel industry in India is still in its infancy and biofuel production in India accounts for around 1 percent of global production. Of the 2.15 billion litres of ethanol produced in 2008, only 280 million litres were used for blending with petrol, and the target of blending petrol with 5 percent ethanol has yet to be achieved. The major reason for this has been the competing demand for ethanol for potable purposes and the chemical and pharmaceutical industries. To address this issue, the Government has recently increased the minimum purchase price of ethanol

from Rs 21.50 to Rs 27.00 per litre of ethanol, hoping that this would increase the availability for blending. Large-scale blending of biodiesel with conventional diesel has not yet started in India. Around 20 biodiesel plants annually produce 140–300 million litres of biodiesel, which is mostly utilized by the informal sector locally for irrigation and electricity generation, and by the automobile and transportation companies for running their experimental projects (USDA, 2010).

The Planning Commission launched the National Biodiesel Mission to promote jatropha, and the first phase (2003–2007) was mostly a demonstration phase. The second phase involved the expansion of the activities of the first phase to make the programme self-sustaining by producing enough biodiesel to meet the 20 percent blending target (NCAER, 2007). Efforts by the different state governments and the federal government to boost the production of feedstocks for biofuels include the announcement of a minimum purchase price for jatropha seed and for biodiesel, subsidy programmes and tax incentives.

Shinoj *et al.* (2011) projected the demand for ethanol and biodiesel (Table 1) for varying levels of blending of biofuels, considering that the annual demand for petrol is increasing at 8.5 percent and diesel at 7.5 percent.

Based on their projections, Shinoj *et al.* (2011), concluded that to achieve its 20 percent blending target

TABLE 1
Projected demand for biofuels in India (million tonne)

Year	Petrol demand	Diesel demand	5% blending		10% blending		20% blending	
			Bio-ethanol	Biodiesel	Bio-ethanol	Biodiesel	Bio-ethanol	Biodiesel
2011–12	14.37	64.19	2.08	3.21	2.80	6.85	4.23	12.84
2016–17	21.61	92.15	2.68	4.61	3.76	9.83	5.92	18.43
2020–21	29.94	123.06	3.31	6.15	4.80	13.13	7.80	24.61

Source: Shinoj *et al.*, 2011.

India has to triple ethanol production or has to go for massive imports, both of which are unlikely due to the plateau in the productivity of sugar cane, demand for land and water for staple crops, import policy and high price of ethanol in international markets. Similarly for biodiesel, the jatropha-based biodiesel production programme is bogged down because of obstacles like slow progress in planting (current plantation area is 0.5 million hectares against the requirement of 26.25 million hectares for 20 percent blending), sub-optimal processing and marketing infrastructure, and under-developed distribution channels (Shinoj *et al.*, 2011).

BIOFUELS FEEDSTOCK AND CO-PRODUCTS

Globally, the major feedstocks for biofuels are maize, sugar cane and oilseeds, as shown in Table 2.

Unlike other countries, which rely heavily on food crops like maize and oilseeds for their biofuel production, India's major biofuel feedstocks are molasses for ethanol, and non-edible oilseed such as jatropha and pongamia for biodiesel. Other minor feedstock include sugar cane juice, sweet sorghum, tropical sugar beet, edible oil wastage and animal fats. Although India is the largest producer of castor, the possibility of using castor oil for biodiesel production has not been explored intensively. In contrast, in Brazil, the third-largest producer of castor, the Brazilian Ministry of Agrarian Development has revived castor production as raw material for biodiesel (Lago, 2009). The co-products of feedstocks, such as bagasse (fibrous residue of sugar cane after juice extraction), oilseed cakes and glycerol, can be used for feeding livestock as sources of roughage, protein or energy. The scope and limitations of biofuel feedstock co-products from castor for livestock feed is discussed briefly here.

CASTOR CAKE PRODUCTION AND UTILIZATION

India is the largest producer of castor seed, followed by China and Brazil, accounting for around 73, 12 and 7 percent of global production, respectively (FAOSTAT data, 2009). Globally, the area under castor bean has not changed significantly over the last two decades, with little change in production (Table 3). The production of castor seed in India, largest producer of castor, has shown a consistent increase. Much of the castor oil produced in India is exported after meeting local demand. Currently castor oil is not being used for biodiesel production, and in the event of its use as biodiesel the local demand for castor oil in India would go up. This is likely to stimulate castor production, as the castor crop has several advantages over other biodiesel crops in terms of availability of high yielding varieties, short production cycle and consistent, superior yields. Castor oil is one of the world's most useful and economically important natural plant oils, with wide applications. Castor is a high-yield oilseed crop producing around 50 percent oil by weight in the seed, out-yielding conventional oilseeds like soybean, rapeseed, groundnut, sunflower and cottonseed. Castor oil obtained from castor seeds has high viscosity, heat and pressure stability; low freezing point; and the ability to form waxy substances after chemical treatments (Conceic *et al.*, 2005), making it a potential candidate for biodiesel. There are different cultivars of castor, and oil content varies from 46 to 55 percent by weight (Ogunniyi, 2006). The residual castor cake obtained after oil extraction is approximately half of the seed weight. Whole seed contains 29 to 31 percent hulls, which are high in fibre and lignin, and de-hulling improves the oil extraction yield by 15–20 percent, besides improving the oil quality (Shashikala and Singh, 1992). De-cortication machines capable of de-shelling castor seeds are used in Brazil with an efficiency

TABLE 2
Distribution of feedstock in major biofuel producing countries

Country or region	Feedstock		Co-products
	Bio-ethanol	Biodiesel	
USA	Maize	Soy (40%), tallow (20%), canola (20%), palm (20%)	Distillers grain, oilseed cake and glycerol
Brazil	Sugar cane	Soy (80%), tallow (10%), other vegetable oils (10%)	Bagasse, oilseed cake and glycerol
EU	Beet/grain	Rapeseed (50%), soybean oil (40%), palm (5%) and tallow (5%)	Distillers grain, oilseed cake
China	Maize	Waste vegetable oils	Distillers grain, Glycerol
Canada	Maize	Tallow	Distillers grain, Glycerol

Source: Anon., 2009.

TABLE 3
Production of castor seed and cropped area

	1995		2000		2005		2009	
	Area	Production	Area	Production	Area	Production	Area	Production
India	789	780	1080	883	864	991	840	1098
China	190	170	290	300	240	220	210	190
Brazil	76	33	195	101	231	169	159	91
World	1237	1083	1769	1373	1586	1497	1481	1484

Notes: Area in thousand hectare; production in thousand tonne.

of 85 percent and an output of 650 kg/hour (Lago, 2009). De-cortication not only helps in improving the protein content and improve the efficiency of extraction but also reduces the fibre and lignin content in the hulls, which adversely effects the quality of the cake.

The composition of castor cake from different countries as reported by different researchers is presented in Table 4. The protein content of residual cake varies from 29 to 60 percent depending upon whether decorticated or corticated seeds are used for extraction (Mottola *et al.*, 1968; Okorie and Anugwa, 1987; Anandan, Anil and Ramachandra, 2005). Alongside its high protein content, castor seed contains highly toxic and allergenic compounds, which severely limit or prevent its use as feed after oil extraction (Thorpe *et al.*, 1988; Audi *et al.*, 2005). The rumen degradability of castor bean meal protein was estimated to be 61.9 percent (Diniz *et al.*, 2011). Furthermore, castor bean meal protein was analysed for its amino acid composition and was found to be deficient in the essential amino acids lysine, tryptophan and methionine (Table 5) (Vilhjálmssdóttir and Fisher, 1971). Currently, it is being used as manure in India due to its high nitrogen, potassium and phosphorus content (Parnerkar *et al.*, 2001). Besides toxic principles, castor cake has high levels of fibre and lignin due to the presence of the seed hulls. High fibre and lignin content of the castor cake in monogastric animals such as poultry and pigs can be an issue, as they have limited ability to digest fibre.

Much research has been carried out to develop detoxification and de-allergination methods so as to be able to

use castor cake as livestock feed, with varying degrees of success. These technologies have not been very successful, as can be judged from the fact that in spite of the huge availability and low cost of castor cake in comparison with high costs of conventional protein supplements in India, castor cake has not been accepted as a feed resource, and it continues to be used as organic fertilizer.

TOXIC PRINCIPLES

Castor cake contains three undesirable constituents: a highly toxic, heat labile protein called ricin; a toxic alkaloid, ricinine; and a powerful and very stable allergen known as Castor bean 1 allergen (CB-1A) (Coulson, Spies and Stevens, 1960; Horton and Williams, 1989). The ricin is easily destroyed by heat and can be inactivated during the de-solventization step following solvent extraction. Ricin is reported to be present to the extent of 1.5 percent in the castor cake (Ambekar and Dole, 1957). The ricinine is present at very low levels, 0.23 percent of cake (Hinkson, Ellinger and Fuller, 1972) and presents no problem in animal feeds provided the feeds do not contain high levels of castor meal. Ricinine is also reported to have goitrogenic activity (Pahuja *et al.*, 1978) but ricinine or its hydrolysates even up to 100 mg/kg body weight were found to be harmless (Rao, 1970). The CB-1A allergen, however, requires a special processing step to de-activate it. CB-1A is a non-toxic, unusually stable protein that exhibits an extraordinary capacity to sensitize individuals exposed to small concentrations of the dust from castor beans or the castor cake. Alilaire was the first to describe human hypersensitivity to

TABLE 4
Chemical composition as percentage of castor cake

Country	DM	CP	CF	EE	Ash	NFE	NDF	ADF	Lignin	Ca	P	References
India	–	39.4	–	1.4	7.6	–	40.0	30.6	–	0.9	0.95	Gowda <i>et al.</i> , 2009.
Nigeria	90.2	29.4	32.0	8.5	6.8	13.5	38.3	21.3	2.1	–	–	Babalola, Apatha and Atteh, 2006.
Brazil	88.1	37.8	–	3.1	–	–	46.5	41.1	4.5	0.78	0.68	de Oliveira <i>et al.</i> , 2010a.
Nigeria	93.1	36.4	37.7	2.2	5.4	11.4	–	–	–	–	–	Okoye <i>et al.</i> , 1987.
Brazil	90.7	35.8	–	1.7	–	–	47.2	35.1	5.1	–	–	Diniz <i>et al.</i> , 2010.
India	–	41.6	26.7	1.6	5.7	24.4	56.6	46.6	7.2	–	–	Anandan, Anil and Ramachandra, 2005.

Notes: DM = dry matter; CP = crude protein; CF = crude fibre; EE = ether extract; NFE = nitrogen-free extract; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; Ca = calcium; P = phosphorus.

TABLE 5
Amino acid composition of castor bean meal protein

Amino acid	as % of protein	Amino acid	as % of protein	Amino acid	as % of protein
Valine	5.44	Methionine	1.51	Tryptophan	0.31
Isoleucine	4.68	Phenylalanine	4.02	Tyrosine	2.82
Leucine	6.42	Lysine	2.68	Cysteine	1.68
Threonine	3.44	Histidine	1.25	Proline	3.74
Glycine	4.31	Alanine	4.26	Asparatic acid	9.67
Serine	5.44	Hydroxyproline	0.28		
Glutamic acid	18.87	Arginine	8.61		

Source: Vilhjálmssdóttir and Fisher, 1971.

castor bean (Jones, 1947). CB-1A is the principal allergen of the castor bean and is a polysaccharidic protein factor. The allergen contents of de-corticated, de-fatted castor beans ranged from 6.1 to 9.0 percent, while the commercial castor cake contained 0.09–4.2 percent of the same (Coulson, Spies and Stevens, 1960).

Ricin is a 62–66 kDa protein consisting of two polypeptide chains, approximately 32 kDa and 34 kDa in size, linked by a disulphide bond (Audi *et al.*, 2005). Ricin (RCA60) is a class II ribosome-inactivating protein; a heterodimeric protein. The A-chain of the ricin molecule is the effective toxin. It works by depurinating specific residues on the rRNA of the 28 S subunit of the ribosome, halting translation (Endo *et al.*, 1987). The B-chain of the ricin molecule is responsible for cell entry. The disulphide link between the chains is not essential for the enzymatic activity of the A-chain, but it is necessary for toxicity, since the A-chain cannot enter the cell without the B-chain (Harley and Beevers, 1982; Lord *et al.*, 2003). Ricin has relatively low toxicity when orally consumed, but when injected or inhaled, the LD₅₀ can be as little as 3–5 µg/kg body weight (Audi *et al.*, 2005). Ricin is also reported to inhibit rumen microbial growth (de Oliveira *et al.*, 2010b). The allergen consists of ricin agglutinin (RCA120), a potentially harmful allergen. Ricin and ricin agglutinin share around 90 percent homology within the A chain of the proteins, meaning that detection of the ricin A-chain is directly linked to detection of the agglutinin when using A-chain-specific antibodies (Pinkerton *et al.*, 1999). The allergens set is composed of albumins 2S, formed by heavy and light subunits with molecular mass of 9 and 4 kDa, respectively (Thoyts, Napier and Millichip, 1996). Biochemical and immunological data relative to nine different fractions of albumins 2S resulted in identification of seven fractions exhibiting allergenic potential (Machado *et al.*, 2003). Allergen is a matter of concern for the people handling the cake, while the animals are unaffected by the allergen (UNIDO, 1989).

DETOXIFICATION AND DE-ALLERGENATION OF CASTOR CAKE

Growing demand for feed resources and the high cost of conventional feed resources in developing countries have prompted researchers to seek alternative feed resources. Although there are claims (Kim, 2001) that the normal extraction and de-solventization processes for meals are capable of total destruction of ricin, the presence of ricin in the solvent-extracted castor cake indicates that the normal processing methods are not capable of destroying the toxin totally. There is therefore a need for proper detoxification before any further use as livestock feed. Current oil extraction procedures utilize solvent extraction, which does not involve heating the meal, leaving the ricin and agglutinin mostly intact (Ogunniyi, 2006). The United Nations

Industrial Development Organization (UNIDO) sponsored a research programme to investigate methods to detoxify castor meal in an economically feasible way to enable utilization of castor meal by the feed industry. The UNIDO work was carried out by Rhee in 1987 and published by UNIDO (1989). Similarly the International Castor Oil Association published a technical bulletin (ICOA, 1989) on detoxification and de-allergenation of castor meal. In addition, a lot of research has been carried out in the past and efforts to develop a suitable processing method for effective utilization of castor cake as livestock feed continue.

A number of different approaches – physical, chemical and biological, alone or in combination – have been tried by different workers. The efficacy has generally been tested based on the actual reduction in the toxic principles before and after, indirect quantification of toxins (using precipitin, neutralization and agglutination) or animal experiments. Autoclaving at various pressures (10–20 psi) and duration (15–60 minutes) was earlier tried to detoxify castor meal (Jaki, 1940; Ambekar and Dole, 1957; Okamoto *et al.*, 1965; Mottola, Mackey and Herring, 1971). Autoclaving highly toxic castor pomace for periods of 15 minutes or more resulted in essentially complete destruction of the toxin, with minimal changes in the physical character of the substrate (Kodras, Robert and MacVicar, 1949). Autoclaving at 125 °C for 15 minutes or at 20 psi for 60 minutes almost completely destroyed ricin with minimum physical changes in oil cake properties (Purushotham, Rao and Raghavan, 1986). Dry heat does not seem to have much effect on reducing toxin levels in the castor cake (Heller, 1932). Ambekar and Dole (1957) reported that the heating of castor bean meal to 150 °C for 3 hours did not reduce the toxin levels, and feeding the heat-treated cake resulted in rat mortality. However, a few reports also exist (Tangl, 1938; Okorie *et al.*, 1985) showing the beneficial effects of heat treatment in various time and temperature combinations in removal of the toxin. Earlier attempts to detoxify castor bean meal by steaming at different temperature and time combinations were not successful (Borchers, 1949; Okorie *et al.*, 1985). The absence of toxic symptoms in chicks fed hot-water-extracted castor cake indicated that the water treatment was more effective (Vilhjálmssdóttir and Fisher, 1971). Dry heating (200 °C [400 °F]) and moist cooking with different chemicals (1–2 percent NaOH, 3–10 percent formaldehyde, 0.9 percent HCl) was effective in reducing the toxin by 98–100 percent as determined by the precipitin test (Gardener *et al.*, 1960). In studies with rats, tannins have been successfully used to neutralize the toxic effect of castor meal extract in rats. This is based on the ability of the tannins to react with proteins to form tannin protein complexes that interfere with digestibility and absorption of the proteins (Gandhi and Mulky, 1994). Of the various chemicals tried, treatment with NaCl at 1 per-

TABLE 6
Effect of different detoxification methods and their efficacy in toxin reduction

Technology	Process	Response	Source
Solid state fermentation (SSF)	Biological detoxification using SSF of castor bean waste by fungus <i>Penicillium simplicissimum</i>	Ricin reduction to undetectable levels. Reduction in allergic activity by 16%	Godoy, Gutarra and Maciel, 2009.
Thermoplastic extrusion	1 or 2% CaO, followed by extrusion	2% was more efficient than 1% CaO. Simultaneous detoxification and de-allergation	Ascheri <i>et al.</i> , 2007.
Two-stage cooking	Cooking at 100 °C for 20, 30, 40, 50 or 60 minutes	Cooking at 50 and 60 minutes resulted in reduction of ricin by 70 and 77%, respectively	Ani and Okorie, 2006.
Lime treatment of castor cake (feed grade @ 4% w/w)	Cake wetted with water containing feed-grade lime (4% of cake weight)	Lime treatment reduced ricin by 58%	Gowda <i>et al.</i> , 2009.
Boiling or autoclaving	Boiling or autoclaving of seeds for 20 minutes before solvent extraction	Promising reduction of chain A (ricin) detected by antibody reaction	Daniel <i>et al.</i> , 2009.
Hot press	Heating the crushed meal to high temperature resulting in meal expelled at 130 °C	No reactivity with antibody, implying effective destruction of ricin	Daniel <i>et al.</i> , 2009.
Physicochemical treatments	Physical: soaking, steaming, autoclaving (15 psi for 30 minutes), heating Chemical: ammonia, formaldehyde, lime (10 & 20%) and tannic acid	Less than 90% (varying from 27 to 90%) reduction in ricin	Anandan <i>et al.</i> , 2005.
Physicochemical treatments	Boiling (30 & 60 minutes), Autoclaving (15 psi 30 minutes), Lime 4%, Sodium hydroxide 10%	Above 91% (varying from 91 to 100%) reduction in ricin	Anandan <i>et al.</i> , 2005.

cent was found to be most effective (Kiran, 1998; Agarwal, 2001) in detoxifying castor cake.

Some of the recent approaches for detoxification and their efficacy as reported by different researchers are summarized in Table 6.

As far as approaches by crop scientists are concerned, there are basically two ways of toxin reduction. The first is a conventional one based on selection and breeding, whereby the varietal differences in toxin levels are exploited. This involves screening and identifying lines with low toxin levels and promoting the low-toxin lines as commercial cultivars. The second one is the biotechnological approach, whereby efforts are made to suppress or knock out the genes involved in toxin production. Work is underway by the crop scientists at the Directorate of Oilseeds Research, Hyderabad, India, to reduce the toxic endosperm proteins, ricin and *Ricinius communis* agglutinin in the seed through post-transcriptional gene silencing approaches (DOR, 2010).

FEEDING STUDIES USING CASTOR CAKE

Feeding studies using laboratory animals or domestic animals to assess efficacy, although costly and time consuming, is always preferred over the chemical quantification of the toxins. Animal response to the processed cakes would be influenced by the efficiency of the detoxification; the level of the cake in the diet; duration of feeding; and the animal species. Studies have been carried out since early 1940 by different workers using differently processed cake at varying levels in different species. Studies in fattening cattle at 10 percent of castor bean meal (CBM) did not have any ill effects. In growing cattle, at 10 percent level,

feed intake and growth were reduced in comparison with the cottonseed fed group (Bris and Algee, 1970). Butter from cattle fed CBM showed slightly increased viscosity and lower iodine value (Popvic, 1968), and it was concluded that incorporation of CBM at a 10 percent level was not economical. Reddy, Reddy and Reddy (1986) observed optimum feed intake with a comparable plane of nutrition in experimental buffaloes fed 30 percent CBM ration compared with those on the control ration. The growth rate and efficiency were depressed in lambs when autoclaved CBM replaced groundnut cake nitrogen beyond 33.3 percent without affecting nutrient intake and digestibility (Purushotham, Rao and Raghavan, 1986). Kiran (1998) noticed a significant depression in the digestibilities of dry matter in sheep fed 26 percent raw castor bean meal, while processed castor bean meal (1 percent NaCl and 0.2 percent NaOH, w/w in 1:2 w/v) resulted in comparable nutrient digestibility. No vital organs revealed any gross or histopathological changes due to feeding of NaCl-treated castor cake at 21 percent of the diet in rabbits (Agarwal, 2001).

The results of the studies using detoxified cakes in different animals as reported by other researchers are summarized in Table 7.

From the animal experiments conducted it shows that ruminants are relatively more tolerant than monogastrics and can withstand higher levels. Interestingly, a few of the studies using untreated cake showed no deleterious effects in ruminants, and this needs to be further investigated. In economic terms, a few of the studies involving the feeding cost of production revealed that feeding treated castor cake in place of conventional oilcakes resulted in either

TABLE 7
Effect of different processing methods of castor cake on production responses in livestock

Processing method	Feeding details		Duration	Animal response	Reference
	Species	Level			
Non ruminants					
Two-stage cooking: Cooking at 100 °C for 50 minutes	Broiler finisher birds	0, 10, 15 and 20% of the diet	4 weeks	Birds fed 10% cake had similar feed intake and weight gain as control, At higher levels there was depression in feed intake and weight gain,	Ani and Okorie, 2009.
Roasting of seeds at 140 °C for 20 minutes	Broiler birds	0, 10, 15, 20 and 25% of the diet	6 weeks	Inclusion of cake reduced the feed intake and weight gains at all levels. Watery faeces, salivation, drooping of the wings, poor feathering, emaciation and death were observed at 20 and 25% levels. Severe congestion of the internal organs and haemorrhages, degeneration of the renal epithelial cells, hepatocytes, bile duct proliferation and lymphocytic depletion in the lymphoid organs were observed at all levels of inclusion, although at 10% level there was no mortality.	Okoye <i>et al.</i> , 1987.
Fermented cake. Water soaked (1:4 ratio) and fermented for 5 days in airtight conditions	Broiler chicks	0, 5, 10 and 15% of the diet	56 days	No deleterious effect on growth response, nutrient digestibility, blood cell counts, serum enzymes and carcass yield in 5% fed groups.	Oso <i>et al.</i> , 2011.
Boiled castor bean meal supplemented with β -xylanase	Broiler birds	0, 10, 15, 20 and 25% of the diet	49 days	Weight gains up to 15% level were comparable to control. At higher levels weight gain reduced and there were changes in haematological values and serum constituents.	Babalola, Apata and Atteh, 2006.
Roasting of seeds at 140 °C for 20 minutes	Ducks	40% of the diet	21 days	Reduced feed intake and weight gain in ducks when fed at 10% of the diet	Okoye <i>et al.</i> , 1987.
Hot water extraction 4 times 10 minutes each (1:5 times water)	Chicks	40% of the diet	21 days	Hot-water-extracted cake was satisfactory, while supplementing with lysine and tryptophan gave comparable results to soybean protein in terms of growth and feed conversion ratio.	Vilhjálmssdóttir and Fisher, 1971.
Boiled castor meal	Growing rabbits	0, 10, 15, 20 and 25% of the diet	14 days	Daily gain and feed conversion ratio was comparable up to 15% inclusion level. Animals in 10 and 15% groups had 33% mortality, while 20 and 25% groups had 100% mortality	Adejebi <i>et al.</i> , 2006.
Ruminants					
CaO treatment at 6% of cake (soaking cake in CaO solution followed by drying)	Crossbred cattle	0, 33, 66 and 100% replacement of soybean meal	14 days	Did not affect the digestive and physiological variables at different levels and treated cake can partially or totally replace soybean meal. The feed cost for unit carcass gain at 66% level was found to be comparable. Even the untreated castor cake gave satisfactory results with regard to digestive and physiological variables.	Diniz <i>et al.</i> , 2010.
Ricin detoxified meal	Dairy cows	10 and 20% meal 0.5% castor oil	14 months	Transfers of ricinine, ricin, hydroxy fatty acids and antigens were at or below detection limits. Milk from cows on long-term castor meal and castor oil intake was not apparently detrimental when fed to calves and rats. No residue accumulation in muscle was observed, and no abnormal conditions were associated with feeding castor meal to the cows.	Robb <i>et al.</i> , 1974.
(i) Expeller cake, (ii) Solvent extracted cake (i) and (ii) treated with 4% lime	Sheep	0 and 15% of the diet	21 days	There were no changes in blood enzyme profile with treated and untreated cakes. Lime treatment improved the efficiency of microbial protein synthesis.	de Oliveira <i>et al.</i> , 2010a.
Alkali treatment (4% lime treatment – technical grade) followed by extrusion	Growing sheep	28% of concentrate mixture (13% of diet)	7 months	Feed intake and weight gain were not affected. Animals were healthy throughout the experiment and there were no pathological changes in visceral organs.	NATP, 2004.
	Milking buffaloes	10% of the concentrate mixture	75 days	Feed intake, nutrient utilization, milk yield and composition were comparable to control group, and the castor-fed group gave greater profit.	
(i) Solvent extracted (ii) Lime treatment (feed grade lime @ 4% of cake, soaked overnight and dried)	Adult sheep	0 and 12.3% of the total mixed diet	150 days	Feeding solvent extracted or lime treated cake had no effect on body weight changes, nutrient digestibility, rumen fermentation pattern and histopathology of visceral organs compared with control group fed soybean meal.	Gowda <i>et al.</i> , 2009.

comparable or lower feeding costs. Differences in the digestive physiology of ruminants versus non-ruminants mean that the high fibre and lignin content of castor cake are likely to affect the performance of monogastric animals. Although at low levels of inclusion the performance was comparable to controls, feed industry and farmers are not yet accepting the technology. Before the technology can be accepted there is probably a need for more focused studies involving the feed industry and farmers on a large scale, with better interaction among stakeholders, namely castor processing industries, researchers, feed industries and livestock farmers.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Ricin is one of the most potent naturally occurring plant toxins, and all care has to be ensured that the detoxification process is foolproof. This will ensure that the detoxified cake can be safely fed to any category of livestock irrespective of species or age. Different researchers have used various approaches to quantifying the toxin and as a result, though some of the methods were effective in neutralizing the toxins completely, this was not necessarily reflected in animal experiments. This is a major limitation: lack of a sensitive and commonly accepted approach. Further, few of the processing methods were limited to neutralization of toxins, and the subsequent animal experiments were not carried out to ascertain the efficacy of the same. There is a need to identify the most reliable and acceptable method that would have high correlation between the chemical quantification and animal response. This would ensure that the selection of an appropriate detoxification method based on toxin quantification would correlate well with the response in animal studies. In addition, most of the studies have been carried out at a laboratory scale, where the conditions are comparatively easy to control. Up-scaling to a commercial level while retaining the same efficiency always presents a problem. Involving the industrial partners in evolving appropriate processing methods at an earlier stage of technology development would facilitate easier adoption of technology. The technology needs to be practical, industry adaptable and inexpensive for detoxifying the ricin and completely inactivating the allergens without affecting the quality of the product. Crop scientists using recent advances in plant breeding and biotechnological approaches could contribute significantly by evolving new varieties with low or negligible toxin levels.

CONCLUSIONS

An increased demand for biofuels based on castor seed would result in availability of large quantities of castor cake, and utilizing this feed resource would add great value to the castor processing industry and livestock

production. At present, the cost of untreated castor cake is 40 to 60 percent cheaper than the conventional protein supplements used in livestock feed in India, and adding the processing cost would not change the price structure drastically, thus making castor seed cake competitive and commercially viable. Incidentally, the major producers of castor – countries like Brazil, China and India – have large livestock populations and a shortage of protein supplements. The technology could have great relevance for regional development. Although a lot of research has been carried out and the research continues to develop an appropriate technology for detoxification, the current technologies have not been adopted by industry or otherwise commercialized. There is a strong need to address this issue, involving the researchers and the industries concerned in successfully translating the knowledge generated into commercially viable technologies. This would only be possible by bringing together all the stakeholders: crop breeders, livestock nutritionists, castor processing industries, feed industries and farmers. A collaborative approach is required all the way from selection of superior cultivars with low anti-nutrients; through selection of effective and economical detoxification method; to scaling up methods for commercial-scale operations; together with large-scale field trials involving plant breeders, livestock nutritionists, feed technologists, castor processing industries, feed mills and farmers. This offers a way forward for successful commercialization and popularization of castor cake as livestock feed.

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Chapter 21

Use of detoxified jatropha kernel meal and protein isolate in diets of farm animals

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ABSTRACT

Jatropha curcas L. (physic nut) is a drought-resistant shrub or tree, which is widely distributed in wild or semi-cultivated areas in Central and South America, Africa, India, China and South East Asia. It is a hardy plant and thrives on degraded land. Jatropha kernels (de-shelled seeds) contain 55–60 percent oil that can be transformed into good quality biodiesel through transesterification and used as a substitute for diesel. The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 60–66 percent crude protein; while jatropha protein isolate obtained from jatropha seed cake (residue obtained after mechanical pressing of the whole seeds) has about 81–85 percent crude protein. The contents of essential amino acids (EAAs) (except lysine) are higher in jatropha kernel meal than in soybean meal (SBM), and higher in jatropha protein isolate than soy proteins isolate. However, presence of toxic factors (phorbol esters) and anti-nutritional constituents (trypsin inhibitors, lectins and phytate) restricts the use of Jatropha meal and protein isolate in animal feed. Phorbol esters are the toxic compounds in *J. curcas*. Kernel meal and protein isolate from *J. curcas* have been detoxified. Another *Jatropha* species, *J. platyphylla* is free of phorbol esters and hence non-toxic; however, its seed kernels and kernel meal contain trypsin inhibitors, lectin and phytate. The kernel meal from *J. platyphylla* obtained after oil extraction contains 65–70 percent crude-protein. Detoxified *J. curcas* kernel meal (DJKM), heated *J. platyphylla* kernel meal (H-JPKM) and detoxified *J. curcas* protein isolate (DJPI) can replace 50, 62.5 and 75 percent of fishmeal protein, respectively, in fish diets without compromising their growth performance and nutrient utilization. In addition, DJKM could also replace 50 percent of fishmeal protein without adversely affecting growth and nutrient utilization in shrimp. Increased DJKM inclusion in diets (>50 percent replacement of fishmeal protein) caused a significant lowering of the digestibility of protein, lipid and energy. No such effects were observed when DJPI was used in fish diets. Feeding DJKM to common carp and H-JPKM to Nile tilapia did not change the energy budget (routine metabolic rate, heat released and metabolizable energy) compared with the fishmeal-fed group. No mortalities, unaffected haematological values and no adverse histopathological alterations in stomach, intestine and liver of fish suggested that they were in normal health.

DJKM has also been fed to turkeys with no significant difference in feed intake and weight gain compared with the SBM-containing diet, with feed efficiency (gain:feed ratio) was higher in the DJKM-fed groups. The pre-caecal amino acid digestibilities of DJKM varied from 0.48 (cystine) to 0.91 (methionine) in turkeys. In pigs, average weight gain and feed:gain ratio were similar for DJKM-fed groups and the SBM-based control group. In addition, the serum and haematological parameters did not differ amongst the groups and values were within the normal range. Histopathological studies revealed that the liver and kidney of pigs fed DJKM and control diets exhibited normal histomorphology. Overall, the DJKM can replace SBM protein in fish, shrimp, turkey and pig diets by as much as 50 percent. DJKM, H-JPKM and DJPI are thus good quality protein sources for animal feeds.

INTRODUCTION

There is an urgent need to increase animal production in order to meet the increasing demand for animal protein driven by increasing human population and the growing economies of developing countries. The rapid world-wide expansion of aquaculture and livestock production strongly

indicates that a crisis is imminent in the livestock and aquaculture feed industries in the near future due to unavailability of good quality feed resources (Spinelli, 1980; Belewu *et al.*, 2009). More than 1000 million tonne of animal feed is produced globally every year, including 600 million tonne of compound feed. In terms of species, use of the compound

MAIN MESSAGES

- Detoxified *Jatropha curcas* kernel meal, heat-treated *J. platyphilla* kernel meal and detoxified *J. curcas* protein isolate can replace 50, 62.5 or 75 percent fishmeal protein, respectively, without compromising growth performance and nutrient utilization in fish, and without adversely affecting fish health, as illustrated by blood parameter evaluation and histopathological investigations on fish organs.
- Detoxified *J. curcas* kernel meal can also replace 50 percent fishmeal protein without any adverse effects on growth and nutrient utilization in shrimp.
- High inclusion (>50 percent fishmeal protein replacement) of detoxified *J. curcas* kernel meal decreases the efficiency of conversion of feed to body mass. No such effects were observed on using detoxified *J. curcas* protein isolate.
- Based on good growth performance, nutrient utilization and high amino acid digestibility, detoxified *J. curcas* kernel meal is valuable protein source for turkeys.
- Detoxified *J. curcas* kernel meal can replace 50 percent soymeal protein in diets of growing pigs.
- Detoxified *J. curcas* kernel meal and heat-treated *J. platyphilla* kernel meal contain approximately 65 percent crude protein, which is similar to the level in fishmeal, and therefore these could substitute for fishmeal on an equal-weight basis.
- The acceptability of DJKM, H-JPKM and DJPI-based diets by fish, as measured by immediate consumption and no waste in the tanks, is good.
- Detoxified *J. curcas* kernel meal, heat-treated *J. platyphilla* kernel meal and detoxified *J. curcas* protein isolate are deficient in lysine. Therefore lysine monohydrochloride should be supplemented at a level of 1.5 percent of these jatropha-based products (w/w) in the diet to compensate for the deficiency.
- Detoxified *J. curcas* kernel meal and heat-treated *J. platyphilla* kernel meal contain approximately 9–10 percent phytate, which is almost 3-fold that in soybean meal. To mitigate its effect, addition of 1500 FTU phytase per kg of diet is suggested.

feed is most for poultry, followed by pigs and then cattle. Although feed production for aquaculture is relatively low (14 million tonne) currently, there is an increasing demand for feed for farmed fish and crustaceans.

Substantial progress has been made towards the use of different plant ingredients, including soybean meal (SBM), lupin, maize, wheat, sorghum, peas, rapeseed meal and sunflower meal in animal feed. Typical compositions of commonly used animal feed ingredients are presented in Table 1. Among plant ingredients, SBM is currently the most commonly used plant protein source in animal feeds because of its reliable supply and high content of protein with a high concentration of essential amino acids (EAAs). On a worldwide basis, soybean supplies over one-quarter of the fats and oils and two-thirds of the protein concentrates for animal feeds, and is three-quarters of the total world trade in high-protein meals (Peisker, 2001; Best, 2011). However, soybean, together with maize, has been a staple food of mankind since ancient times. In human diets, soybean has been used as a protein source for over 5 000 years (Peisker, 2001). A vast array of products can be derived from soybean and these are found nowadays in more than 20 000 items on the food shelves of supermarkets worldwide. Also, nutrition of high performing animals is unthinkable without soy

products (Peisker, 2001). Soybean competes with human food and hence there is a need to identify other protein-rich plant resources that could be used in animal diets. The world is becoming increasingly aware of the looming food scarcity, and hence the possibility of raising animals on unconventional but easily sourced and available feedstuffs in the tropics and subtropics deserves more attention (Belewu *et al.*, 2009). Worldwide, the growing scarcity of conventional animal feed has therefore motivated nutritionists to find alternative sources of protein for livestock.

JATROPHA

Botanical and agro-climatic description

The genus *Jatropha* belongs to the tribe Joannesieae of Crotonoideae in the Euphorbiaceae family (well known for its toxicity) and contains approximately 175 known species. It is considered to have originated in Central America, most probably Mexico. *Jatropha* species for which the toxicity has been widely studied are *Jatropha curcas*, *J. elliptica*, *J. glauca*, *J. gossypifolia*, *J. aceroides*, *J. tanoresisi*, *J. macarantia*, *J. integerrima*, *J. glandulifera*, *J. podagrica* and *J. multifida* (Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a). Among these, *J. curcas* (toxic genotype) is the most studied as

TABLE 1
Typical composition of commonly used animal feed ingredients

Ingredient	DM	Proximate composition (g/kg DM except Gross energy)			Essential Amino Acids (g/kg DM)									
		CP	Total lipid	Ash	GE	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Fishmeal ⁽¹⁾	917	770	68	142	21.3	43	25	28	55	46	21	29	32	34
Fishmeal ⁽²⁾	920	720	84	104	21.6	38	19	25	46	43	18	23	26	32
SE soybean meal	909	518	47	69	19.6	42	14	23	44	28	9	27	24	24
SE canola meal	962	431	22	86	19.6	32	26	3	25	41	30	27	16	78
EX canola meal	898	381	136	66	23.1	39	28	3	28	46	37	29	18	66
Yellow lupin ⁽³⁾	903	547	87	44	20.9	61	15	20	45	23	4	21	20	19
NL lupin	885	415	53	33	20.4	47	10	15	29	14	3	16	16	14
Groundnut meal	928	481	13	58	20.3	67	16	19	32	20	5	29	14	20
Sunflower meal	930	422	29	76	20.7	36	11	17	26	12	7	20	13	23
Maize gluten meal	900	602	18	21	21.1	19	13	25	102	10	14	38	21	28
Cotton seed meal	900	414	18	64	20.6	45	12	13	25	17	7	22	14	18
Pea meal	928	252	13	38	16.4	19	11	14	41	27	6	19	17	14
Rapeseed meal	900	385	39	67	20.8	21	11	14	24	20	8	16	15	17
Wheat gluten	937	856	13	9	21.1	43	21	43	69	16	17	49	24	43
Wheat meal	941	145	16	14	18.7	6	3	4	9	3	7	2	4	5
DJKM	945	665	11	137	18.3	70	22	27	47	23	11	30	22	32
PPC	910	738	15	20	20.9	38	17	71	76	58	17	49	43	49
JPI	945	808	97	10	21.3	86	24	34	56	19	39	12	26	59
LPC	942	690	93	31	22.2	78	15	27	51	25	5	28	23	23
SPC	939	590	54	79	20.3	45	15	26	48	28	9	30	25	27
SPI	957	922	10	38	22.0	69	24	36	68	52	43	12	31	37

Notes: (1) Chilean anchovetta meal; (2) Herring; (3) *Lupinus luteus* (cv. Wodjil) kernel meal. DM = dry matter; CP = Crude Protein; GE = Gross energy expressed as MJ/kg; NL = Narrow-leaf lupin (*Lupinus angustifolius*) (mixed cultivars) kernel meal; LPC = *Lupinus angustifolius* (mixed cultivars) protein concentrate. SE = solvent extracted; EX = expeller; DJKM = detoxified jatropha kernel meal; SPC = soybean protein concentrate; SPI = soy protein isolate; JPI = jatropha protein isolate; PPC = potato protein concentrate.

Sources: Miller and Young, 1977; Nwokolo, 1987; NRC, 1983, 1998; Glencross, Booth and Allan, 2007; Makkar, Francis and Becker, 2008; Makkar and Becker, 2009a.

TABLE 2
Common and vernacular names of *Jatropha curcas*

Language or country	Common name
Angola	Mupuluka
Arabic	Dand barrī, habel meluk
Brazil	Mundubi-assu
Chinese	Yu-lu-tzu
Costa Rica	Coquillo, template
Côte d'Ivoire	Bagani
Dutch	Purgeernoot
English	Physic nut, purging nut, pulza
French	Pourghère, pignon d'Inde
German	Purgiernuß, Brechnuß
Guatemala	Pinón
Hindi (India)	Ratanjyot, bagbherenda, jangli arandi, safed arand, bagaranda
Indonesia	Jarak budeg
Italian	Fagiola d'India
Mexico	Piñoncillo
Nepal	Kadam
Nigeria	Butuje
Peru	Piñol
Philippines	Túbang-bákod, tuba-tuba
Portuguese	Turgueira
Puerto Rico	Tártago
Sanskrit	Kanananaeranda, parvataranda
Senegal	Tabanani
Tanzania	Makaen
Thailand	Sabudam
Togo	Kpoti

Sources: Schultze-Motel, 1986; Münch, 1986; Divakara *et al.*, 2010; Mabberley, 1987.

a result of its oil (as a source of biofuel) and associated co-product utilization (Makkar and Becker, 2009a, b). A non-toxic genotype of *J. curcas* has also been recorded, which is found only in Mexico (Makkar and Becker, 2009a). *Jatropha curcas* (toxic genotype) is found in parts of tropical America (central and southern regions) and many tropical and subtropical regions of Africa and Asia. It is believed that *Jatropha* species were introduced into other regions from the Caribbean, where it was used during the Mayan period (Schmook and Seralta-Peraza, 1997; Gaur, 2009), by sailors on Portuguese ships travelling via the Cape Verde islands and Guinea Bissau (Heller, 1996). The name *Jatropha curcas* (Euphorbiaceae) was first given by Linnaeus (Linnaeus, 1753). The genus name *Jatropha* derives from the Greek words *jatr'os* (doctor) and *troph'è* (food), which implies its medicinal uses. Table 2 presents some vernacular names of *J. curcas*. *J. curcas* is monoecious, flowers are unisexual but occasionally hermaphrodite flowers occur, each inflorescence yielding a bunch of approximately 10 or more ovoid fruits (Dehgan and Webster, 1979; Kumar and Sharma, 2008). The young *J. curcas* plant with both flowers and developing seed pods is shown in Photo 1A. Photo 1B shows the *J. curcas* inflorescence containing both male staminate flowers and female pistillate flowers. The seeds of *J. curcas* form within seed pods. Each seed pod

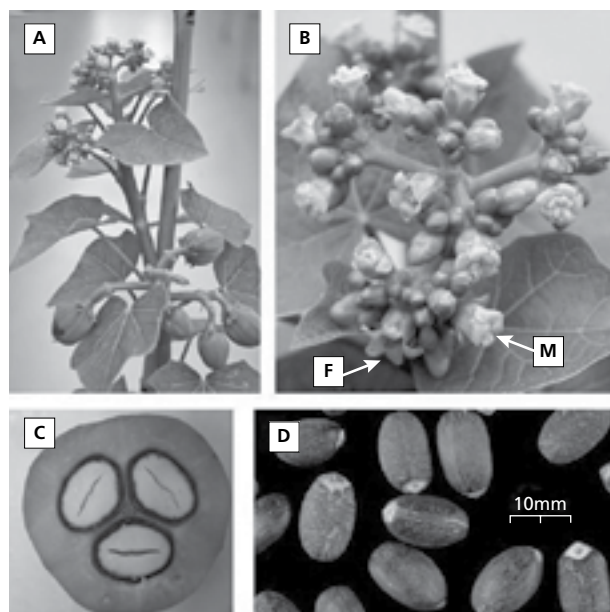


Photo 1

Images of Jatropha curcas

Notes: (A) Young *J. curcas* plant with both flowers and developing seed pods. (B) *J. curcas* inflorescence containing both male staminate flowers (M) and female pistillate flowers (F). (C) Cross-section of a *J. curcas* seed pod containing three developing seeds. (D) Mature seeds of *J. curcas*. Source: King *et al.*, 2009.

typically contains three seeds (Photo 1C) (King *et al.*, 2009). The seeds mature 3–4 months after flowering. Mature seeds of *J. curcas* are presented in Photo 1D. The plant can be easily propagated from seeds or cuttings. It grows under a wide range of rainfall regimes, from 250 to over 1200 mm per annum (Katwal and Soni, 2003; Kumar and Sharma, 2008). The trees are deciduous, shedding their leaves in the dry season. One major trait associated with the plant is its hardiness and sustainability in warm and arid climates. It prefers well-drained alkaline soil (pH 6–9) (Kumar and Sharma, 2008). It is a small perennial tree or large shrub, which normally reaches a height of 3–5 m, but can attain 8–10 m under favourable conditions (Gaur, 2009). Seed yields of 5–8 tonne/ha have been reported (Gübitz, Mittelbach and Trabi, 1999).

A new, non-toxic, species of *Jatropha*, *Jatropha platyphylla*, has been identified (Makkar *et al.*, 2011). *J. platyphylla* (locally called 'sangregrado' in Mexico) is a drought-resistant shrub or tree, 2–5 m high, almost glabrous. The species is restricted to warm areas (average temperature 20–29 °C) on the Pacific coast from Sinaloa to Michoacán, including Nayarit and Jalisco states in Mexico, and is usually found in and around deciduous forests. It has thick succulent branches, large peltate glabrous leaves (25–35 cm) on long petioles, and white urceolate flowers that are held on a long and branched florescence (Dehgan, 1982). The kernel (white portion after removal of shells) contains about 50–60 percent oil, which can be used as edible oil or can be converted into biodiesel of high quality (Makkar *et al.*, 2011). The kernel meal obtained



Photo 2
Seeds of (A) *Jatropha platyphylla* and
(B) *Jatropha curcas*
Source: Makkar *et al.*, 2011.

after oil extraction is an excellent source of nutrients and contains 60–65 percent crude protein (Makkar *et al.*, 2011). The levels of EAAs (except lysine) are higher in defatted *J. platyphylla* kernel meal than in SBM (Makkar *et al.*, 2011). In addition, *J. platyphylla* kernel meal is free of phorbol esters, the main toxin present in most *Jatropha* species (Makkar *et al.*, 2011). However, anti-nutrients, e.g. a trypsin inhibitor, lectin and phytate, are present in the meal at high levels (Makkar *et al.*, 2011). Heat labile anti-nutrients, protease inhibitors and lectins are easy to inactivate by moist heating, and phytase could be incorporated into the diet for degradation of phytate.

Applications of jatropha

Jatropha seeds have been extensively investigated as a source of oil. *J. curcas* seeds contain 25–35 percent crude oil (Makkar and Becker, 2009a; King *et al.*, 2009). The oil contains 21 percent saturated fatty acids and 79 percent unsaturated fatty acids (Gübitz, Mittelbach and Trabi, 1999; Makkar and Becker, 2009a). *Jatropha* oil fatty acid composition includes 14–16 percent palmitate (16:0), 5–8 percent stearate (18:0), 34–46 percent oleic acid (18:1), 29–44 percent linoleic acid (18:2) and a trace of longer-chain saturated fatty acids (Foidl *et al.*, 1996; Gübitz, Mittelbach and Trabi, 1999; King *et al.*, 2009). *Jatropha curcas* oil has good feedstock qualities for biodiesel production, the biodiesel meeting the European Union (EN14214) and North American standards (ASTM D6751) (Makkar and Becker, 2009a; King *et al.*, 2009). A number of countries, including India, Pakistan, China, Mexico, Brazil, Nigeria, Indonesia, Madagascar, Mali, Thailand, Ghana, Bangladesh, Kenya, Zimbabwe and Cape Verde, have initiated programmes for planting *J. curcas* as an energy plant. The cultivation of *Jatropha* species as a source of oil for biodiesel production will in turn produce a number of by-products and co-products. The utilization of these products may increase the overall value of the *jatropha* biodiesel production chain. However, the presence of toxic components limits the utilization of many unprocessed *jatropha*-based products. *Jatropha* and

its components have several uses, which are summarized in Table 3.

Comparative physical and chemical characteristics of seeds and kernel meals from toxic and non-toxic *Jatropha curcas* genotypes and *Jatropha platyphylla*

The seeds of *J. curcas* (toxic and non-toxic genotypes) are elliptical whereas seeds of *J. platyphylla* are almost circular (Photo 2) (Makkar *et al.*, 2011). The seed, shell and kernel masses are similar for both the toxic and non-toxic genotypes (Table 4). Composition of *jatropha* seed is presented in Figure 1. The seeds are rich in crude protein and lipids. The chemical composition of seeds of these two *Jatropha* species – *J. curcas* and *J. platyphylla* – is similar (Table 4). Sugar and starch contents and the mineral composition (except

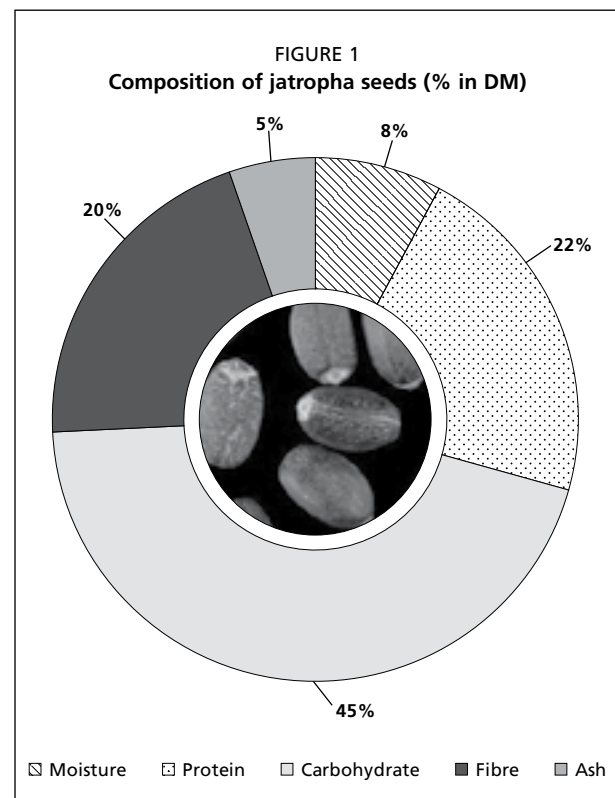


TABLE 3
Uses of jatropha-based products

Plant part and use	References
Use in ethnomedicine	
Latex Contains an alkaloid Jatrophine, which has anti-carcinogenic properties, and latex also strongly inhibits the watermelon mosaic virus	Parajuli, 2009; Devappa, Makkar and Becker, 2011a.
Leaves and sap Used to control parasites. Sap is used for staining linen. Sometimes used for marking and labelling	Rug <i>et al.</i> , 1997; Kisangau <i>et al.</i> , 2007; Devappa <i>et al.</i> , 2010b.
Leaf extracts Used to clean sores, treat skin rashes and oral candidiasis. It is also used for fever, mouth infections, jaundice, guinea-worm sores, and joint rheumatism	Kisangau <i>et al.</i> , 2007; Devappa, Makkar and Becker, 2010b.
The juice of the whole plant Used for stupefying fish	Gübitz, Mittelbach and Trabi, 1999.
Emulsion of the twig sap with benzyl benzoate Effective against scabies, wet eczema and dermatitis	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009.
Roots Acts as an antidote to treat snake-bite. Used as mouthwash for bleeding gums and toothache. Applied on skins to treat eczema, ringworm and scabies. Used to treat dysentery and venereal diseases like gonorrhoea, leprosy	Irvin, 1961; Oliver-Bever, 1986; Devappa, Makkar and Becker, 2010b.
Root oil (yellow in colour) Used as strong anthelmintic. Has wound healing and anti-inflammatory effects	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009; Nath and Dutta, 1997; Staubmann <i>et al.</i> , 1997; Kumar and Sharma, 2008. Gübitz, Mittelbach and Trabi, 1999.
Seeds Acts as an anthelmintic	Gübitz, Mittelbach and Trabi, 1999.
Seed oil Used to treat rheumatism, eczema and skin diseases and, also reported to be abortifacient and efficacious in dropsy, sciatic and paralysis	Heller, 1996; Gübitz, Mittelbach and Trabi, 1999; Kumar and Sharma, 2008; Parajuli, 2009.
Jatropha seeds enzyme (β-1,3-glucanase) Antifungal against <i>Rhizoctonia solani</i> Kuha and <i>Gibberelle zeae</i> Schw.	Wei <i>et al.</i> , 2005; Makkar and Becker, 2009a.
Use as source of phytochemicals and its agro-pharmaceutical importance	
Phorbol esters Tumour-promoting, irritant, cytotoxic, anti-inflammatory, antitumour, molluscicidal, insecticidal and fungicidal activities	Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a.
Pesticidal effects against <i>Sitophilus zeamays</i> and <i>Callosobruchus chinensis</i>	
Kills snails of the <i>Physa</i> species, which are also known to be intermediary hosts of schistosomes that causes the deadly disease schistosomiasis in humans	
Compound (12-deoxyphorbol-13-phenylacetate) synthesized from phorbol ester Acts as antidote against HIV by inhibiting the HIV entry into target cells	Wender, Kee and Warrington, 2008; Makkar and Becker, 2009a.
A proteolytic enzyme, curcain from jatropha latex Wound-healing properties	Nath and Dutta, 1997.
Biologically active cyclic peptides Mahafacyclin, pohlianin, chevalierin and curcacyclin have anti-malarial properties Jatrophidin has antifungal activity Labaditin and biobollien have immuno-modulatory effects	Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a
Phytates from seeds Cancer prevention, hypercholesterolemic effects, reduction in iron-induced oxidative injury and reversal of colorectal tumorigenesis initiation, and prevention of lipid peroxidation	Singh, Bhat and Singh, 2003; Kumar <i>et al.</i> , 2010a.
Other uses	
Bark Yields a dark blue dye which is reported to be used in Philippines for colouring cloth, finishing nets and lines	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009.
Jatropha proteins (approximately 50 kDa) Production of wood/paper adhesive – polyketone-based wood adhesive formulations	Hamarneh <i>et al.</i> , 2010.
Jatropha wood and husks/shells Jatropha seed shells have 45–47% lignin and has a high energy value (ca 19.5 MJ/kg)	Makkar and Becker, 2009.
Jatropha-derived biodiesel Mixed with jet fuel and used as an aviation fuel	Gaur, 2009.
Jatropha oil Used for making soap and candles in addition to direct use as energy and as biodiesel	Gübitz, Mittelbach and Trabi, 1999.
Oil with iron oxide Preparation of varnish	Gübitz, Mittelbach and Trabi, 1999.
Jatropha seed cake Fertilizer Briquettes for use as fuel Production of biogas Raw material for plastics and synthetics fibres As a substrate for solid state fermentation to produce: (a) proteases and lipases using <i>Pseudomonas aeruginosa</i> and (b) xylanase using <i>Scytalidium thermophilum</i> Source of fermentable sugars and solubilized proteins	Gübitz, Mittelbach and Trabi, 1999; Vyas and Singh, 2007; Singh, Bhat and Singh, 2003; Sharma and Singh, 2008; Carels, 2009; Mahanta, Gupta and Khare, 2008; Makkar and Becker, 2009a; Ali, Kurchania and Babel, 2010; Joshi and Khare, 2011; Liang <i>et al.</i> , 2010.

TABLE 4

Physical and chemical parameters of *Jatropha curcas* (toxic and non-toxic genotypes) and *J. platyphylla* seeds and kernel meals

	<i>Jatropha curcas</i>		<i>Jatropha platyphylla</i>
	Toxic	Non-toxic	
Seed weight (g)	0.80 ± 0.08	0.73 ± 0.09	1.80 ± 0.15
Shell weight (g)	0.31 ± 0.05	0.26 ± 0.03	0.92 ± 0.01
Kernel weight (g)	0.49 ± 0.0.7	0.47 ± 0.07	0.85 ± 0.13
Proximate composition (g/kg DM) of kernel			
Crude protein	266 ± 11.2	268 ± 12.4	271 ± 20
Oil	574 ± 5.0	575 ± 6.9	603 ± 354
Ash	40 ± 6.7	45 ± 5.6	39 ± 0.9
Nutrients in defatted kernel meal (g/kg on DM basis)			
Crude protein	637 ± 11	624 ± 26	664 ± 20
Crude lipid	11.4 ± 0.52	12.1 ± 0.41	11.4 ± 0.29
Crude ash	94 ± 10.1	91 ± 10.4	90 ± 5.8
Neutral-detergent fibre	182	180	–
Total sugar	7.7–10.3	10.2	–
Starch	9.4–11.2	10.6	–
Minerals in defatted kernel meal (mg/kg on DM basis)			
Boron	14.0–15.0	23.1–25.6	41.5–43.1
Calcium	8 995–9 769	6 660–7 077	6 771–7 396
Copper	48–52	40–44	48–53
Iron	304–344	251–278	209–231
Potassium	19 882–21 064	21 381–22 878	22 965–24 259
Magnesium	17 947– 19 452	14 432–15 715	15 094–13 801
Manganese	69–74	53–57	76–84
Sodium	26 652– 27 190	219–226	24–28
Phosphorus	21 171–22 676	17 533–18 815	21 456–23 288
Zinc	105–114	80–89	116–135

Sources: Makkar and Becker, 2009a; Makkar *et al.*, 2011.

sodium) in kernel meals of *J. platyphylla* and toxic and non-toxic genotypes of *J. curcas* are almost similar (Table 4). The amino acid composition of *J. platyphylla* and *J. curcas* (toxic and non-toxic genotypes) kernel meal is almost identical (Table 5). The levels of EAAs (except lysine) are higher than those quoted in the FAO reference protein for a growing child of 2–5 years of age (Makkar and Becker, 2009a). The amino acid composition of jatropha kernel meal and SBM is similar (except lysine and the sulphur-containing amino acids cystine and methionine); lysine is less and sulphur-containing amino acids are more in the jatropha kernel meal compared with SBM. EAA contents in jatropha kernel meals are higher than or similar to those in castor bean meal (Makkar, Aderibigbe and Becker, 1998; Makkar and Becker, 2009a). Jatropha kernel meal contains low level of non-protein nitrogen (9.0 percent of total nitrogen), suggesting a high level (91 percent) of true protein (Makkar, Aderibigbe and Becker, 1998; Makkar and Becker, 2009a). When a non-toxic genotype from *J. curcas* kernel meal (JCM) was fed to fish and rats, high growth rate and good protein utilization were observed, suggesting that the quality of protein in jatropha kernel meal is good (Makkar and Becker, 1999, 2009a).

Jatropha kernel meal (heated to 121 °C at 66 percent moisture for 30 minutes) from toxic and non-toxic geno-

types has similar digestibility and metabolizable energy; however, these meals have lower digestibility and metabolizable energy than SBM (Table 6) (Menke *et al.*, 1979; Makkar and Becker, 2009a). The pepsin plus trypsin digestibilities of jatropha kernel meal protein were similar to those of the heated SBM, whereas the *in vitro* rumen digestibility of proteins in the kernel meal of the non-toxic jatropha genotype was lower (ca 50 percent) compared with that of SBM, suggesting that the former meal has substantial amounts of rumen undegradable protein, which could be used post-rationally. These results demonstrate that kernel meal from the non-toxic jatropha genotype can be used as a good quality protein source in animal nutrition (Makkar and Becker, 2009a). Furthermore, it is inferred from these results that a similar level of application could also be expected of jatropha kernel meal from the toxic genotype, provided it is detoxified.

Constraints: toxic component and antinutrients in *Jatropha curcas*

Makkar and Becker (1997) unequivocally established that the main toxic factor in *J. curcas* seeds, oil and cake, is the diterpene derivatives of a tiglane skeleton classified as phorbol esters. A number of anti-nutrients are present in

TABLE 5
Amino acid composition (g/16 g nitrogen) of kernel meals of *Jatropha curcas* (toxic and non-toxic genotypes), *J. platyphylla* and SBM, versus FAO reference dietary protein requirement values

Amino acid	<i>Jatropha curcas</i>		<i>J. platyphylla</i>	SBM	FAO reference protein (2–5-year-old child)
	Toxic	Non-toxic			
Essential					
Methionine	1.56–1.91	1.38–1.76	1.58	1.32	2.50 ⁽¹⁾
Cystine	1.77–2.24	1.58–1.81	1.55	1.38	
Valine	4.35–5.19	3.79–5.30	6.91	4.50	3.50
Isoleucine	3.93–4.53	3.08–4.85	4.10	4.16	2.80
Leucine	6.55–6.94	5.92–7.50	6.68	7.58	6.60
Phenylalanine	4.08–4.34	3.93–4.89	4.71	5.16	6.30 ⁽²⁾
Tyrosine	2.45–2.99	2.62–3.78	2.69	3.35	
Histidine	2.81–3.30	2.65–3.08	2.66	3.06	1.90
Lysine	3.63–4.28	3.40–3.49	3.16	6.18	5.80
Threonine	3.33–3.96	3.15–3.59	3.64	3.78	3.40
Tryptophan	1.31	ND	1.06	1.36	1.10
Non-essential					
Serine	4.67–4.80	4.59–4.91	5.05	5.18	–
Arginine	11.8–12.2	11.4–12.90	12.46	7.64	–
Glutamic acid	14.68–16.7	15.91–16.50	16.21	19.92	–
Aspartic acid	9.49–11.8	9.92–11.7	9.33	14.14	–
Proline	4.13–4.96	3.80–4.21	5.16	5.99	–
Glycine	4.40–4.92	4.18–4.61	4.56	4.52	–
Alanine	4.36–5.21	4.26–4.94	4.04	4.54	–

Notes: (1) Methionine plus cystine; (2) Phenylalanine plus tyrosine. ND = not detected. Sources: Makkar and Becker, 2009a; Makkar et al., 2011.

TABLE 6
Pepsin plus trypsin digestibilities, available lysine, digestible organic matter, metabolizable energy and rumen-degradable nitrogen of heat-treated *jatropha* kernel meals

	<i>Jatropha curcas</i>		<i>Jatropha platyphylla</i>	SBM
	Toxic	Non-toxic		
Pepsin plus trypsin digestibility (% of total nitrogen)	89	90	97.1	91
Available lysine (mg/100 mg sample)	3.10	3.16	3.29	–
Available lysine (g/16 g N)	4.87	5.06	4.95	–
Digestible organic matter (%)	78	77.3	–	87.9
Metabolizable energy (MJ/kg)	10.9	10.7	–	13.3
24-hour <i>in vitro</i> rumen-degradable nitrogen (% of total nitrogen)	43.3	28.9	–	80.9

Notes: SBM = Soybean meal. Sources: Makkar and Becker, 2009a; Makkar et al., 2011.

defatted kernel meal obtained from *J. curcas* genotypes (toxic and non-toxic) and these are listed in Table 7.

Phorbol esters are naturally-occurring compounds that are widely distributed in plant species in the Euphorbiaceae and Thymelaeaceae. They are tetracyclic diterpenoids of phorbol type and esters of tiglane diterpenes (Evans, 1986; Devappa, Makkar and Becker, 2010b, 2011a). Six phorbol esters (*jatropha* factors C1–C6) have been characterized from *J. curcas* seed oil (Haas, Sterk and Mittelbach, 2002; Devappa, Makkar and Becker, 2010b, 2011a) and designated as C1 (A), C2 (B), C3 (C), epimers C4 (D), C5 (E) and C6 (F), with the molecular formula $C_{44}H_{54}O_8Na$ (MW 733.37) (Figure 2). The phorbol esters are lipophilic, present mainly in oil or kernel, and are not affected by heat treatment. The concentration of phorbol esters varies from 1 to 3 mg/g kernel meal and from 2 to 7 mg/g oil (Makkar and Becker, 1997, 2009a; Devappa, Makkar and Becker,

2010b, 2011a). Table 8 shows phorbol ester content of different parts of a toxic *J. curcas* plant. Figure 3 represents the phorbol ester content in different part of the toxic *J. curcas* kernel (Devappa, Makkar and Becker, 2011b).

Rumen microbes cannot degrade phorbol esters (Makkar and Becker, 2010b) and they cause as severe toxic symptoms in ruminants as they do in monogastric animals. These mimic the action of diacylglycerol, an activator of protein kinase C which regulates different signal transduction pathways (Devappa, Makkar and Becker, 2010a, b, 2011a). Phorbol esters affect a number of processes including phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression. These are considered to be a co-carcinogen and have strong purgative and membrane-irritant effects (Goel et al., 2007; Devappa, Makkar and Becker, 2010a, b, 2011a).

TABLE 7
Levels of toxic and anti-nutritional factors in unheated kernel meals of *Jatropha curcas* (toxic and non-toxic genotypes) and *J. platyphylla*

Component	<i>Jatropha curcas</i>		<i>Jatropha platyphylla</i>
	Toxic	Non-toxic	
Phorbol esters (mg/g kernel) ⁽¹⁾	2.79	ND	ND
Total phenols (% tannic acid equivalent)	0.36	0.22	0.33
Tannins (% tannic acid equivalent)	0.04	0.02	0.17
Condensed tannins (% leucocyanidin equivalent)	ND	ND	ND
Phytate (% DM)	9.4	8.9	8.7
Saponins (% diosgenin equivalent)	2.6	3.4	1.9
Trypsin inhibitor (mg trypsin inhibited per g sample)	21.3	26.5	20.8
Lectin activity (inverse of mg meal per mL of the assay that produced haemagglutination)	51–102	51–102	51–102
Glucosinolates	ND	ND	ND
Cyanogens	ND	ND	ND
Amylase inhibitor	ND	ND	ND
Non-starch polysaccharides (% in DM)			
Rhamnose	0.2	0.2	0.3
Fucose	0.1	0.1	0.1
Arabinose	2.5	2.7	3.1
Xylose	1.2	1.4	2.0
Mannose	0.3	0.3	0.5
Galactose	1.2	1.2	1.4
Glucose	4.7	4.7	5.7
Glucuronic acid	0.9	0	0
Galacturonic acid	2.6	3.0	3.0
Total non-starch polysaccharides	13.7	13.6	16.0

Notes: (1) As phorbol-12-myristate 13-acetate equivalent. ND = not detected. Sources: Makkar and Becker, 2009a; Makkar et al., 2011.

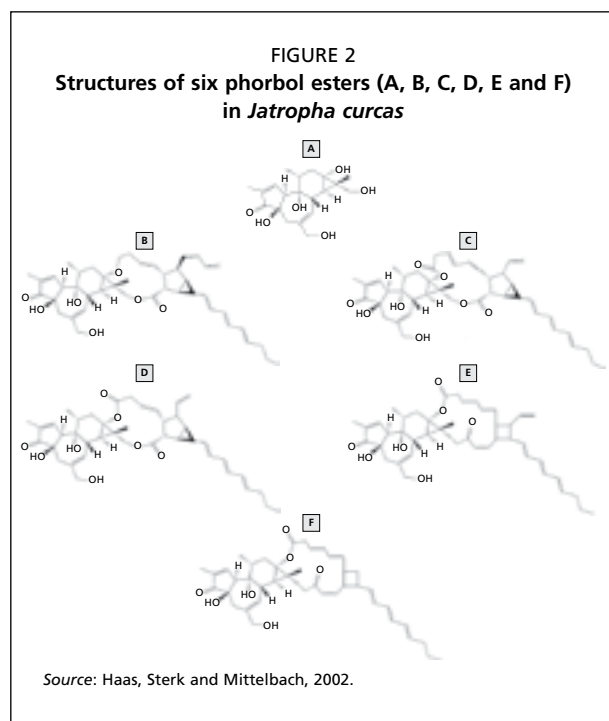


TABLE 8
Phorbol esters in different parts of toxic *J. curcas* plants

Plant part	Phorbol esters (mg/g DM) ⁽¹⁾
Kernel	2.00–6.00
Leaves	1.83–2.75
Stems	0.78–0.99
Flower	1.39–1.83
Buds	1.18–2.10
Roots	0.55
Latex	Not detected
Bark (outer brown skin)	0.39
Bark (inner green skin)	3.08
Wood	0.09

Notes: (1) As phorbol-12-myristate 13-acetate equivalent. Source: Makkar and Becker, 2009a.

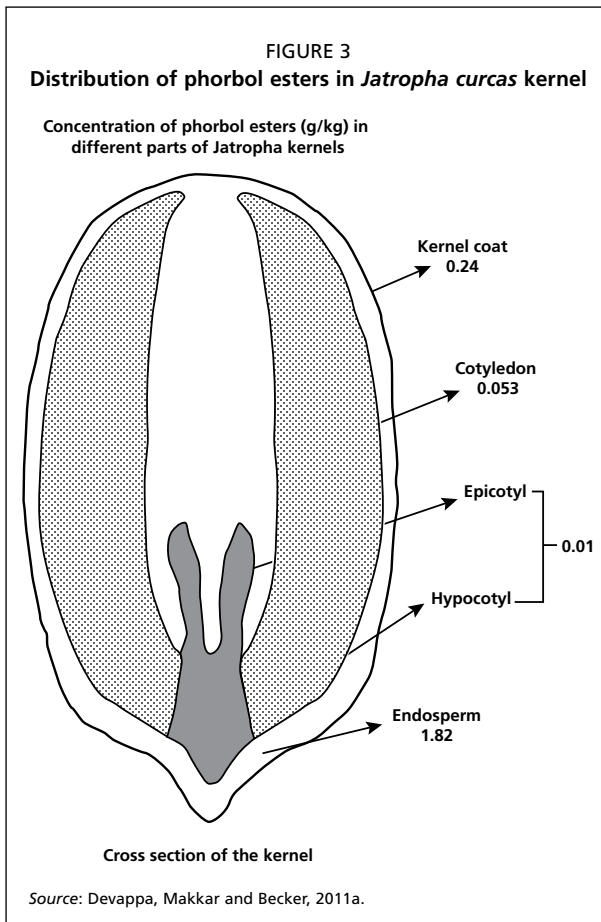
activated by heat treatment, and the adverse effects of phytate can be mitigated by supplementation with phytase enzyme. However, the main toxic compounds, the phorbol esters, are heat stable to a large extent. Other strategies must therefore be applied for their removal.

Different approaches evaluated for detoxification of *Jatropha curcas* products

In the past two decades, several approaches (active chemicals or organic solvents) have been tried for detoxifying defatted cake and kernel meal. Makkar and Becker (1997) reported that ethanol (80 percent) or methanol (92 percent) [1:5 w/v] reduced both the saponins and phorbol

The main antinutrients present in the seeds of kernel meal are curcins, trypsin inhibitors and phytate.

For effective utilization of kernel meal the removal of antinutrients and toxic principles is necessary. Antinutrients such as trypsin inhibitors and lectin (curcins) can be de-



esters by 95 percent after four extractions. Heat treatment in presence of alkali was also effective in reducing phorbol esters. Martinez-Herrera *et al.* (2006) studied the effect of various treatments, such as hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with NaHCO_3 , and ionizing radiation, to inactivate the anti-nutritional factors in jatropha kernel meal. Trypsin inhibitors were easily inactivated with moist heating at 121 °C for 20 minutes (Makkar and Becker, 1997). Extraction with ethanol, followed by treatment with 0.07 percent NaHCO_3 considerably reduced lectin activity. The same treatment also decreased the phorbol ester content by 97.9 percent. Chivandi *et al.* (2004) reported that petroleum ether extraction reduced phorbol ester content in kernels of *J. curcas* seeds by 67.7 percent, and double solvent extraction followed by moist heat treatment reduced phorbol esters by 70.8 percent. Double solvent extraction accompanied with wet extrusion, re-extraction with hexane and moist-heat treatment diminished phorbol ester content by 87.7 percent. Rakshit and Bhagya (2007) reported that up to 90 percent of the phorbol esters could be removed by treating the meal with 20 g/L of calcium hydroxide. Gaur (2009) developed a process that obtains high yields of jatropha oil and detoxifies the defatted (oil-free) jatropha meal. The principle of solid-liquid extraction was utilized to

detoxify the meal. Various organic solvents were used for the extraction. Extraction of ground jatropha seed kernels in a Soxhlet apparatus involving a sequential combination of hexane, followed by methanol proved highly efficient in detoxifying the meal. Phorbol ester content was reduced by 99.6 percent from 6.05 mg/g in untreated meal to about 0.06 mg/g in solvent-treated meal.

Chivandi *et al.* (2006) detoxified defatted *J. curcas* kernel meal (JCM) using 95 percent ethanol at 35 °C to remove most of the highly lipo-soluble phorbol esters in the kernels. The ethanol-extracted meal was heated with pressurized steam at 90 °C for 30 minutes to distill off the ethanol, after which the meal was sun-dried. The re-extracted meal was autoclaved at 121 °C for 30 minutes to inactivate the heat-labile antinutrients. This “detoxified” JCM was then fed to pigs for 8 weeks. Haematological and biochemical parameters were measured and it was found that dietary ‘detoxified’ JCM caused severe adverse effects in pigs. This demonstrates that the detoxification procedure had failed to remove and/or neutralize the toxic factors in the JCM. Some of the toxicity observed could be ascribed to the residual phorbol esters in the JCM. In the study of Belewu, Belewu and Ogunsol (2010), autoclaved (121 °C, 15 psi for 30 minutes) *J. curcas* seed cake was treated with fungi (*Aspergillus niger* and *Trichoderma longibrachiatum*) and fed to West African dwarf goats for 70 days. Phorbol ester content was reported for neither the treated nor untreated *J. curcas* kernel cakes. The growth and nutrient utilization was lower in *J. curcas* cake-fed groups compared with the control, implying that the cake was not detoxified and could not be used as a component in animal feed.

The solid-state fermentation (SSF) of seed cake using the white-rot fungi *Bjerkandera adusta* and *Phlebia rufa* decreased phorbol ester content by 91 and 97 percent, respectively, under optimized laboratory conditions (28 °C for 30 days) (de Barros *et al.*, 2011). Similarly, SSF using *Pseudomonas aeruginosa* PseA strain decreased phorbol esters to an undetectable level within nine days under optimized conditions (30 °C, pH 7.0 and relative humidity 65 percent) (Joshi, Mathur and Khare, 2011). Animal studies have not been conducted using material treated thus.

In Hohenheim, Germany, a new method has been developed to detoxify jatropha kernel meal and protein isolate (Makkar and Becker, 2010a). This detoxification of kernel meal and protein isolate is based on extraction of phorbol esters using organic solvents (alkaline methanol) and inactivation of trypsin inhibitors and lectin by heat treatment. Furthermore, these authors reported a one-step detoxification method in which the proteins from mechanically pressed jatropha seed cake were solubilized at pH 11, and then the solubilized proteins were precipitated and detoxified using ethanol at pH 8. These procedures are available in patent (WIPO Patent, WO/2010/092143). The detoxified

JCM and protein isolate obtained using this process have been intensively investigated as soybean and fishmeal protein replacers in diets of a number of farm animal species, and these studies are discussed below.

DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL AS A PROTEIN SOURCE IN AQUA FEED

Aquaculture continues to grow at a faster pace than the farming of terrestrial animals. For fish and shrimp feeds, the most pressing need is to find alternative protein sources. Several studies performed on partial replacement of protein sources, especially fishmeal, by detoxified *J. curcas* kernel meal (DJKM), heated *J. platyphylla* kernel meal (H-JPKM) and detoxified jatropha protein isolate (DJPI) in fish and shrimp diets are presented.

Use of detoxified jatropha kernel meal in common carp (*Cyprinus carpio* L.) diet Feed intake, feed utilization and growth performance

Two experiments were performed by Kumar, Makkar and Becker (2011a) wherein 50 and 75 percent (Table 9), and 50 and 62.5 percent (Table 11) of fishmeal protein was

replaced by DJKM, with synthetic lysine added in the DJKM-containing diets. Based on visual observations during feeding time, acceptability and palatability of the DJKM-based feeds was similar to the control diet (Kumar, Makkar and Becker, 2011a). High inclusion (>50 percent replacement of fishmeal protein) of the detoxified meal resulted in reduced protein utilization, measured as protein efficiency ratio and protein productive value (Kumar, Makkar and Becker, 2011a, 2010c). These results showed that ≤50 percent replacement of fishmeal protein by DJKM in common carp diet met the dietary demands for protein and energy.

The blends of DJKM with fishmeal, at different levels, were found to have excellent protein, lipid and energy digestibilities in common carp (Kumar, Makkar and Becker, 2011a). Protein and energy digestibilities were statistically similar ($P > 0.05$) for the control and the group in which 50 percent fishmeal protein was replaced by DJKM, and these values were higher ($P < 0.05$) than those for the group fed a diet in which 75 percent fishmeal protein was replaced by DJKM. The digestibility of the DJKM protein fraction was greater than 90 percent (Kumar, Makkar and Becker, 2011a). The protein digestibility coefficients compare favourably with those of any other high-quality

TABLE 9

Growth performance, nutrient utilization, digestibility measurement, digestive enzymes activity and haematological parameters of common carp fed detoxified jatropha kernel meal (DJKM)-based diets

Parameter	Control	Jatropha inclusion	
		50% (J_{50})	75% (J_{75})
Initial body mass (g)	3.2 ± 0.1	3.2 ± 0.10	3.2 ± 0.10
Final body mass (g)	32.0 ^a ± 1.96	33.3 ^a ± 0.64	28.3 ^b ± 1.21
Feed conversion ratio	1.00 ^b ± 0.05	1.01 ^b ± 0.02	1.21 ^a ± 0.24
Protein efficiency ratio	2.6 ^a ± 0.12	2.6 ^a ± 0.04	2.2 ^b ± 0.37
Protein productive value (%)	38.8 ^b ± 2.41	41.3 ^a ± 0.88	34.4 ^b ± 5.56
Digestibility measurements, relative intestinal length, digestive and metabolic enzymes activity			
Protein digestibility (%)	92.3 ^a ± 0.45	92.2 ^a ± 0.39	90.6 ^b ± 0.07
Lipid digestibility (%)	97.2 ^a ± 0.68	95.0 ^b ± 0.91	92.1 ^c ± 0.90
Energy digestibility (%)	87.7 ^a ± 1.33	87.6 ^a ± 1.11	83.1 ^b ± 0.95
Relative intestinal length (mm/g)	2.55 ^b ± 0.13	2.93 ^{ab} ± 0.10	3.30 ^a ± 0.18
Amylase (U/g protein)	14.2 ^a ± 1.71	11.6 ^b ± 1.80	11.0 ^b ± 1.59
Protease (U/g protein)	31.1 ^a ± 5.04	24.8 ^b ± 1.92	20.1 ^c ± 2.37
Lipase (U/g protein)	4.9 ^a ± 0.74	4.4 ^{ab} ± 0.38	4.2 ^b ± 0.62
Alkaline phosphatase (U/L)	85 ^a ± 55.1	115 ^a ± 52.1	75 ^a ± 32.5
Alanine transaminase (U/L)	92.0 ^a ± 6.63	85.7 ^b ± 4.57	80.2 ^b ± 10.2
Blood parameters			
Red blood cells (10 ⁶ cells/mm ³)	1.58 ^b ± 0.10	1.74 ^{ab} ± 0.20	1.85 ^a ± 0.13
Albumin (mg/dl)	1.98 ^a ± 0.15	1.73 ^{ab} ± 0.13	1.63 ^b ± 0.35
Globulin (mg/dl)	0.88 ^c ± 0.17	1.03 ^b ± 0.19	1.20 ^a ± 0.12
Lysozyme activity (IU/ml)	336.4 ^a ± 32.0	447.7 ^a ± 172.9	401.8 ^a ± 186.7
Glucose (mg/dl)	73 ^b ± 4.03	88 ^b ± 4.03	98 ^a ± 5.63

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Jatropha inclusions levels are 50% (J_{50}) and 75% (J_{75}) fishmeal protein replaced by DJKM. Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A₂₈₀ per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 microequivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 at 37 °C. Alkaline phosphatase and Alanine transaminase expressed as 1 U = 16.66 nKat/L; nKat = amount of glandular kallikrein that cleaves 0.005 mmol of substrate per minute. Lysozyme activity IU is the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25 °C, using a suspension of *Micrococcus lysodeikticus* as the substrate.

Sources: Kumar et al., 2010b; Kumar, Makkar and Becker, 2011a.

protein feedstuff, such as fishmeal (NRC, 1993). High availability of amino acids from DJKM for this fish species is expected. In general, the digestibility coefficients obtained for various jatropha constituents have been high, indicating that a large percentage of those constituents are digested and absorbed by the fish for further metabolism. Lipid digestibility of DJKM-based diets ranged from 74 to 90 percent (Kumar, Makkar and Becker, 2011a). High inclusion levels of DJKM (>50 percent fishmeal protein replacement) decreased lipid digestibility probably because of its high content of non-starch polysaccharides (NSPs) (Kumar, Makkar and Becker, 2011a, b).

Intestinal amylase, protease and lipase activities for the control group were significantly higher ($P < 0.05$) than for DJKM-fed groups (Kumar, Makkar and Becker, 2011a). Heat labile antinutrients, such as trypsin inhibitors and lectins, are absent in the DJKM, whereas a heat stable antinutrient (phytate) is present. Phytate is known to inhibit digestive enzymes such as pepsin, trypsin and α -amylase (Robaina *et al.* 1995; Alarcon, Moyano and Diaz, 1999). It also forms complexes with minerals (Teskeredzic *et al.*, 1995; Sugiura *et al.*, 1999) and proteins (Lopez *et al.*, 1999), thereby modifying digestion processes and impairing intestinal absorption. Kumar, Makkar and Becker (2011a) observed a decrease in digestive enzyme (amylase, protease and lipase) activity in the intestine on inclusion of DJKM in the common carp diets, which might be caused by the presence of phytate in the DJKM-based diets. However, this study used phytase 500 FTU phytase per kg for DJKM-based feeds, which might not be sufficient because of the high phytate content (9–10 percent) in DJKM. An increase in the level of DJKM in the diets led to a decrease in protein availability, probably caused by the presence of unhydrolysed phytate.

Effects of DJKM-containing feeds on growth performance, nutrient utilization, digestibility measurement, digestive enzymes activity and haematological parameters of common carp are presented in Table 9. Based on these results it is concluded that 50 percent of the fishmeal protein can be replaced by DJKM in common carp diets without compromising growth and nutrient utilization. However, >50 percent replacement of fishmeal protein by DJKM leads to significantly lower growth and higher feed conversion ratio (feed/body mass gain) in common carp, which could be attributed to factors such as:

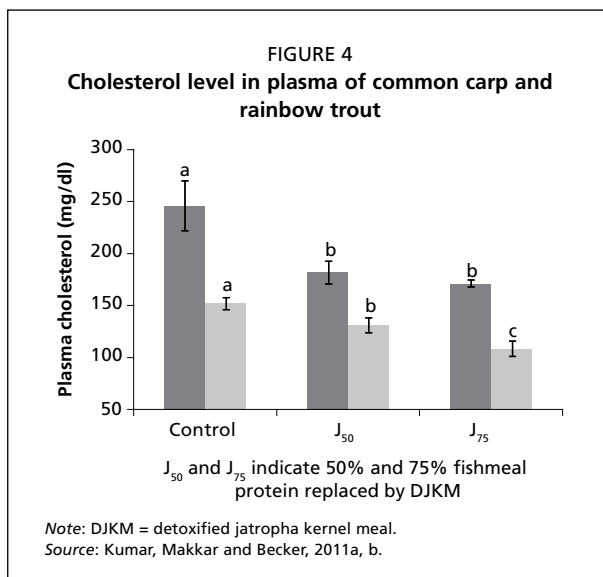
- Lower digestibilities of protein and energy in the diets, leading to lower protein and energy availability from DJKM (plant protein structures in general are much more compact than fishmeal protein, so digestive enzymes act slowly on DJKM proteins).
- The DJKM contains large concentrations of antinutrients such as phytate and non-starch polysaccharides (NSPs), and these could adversely affect feed utilization.

- The digestibility of synthetic lysine, which was added as a supplement to the diets, may be less than that of the natural amino acid present in the feed ingredients.

Retention of nutrients in the whole body

The efficiency with which nutrients and energy are retained from feeds provides a useful assessment of the efficiency of nutrient utilization from diets (Cho and Kaushik, 1990; Booth and Allan 2003; Glencross *et al.*, 2004). Feeding trials performed by Kumar, Makkar and Becker (2011a, 2010c) showed that inclusion of DJKM in a common carp diet exhibited significantly higher lipid deposition in the whole body than in the control group. The increase in whole body fat content on using dietary DJKM-based diets could be due to the higher content of total carbohydrate in these diets. Carbohydrates can be converted to lipids in the body by lipogenesis (Kumar, Makkar and Becker, 2011a). There is evidence that replacement of fishmeal protein by plant protein sources such as maize gluten meal and soy protein concentrates increases hepatic lipogenic enzyme activities in fish (Dias 1999; Kaushik *et al.*, 2004), leading to higher whole body lipid. In fish (salmonids), increases found in whole body fat content with the use of dietary plant proteins, were explained by imbalances in amino acid concentrations (Kaushik *et al.*, 2004; Bjerkeng *et al.*, 1997). Furthermore, it is suggested that unbalanced amino acid composition influences energy metabolism. Vilhelmsson *et al.* (2004) found an up-regulation of several proteins involved in energy metabolism in fish liver when fed plant proteins (maize gluten meal, wheat gluten, extruded whole heat, extruded peas and rapeseed meal) and concluded that the plant proteins increase energy demands of fish. Another possible reason could be a greater supply of some of the dispensable amino acids, such as glutamic acid, in excess by the DJKM proteins that could have led to higher lipid retention. Involvement of possible metabolic or endocrine mechanisms in eliciting such differences in whole body lipid deposition is suggested (Kumar, Makkar and Becker, 2011a, b). Proficient protein synthesis requires adequate availability of all EAAs. Unbalanced amino acid concentrations in a common carp diet resulted in increased protein degradation, and thereby increased protein turnover (Langar *et al.*, 1993; Kumar, Makkar and Becker, 2011a, b; Martin *et al.*, 2003). Generally, the plant protein-based diets decreased nitrogen retention in fish and shrimp because these diets have less digestible energy and an amino acid profile that is sub-optimal for muscle growth. Interestingly, Kumar, Makkar and Becker (2011a) showed that when compared with fishmeal, feeding DJKM to common carp led to higher whole body crude protein content, showing that DJKM contains optimum digestible energy and has a balanced amino acid profile ideal for fish growth.

Dietary inclusion of DJKM reduced the cholesterol level



in plasma and muscle when compared with the fishmeal-fed group (Kumar *et al.*, 2010b). As DJKM level increased in the common carp diets the cholesterol level in muscle and plasma decreased (Figure 4). This hypocholesterolaemia in response to increasing dietary DJKM supply could be due either to an increased excretion of bile salts, to an inhibition of cholesterol intestinal absorption, or just to the withdrawal of fishmeal, rather than to the direct effects of plant protein (Kaushik *et al.*, 2004; Kumar *et al.*, 2010b). Further, fibre and antinutritional factors (NSPs and phytate) reduce absorption of total fat, including cholesterol, when these factors are increased in the diet (Krogdahl, Bakke-McKellep and Baeverfjord, 2003; Hansen, 2009). Faecal excretion of steroids (bile acids) is the major pathway for elimination of cholesterol from the body (Hansen, 2009).

Energy budget and metabolic efficiency

Growth and production can be described in terms of partition of dietary energy between catabolism as fuels and anabolism as storage in tissues. Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure. Kumar,

Makkar and Becker (2010c) reported that common carp fed DJKM and fishmeal-based diets exhibited similar values for routine metabolic rate (Table 10). These observations suggest that energy requirement for digestion and absorption of nutrients from DJKM and fishmeal are similar, and that DJKM is a promising good quality protein source for incorporation in feed for common carp. An energy budget was constructed by Cui and Liu (1990) for fish fed ad libitum and found that heat loss was always the largest component, 50–69 percent of the consumed energy, whereas the energy used for growth was much smaller, 21–35 percent. Kumar, Makkar and Becker (2010c) also observed that for DJKM-fed fish, energy retained for growth was 37 percent and energy expenditure was 41.4 percent of the gross energy fed. Metabolizable energy of the DJKM-based diet and metabolizable energy for growth in common carp were 78 percent and 47 percent, respectively. Metabolizable energy and energy expenditure per gram of protein retained in the fish body for growth in fish was also similar for DJKM- and fishmeal-fed groups (Table 10). It is evident that the protein quality of DJKM is equivalent to fishmeal protein, and both these protein sources result in similar growth performance, energy expenditure and energy retention (Kumar, Makkar and Becker, 2010c).

Impact of feeding detoxified jatropha kernel meal on common carp health

Haematological, biochemical and histological measurements are an integral part of evaluating the health status of commercially important fish. The activities of alkaline phosphatase (ALP) and alanine transaminase (ALT) in blood are used as indicators of liver cell condition. Usually, the level of ALP and ALT rises in blood during acute liver damage (Goel, Kalpana and Agarwal, 1984). Feeding DJKM and fishmeal did not change ($P > 0.05$) levels of ALP and ALT activity in the blood (Kumar *et al.*, 2010b; Kumar, 2011) (Table 9). In addition, ALP and ALT levels in all groups were in the normal range as reported by Zhang *et al.* (2009) for healthy fish. Other health-related blood parameters, such as blood urea nitrogen, total bilirubin and creatinine contents, which

TABLE 10
Energy budget of common carp (*Cyprinus carpio* L) fed fishmeal (Control) and DJKM-based diet

Parameter	Control	DJKM
Initial body mass (g)	11.2 ± 1.14	10.6 ± 0.63
Final body mass (g)	49.0 ^a ± 7.9	48.3 ^a ± 3.0
Energy expenditure (EE; % of GE fed)	44.3 ^a ± 8.4	41.5 ^a ± 0.9
Energy retention (ER; % of GE fed)	33.5 ^a ± 0.7	36.9 ^a ± 1.5
Apparently unmetabolized energy (AUE; % of GE fed)	22.2 ^a ± 8.2	21.6 ^a ± 1.10
Efficiency of energy retention (ER/EE)	0.77 ^a ± 0.13	0.89 ^a ± 0.05
Average metabolic rate (mg O ₂ kg ^{0.8} /hour)	363 ^a ± 83.3	442 ^a ± 120.9
Energy expenditure (EE)/g protein fed	19.6 ^a ± 3.9	18.9 ^a ± 1.1

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). DJKM diet had 75% fishmeal protein replaced by DJKM. Source: Kumar, Makkar and Becker, 2010c.

are indicators of liver, kidney and gill function (Stoskopf, 1993; Tietz, 1986) were also in the normal range (Kumar *et al.*, 2010b). This suggested that the liver, kidney and gills of the common carp were in a normal functional condition in the DJKM-fed groups (Kumar *et al.*, 2010b). Also when common carp were fed DJKM as a protein source (Kumar *et al.*, 2010b; Kumar, 2011), haematology [haematocrit, haemoglobin and red blood cell count] values were within normal ranges (Ghittino, 1983; Rosenlund *et al.*, 2004).

One of the few unusual effects observed was a significant reduction in blood cell size (measured as mean cell volume) as the content of DJKM proteins increased in common carp diets (Kumar *et al.*, 2010b). As this observation appeared to coincide with increased spleen size, it was suggested that some of the plant ingredients may have caused early release of immature erythrocytes (Kumar *et al.*, 2010b). It may be noted that the spleen was larger in DJKM protein-fed groups than in the control group (fishmeal-based diet) (Kumar *et al.*, 2010b). Blood protein is considered a basic index for health and nutritional status in fish (Martinez, 1976). Among the blood proteins, albumin and globulin are the major proteins, which play a key role in the immune response. Lysozyme is regarded as the first line of defense, with high activity in mucus, serum, gills and the alimentary tract (Lie *et al.*, 1989). Feeding DJKM to common carp led to significantly higher ($P < 0.05$) albumin, globulin and total protein concentrations in blood, and numerically higher lysozyme activity in serum, than in the control diet, indicating an immuno-stimulatory effect of DJKM on the common carp (Kumar *et al.*, 2010b; Kumar, 2011). Albumin, globulin and total protein concentrations in blood were within the normal range for DJKM-fed groups (Wedemeyer and Chatteron, 1970; Sandnes, Lie and Waagbo, 1988). Also DJKM diets exhibited no abnormal changes in intestine and liver (Kumar *et al.*, 2010b). The intestinal mucosa was well developed, no morphological alteration was found, and the intestinal mucosa appeared to be normal for common carp. Liver also showed no pathological alteration or signs of steatosis or hepatic lipidosis in the DJKM-fed group (Kumar *et al.*, 2010b).

Based on the above findings, it was concluded that DJKM can replace 50 percent fishmeal protein without comprising growth, nutrient utilization or health of the fish.

Use of detoxified jatropha kernel meal in rainbow trout (*Oncorhynchus mykiss*) diet **Impacts on growth and feed utilization**

The utilization of detoxified jatropha kernel meal (DJKM) as a protein source in a carnivorous fish species, rainbow trout (*Oncorhynchus mykiss*), was investigated (Kumar, Makkar and Becker, 2011b). In this study, 50 percent (J_{50}) and 62.5 percent ($J_{62.5}$) fishmeal protein was replaced by DJKM. Palatability and acceptability of DJKM-based diets were simi-

lar to that of the fishmeal-based diet. Growth performance, and nutrients and energy digestibilities were statistically similar ($P > 0.05$) for control and J_{50} group, but were higher ($P < 0.05$) than for $J_{62.5}$ group (Table 11). Feed conversion ratio, protein efficiency ratio, protein productive value and energy retention were similar for control and DJKM-fed groups (Table 11). The lower growth response of the $J_{62.5}$ group could be due to lower protein and energy availability from the DJKM, poor availability of crystalline lysine added to DJKM-containing diets to equalize lysine content, or the presence of antinutrients such as phytate and NSPs, which are present in high amounts in the kernel meal. According to NRC (1983) the sulphur-containing amino acid (methionine and cystine) requirement of rainbow trout is 13 g/kg diet. In the $J_{62.5}$ diet the total sulphur amino acid was 11.3 g/kg, which is slightly lower than the optimum requirement. This could have led to lower growth performance in this group. Another constraint related to the digestion of DJKM-based diets is its relatively high carbohydrate content; carbohydrates are generally not well digested by rainbow trout (Kumar, Makkar and Becker, 2011b).

Retention of protein and lipid in the whole body of rainbow trout was higher in DJKM-fed groups compared with control groups, suggesting that DJKM contains optimum digestible energy and a balanced amino acid profile optimal for rainbow trout growth.

As DJKM increased in the rainbow trout diets the activity of digestive enzymes (amylase, protease and lipase) in the intestine significantly decreased ($P < 0.05$), which might be because of phytate present in the DJKM-based diets (Kumar, Makkar and Becker, 2011b) (Table 11). Phytate is well known for inhibition of digestive enzymes. It also forms complexes with minerals and proteins, thereby modifying digestion processes and impairing intestinal absorption in rainbow trout. It is known that carnivorous fish require more time to digest plant protein-based diets compared with animal protein-based diets (Buddington, Krogdahl and Bakke-McKellep, 1997). A direct relationship between the amount of dietary plant protein and relative intestine length (RIL; mm/g) has been reported in fish (Kramer and Bryant, 1995). In rainbow trout, DJKM-based diets exhibited higher ($P < 0.05$) RIL than the control group (Table 11). The RIL value increased as the DJKM inclusion increased. From a physiological view point, a greater RIL would facilitate an increase in digestibility and retention time by enhancing contact time of the digestive enzymes and the feed components, resulting in increases in their digestion and absorption. Carnivorous fish species like rainbow trout showed compensation mechanisms, such as an increase in RIL and as a result an increase in digestive activity, to achieve a digestive balance and growth rates similar to those observed for control groups (Kumar, Makkar and Becker, 2011b).

TABLE 11
Growth performance, nutrient utilization, digestibility measurements, digestive enzyme activities and haematological parameters of rainbow trout (*Oncorhynchus mykiss*) and common carp over an experimental period of 16 weeks

Parameters	Rainbow trout			Common carp		
	Control	Jatropha inclusion level		Control	Jatropha inclusion level	
		50%	62.5%		50%	62.5%
Initial body mass (g)	4.12 ± 0.26	4.17 ± 0.49	4.31 ± 0.54	21.5 ± 0.74	21.6 ± 1.04	21.8 ± 1.03
Final body mass (g)	61.0 ^a ± 9.90	61.8 ^a ± 6.88	54.6 ^b ± 5.18	149 ^a ± 23.0	128 ^a ± 8.0	104 ^a ± 31.6
FCR	1.3 ^a ± 0.10	1.2 ^a ± 0.10	1.3 ^a ± 0.20	1.7 ^a ± 0.26	1.8 ^a ± 0.05	2.2 ^a ± 0.48
PER	1.6 ^a ± 0.10	1.7 ^a ± 0.11	1.6 ^a ± 0.40	1.6 ^a ± 0.16	1.4 ^a ± 0.02	1.2 ^a ± 0.26
PPV (%)	22.8 ^a ± 1.10	26.3 ^a ± 2.37	25.2 ^a ± 4.59	26.5 ^a ± 4.26	26.1 ^a ± 0.29	21.9 ^a ± 3.88
Digestibility measurements, relative intestine length (RIL), digestive and metabolic enzymes activity						
PD (%)	89.8 ^a ± 0.55	89.7 ^a ± 0.83	84 ^b ± 1.41	85.9 ^a ± 0.98	83.2 ^b ± 0.80	78.6 ^c ± 0.37
LD (%)	95.2 ^a ± 0.43	95.2 ^a ± 0.80	89.8 ^b ± 0.86	89.6 ^a ± 0.51	86.2 ^b ± 0.76	80.1 ^c ± 1.65
ED (%)	86.8 ^a ± 0.83	86.1 ^a ± 0.71	81.8 ^b ± 1.13	82.0 ^a ± 1.62	77.7 ^b ± 1.83	73.4 ^c ± 0.49
Amylase (U/g protein)	4.6 ^a ± 0.40	3.2 ^b ± 0.20	2.5 ^c ± 0.15	17.4 ^a ± 1.32	13.6 ^b ± 0.83	11.0 ^c ± 0.76
Protease (U/g protein)	50.3 ^a ± 3.59	41.0 ^b ± 2.16	32.5 ^c ± 1.29	36.5 ^a ± 1.31	28.1 ^b ± 0.83	20.7 ^c ± 1.24
Lipase (U/g protein)	13.9 ^a ± 1.02	10.8 ^b ± 0.38	8.6 ^c ± 0.48	6.8 ^a ± 0.29	5.3 ^b ± 0.32	4.3 ^c ± 0.22
RIL (mm/g)	0.47 ^c ± 0.02	0.56 ^b ± 0.02	0.63 ^a ± 0.01	2.24 ^c ± 0.07	2.78 ^b ± 0.10	3.17 ^a ± 0.10
Alkaline phosphatase (U/L)	101 ^a ± 8.3	96 ^a ± 25.0	84 ^a ± 30.7	60.8 ^a ± 5.6	64.8 ^a ± 29.6	84.8 ^a ± 24.4
Alanine transaminase (U/L)	48.4 ^a ± 13.1	75.2 ^a ± 41.8	71.0 ^a ± 35.5	80.3 ^a ± 17.2	61.0 ^a ± 6.1	64.8 ^a ± 13.1
Blood parameters						
RBC (10 ⁶ cells/mm ³)	0.96 ^a ± 0.05	0.97 ^a ± 0.07	1.05 ^a ± 0.08	1.32 ^c ± 0.02	1.41 ^b ± 0.06	1.52 ^a ± 0.06
Albumin (mg/dl)	2.18 ^b ± 0.28	2.66 ^a ± 0.15	2.36 ^b ± 0.53	2.25 ^a ± 0.48	2.05 ^a ± 0.71	2.13 ^a ± 0.05
Total protein (mg/dl)	3.8 ^b ± 0.20	4.1 ^a ± 0.30	3.8 ^b ± 0.50	2.78 ^a ± 0.39	2.83 ^a ± 0.50	2.88 ^a ± 0.13
Creatinine (mg/dl)	1.70 ^a ± 0.86	0.98 ^{ab} ± 0.22	0.34 ^b ± 0.21	1.55 ^a ± 1.12	0.28 ^b ± 0.15	0.20 ^b ± 0.00

Notes: Values are mean ± standard deviation. For each species, mean values in the same row with different superscript differ significantly ($P < 0.05$). Jatropha inclusion levels are 50 and 62.5% of fishmeal protein replaced by DJKM. FCR = feed conversion ratio; PER = protein efficiency ratio; PPV = protein productive value; PD = protein digestibility; LD = lipid digestibility; ED = energy digestibility; RBC = red blood cells. Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A₂₈₀ per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 micro-equivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 and 37 °C. ALP and ALT expressed as 1 U = 16.66 nKat/l; nKat = amount of glandular kallikrein that cleaves 0.005 mmol of substrate per minute. Sources: Kumar, Makkar and Becker, 2011b; Kumar, 2011.

Inclusion of DJKM in rainbow trout diets decreased cholesterol level in muscle and plasma (Figure 6) (Kumar, Makkar and Becker, 2011b). This could be due to an increased excretion of bile salts, an inhibition of cholesterol intestinal absorption, or just the taking away of fishmeal rather than to the direct effects of DJKM (Kumar, Makkar and Becker, 2011b). Higher ($P < 0.05$) concentrations of phosphorus and calcium ions in blood of rainbow trout were observed for DJKM-fed groups. The DJKM-based diets were supplemented with phytase (500 FTU/kg feed), which would increase release of phosphorus and calcium from feed and make them available for rainbow trout.

Impacts on health of fish

Metabolic enzyme (ALP and ALT) activities, metabolites (urea nitrogen, total bilirubin and creatinine) and ion concentrations in blood were in the normal range in the groups in which 50 percent and 62.5 percent of fishmeal protein was replaced by DJKM. Blood parameters such as red blood cell (RBC) and white blood cell counts, haematocrit and haemoglobin level were also not affected ($P > 0.05$) by dietary treatments (Kumar, Makkar and Becker, 2011b) and their ranges were also in the normal range reported by Blaxhall and Daisley (1973) for healthy trout.

After feeding DJKM as a protein source to the rainbow trout, no signs of histopathological lesions were observed in the organs (Kumar, 2011). The gastric glands were well developed and the epithelium lining the luminal surface that consists of highly columnar cells and produces protective mucous was not altered. The case for the branched tubular glands was similar, as they were also well developed. There was no change in the shape and cellular morphology of pepsin- and hydrochloric acid-producing cell-types (oxyntopeptidic cells), indicating no leucocyte immigration and therefore no signs of inflammation (Kumar, 2011). In addition, there was no alteration in intestinal loops, pyloric appendices, the terminal hind gut, and the villi of the appendices or terminal intestine. There was also no sign of hepatic steatosis or lipidosis in rainbow trout when fed with DJKM as a protein source.

Conclusively, DJKM can replace 50 percent fishmeal protein without compromising the growth, feed utilization and health of rainbow trout.

USE OF DETOXIFIED JATROPHA KERNEL MEAL AS A PROTEIN SOURCE IN WHITE LEG SHRIMP FEED

Growth performance and nutrient utilization parameters on incorporation of detoxified jatropha kernel meal (DJKM)

TABLE 12
Growth performance and nutrient utilization of white leg shrimp (*Litopenaeus vannamei*) fed control and DJKM-based diets for eight weeks

Treatment	Initial body mass (g)	Final body mass (g)	MGR (g kg ^{0.8} day ⁻¹)	Feed conversion ratio	Protein efficiency ratio
Control	4.46 ± 0.60	10.54 ^b ± 3.17	5.51 ^b ± 0.70	3.18 ^a ± 0.37	1.01 ^b ± 0.11
JC ₂₅	4.47 ± 0.64	12.59 ^a ± 3.98	6.67 ^a ± 0.38	2.46 ^b ± 0.28	1.24 ^a ± 0.21
JC ₅₀	4.45 ± 0.69	13.60 ^a ± 3.18	7.22 ^a ± 0.75	2.28 ^b ± 0.39	1.39 ^a ± 0.23

Notes: JC₂₅ and JC₅₀ are 25% and 50% of fishmeal protein replaced by DJKM. MGR = metabolic growth rate. Values are mean (n = 4) ± standard deviation. Mean values in the same column with different superscript differ significantly ($P < 0.05$). Source: Harter *et al.*, 2011.

in the diet of white leg shrimp are presented in Table 12. Greater growth response and nutrient utilization were observed in DJKM-fed groups (25 or 50 percent fishmeal protein replaced by DJKM) compared with fishmeal-fed group in white leg shrimp (*Litopenaeus vannamei*) (Harter *et al.*, 2011). There is a possibility of synergistic effects between the feed ingredients used (fishmeal and DJKM), being complementary to each other in their amino acid composition. The DJKM protein in combination with fishmeal protein gave excellent nutrient and energy digestibility, leading to higher growth performance and nutrient utilization (Harter *et al.*, 2011). These results, along with the amino acid composition of the diets tested, indicated that the requirements of shrimp (Akiyama and Tan, 1991; Van Wyk, 1999) for amino acids were met.

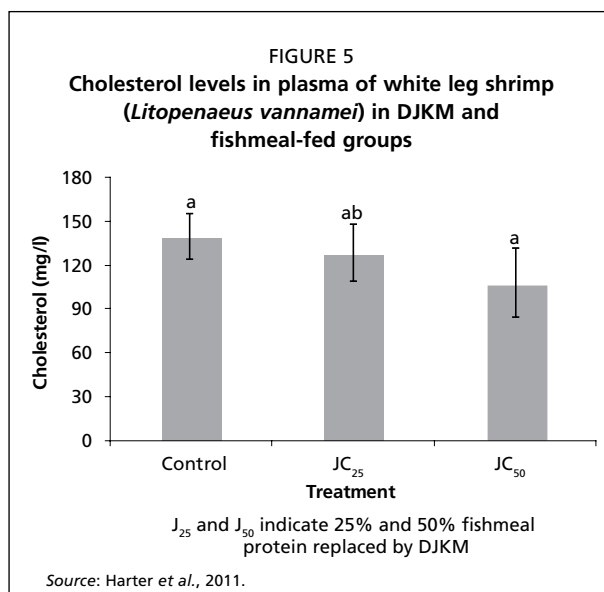
In the whole body of the shrimp, there was no significant effect ($P > 0.05$) on lipid deposition after feeding DJKM, whereas protein and energy deposition were significantly higher ($P < 0.05$) in the control group compared with DJKM-fed groups (Harter *et al.*, 2011). Cholesterol is reported to be an essential nutrient for growth and survival of all crustacean species (Kanazawa *et al.*, 1971). Usually, shrimp diets are supplemented with cholesterol, because they like other crustaceans cannot synthesize cholesterol *de novo* (Teshima and Kanazawa, 1971). Cholesterol levels in plasma of white leg shrimp decreased when fed with

DJKM-based diets (Figure 5). Reduction in plasma cholesterol level in shrimp as dietary fishmeal levels decreased and DJKM levels increased was a consequence of the reduced amount of cholesterol available in the diet (Kaushik *et al.*, 1995; Harter *et al.*, 2011).

Overall, growth performance and nutrient utilization in white leg shrimp for DJKM-fed groups were better ($P < 0.05$) than for the control group, which suggests that white leg shrimp, can efficiently use DJKM as a good quality protein source. Additional studies with DJKM-based diets at a larger scale and under commercial pond conditions are suggested.

USE OF JATROPHA CURCAS KERNEL MEAL OF A NON-TOXIC JATROPHA GENOTYPE IN AQUA FEED

Heat-treated (121 °C at 66 percent moisture for 15 minutes) and unheated *J. curcas* kernel meals of a non-toxic variety were used as protein sources for common carp diet. The heat treatment was done to inactivate trypsin inhibitor and lectins. Similar growth performances were observed for both the groups, suggesting no physiological relevance of heat-labile factors such as trypsin inhibitor and lectins in jatropha meal and of the heat-stable factors such as antigenic proteins, if any, for common carp. Incorporation of jatropha kernel meal that was subjected to heat treatment for >15 min decreased growth performance of common carp. Lower growth performance and nutrient utilization were also observed with 30- and 45-minute-heated jatropha kernel meal compared with the unheated group (Makkar and Becker, 1999). These findings imply the loss of amino acids and their lower availability due to Maillard reaction products or heat-induced changes in the structure of jatropha proteins, or a combination, which are less digestible by the fish intestinal proteases. The energy retained in the fish was also lower in the 30- and 45-minute-heated jatropha meal-fed groups compared with the unheated meal-fed group. However, heat treatment has been shown to increase protein digestibility of jatropha protein by rumen proteases (Aderibigbe *et al.*, 1997) and also to inactivate the trypsin inhibitor and lectin (Makkar and Becker, 1997). Nutrient retention in the whole body was similar for control, unheated and heated (15, 30 or 45 minutes at 121 °C) jatropha meal-fed groups. It would be interesting to investigate the effects of incorporating unheated jatropha kernel



meal from the non-toxic jatropha genotype in the diets of other fish species.

The results of Makkar and Becker (1999) demonstrate that the availability of protein from the unheated jatropha meal is higher than from heat-treated jatropha meal. Furthermore, the nutritional value of jatropha meal of the non-toxic genotype is high, and potential exists for its incorporation into the diets of monogastrics and aquaculture species.

USE OF JATROPHA PLATYPHYLLA KERNEL MEAL AS A PROTEIN SOURCE IN AQUA FEED

Impacts of *Jatropha platyphylla* kernel meal (heat treated) on growth performance of Nile tilapia

The kernel meal of *J. platyphylla* contains 65–70 percent crude-protein with a well-balanced EAA profile, in addition to heat labile antinutritional factors, trypsin inhibitor and lectin. The heat treated (121 °C at 66 percent moisture for 15 minutes) kernel meal (H-JPKM) was fed to Nile tilapia (*Oreochromis niloticus* L.) for 12 weeks with two levels of replacement (50 percent and 62.5 percent) of fishmeal protein). H-JPKM-based diets were supplemented with phytase (500 FTU per kg feed) to mitigate the adverse effects of phytate. The utilization of proteins from H-JPKM and fishmeal was similar ($P > 0.05$). Also, growth performance of H-JPKM-fed groups was similar; indicating that availability of protein (amino acids) from the H-JPKM for protein synthesis was similar to that from fishmeal. These findings showed that H-JPKM is a good quality dietary protein source for Nile tilapia feed. The level of NSPs in H-JPKM was about 16 percent; however, no detrimental effects were observed. The anti-nutritional effects of NSPs are not yet fully understood in fish. However, these compounds are assumed to cause increased intestinal viscosity in fish similar to that in poultry. Usually, NSPs in diets for Atlantic salmon tended to reduce digestibility of protein and lipid due to increased intestinal viscosity and reduced diffusion and activity of the digestive enzymes (Refstie *et al.*, 2000). However, Makkar *et al.* (2011), Kumar *et al.*, (2011c) and Akinleye *et al.* (2011) did not observe any such adverse effects in Nile tilapia after feeding H-JPKM as the protein source. Retention of protein and lipid in the whole body of Nile tilapia were similar ($P > 0.05$) for H-JPKM-fed and fishmeal-fed groups (Kumar *et al.*, 2011c; Akinleye *et al.*, 2011). These results suggest that H-JPKM containing diets were ideal for fish growth.

Impacts of *Jatropha platyphylla* kernel meal (heat treated) on energy budget and health of Nile tilapia

In a feeding trial performed by Kumar *et al.* (2011c) where-in Nile tilapia were fed H-JPKM-based diet (62.5 percent

fishmeal protein replaced by H-JPKM) and a control diet (fishmeal-based diet), no significant difference ($P > 0.05$) for oxygen consumption, average metabolic rate, energy retention, energy expenditure and metabolizable energy were observed among the groups. The energy retention for growth was 35 percent, energy expenditure 40 percent and metabolizable energy 74 percent for the H-JPKM-based diet (Kumar, Makkar and Becker, 2011c). This finding suggests that dietary protein sources H-JPKM can be efficiently utilized for growth of Nile tilapia, and the efficiency is as high as that for fishmeal.

Inclusion of H-JPKM in the diet elicited no adverse effects on biochemical changes such as metabolic enzymes (ALP and ALT) and electrolytes and metabolites (urea nitrogen, bilirubin, calcium, potassium and sodium in the blood) (Akinleye *et al.*, 2011). The prominent changes include increased RBC count, haematocrit content and blood glucose concentrations, and decreased cholesterol concentration in plasma when compared with the control group (Akinleye *et al.*, 2011). However, haematological parameters were within normal ranges for fish (Ghittino, 1983; Rosenlund *et al.*, 2004).

The results showed that H-JPKM can replace fishmeal with no negative impacts on growth, feed utilization and physiological parameters (Makkar *et al.*, 2011; Kumar, Makkar and Becker, 2011c; Akinleye *et al.*, 2011). Conclusively, the H-JPKM can replace fishmeal protein up to 62.5 percent in the diet of Nile tilapia without any unfavorable effects on growth performance, nutrient utilization, energy budget and biochemical activities in the fish, and it can be utilized in Nile tilapia diet as a good quality protein source. Further research should be conducted to examine the possibility of increasing the inclusion of H-JPKM beyond 62.5 percent fishmeal protein replacement in the diet of Nile tilapia. Also studies on the utilization of H-JPKM in other fish species are required.

USE OF DETOXIFIED JATROPHA CURCAS PROTEIN ISOLATE IN COMMON CARP FEED

Impacts on feed intake and growth performance

Kumar, Makkar and Becker (2011d) observed that detoxified jatropha protein isolate (DJPI)-based diets had excellent palatability for common carp and there was no wastage of feed during the experiment. Common carp fed a diet containing a lower level of DJPI (50 percent replacement of fishmeal protein) grew significantly better ($P < 0.05$) than those on the fishmeal-based control diet (Kumar *et al.*, 2011d; Nepal *et al.*, 2010). However, a higher level (75 percent replacement of fishmeal protein) of DJPI exhibited growth performance similar ($P > 0.05$) to that with the control diet (Table 13) (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010). Since overall growth performance, and protein

TABLE 13
Growth performance, nutrient utilization, digestibility measurements, digestive enzyme activities and haematological parameters of common carp (*Cyprinus carpio* L) fed DJPI-based diets

Parameters	Control	Jatropha	
		50% (J ₅₀)	75% (J ₇₅)
Initial body mass (g)	20.3 ± 0.12	20.3 ± 0.11	20.2 ± 0.08
Final body mass (g)	124 ^a ± 9.0	118 ^a ± 13.5	118 ^a ± 13.5
Feed conversion ratio	1.36 ^a ± 0.06	1.31 ^a ± 0.03	1.39 ^a ± 0.10
Protein efficiency ratio	1.91 ^a ± 0.11	2.01 ^a ± 0.05	1.86 ^a ± 0.14
Protein productive value (%)	30.4 ^c ± 2.84	34.2 ^b ± 1.37	33.7 ^b ± 2.83
Nutrient digestibility and digestive and metabolic enzymes activity			
Protein digestibility (%)	90 ^b ± 1.03	93 ^a ± 1.57	89 ^b ± 2.01
Lipid digestibility (%)	94 ^a ± 1.58	95 ^a ± 1.67	94 ^a ± 2.53
Energy digestibility (%)	88 ^b ± 1.28	91 ^a ± 1.64	89 ^b ± 2.08
Amylase (U/g protein)	20.1 ^a ± 3.36	18.6 ^a ± 5.81	18.4 ^a ± 4.40
Protease (U/g protein)	40.0 ^a ± 2.82	37.1 ^a ± 3.91	32.7 ^a ± 3.04
Lipase (U/g protein)	7.8 ^a ± 1.39	8.4 ^a ± 0.46	8.5 ^a ± 0.85
Alkaline phosphatase (U/L)	65.7 ^a ± 13.3	62.7 ^a ± 3.5	68.3 ^a ± 7.4
Alanine transaminase (U/L)	74.3 ^a ± 17.2	79.0 ^a ± 11.8	68.0 ^a ± 10.1
Blood parameters			
Red blood cells (10 ⁶ cells/mm ³)	1.1 ^c ± 0.02	1.3 ^b ± 0.03	1.4 ^a ± 0.01
Albumin (mg/dl)	2.1 ^b ± 0.2	2.5 ^a ± 0.1	2.6 ^a ± 0.3
Globulin (mg/dl)	0.6 ^b ± 0.2	0.8 ^a ± 0.2	0.9 ^a ± 0.1
Lysozyme activity (IU/ml)	384 ^b ± 24	419 ^a ± 18	431 ^a ± 34
Cholesterol (mg/dl)	139 ^a ± 14	116 ^b ± 22	93 ^c ± 15
Glucose (mg/dl)	67.0 ^a ± 8.0	61.0 ^a ± 11.8	87.0 ^a ± 25.2

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscript differ significantly ($P < 0.05$). Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A₂₈₀ per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 micro-equivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 at 37 °C. ALP and ALT expressed as 1 U = 16.66 nKat/l; nKat = amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute. Lysozyme activity IU expressed as the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25 °C, using a suspension of *Micrococcus lysodeikticus* as the substrate.

Sources: Kumar *et al.*, 2011d; Nepal *et al.*, 2010.

and energy utilization of this group were similar to those of the fishmeal-fed group, it is plausible to surmise that a high replacement level (up to 75 percent replacement of fishmeal protein) of fishmeal by a single plant protein-based source such as DJPI is possible in common carp diet. Higher levels of replacement of fishmeal (up to 100 percent) by DJPI should be investigated in future studies.

Impacts on digestive physiology

DJPI in combination with fishmeal protein showed excellent nutrient and energy digestibilities in common carp (Table 13) (Kumar *et al.*, 2011d). Compared with fishmeal protein, DJPI had similar ($P > 0.05$) apparent protein and lipid digestibility, which could be attributed to the absence of a trypsin inhibitor and lectin, the presence of lower levels of NSPs (NSPs in DJPI were 10 percent), and the addition of phytase to mitigate the effects of phytate, if any (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010). Energy digestibility of the DJPI protein-based diets was considerably lower than protein digestibility (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010).

Dietary inclusion of DJPI did not ($P > 0.05$) alter the intestinal digestive enzyme (amylase, protease and lipase) activities (Table 13). Phytate content in DJPI was 2.9 percent,

and the DJPI-based feeds were supplemented with 500 FTU phytase/kg. This level of phytase appears to be sufficient to hydrolyse the phytate in the DJPI-based diets. No change in activities of digestive enzymes could be attributed to the absence of trypsin inhibitors and lectins and addition of phytase (500 FTU phytase/kg) in the DJPI-based diets (Kumar, Makkar and Becker, 2011d).

Impacts on nutrients retentions

Inclusion of DJPI in feed exhibited higher ($P < 0.05$) lipid retention in the whole body of fish compared with control (fishmeal) fish. As DJPI protein increased in the common carp diet, lipid retention in the whole body also increased, which led to a higher value of lipid productive value and energy productive value (Kumar, Makkar and Becker, 2011d). DJPI could have increased the lipogenic enzyme activities in fish. Protein deposition in the whole body of common carp was more pronounced ($P < 0.05$) in DJPI-fed groups compared with the fishmeal-fed group, which concurs with the higher value of protein productive value in the former group (Table 13). Protein synthesis in the body requires an optimum level of dietary EAAs. Unbalanced amino acid concentrations in a diet or different availability of individual amino acids results in increased protein

degradation, leading to increased protein turnover. Usually plant protein sources such as soy protein decrease protein retention because soy protein-based diets are not able to provide well balanced EAAs and energy for growth (Cheng, Hardy and Usry, 2003). Interestingly, Kumar Makkar and Becker (2011d) found that protein retention in the body of common carp was significantly higher ($P < 0.05$) in DJPI-fed groups than the control group. This finding reveals that that DJPI-containing diets have optimum digestible energy and a balanced amino acid profile for optimum growth and optimum nutrient deposition in fish.

Impacts on biochemical parameters and haemato-immunology

Feeding DJPI as a protein source significantly decreased ($P < 0.05$) cholesterol level in plasma and muscle compared with the control group (Table 13) (Kumar *et al.*, 2009; Nepal *et al.*, 2010). The hypocholesterolaemic effect of plant proteins compared with animal proteins is well documented (Forsythe, 1995) and could be due to increased excretion of the bile salts, resulting in inhibition of cholesterol absorption through the intestine (Kumar, Makkar and Becker, 2009; Kumar *et al.*, 2010b; Nepal *et al.*, 2010). The amino acid composition of dietary protein was partially responsible for its effect on cholesterol concentration (Tasi and Huang, 1999). These authors observed a significant positive correlation between the lysine/arginine ratio of the diet and serum cholesterol concentration. Nepal *et al.* (2010) and Kumar Makkar and Becker (2009) also observed that the lysine/arginine ratio in DJPI-based diets was positively correlated with cholesterol concentration in plasma of common carp.

RBC counts increased with increased level of DJPI in common carp diet (Table 13) (Kumar, Makkar and Becker, 2009; Nepal *et al.*, 2010); however these values were in the normal range ($1.10\text{--}2.20 \times 10^6/\text{mm}^3$) for healthy carp (Ghittino, 1983). Higher RBC in the DJPI-fed group might be due to a higher proportion of immature erythrocytes released from the spleen (Härdig and Hoglund, 1983). Kumar *et al.* (2009) and Nepal *et al.* (2010) also observed higher haematocrit value in the DJPI protein-fed groups than the control group due to higher RBC count in these groups. Haematocrit level in all groups was in the normal range of 44–59 percent for common carp (Radu *et al.*, 2009).

Significantly higher ($P < 0.05$) albumin and globulin concentrations in blood and lysozyme activity in serum were observed for DJPI-fed groups (Table 13), which suggest an immuno-stimulatory effect of DJPI in common carp (Kumar, Makkar and Becker, 2009; Nepal *et al.*, 2010). Albumin and globulin concentrations in blood for DJPI-fed groups were within the normal range (Wedemeyer and Chatterton, 1970; Sandnes, Lie and Waagbo, 1988). Feeding DJPI as a protein to common carp exhibited levels of metabolic enzyme (ALP and ALT) activities similar ($P > 0.05$) to those in

the control group, suggesting normal organ function and absence of toxic factors in DJPI (Nepal *et al.*, 2010). Blood glucose concentration was unaffected ($P < 0.05$) by dietary inclusion of DJPI in common carp (Nepal *et al.*, 2010).

An overview of the results of jatropha-based feed ingredients when used to replace fishmeal in fish and shrimp diets are presented in Table 14.

CONCLUSIONS REGARDING USE OF DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* AS AQUA FEED Effects on growth and nutrient utilization

- Detoxified jatropha kernel meal, H-JPKM and DJPI can replace 50, 62.5 or 75 percent fishmeal protein, respectively, without compromising growth performance and nutrient utilization in fish. In addition, DJKM can also replace 50 percent fishmeal protein without any adverse effects on growth and nutrient utilization in shrimp. If the replacement levels are exceeded, the nutrient profile of the feeds must be carefully examined to ensure that desired production levels can be achieved and fish and shrimp health maintained.
- High inclusion (>50 percent fishmeal protein replacement) of DJKM decreases the efficiency of conversion of feed to body mass. No such effects were seen on using DJPI in common carp diets.
- Increased DJKM inclusion (>50 percent fishmeal protein replacement) in diets caused a significant lowering of protein, lipid and energy digestibilities. No such effects were observed when DJPI was used in common carp diets.

Effects on energy budget

- Feeding DJKM and H-JPKM to common carp and Nile tilapia respectively did not change the major components of the energy budget (routine metabolic rate, heat released and metabolizable energy) compared with fishmeal and SBM-fed groups. These results showed that, as dietary protein sources, DJKM and H-JPKM can be efficiently utilized for growth by common carp and Nile tilapia respectively, and as good as soymeal and fishmeal.

Effects on clinical health parameters and gut health

- No mortalities and unaffected haematological values suggested that the fish were in normal health. ALP and ALT activities, urea nitrogen, bilirubin and creatinine concentrations in blood were in the normal ranges, showing no liver or kidney dysfunction.
- The plasma nutrient levels measured gave no indications of stress, but increasing the level of plant protein in the diet decreased plasma cholesterol. A decrease in muscle

TABLE 14

An overview of the results of replacing fishmeal with jatropha-based feed ingredients in fish and shrimp diets

Jatropha-based ingredient	Species	Inclusion level in diet (%)	CP in diet (%)	Experimental period (weeks)	Fishmeal protein replaced in diet (%)	Biological effects	References
Detoxified jatropha kernel meal (DJKM)	Common carp (<i>Cyprinus carpio</i>)	24 and 36	38	8	50 and 75	At up to 50% replacement level growth performance and nutrient utilization were similar to those in control; >50% replacement level decreased performance. Inclusion of DJKM in the diets did not change blood metabolite, ion and enzyme levels. Also no adverse histopathological changes were observed.	Kumar <i>et al.</i> , 2010b; Kumar, Makkar and Becker, 2011a.
DJKM	Common carp	38	38	6	75	Growth performance, nutrient utilization, oxygen consumption and metabolic rate were similar to those in control.	Kumar, Makkar and Becker, 2010c.
DJKM	Common carp	25 and 31	38	16	50 and 62.5	No significant difference in growth rate among the groups.	Kumar, 2011.
DJKM	Rainbow trout (<i>Oncorhynchus mykiss</i>)	34 and 43	45	12	50 and 62.5	Growth rate and feed efficiency for 50% replacement group were similar to those for control; 62.5% replacement significantly depressed these parameters.	Kumar, Makkar and Becker, 2011b.
DJKM	White leg shrimp (<i>Litopenaeus vannamei</i>)	12.5 and 25	35	8	25 and 50	Shrimp on DJKM-based diet grew better than control; nutrient deposition in the body was similar; hypocholesterolaemic effect observed in fish fed DJKM-based diet.	Harter <i>et al.</i> , 2011.
Detoxified jatropha protein isolate (DJPI)	Common carp	20 and 30	38	12	50 and 75	Growth performance, nutrient utilization and digestive enzyme activity were similar to those in control, and improved protein utilization in DJPI-fed group. Blood parameters were in the normal range. Also no adverse histopathological changes were observed.	Kumar, Makkar and Becker, 2011d.
Heated <i>Jatropha platyphylla</i> kernel meal (H-JPKM)	Nile tilapia (<i>Oreochromis niloticus</i>)	20 and 25	36	12	50 and 62.5	No differences in growth rate, feed utilization, oxygen consumption and average metabolic rate among the H-JPKM diets and control.	Makkar <i>et al.</i> , 2011; Akinleye <i>et al.</i> , 2011; Kumar <i>et al.</i> , 2011c.

cholesterol level is also expected, which could be considered good for human health.

- Histopathological evaluation showed no damage to stomach, intestine or liver of common carp or rainbow trout.

USE OF DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL IN POULTRY FEED

Soybean and canola meals (i.e. rapeseed meal) are the major protein meals used worldwide in poultry feed (USDA, 2010). However, SBM competes with human food and there is a need to search for alternative plant-protein sources for poultry feed. Recent research with fish species has shown that detoxified *J. curcas* kernel meal (DJKM) can be an excellent source of dietary protein in animal feeds, especially in situations where fishmeal and conventional protein-rich feed ingredients are in short supply and expensive (Makkar and Becker, 2009a; Kumar, Makkar and Becker, 2011a, b). The nutrient and energy concentrations of DJKM compare well with that of SBM, with a higher content of EAAs (except lysine).

Boguhn *et al.* (2010) evaluated the nutritional quality of DJKM in turkeys (3-week-old) by including at levels of 0 (control), 10 (J_{10}) or 20 percent (J_{20}) into a basal diet based on maize, SBM and wheat gluten, at the expense of maize starch. Body mass gains were 42, 54 and 57g/day for control, J_{10} and J_{20} groups respectively. Feed efficiency (gain:feed ratio) was significantly higher ($P < 0.05$) in DJKM-fed groups (0.81 and 0.82 vs 0.70). Precaecal amino acid digestibilities of amino acid from DJKM varied from 0.48 (cystine) to 0.91 (methionine) (Table 15). Mean digestibility of the non-essential amino acids was 80 percent, while that of EAAs was 83 percent.

Considering growth performance, nutrient utilization and amino acid digestibility of DJKM, it can be concluded that DJKM is valuable protein source for turkeys.

USE OF DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL IN PIG FEED

The most commonly used source of supplemental protein in diets for non-ruminants is SBM because of its excellent

TABLE 15
Amino acid content of detoxified *Jatropha* kernel meal (g/kg DM) and calculated coefficients of their precaecal digestibility (mean \pm standard error)

	Detoxified <i>Jatropha</i> kernel meal	Precaecal digestibility coefficient
Crude protein	630	0.83 \pm 0.042
Alanine	31.0	0.85 \pm 0.037
Arginine	74.0	0.90 \pm 0.034
Aspartic acid	62.0	0.70 \pm 0.033
Cystine	4.9	0.48 \pm 0.057
Glutamic acid	103.3	0.85 \pm 0.056
Glycine	29.2	0.79 \pm 0.035
Isoleucine	25.2	0.88 \pm 0.049
Leucine	45.2	0.86 \pm 0.049
Lysine	21.7	0.87 \pm 0.068
Methionine	10.7	0.91 \pm 0.083
Phenylalanine	29.4	0.89 \pm 0.041
Proline	29.0	0.83 \pm 0.072
Serine	33.1	0.79 \pm 0.041
Threonine	24.1	0.83 \pm 0.051
Tryptophan	6.9	0.83 \pm 0.042
Valine	29.0	0.88 \pm 0.041

Source: Boguhn *et al.*, 2010.

amino acid profile and dependable supply. In a typical pig diet, soybean supplies about 50 percent of the protein and amino acids and about 25 percent of the metabolizable energy. Wang *et al.* (2011) investigated the effects of replacing SBM by detoxified *J. curcas* kernel meal (DJKM) in the diet of the growing pig. The DJKM protein replaced 0, 25 or 50 percent of SBM protein in the diets, and the DJKM-containing diets were supplemented with lysine

(~2 percent of DJKM inclusion). There were no significant differences ($P > 0.05$) in growth performance and feed utilization on substituting 25 or 50 percent of SBM protein with DJKM (Table 16). These results show that the nutrient value of a DJKM-supplemented diet containing additional lysine is comparable with that of SBM for growing pigs. Dietary inclusion of DJKM did not ($P > 0.05$) affect carcass weight, dressing percentage, back fat thickness or visceral organ weight and its ratio to body weight when compared with the control group.

Also, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase and ALP activities and the concentrations of albumin, urea, glucose and triglycerides in serum did not change ($P > 0.05$) in growing pigs. There were no histopathological changes in liver and kidney of growing pigs fed DJKM diets (Wang *et al.*, 2011).

The above data show that incorporation of DJKM had no ill effects on health, and it can replace 50 percent soymeal protein in diets of growing pigs.

CHALLENGES AND OPPORTUNITIES IN USING AS LIVESTOCK FEED BY-PRODUCTS OBTAINED DURING THE PRODUCTION OF BIODIESEL FROM JATROPHA OIL

During the process of biodiesel production, acid gum and fatty acid distillate are produced during the de-gumming and de-odorization processes, respectively, before the transesterification process; and glycerol is produced during the transesterification process. Amongst these by-products, glycerol is recovered in substantial amounts (10 percent of

TABLE 16
Growth performance, nutrient utilization and health parameters of pigs fed DJKM-based diets

Parameter	Control	Jatropha		SEM
		25% (J ₂₅)	50% (J ₅₀)	
Growth and feed utilization				
Initial body mass (kg)	21.45	21.43	21.44	0.244
Final body mass (kg)	38.76	38.40	39.24	0.695
Feed to gain ratio	2.167	2.127	2.150	0.098
Biochemical				
Total protein (g/L)	60.5 ^{ab}	58.0 ^b	63.6 ^a	1.46
Albumin (g/L)	34.6	33.3	36.3	0.94
Urea nitrogen (mmol/L)	2.89	2.80	3.04	0.146
Glucose (mmol/L)	5.40	4.99	4.95	0.350
Triglyceride (mmol/L)	0.39	0.35	0.36	0.026
Enzyme activities				
Superoxide dismutase (U/mL)	148.6 ^{ab}	138.7 ^b	157.3 ^a	4.25
Lactate dehydrogenase (U/mL)	14.0	15.1	14.7	1.02
Lysozyme (U/mL)	70.0	62.8	70.3	4.22
Glutamic-pyruvic transaminase (U/L)	11.7	12.3	12.5	0.81
Glutamic-oxalacetic transaminase (U/L)	10.8	11.4	10.9	1.02
Alkaline phosphatase (U/100 mL)	20.9	18.1	20.6	1.15
Acid phosphatase (U/100 mL)	11.6	10.8	11.7	0.81

Notes: Means with different superscripts within a rows differ significantly ($P < 0.01$). J₂₅ and J₅₀ = 25 and 50% of SBM protein replaced by detoxified *Jatropha curcas* kernel meal, respectively. SEM = standard error of the mean. Source: Wang *et al.*, 2011.

the biodiesel). In the study of Makkar and Becker (2009a) no phorbol esters were detected in the glycerol fraction; however phorbol esters were detected in glycerol samples obtained from two different laboratories associated with biodiesel producing companies that produced biodiesel from jatropha oil (company #1: 0.58–0.97 mg/g glycerol; company #2: 0.061 mg/g glycerol). Similarly, fatty acid distillate produced by the procedure adopted by Makkar and Becker (2009a) was free of phorbol esters; however, an earlier study (Haas and Mittelbach, 2000) reported the presence of phorbol esters in the fatty acid distillate fraction. Compared with the Makkar and Becker (2009a) study, the study of Haas and Mittelbach (2000) used mild conditions during the stripping or de-odorizing step that gives fatty acid distillate. These observations suggest that the process conditions used in the Makkar and Becker (2009a) study led to destruction of phorbol esters, and that process parameters for biodiesel production could be established that gives glycerol and fatty acid distillate fractions free of phorbol esters. It should be noted that at present no information is available on the nature of the phorbol ester degraded products and their possible toxicity. There is need for further research to evaluate the innocuous nature of fatty acid distillate and glycerol so produced. The acid gum fraction obtained during the de-gumming stage was rich in phorbol esters (2.02 mg/g) (Makkar and Becker, 2009a) and hence not usable in animal feeds. At the same time, these fractions obtained during biodiesel production from the oil from the non-toxic *J. curcas* would be safe for inclusion in livestock diets.

To enable safe use of these by-products, a process was needed for isolation of phorbol esters from the toxic jat-

ropha oil before the oil goes for biodiesel production, and efforts in this directions have been successful (Devappa, Makkar and Becker, 2010c; Devappa *et al.*, 2010d). The phorbol esters isolated could be used for various agricultural and pharmaceutical applications since they have strong molluscicidal and pesticidal activities (Makkar and Becker, 2009a).

GUIDELINES FOR USING DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* AS A PROTEIN SOURCE IN ANIMAL FEED

Based on our studies, the detoxified jatropha kernel meal and detoxified jatropha protein isolate should have the traits presented in Table 17.

Detoxified jatropha kernel meal, H-JPKM and DJPI can replace 50, 62.5 and 75 percent fishmeal protein, respectively, in fish diets, without sacrificing growth and nutrient utilization, and without affecting physiological and haematological parameters. For shrimp, 50 percent fishmeal protein could be replaced by DJKM. The guidelines described below would increase the efficiency of DJKM, H-JPKM and DJPI utilization in fish and shrimp.

- Take into account that DJKM and H-JPKM contain approximately 65 percent crude protein, which is similar to the level in fishmeal, and can therefore substitute for fishmeal on an equal weight basis.
- The acceptability of DJKM, H-JPKM and DJPI-based diets by fish, as measured by immediate consumption and no waste in the tanks, is good.
- DJKM, H-JPKM and DJPI are deficient in lysine. Therefore lysine monohydrochloride should be supplemented at a

TABLE 17

Recommended quality parameters for detoxified jatropha kernel meal and detoxified jatropha protein isolate

	Detoxified jatropha kernel meal	Detoxified jatropha protein isolate
Protein (%), minimum	60–66	81–88
Fat (%), minimum	0.9–1.2	0.8–1.0
Fibre (%), maximum	8–9	1
Ash (%)	< 11%	< 3%
Gross energy (KJ/g)	18.5	21.4
Moisture (%)	6–8	4–6
Non-starch polysachharides (%), maximum	16	10
Lysine (%)	> 2.3	> 2.5
Available lysine	Near 100%	Near 100%
Pepsin plus trypsin digestibility (% of total nitrogen)	> 92.0	> 97.0
Protein dispersibility index	15–40%	-
Trypsin inhibitor (mg trypsin inhibited per g sample)	Not detectable	Not detectable
Lectin activity ⁽¹⁾	Not detectable	Not detectable
Texture	Homogenous, free flowing, no lumps, not dusty	Homogenous, free flowing, no lumps, not dusty
Taste	Bland	Bland
Contaminants	Free of PEs Free of urea Free of ammonia Free of mycotoxins and mould	Free of PEs Free of urea Free of ammonia Free of mycotoxins and mould

Notes: (1) Based on haem-agglutination. PEs = phorbol esters (sensitivity: <3 µg/g material).

level of 1.5 percent of the DJKM, H-JPKM and DJPI (w/w) inclusion in the diet to compensate for the deficiency.

- DJKM and H-JPKM contain approximately 9–10 percent phytate, which is almost 3-fold that in SBM. To mitigate its effect, add 1500 FTU phytase per kg of diet (Kumar *et al.*, 2011f).
- Detoxified jatropha kernel meal-, H-JPKM- and DJPI-based diets could be fed to fish at 5 times maintenance requirements. Single maintenance requirement equals 3.2 g feed/kg metabolic body mass (kg^{0.8}) per day. Shrimp (juveniles, >10 g) should be fed 3–4 percent of the total body weight per day.

POTENTIAL CHALLENGES IN USING DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* IN FEEDS

- Inadequately heated jatropha kernel meal that contains significant amounts of trypsin inhibitor and lectin could reduce the performance of monogastric animals. Similarly, inadequately detoxified material containing phorbol esters could cause adverse effects. Phorbol esters must be below the detectable limit (<3µg/g meal).
- Overheating jatropha kernel meal could increase the portion of 'bound protein', which is indigestible. A laboratory measurement of ADIN (acid-detergent insoluble nitrogen) could be used as an indicator of the extent of bound protein.
- Incorporating a high level of DJKM into a diet requires rebalancing the ingredients in order to maintain a proper ratio of energy to other nutrients, especially protein.
- Fish and shrimp fed high levels of plant protein such as DJKM or DJPI could deposit more fat in their fish muscles, which could be more unsaturated and hence more susceptible to oxidation.

ENVIRONMENTAL CONSIDERATIONS

Detoxified jatropha kernel meal and DJPI contain almost three times the phytate content of SBM and soy protein isolate, respectively. Detoxified jatropha kernel meal-based feeds have higher phosphorus concentrations than traditional feeds. Feeding large quantities of these feeds increases the amount of phosphorus excreted by the aquatic organism. In any given aquaculture enterprise, the excess phosphorus would increase the acreage needed for spreading waste in order to comply with waste management regulations, which could potentially limit future expansion. To minimize these potential problems, it is suggested that supplemental phosphorus should not be included in the diet when DJKM-based feeds containing phytase are fed. These diets would provide adequate phosphorus and meet the requirements indicated in National Research Council (NRC) 1993, recommendations without further supplementation. Several studies have demonstrated that feeding

excess phosphorus does improve neither the reproduction efficiency nor health of fish or shrimps. When phosphorus is fed in excess of NRC recommendations, additional calcium may be required to maintain normal calcium-phosphorus ratios in the diet.

FUTURE STUDIES

Further studies are warranted on the utilization of DJKM and DJPI in other fish (e.g. Nile tilapia (*Oreochromis niloticus*); major Indian carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*); Atlantic Salmon (*Salmo salar*); and shrimp species (e.g. freshwater prawn (*Macrobrachium rosenbergii*) and giant tiger prawn (*Penaeus monodon*). In addition, long-term feeding trials at farm level should be conducted on fish, shrimp, turkey, poultry, pigs and other domestic animals. Organoleptic properties, while not a common assessment, have been used to evaluate the potential impact of novel ingredients on product quality. Such assessments could also be conducted. Other related studies that would enhance the utilization of jatropha-based products as animal feed are: comparative evaluation of toxic and non-toxic genotypes of jatropha with respect to yields, disease susceptibility and nutrient and water requirements; and breeding studies to produce jatropha cultivars that are adapted to different agroclimatic conditions and with reduced toxins.

FINAL COMMENTS

The animal feed industry is growing at a rapid pace, and production is switching to intensively managed high-input systems. The main input in any livestock production system is feed, and demand for feed for aquatic organisms is expected to nearly triple by the end of the decade. This growth dictates greater use of protein sources other than fishmeal or soymeal. Jatropha products could be leading candidates to supply a large amount of the protein required for the expanding feed market. The work reported in this chapter enlarges the portfolio of feed ingredients available to the feed industry. It also highlights synergies between the bio-energy and feed industries, and shows how food and energy security can be better integrated.

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Chapter 22

Use of *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes for feeding livestock

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ABSTRACT

India, with the world's largest livestock population, intertwined intimately with its crop production, has immense potential for future growth and development of the livestock sector. However, chronic shortage of protein- and energy-rich feeds, shrinking grazing land and liberalized export policies are posing serious threats to developing the livestock industry into an economic enterprise. As the conventional protein-rich feeds are costly, the poor and marginal farmers of the country are unable to incorporate them in livestock diets to obtain optimum production. Animal nutritionists constantly seek cheaper and easily available non-competitive unconventional agroforestry-based industrial co-products for feeding livestock. Agroforestral industrial co-products include *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes, commonly used only as manure, which is highly uneconomical and almost unethical in a country having the largest livestock population in the world and facing chronic shortage of good quality feeds for them. In India, 1.3 million tonne of karanj cake and 0.9 million tonne of neem seed cake are available annually. The toxic principles present in these cakes make them unpalatable, but these toxins can be removed by various techniques. Industry and progressive farmers are being recommended to include these cakes in the diet of animals after partial or complete detoxification. De-oiling of karanj cake results in complete removal of fat-soluble toxic compounds, and water washing of karanj cake and neem seed cake can detoxify them partially. Therefore, these treated cakes might replace conventional oil cakes (soybean meal or groundnut cake) at up to 50 percent of the nitrogen in diets, without any adverse effect on nutrient metabolism, growth and health of the animals.

INTRODUCTION

Protein-rich feeds are one of the costliest feed ingredients in animal diets. The roughage-based diets that are the primary ruminant feed in India are deficit in protein. Poor dietary supply of proteins results in low rates of reproduction and production, as well as increased susceptibility of livestock, including poultry, to metabolic disorders and infectious diseases. The strategy for improving livestock production has therefore been to maximize the efficiency of utilization of the available feed resources in the rumen by providing optimum conditions for microbial growth and by supplementation with protein-rich feeds like oil seed cakes, thus optimizing the ratio of energy to protein. Animal nutritionists constantly seek cheaper, easily available, non-competitive (with human food), unconventional agroforestral and industrial co-products for feeding to ruminants and poultry. The feed deficit in India is currently 11 percent for dry fodder, 28 percent for concentrate and 35 percent for green fodder (NIANP, 2005).

Oilseed cakes are commonly used as protein supplements in India. Dikshit and Birthal (2010) estimated the feed consumption rates for different livestock species and the generated demand for different types of feeds by the year 2020, when India is forecast to require 56×10^6 tonne of concentrate feeds, comprising 27.4×10^6 tonne of cereals, 4.0×10^6 tonne of pulses, 20.6×10^6 tonne of oilseeds, oilcakes and meals and 3.6×10^6 tonne of manufactured feed. The present requirement for concentrate feeds in the country is 47.3×10^6 tonne (Dikshit and Birthal, 2010), but the availability is only 34×10^6 tonne. There has been serious concern over the question of protein scarcity emerging as the major constraint to dairy development in the coming years. Several strategies have been examined by scientists, policy-makers and administrators, amongst which are:

- restricting oilseed meal exports as a means of increasing domestic availability of oilseed proteins;
- developing ways to increase rapidly the cultivated area under high-protein green fodders;

MAIN MESSAGES

- By 2020, India can be expected to have an annual requirement for 56×10^6 tonne of concentrate feeds, comprising 27.4×10^6 tonne of cereals, 4.0×10^6 tonne of pulses, 20.6×10^6 tonne of oilseeds, oilcakes and meals, and 3.6×10^6 tonne of manufactured feed.
- Conventionally, different parts of *Pongamia glabara* are used for different purposes: the oil is used as a lubricant, water-paint binder, pesticide, in soap making, for tanning, fuel for cooking and lamps, in rheumatism, herpes, enhancing the pigmentation of skin affected by leucoderma or scabies; and the leaf juice is used in treating colds, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy, and as an anthelmintic, digestive and laxative aid.
- Neem oil and other products of the neem tree are used traditionally for making cosmetics (soaps, mild detergents, creams, tooth cleanser) and in traditional Indian medicine (skin infections, inflammations, fever, leprosy, malaria, tuberculosis, worm infestation, eczema, etc.), in addition to use as an anti-bacterial and anti-fungal agent in bio-manure and in plant protection.
- Karanj and neem seed cakes are rich in protein. The crude protein content of rotary-pressed karanj cake ranges from 6 to 24 percent, while it varies from 22.0 to 28.7 percent in expeller-pressed karanj cake and 30.0 to 34.0 percent in solvent-extracted karanj cake. On a dry matter basis, neem seed cake contains 12.4 to 19.6 percent crude protein, de-oiled NSC contains 17.9–18.4 percent crude protein, and neem seed kernel cake contains 33.5–40.8 percent crude protein.
- Karanj cake toxins include furanoflavones (karanj, pongamol, pongapin, pongaglabron, kanjone, isopongaglavone lanceolatin B), tannins and trypsin inhibitors. Karanj and pongamol are the most important toxic factors, and its bitterness is attributed to these two compounds.
- Neem seed cake contains toxic triterpenoids (azadirachtin, salanin, nimbin, nimbidiol) and its bitterness is attributed to these compounds.
- The anti-nutritional factors of karanj cake are soluble in oil. Complete removal of oil from cake appears to be more effective than other treatment methods.
- Water-washed or de-oiled karanj cake and water-washed neem seed cake may be incorporated at up to 50 percent of the nitrogen moiety of conventional protein supplements like soybean meal or groundnut cake without any adverse effect on nutrient metabolism, growth or health of animals.

- developing technical interventions to improve utilization of existing protein sources in the rumen through protection of degradable proteins; and
- identifying unconventional oil cake sources, and their detoxification for use as animal feed.

In the present chapter, efforts have been made to consolidate information available on *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes with respect to their chemical composition, toxic compounds present, detoxification and the effects of their inclusion in the diets of ruminants and poultry on the physiology and health of these animals and the quality of their products.

KARANJ (*PONGAMIA GLABRA*) CAKE

The ambitious National Biodiesel Mission aims to meet 20 percent of India's diesel requirements through bio-diesel by 2016–2017. Since the demand for edible vegetable oil exceeds supply, the government has decided to use non-edible oil seeds as biodiesel feedstock. Biofuels offer a number of environmental, social and economic advantages, including lower emissions of harmful pollutants; decreased greenhouse gas emissions; increased employment opportunities; increased energy security, especially in rural areas; decreased dependence on oil imports; and good fuel

properties for vehicles. The national mission on biofuels has already been launched in two phases. Under the first phase, jatropha and karanj plantations would be established on 400 000 ha of government-owned land. Among the various agroforest based industrial co-products, the current use of karanj cake primarily as manure is highly uneconomical and almost unethical in a country having the largest livestock population in the world and facing chronic shortage of quality feeds for them. Karanj cake will be available as a co-product from the biodiesel plants in appreciable quantities in various parts of the country, and could be used as a source of protein for economic livestock production.

Availability and conventional uses of *Pongamia glabara*

Pongamia glabra (syn. *Pongamia pinnata*), commonly known as karanj (pongam oil tree), belongs to the family Leguminosae. It is a medium-sized, deciduous, glabrous, fast growing tree with a spreading crown of up to 25 m, and capable of growing under a wide range of agroclimatic conditions (Parmar, Sahrawat and Mukherjee, 1976). In India, it is found abundantly in Andhra Pradesh, Bihar, Jharkhand, Karnataka, Maharashtra, Tamil Nadu and West Bengal. The tree is adapted to humid and subtropical envi-

TABLE 1
Conventional uses of *Pongamia glabra*

Plant part	Conventional uses
Oil	Commonly used as a lubricant, water-paint binder, pesticide, in soap-making, for tanning, as fuel for cooking and lamps, as medication in rheumatism, for itches, herpes, effective in enhancing the pigmentation of skin affected by leucoderma or scabies.
Leaf juice	Medicinally for colds, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy, and as an anthelmintic, digestive and laxative aid.
Roots	Cleaning gums, teeth, and ulcers.
Bark	Bleeding piles.
Flowers	Biliousness and diabetes.
Fruit and seed	Keratitis, piles, urinary discharges and diseases of the brain, eye, head and skin.
Medicinal activity of various products	
70% ethanolic extract of leaves	Anti-inflammatory activity without any side effects; anti-pyretic action
Decoction of leaves	Anti-diarrhoeal action.
Ethanolic extract of flowers	Anti-hyperglycaemic and anti-lipidperoxidatives effects.

Sources: Adapted from Chopade *et al.*, 2008; Srinivasan, Muruganandan and Lal, 2001; Brijesh, Daswani and Tetali, 2006; Punitha and Manoharan, 2006.

ronments and thrives in areas having an annual rainfall of 500–2500 mm. In its natural habitat in India, the maximum temperature ranges from 27 to 38 °C, with a minimum of 1 to 16 °C. Mature trees can withstand waterlogging and slight frost. These trees can grow even at a height of about 1200 masl, although in the Himalayan foothills it is not found above 600 masl (Government of India, 1983).

It is known by different names in different Indian languages: 'karanj' in Hindi, 'pongam' and 'punnai' in Tamil, 'honge' in Kannada, 'Indian beech' in English, 'kanuga' in Telugu and 'karanja' in Bengali. It bears compound pinnate leaves consisting of 5–7 leaflets arranged in 2 or 3 pairs. Leaflets are 5–10 cm long, 4–6 cm wide and pointed at the tip. Flowers are borne on racemes, and are pink, light purple or white in colour. Pods are elliptical, measuring 3–6 cm in length and 2–3 cm in width, with a thick wall and usually containing a single seed. Seeds are 1–2 cm long or oblong and light brown in colour.

Karanj thrives on all sorts of soils, ranging from stony, through sandy to clayey, including vertisols, but prefers well-drained light porous soil. It is a common sight to find the tree near perennial water sources, on the banks of rivers, streams, tanks, canals and lakes. It is also a well-known avenue tree, grown in parks, gardens and roadsides. It is highly tolerant of salinity and hence it is common along waterways or seashores, with its roots in fresh or salt water. It does not do well on dry sands, and the highest growth rates are observed on well-drained soils with assured moisture.

The tree starts bearing seeds at 4–7 years. The fruit come to harvest at different periods of the year in different parts of the country, but the harvest season extends in general from November–December to May–June. The pods are collected and the shells are removed either by hand or separated by a decorticator before oil extraction. The seed yield ranges from 10 kg to more than 90 kg per tree (Anon., 1969). Mature seed contains 5 percent shell and 95 percent oleaginous kernel. Pressing the seed produces 25 percent

oil and 70 percent residue, known as cake, assuming 5 percent of losses. This is a high oil yield compared with other oil seeds. The main drawback is that the cake is non-edible as such, due to its toxicity.

Karanj seed production in India is 110 000 to 130 000 tonne annually (Ministry of Agriculture, 1992; NOVODB, 1995), of which about 85 500 tonne go uncollected. Seeds are mainly used for oil extraction and production is nearly 30 000 tonne per annum (De *et al.*, 1998). The oil is dark in colour, with an unpleasant odour. Technology has been developed to upgrade oil quality for soap manufacture and other industrial purposes.

Different plant parts of the karanj tree have different uses, and their extracts have medicinal values, as listed in Table 1. The karanj oil has varied uses in industry (leather dressing, soap making, lubrication, bio-diesel, illumination, etc.), as an insecticide or in medicine. The oil is known for its curative effect on skin problems, such as leucoderma, psoriasis, scabies and skin itches (Bringi and Mukerjee, 1987). At present, the use of karanj oil for the production of biodiesel is being explored (De and Bhattacharyya, 1999; Srivastava and Prasad, 2000; Vivek and Gupta, 2004; Meher *et al.*, 2006). Increased production of biodiesel from karanj may enhance the availability of karanj cake, which is the residue left after oil extraction. The cake, which is bitter and pungent, is used as manure, fungicide or insecticide. Although the cake is a protein-rich co-product potentially of great value for animal feeding, it is seldom used in animal feeding due to its poor palatability and the presence of various toxic constituents.

Chemical composition of karanj cake

Three main types of karanj cakes are available, namely rotary pressed, expeller-pressed (EKC) and solvent-extracted (SKC), the composition of which depends on the degree of decortication and method of oil extraction. The crude protein content of rotary-pressed karanj cake (Natanam, Kadirvel and Balagopal, 1988; Chandrasekaran, Kadiravel

TABLE 2
Chemical composition of karanj cake (as % of DM)

Parameter	Kernel ⁽¹⁾	EKC ⁽²⁾	SKC ⁽³⁾	Rotary pressed ⁽⁴⁾
Crude protein	20.5–24.1	24.1–28.7	30.0–34.0	24.6
Ether extract	33.2–43.3	6.1–14.2	0.1–2.2	14.0
Crude fibre	3.8–4.6	3.9–10.7	5.0–5.6	6.0
NFE	24.5–39.7	49.9–56.4	54.3–59.9	–
Total ash	2.8–3.5	3.2–7.1	4.4–6.9	5.2
NDF	–	18.2	18.0–28.2	37.7
ADF	–	10.6	1.7–20.0	11.3
Karanjin	–	0.29–0.32	0.01–0.19	–
Ca	0.5	0.6–0.8	0.6–0.9	0.74
P	0.38	0.48–0.70	0.55–0.61	0.89

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; NFE = nitrogen-free extract; ADF = acid-detergent fibre; NDF = neutral-detergent fibre. Sources: (1) Natanam, Kadirvel and Ravi, 1989; Vinay and Kanya, 2008. (2) Gupta *et al.*, 1981; Natanam, Kadirvel and Ravi, 1989; Ravi *et al.*, 2000; Prabhu, 2002; Panda, 2004; Soren *et al.*, 2007. (3) Mandal, Banerjee and 1974; Konwar, Banerjee and Mandal, 1984; Konwar and Banerjee, 1987; Gupta *et al.*, 1981; Ravi *et al.*, 2000; Prabhu, 2002; Panda, 2004; Soren, 2006. (4) Chandrasekaran, Kadiravel and Viswanathan, 1989.

TABLE 3
Amino acid composition (g/16 g N) of karanj cakes compared with soybean meal

Amino acid	SKC ⁽¹⁾	EKC ⁽²⁾	SBM ⁽³⁾
Histidine	2.07	5.11	1.13
Isoleucine	3.59	5.95	2.56
Lysine	5.60	3.34	2.96
Leucine	7.72	7.87	3.47
Methionine	0.99	1.43	0.62
Arginine	4.33	4.62	3.27
Phenylalanine	5.22	3.64	2.24
Tryptophan	0.61	–	0.60
Threonine	2.64	4.13	1.73
Valine	5.26	6.44	2.44
Aspartic acid	10.92	6.19	–
Serine	4.53	3.98	–
Glutamic acid	13.45	17.56	–
Proline	5.01	2.90	–
Glycine	3.85	3.39	3.27
Alanine	5.10	2.31	–
Cystine	6.06	1.23	0.69
Tyrosine	3.67	1.27	1.43

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; SBM = soybean meal. Sources: (1) Mandal and Banerjee, 1975. (2) Natanam, Kadirvel and Ravi, 1989. (3) Adapted from Kellem and Church, 1998.

and Viswanathan, 1989) ranges from 6 to 24 percent, while it is 22.0–28.7 in EKC and 30.0–34.0 percent in SKC. The crude fibre (CF) level varies from 3.9 to 5.6 percent. The variation in ether extract (EE) content is mainly due to method of oil extraction. Usually, cakes obtained by expeller extraction have a higher EE value (9–14.5 percent), while lower levels (0.1–2.2 percent) are found in the cake produced by solvent extraction. A summary of various reports by different workers regarding the chemical composition of various types of karanj cake are presented in Table 2.

Amino acid composition is very important in determining the protein quality of any cake. Values for 18 amino acids, including essential amino acids, have been reported by various workers (Mandal and Banerjee, 1975; Parmar, Sahrawat and Mukherjee, 1976). Mandal and Banerjee

TABLE 4
Mineral composition of Karanj cake compared with soybean meal

Mineral	Seed kernel	EKC	SKC	SBM
Macro minerals (as % in DM)				
Ca	0.51	0.76	0.87	0.38
P	0.38	0.48	0.55	0.71
Na	0.29	–	0.49	0.48
K	0.27	0.23	0.20	0.15
Mg	0.47	0.27	–	0.28
Trace minerals (ppm)				
Cu	1.37	1.96	1.97	20.0
Fe	3.28	14.32	17.81	150.0
Co	0.27	0.09	0.15	0.16
Mn	35.78	76.21	70.82	22.0
Pb	0.11	0.73	1.00	–

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; SBM = soybean meal. Sources: Natanam, Kadirvel and Ravi, 1989; Georgievskii, Annenkov and Samokhin, 1982.

(1975) observed that the amino acid composition of karanj cake was almost similar to that of sesame, groundnut cake (except for lysine, which was higher in karanj cake) and soybean meal (SBM) (except for methionine, which was lower in karanj cake). Further, karanj cake was found to be rich in cystine. Amino acid values reported by various workers are presented in Table 3. The mineral composition of karanj cake (Natanam, Kadirvel and Ravi, 1989) is presented in Table 4. In comparison with SBM, karanj cake has higher calcium, phosphorus and sodium; however, the concentrations of copper and iron are very low.

Toxic compounds in karanj cake

On the basis of chemical nature, the toxic compounds in karanj cake can be grouped into three categories, viz. furanoflavones, tannins and trypsin inhibitors.

Furanoflavones

The furanoflavones present in karanj seed include karanjin, pongamol, pongapin, pongaglabron, kanjone and isopo-

ngaflavone lanceolatin B (Limaye, 1925; Rangaswami and Seshadri, 1940; Roy, Sharma and Khanna, 1977). Among them, karanjin and pongamol are the most important toxic factors due to their potency. The bitter taste of karanj cake is attributed to the presence of these two compounds.

Karanjin

Karanjin ($C_{18}H_{12}O_4$; 3-Methoxyfurano-(2',3':7,8)-flavone) is the first flavonoid compound that was isolated, identified and characterized, and hence it is the earliest known furanoflavone and the most important physiologically active factor of *Pongamia* sp. Its concentration in karanj oil is approximately 1.25 percent. Limaye (1925, 1926) isolated karanjin from pongamia oil. Seshadri and Venkateshwarlu (1943) identified, characterized and established the structure of karanjin. Its melting point is 158–159 °C. In the seeds of *P. glabra*, pongamol was subsequently identified along with karanjin (Rangaswami and Seshadri, 1940). The karanjin content of the karanj oil has been reported to be 147 mg/100 ml and 10–15 mg/100 g in SKC (Punj, 1988). Similarly, karanjin content of EKC was found to be in the range of 0.19 to 0.324 percent and 0.01–0.132 percent in SKC (Prabhu *et al.*, 2002; Panda *et al.*, 2006; Soren, 2006). Vinay, Appu Rao and Sindhu Kanya (2006) reported karanjin content in the whole karanj seed to be 1.95 percent.

Of the 24 furanoflavonols isolated, karanjin has been studied extensively and found to be hypoglycaemic. Oral administration at a dose of 2 mg/kg per day for 7 days caused a reduction in blood glucose level both in normal and alloxan-induced diabetic rats (Mandal and Maity, 1987). It also showed antitubercular (suppressing growth of *Mycobacterium tuberculosis*; Ramaswamy and Sirsi, 1960), antifungal (Pan *et al.*, 1985), antibacterial, phytotoxic (Simin *et al.*, 2002), and central nervous system stimulant activities (Mahali *et al.*, 1989). Apart from these activities, karanjin is also a nitrification inhibitor (Majumdar, 2002), juvenomimetic (Mathur *et al.*, 1990) and synergist to insecticides (Sighamony, Naidu and Osmani, 1983). It was found to haemolyse red blood cells, with release of LDH (lactate dehydrogenase) (Gandhi and Cherian, 2000).

Pongamol

Pongamol, a crystalline compound from the oil of *P. glabra*, was identified by Rangaswami and Sheshadri (1942). Compared to karanjin, it is a minor component, far more soluble in oils. It has the molecular formula $C_{18}H_{14}O_4$ and contains a methoxyl group. Demethylation with aluminium chloride yields nor-pongamol, whereas treatment with HCl gives rise to a product which is probably isomeric but does not possess a phenolic group. Oxidation with $KMnO_4$ or decomposition with alkali yields benzoic acid. These properties and colour reaction suggest that pongamol is a flavone derivative (Rangaswami

and Sheshadri, 1942). Narayanaswamy, Rangaswami and Seshadri (1954) established the structure of pongamol as benzoyl-O-methyl karanjyl methane (S-benzolacetyl-4-methoxy benzofuran). It is the first example of a naturally occurring diketone related to flavones. Pongamol melts at 128 °C and has relatively less bitterness compared with karanjin. Among the flavanoid diketones, pongamol has been explored extensively and found to have sedative and depressant effects (Mahali *et al.*, 1989). It is commercially used in cosmetic and sun-screen preparations (Noriaki, Masamichi and Masanori, 2001).

Tannins

Apart from karanjin, the cake is also reported to contain tannins to the extent of 3.2–3.4 percent (Natanam, Kadirvel and Ravi, 1989). Tannins are a naturally occurring group of phenolic compounds with a molecular weight of 500–3000 daltons (Haslam, 1966). These have alkaloids, gelatins and protein precipitation properties. However, the total tannins and condensed tannins content of karanj cake was reported to be lower and protein-precipitation capacity was not detected by Makkar, Singh and Negi (1990), suggesting that these co-products could be safe for incorporation in livestock feed. The tannin content was found to be slightly higher in SKC than in the expeller cake (Panda *et al.*, 2006). The SKC contained 0.94 percent tannins, along with other antinutritional factors, such as phytate (0.65 percent) and trypsin inhibitors (31 units/mg) (Vinay and Kanya, 2008).

Protease inhibitors

Protease inhibitors are well known anti-nutrients that are responsible for lower digestibility of plant proteins. Protease inhibitors, namely trypsin and chymotrypsin inhibitors, are found in karanj oil seed residue (Rattansi and Dikshit, 1997). These are a group of anti-nutritional factors, protein in nature, with a molecular weight between 6000 and 25000 daltons, and are generally present in leguminous seeds (Birk, 1976; Liener, 1979). The expeller- and solvent-extracted cakes contain trypsin inhibitor up to 8.7 and 8.2 percent of protein, respectively (Natanam, Kadirvel and Ravi, 1989). The adverse effect of trypsin inhibitors is mainly on the pancreas, which responds to the inhibitors by enhanced synthesis and secretion of proteolytic enzymes. The pancreatic enzyme secretion is regulated by a negative feedback mechanism mediated by intestinal trypsin and chymotrypsin, and complex formation of trypsin with the inhibitors leading to a reduction of free trypsin in the small intestine (Alumot and Nistan, 1961). This reduction activates the pancreas-stimulating hormone, cholecystokinin, the release of which from the intestinal mucosa is inhibited by free trypsin (Wilson *et al.*, 1978). As a consequence of cholecystokinin action, the pancreas becomes hyperactive.

Use of karanj cake as ruminant feed

The karanj cake can be used as livestock feed as it contains a fairly good amount of crude protein. However, its use as protein supplement is limited due to the presence of toxic compounds. The detoxification processing of karanj cake significantly reduces the toxic effects and therefore its use as a livestock feed has been tested. The results of some of these experiments are summarized in this section.

Detrimental effects of feeding karanj cake in ruminants

Feeding of EKC has been reported to depress feed intake, cause histopathological changes in vital organs and produce toxicity symptoms. A concentrate mixture containing 4 percent EKC was found to be unpalatable to buffalo calves, and the animals developed symptoms like loss of appetite and weight, frequent and strong-coloured micturition, swelling in the intermaxillary spaces and facial muscles, discoloration of skin and loss of hair, watery to sticky lacrimation, and gangrene of tail, followed by its sloughing (Gupta *et al.*, 1981). Konwar and Banerjee (1987) found no harmful effect on red (RBC) and white blood cell (WBC) counts, packed cell volume (PCV) and haemoglobin, Fe, Ca and P content in growing calves, except for blood plasma protein concentration, which was significantly lowered at 75 percent of the level of de-oiled karanj cake (DKC) incorporation in the diet.

Detoxification of karanj cake

Karanjin and pongamol are soluble in oil and their levels vary in cake depending on the residual oil content in it. Both are insoluble in water but easily soluble in organic solvents like ethyl alcohol, methyl alcohol or benzene. Some karanjin is removed by water washing due to washing away of EE attached with other ingredients of the SKC, which is evident from the lowered EE of the water-washed SKC (Soren *et al.*, 2007). Dilute acid treatment causes change in the nature of oil due to hydrolysis of oil and production of fatty acids, which render karanjin and pongamol insoluble in it, and these compounds precipitate. Alkaline decomposition of karanjin yields four products: C-acetyl coumarone ($C_{11}H_{10}O_4$), karanjic acid ($C_9H_6O_4$), kanjol ($C_8H_6O_2$) and benzoic acid (Limaye, 1926); whereas alkaline decomposition of pongamol yields a single product, namely benzoic acid (Rangaswami and Sheshadri, 1942). All these intermediate decomposition products are said to be non-bio-active and non-toxic.

Various attempts have been made so far to detoxify the cake, initially without specifically targeting any of the particular toxins, and later targeting specific toxins, namely karanjin, tannin, trypsin inhibitors and phytates. Non-specific detoxification attempts include de-oiling (Konwar and Banerjee, 1987), oven drying (100 °C for 24 hours), autoclaving (242 kPa pressure, 30 minutes), cold water

extraction (1:3, w/v, 24 hours) (Natanam, Kadirvel and Ravi, 1989), hot water extraction (60 °C) and toasting (15 minutes) (Mandal and Banerjee, 1974). Prabhu *et al.* (2002) detoxified karanj cake by various physico-chemical methods, including solvent extraction, water washing, pressure cooking, alkali and acid treatments. They found that de-oiling of karanj cake was the best method of detoxification as it substantially reduced the karanjin content (from 0.19 down to 0.01). Panda *et al.* (2006) observed that pressure cooking (30 minutes), treatment with alkali (1.5 percent NaOH) and lime (3.0 percent) were effective in reducing the karanjin content in SKC. Similarly, various methods, namely water washing, water soaking, dry heat treatment, pressure cooking, urea ammoniation, alkali (calcium hydroxide, potassium hydroxide, sodium hydroxide and sodium bicarbonate) treatments, biological treatments (*Saccharomyces cerevisiae*, *Aspergillus oryzae*) and toxin binder (HSCAS) were tried to reduce karanjin content of cake by Soren *et al.* (2009). They reported that pressure cooking was found to be the most effective method, followed by sodium hydroxide treatment, for removing karanjin. However, none of the treatments removed karanjin completely from the cake.

Effect on rumen fermentation

In vitro studies involving incubation of karanj cake with rumen liquor for 48 hours resulted in disappearance of 92.3 percent of DM and 93.5 percent of organic matter (OM) (Chandrasekaran, Kadiravel and Viswanathan, 1989). However, in buffaloes, *in sacco* DM degradation was reported to be 49.5 percent and protein degradation 22.2 percent (Paul *et al.*, 1995). Decreased degradability of DM, OM and neutral-detergent fibre (NDF) was reported when SKC and EKC were incorporated in the concentrate mixture at a 20 percent level (Saha *et al.*, 2004a, b). The *in vitro* DM degradability due to inclusion of raw and treated SKC were comparable to control, although NDF degradability was significantly lower in the raw-SKC-containing concentrate mixture (Soren, 2006).

Effect on rumen fermentation

Ravi *et al.* (2001) reported a significantly higher pH in growing lambs fed EKC, whereas the pH in the SKC-fed group was comparable with the control. Though other nitrogen fractions were comparable, ammonia nitrogen was significantly lowered in karanj cake-fed groups (Table 5). Contrary to the above finding, the pH and concentration of NH_3 -N, total N and trichloroacetic acid ppt.-N (TCA) was found similar in sheep given isonitrogenous diets containing de-oiled groundnut cake (GNC), SKC and EKC (replacing 50 percent N of de-oiled groundnut cake (DGNC) in full) for 210 days of experimental feeding, while total volatile fatty acid (TVFA) concentration was lower in the karanj cake-fed group (CASAN, 1999–2000).

TABLE 5
Effect on ruminal metabolites in lambs of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake

Parameter	Groups		
	DGNC	EKC	SKC
pH	6.20 b	6.54 a	6.28 b
TVFA (mEq/dl)	7.86	7.24	8.04
Total-N (mg/dl)	135.0	115.0	127.5
TCA-ppt-N (mg/dl)	85.00	69.17	80.28
Ammonia-N (mg/dl)	18.05 a	14.35 b	14.50 b

Notes: TVFA = total volatile fatty acids; DGNC = de-oiled groundnut cake; TCA = trichloroacetic acid. Means with different letters in a row differ significantly ($P < 0.05$). Source: Ravi *et al.*, 2001.

Prabhu (2002) and Soren (2006) reported comparable pH and concentrations of $\text{NH}_3\text{-N}$, total volatile fatty acid (TVFA) and other nitrogen fractions in rumen liquor of lambs fed either soybean- or karanj cake-based isonitrogenous supplements replacing 50 percent N of DGNC with EKC and SKC. TVFA concentration was comparable except in binder-treated cake, in which it was lower than control. TCA-ppt.-N was significantly higher in the control group than in the test groups. Urinary excretion of purine derivatives and microbial protein synthesis did not differ significantly among diets. However, urinary excretion of creatinine was lower ($P < 0.05$) in lambs fed control and water-washed diet as compared with LM (lime treated) and BN (binder treated) treated SKC diets (Table 6) (Soren and Sastry, 2009). The higher excretion of creatinine in the LM and BN groups might be due to catabolism of body protein to meet energy needs and the residual karanj in the processed cake might have affected protein anabolism.

TABLE 6
Urinary excretion of purine derivatives (PD) and creatinine, absorption of PD and microbial nitrogen supply in lambs fed processed karanj cake

Variables	Diets			
	Control	WW	LM	BN
Urinary excretion of PD (mmol/day)				
Allantoin	3.88	3.44	3.70	2.87
Uric acid	1.29	1.67	1.25	1.08
Xanthine	0.38	0.20	0.38	0.43
Hypoxanthine	0.16	0.19	0.20	0.12
Total PD	5.73	5.51	5.54	4.51
Creatinine	1.53 b	1.48 b	2.94 a	2.46 a
PD absorption (mmol/day)	6.50	6.26	6.31	4.96
Microbial N synthesis (g N/day)	4.72	4.55	4.58	3.60
Efficiency of microbial N synthesis				
g N/kg DOMI	15.65	20.33	23.88	21.34
g N/kg DOMR	24.08	31.28	36.73	32.83

Notes: Control = soybean meal; WW = water-washed SKC; LM = 25 g/kg lime-treated SKC; BN = 4 g/kg binder-treated SKC; SKC = solvent-extracted karanj cake; DOMI = digestible organic matter intake; DOMR = digestible organic matter. Means with different letters in a row differ significantly ($P < 0.01$). Source: Soren and Sastry, 2009.

Palatability and voluntary feed intake

Karanj cake as sole feed is highly unpalatable. A palatability trial conducted with male buffalo calves given EKC-based supplement revealed that even at a 4 percent level it was quite unpalatable and led to development of toxic symptoms (Gupta *et al.*, 1981), although they found that de-oiling could completely remove the bitter taste and pungent smell of the karanj cake, and SKC could be included at up to 32 percent, replacing 80 percent of mustard cake without any adverse effect on DM intake (DMI). In general, growing animals are expected to be more sensitive than adults in accepting any unconventional feed. Complete replacement of de-oiled GNC with SKC significantly reduced feed intake, however. Konwar and Banerjee (1987) reported that absolute replacement of de-oiled GNC nitrogen with SKC did not affect DMI in growing cross-bred bulls. In another study involving growing calves, Konwar, Banerjee and Mandal (1984) found no adverse effect on feed intake when SKC was incorporated at up to 25 percent in the concentrate mixture. Srivastava *et al.* (1990) also found no difference in DMI of kids when de-oiled GNC nitrogen was replaced with de-oiled karanj cake at the 40 percent level.

Ravi (1999) reported significantly lower DMI in lambs given concentrate mixture containing EKC at 24 percent level, although comparable DMI was observed when they fed a concentrate mixture incorporating 20 percent SKC (Ravi *et al.*, 2000). The reduced intake on EKC-containing diets might be due to the presence of bitter and pungent compounds (Parmar, Sahrawat and Mukherjee, 1976) in the oily portion of the karanj cake, rendering it unpalatable. Prabhu (2002) also observed comparable DMI in growing lambs fed diets incorporating 16.5 percent raw and alkali-treated SKC in a 180-day feeding trial.

Nutrient digestibility

Only a few reports are available on the effect on the digestibility of nutrients of feeding EKC, which may be due to the fact that animals did not readily consume EKC because of its pungent and repulsive smell. Chandrasekaran, Kadiravel and Viswanathan (1989) determined the nutritive value of EKC in adult ewes and reported that it could be used in the concentrate mixture by replacing a maximum of 75 percent N of GNC as a temporary short-term feed source without any adverse effect. They further reported *in vitro* digestibility coefficients of 0.853, 0.797, 0.859, 0.308 and 0.756 for DM, OM, CP, CF and EE, respectively.

Konwar and Banerjee (1987) reported digestibility coefficient of 0.593, 0.622, 0.607, 0.580, 0.698 and 0.615 for DM, OM, CP, CF, EE and NFE, respectively, in growing calves fed rations containing 25 percent de-oiled karanj cake (DKC). They further reported no adverse effect on nutrients digestibility up to 25 percent DKC inclusion. Feeding of rations containing 0, 6, 9 or 12 percent DKC showed no

TABLE 7
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on nutrient utilization in lambs

Parameter	Diet group		
	DGNC	EKC	SKC
Body weight (kg) during trial period	13.4	12.8	13.6
Metabolic body weight (kg W ^{0.75})	7.0	6.7	7.0
Dry matter intake (g per day)	525.6	490.6	540.0
Daily DMI (g/kg W ^{0.75})	74.9	71.9	75.9
Digestibility (%)			
DM	59.1a	53.5 b	59.1 a
OM	61.9 a	56.2 b	61.6 a
CP	55.9 a	46.7 b	53.0 ab
NDF	51.5 a	44.3 b	49.4 ab
ADF	47.9 a	38.7 b	48.3 a
Daily intake of nutrients (g/kg W ^{0.75})			
TDN	42.6	37.3	43.0
DCP	5.37	4.26	5.0

Notes: DMI = Dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; TDN = total digestible nitrogen; DCP = digestible crude protein; DGNC = concentrate mixture containing de-oiled groundnut cake as major protein source; EKC = concentrate mixture replacing 50 percent of DGNC N by EKC; SKC = concentrate mixture replacing 50 percent of DGNC N by SKC; W^{0.75} = metabolic weight (size). Means with different letters in a row differ significantly ($P < 0.05$). Source: Ravi *et al.*, 2000.

significant difference with respect to digestibility coefficients of DM, CF and NFE amongst kids in different dietary groups (Srivastava *et al.*, 1990), although they found that the digestibility of CP and EE was significantly ($P < 0.05$) depressed by feeding 12 percent DKC.

Ravi *et al.* (2000) reported significant reduction in digestibility of various nutrients in lambs fed concentrate mixture containing 24 percent EKC, while SKC inclusion up to 20 percent did not adversely affect the digestibility of proximate principles and fibre fractions. Prabhu (2002) observed no adverse effect on the digestibility of various nutrients by feeding raw and processed SKC at the 16.5 percent level in the diet of growing lambs. Digestibility of fibre fractions (NDF, ADF, hemicellulose and cellulose) were also found to be comparable among the control and SKC-fed groups (Table 7). Similarly, no significant difference was observed between the digestibility of DM, OM and CP in the control feed (27 percent SBM in concentrate) and the processed SKC feed (22.5 percent in concentrate) (Soren and Sastry, 2009).

Nutrient balance

The balance of various nutrients is an important parameter when assessing the suitability of any feed in the diet of farm animals. Srivastava *et al.* (1990) reported a significant reduction in the retention of N and Ca with inclusion of more than 9 percent SKC in the concentrate mixture for growing kids, although no adverse effect was noticed with respect to the balance of P. Nutrient balance studies conducted with growing calves showed similar Ca and P retention when SKC was incorporated at up to 17 per-

TABLE 8
Effect on intake and balances of N, Ca and P in lambs of feeding processed karanj cake

Variables	Diets			
	Control	WW	LM	BN
N balance				
Daily N intake	12.24 a	9.86 b	8.59 bc	7.19 c
N retained	4.78 a	3.71 b	2.71 bc	1.87 c
Ca				
Daily Ca intake	3.60 a	3.16 ab	2.47 b	2.54 b
Ca retained	0.34 b	0.65 a	0.29 b	0.12 b
P				
Daily P intake	4.06 a	2.40 b	2.09 c	1.94 c
P retained	2.67 a	1.22 b	1.09 b	0.96 b

Notes: Control was a soybean meal diet; WW = water-washed SKC; LM = 25 g/kg lime treated SKC; BN = 4 g/kg binder-treated SKC; SKC = solvent-extracted karanj cake. Means with different letters in a row differ significantly ($P < 0.05$). Source: Soren and Sastry, 2009.

cent in the concentrate mixture, though at the 25 percent level SKC resulted in significantly lowered retentions (Konwar and Banerjee, 1987). Chandrasekaran, Kadiravel and Viswanathan (1989) reported positive N (1.6 g), Ca (0.7 g) and P (0.3 g) balances in ewes fed EKC. Lactating cows given a supplement replacing 25 percent protein of mustard oil meal with DKC protein showed no adverse effect on the balances of N, Ca and P (Dutta *et al.*, 1984).

In a short-term 98-day study with lambs, Ravi *et al.* (2000) observed no adverse effect on retention of N, Ca and P with inclusion of 20 percent SKC in the concentrate mixture, though inclusion of EKC at 24 percent adversely affected the retention of N and Ca. Similarly, Prabhu (2002) and Soren and Sastry (2009) observed no adverse effect on the balance of N, Ca and P in lambs fed concentrate mixtures replacing 50 percent of SBM of the control concentrate mixture with SKC and alkali-treated SKC for a period of 90–180 days (Table 8).

Growth and feed conversion efficiency

Studies (Srivastava *et al.*, 1990) with small ruminants, viz. lambs and kids, have shown diverse responses in growth rates due to karanj cake feeding as compared with conventional protein supplements. An average daily gain (ADG) of 35–38 g was obtained in kids when SKC was fed at up to 9 percent, while further incorporation up to 12 percent resulted in a significant reduction in gain (ADG of 22 g). Srivastava *et al.* (1990) further reported comparable feed conversion efficiency (FCE) of 10.1 when feeding levels of up to 6 percent in the diet; however, beyond a 6 percent level, inclusion of SKC lowered the FCE (15.9).

Gupta *et al.* (1981) reported a reduced growth rate (236 g/day vs 409 g/day) in growing calves fed diets replacing mustard cake protein with more than 40 percent SKC. Corresponding values of ADG and FCE were observed by

TABLE 9
Effect on body weight changes in lambs of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake

Parameter	Groups		
	DGNC	EKC	SKC
Initial body weight (kg)	11.2	9.9	10.7
Final body weight	17.1	14.7	16.5
Average daily gain (g)	60.5 a	48.8 b	59.6 a
Total DMI in 98 days (kg)	54.8	50.5	54.1
Feed conversion efficiency	9.2	10.6	9.3

Notes: DMI = dry matter intake; DGNC: concentrate mixture containing de-oiled groundnut cake as major protein source; EKC = concentrate mixture replacing 50% of DGNC-N by EKC; SKC = concentrate mixture replacing 50% of DGNC-N by SKC. Means with different letters in a row differ significantly ($P < 0.05$). Source: Ravi *et al.*, 2000.

TABLE 10
Effect of feeding processed karanj cake on body weight changes, intake and digestibility of nutrients in lambs

Variables	Diets			
	Control	WW	LM	BN
Initial body weight (kg)	12.6	12.9	12.8	12.9
Final body weight (kg)	23.7 a	22.4 a	17.5 b	17.0 b
Body weight change	10.7 a	9.5 a	4.7 b	4.1 b
Dry matter intake (g/day)	536 a	402 b	357 b	332 b
Apparent digestibility				
Dry matter	0.54	0.59	0.53	0.54
Organic matter	0.58	0.61	0.56	0.57
Crude protein	0.58	0.58	0.55	0.53

Notes: Control was a soybean meal concentrate; WW = water-washed SKC; LM = 25 g/kg lime-treated SKC; BN = 4 g/kg binder-treated SKC; SKC = solvent-extracted karanj cake. Means with different letters in a row differ significantly ($P < 0.01$). Source: Soren *et al.*, 2009.

Ravi *et al.* (2000) in growing lambs when SKC was fed at up to 20 percent in concentrate mixture replacing 50 percent de-oiled GNC; however, EKC inclusion at 24 percent in the same experiment resulted in lowered ADG without any effect on FCE (Table 9).

Prabhu (2002) observed similar ADG and FCE in lambs fed SBM and raw or processed SKC containing diets up to 16.5 percent level. Similar growth was reported by Soren *et al.* (2009) in lambs fed either a SBM control or water-washed SKC-based diets, although growth was significantly reduced in lime- and toxin-binder (HSCAS)-treated SKC-based diets (Table 10).

Blood biochemistry

Blood parameters are important indicators to assess the wholesomeness and suitability of any unconventional feed for the presence of negative factors. Short-term or longer duration feeding may have immediate effects on the blood picture. Assessment of serum enzymes gives a clear status of different organs. Thus greater transaminases is normally observed due to liberation of enzymes into the circulating blood stream because of cell destruction (La Due, Wroblewski and Karmen, 1954).

TABLE 11
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on blood bio-chemicals in lambs

Parameter	Diet groups		
	DGNC	EKC	SKC
Glucose (ml/dl)	37.50	39.75	44.05
Urea-N (ml/dl)	12.61 a	16.06 b	14.20 ab
AST (IU/L)	66.34	70.02	65.87
ALT (IU/L)	17.72	20.29	18.00
SDH (IU/L)	16.38	17.34	15.69

Notes: DGNC = concentrate mixture containing de-oiled groundnut cake as the major protein source; EKC = concentrate mixture replacing 50% of DGNC N by EKC; SKC = concentrate mixture replacing 50% of DGNC N by SKC; AST = aspartate aminotransferase; ALT = alanine aminotransferase; SDH = sorbitol dehydrogenase. Means with different letters in a row differ significantly ($P < 0.05$). Source: Ravi *et al.*, 2000.

Incorporation of 16.6 and 25 percent de-oiled karanj cake to substitute 50 and 75 percent de-oiled GNC nitrogen had no adverse effect on RBC and WBC counts, packed cell volume (PCV), haemoglobin, iron, calcium and phosphorus concentration of blood in growing calves, however, blood plasma protein content was significantly reduced at 75 percent level of de-oiled karanj cake incorporation (Konwar and Banerjee, 1987). Similarly, Ravi *et al.* (2001) observed no adverse effect on serum glucose and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) in lambs fed SKC, though level of serum urea was significantly increased when EKC was incorporated up to 24 percent in the diets of lambs (Table 11).

Prabhu (2002) also found no adverse effect on blood glucose, total protein, globulin, albumin, urea nitrogen and creatinine in lambs fed either SKC or alkali-processed SKC. They further reported statistically similar antibody titre among lambs sensitized with sheep pox virus on various diets, though antibody titres in general were lower in lambs fed raw SKC. Similarly, Soren (2006) observed comparable haemoglobin, serum glucose, calcium, albumin, globulin, A:G ratio, blood urea nitrogen and activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase in lambs fed either SBM based or the processed SKC diets, while LDH activity was increased significantly in groups fed SKC compared with the control. Singh *et al.* (2006) reported lower ($P < 0.05$) levels of SGOT, LDH and acetylcholine esterase in sheep fed EKC and SKC diets versus sheep fed a DGNC (control) diet.

Carcass characteristics

The results of a study in which the animals were slaughtered after 280 days of experimental feeding with karanj cake (CASAN, 1999–2000) revealed that the fasted live weight and weight of hot carcass was significantly ($P < 0.05$) reduced in sheep fed EKC based diet compared with diets containing de-oiled GNC and SKC. The comparable weights

TABLE 12
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on carcass characteristics in various groups

Parameter	Treatment group		
	DGNC	EKC	SKC
Live body weight (after 12 h fasting) (kg) **	19.5 b	13.5 a	16.2 ab
Dressed hot carcass weight (kg) *	7.74 b	5.67 a	6.52 ab
Weight of skin (kg)	2.35	1.65	1.82
Weight of head (kg)	1.64	1.22	1.37
Weight of gastro-intestinal tract (kg) **	6.12	3.77 a	5.41 ab
Weight of hooves (g)	495	412	462
Weight of vital organs (g)			
Liver **	270 a	375 b	309 ab
kidneys *	68 a	85 a	80 ab
Heart	80	87	75
Testes **	170 b	87 a	91a
Spleen	28	25	27
Lungs and trachea	275	287	294
Weight of various cuts (g)			
Loin *	910 b	600 a	650 a
Breast and shank *	1620 b	1325 a	1512 ab
Neck *	566 b	337.5 a	387 a
Nack *	1175 b	925 a	1019 ab
Thigh (hind legs) *	2820 b	1825 a	2415 ab

Notes: DGNC = de-oiled groundnut cake. a,b = Means with same suffixes in a row differ significantly. * = $P < 0.05$, ** = $P < 0.01$.
Source: Singh *et al.*, 2006.

of various meat cuts between de-oiled GNC and SKC diets were significantly ($P < 0.05$) heavier than in sheep on an EKC diet. The organoleptic evaluation of pressure-cooked meat (with 1.5 percent salt) revealed no untoward taste due to karanj cake feeding.

Prabhu (2002) observed that dietary variation did not affect the physical and chemical characteristics of the carcass in lambs fed raw or alkali-processed SKC replacing 25 and 50 percent nitrogen of a SBM-based diet. Contrary to earlier studies, Soren (2006) reported significantly higher carcass weight in lambs fed SBM or water-washed SKC diets compared with lime- and binder-treated SKC-based diets, although the yields of bone and meat, the meat:bone ratio and the yields of wholesale cuts in the control and test diets did not differ significantly.

Singh *et al.* (2006) reported that the fasted live weight and weight of hot carcass ($P < 0.05$) was significantly lower in sheep fed a diet containing EKC compared with weights in sheep receiving DGNC and SKC diets. However, weight of skin, head and hooves did not differ significantly among the three groups (Table 12). The weights of various primal cuts such as loin, breast and shank, neck, rack and thigh were significantly higher ($P < 0.05$) in sheep fed DGNC and EKC diets.

Gross pathology and histopathological changes

After feeding karanj cake-based diets for 280 days (CASAN, 1999–2000), no gross pathological lesions were found in the vital organs of sheep. However, karanj cake feeding interfered with normal spermatogenesis, due to testicular degeneration. Spleens also showed moderate haemosi-

derosis. Similarly, Prabhu (2002) observed no gross or histopathological lesions in the tissues of vital organs in lambs fed for 180 days on diets containing SKC replacing the nitrogen moiety of SBM at either 25 or 50 percent in the concentrate mixture. In the same study, lambs fed 8.25 percent SKC and 16.50 percent processed SKC showed loss of striations, and thin and increased intermyofibrillar space due to oedema.

Long-term feeding of karanj cake (EKC, 24 percent) resulted in abnormalities of long bones and lameness. The radiographic examination of radius, carpal and metacarpal bones of lambs revealed thinner and less dense cortices, widened medullary cavity of the radius and presence of swollen soft tissues around carpal joints, similar to osteoporosis (Sastry *et al.*, 2000). No gross histopathological lesions could be noticed in the vital organs of the lambs fed an SBM-based control diet. Histopathological lesions were found in testes, epididymis, parathyroid, liver and small intestine of some lambs fed processed SKC-based diets (Soren, 2006), who also observed reduced length of long bones (metacarpals and metatarsals), reduced radiographic density, thinner cortices and increased diameter of the medullary cavity with binder (HSCAS)-treated SKC diets, and thicker cortices and narrowing of the medullary cavity in lime-treated SKC-fed lambs, all suggestive of poor mineralization of the bones, while mineralization of the long bones was normal in the control group and the group fed water-washed SKC.

On the basis of the above results, it can be inferred that long-term feeding of expeller-pressed, and to some

extent also solvent-extracted, karanj cake had deleterious effects on nutrient utilization, blood biochemical profile, rumen fermentation pattern and carcass characteristics, with clinico-pathological changes in bones and vital organ tissues. Water-washing and de-oiling of karanj cake may be the most feasible detoxification methods. Water-washed karanj cake may be incorporated at up to 22.5 percent in concentrate mixtures by replacing 50 percent of the nitrogen moiety of a conventional protein supplement like SBM, without any adverse effect on nutrient metabolism, growth and health of lambs.

Karanj cake as poultry feed

Detoxification

As discussed above, various techniques have been used for detoxification of karanj cake for the feeding of ruminants. Some of these techniques could also be applicable in the case of poultry, but the levels of incorporation in the diet will have to be standardized for each of the animal categories. In addition to the detoxification studies of Natanam, Kadirvel and Ravi (1989), Prabhu *et al.* (2002) and Panda *et al.* (2006) tried 36 possible detoxification methods for karanj cake. Based on reduction in karanjin, tannin and trypsin inhibitor activities, two methods of detoxification of SKC (1.5 percent NaOH or 3 percent Ca(OH)₂, w/w) and one for EKC (2 percent NaOH, w/w) have been recommended.

Palatability

Karanj cake as such is unpalatable to poultry as a sole feed. Substitution of 25 percent black til cake (black-seeded *Sesamum indicum*, commonly used as a protein source) with raw EKC in chick starter ration drastically reduced the feed intake with 50 percent mortality at 4 weeks old. However, incorporation of SKC to replace 25 percent black til cake on an isonitrogenous basis did not affect the quantity of feed consumed, with no mortality, and the feed conversion ratio (FCR) was found to be equivalent to the control group (Mandal and Banerjee, 1974). Inclusion of 5 percent raw karanj kernels in diets of male White Leghorn chicks up to 4 weeks of age reduced feed consumption by about 50 percent (Natanam, Kadirvel and Ravi, 1989), while the inclusion of either EKC or SKC at the 10 percent level did not result in any significant variation from control diet with respect to feed intake by White Leghorn pullets (Natanam, Kadirvel and Vishwanathan, 1989). In another study involving quail chicks, substitution of 20 percent red til cake (red-seeded *Sesamum indicum*) with de-oiled karanj cake up to 4.45 percent of the ration had no adverse effect on feed intake (Dhara *et al.*, 1997), but increasing the level of DKC beyond this resulted in poor growth and reduced feed consumption. The results obtained from different laboratories working on karanj cake co-products indicate that EKC or SKC had no adverse

effect, while the raw products affected intake and nutrient utilization adversely, confirming that the anti-nutritional factors in karanj are either fats or fat-soluble.

Growth and feed efficiency

Twenty-five per cent substitution of til cake with SKC on an equi-protein basis did not result in any significant difference with respect to average daily gain (212 g vs 206 g) in broiler chicks up to 4 weeks old (Mandal and Banerjee, 1974). Mandal and Banerjee (1982a) also reported that de-oiled karanj cake could replace black til cake up to 30 percent in the diet of pullets from 9–18 weeks of age without affecting growth rate and feed conversion efficiency. The inclusion of either EKC or SKC at 10 percent level in the diets of White Leghorn pullets did not show any significant variation with respect to body weight gain and feed intake compared with those fed a control diet. However, birds fed on EKC had maturity delayed by 14–17 days (Natanam, Kadirvel and Vishwanathan, 1989). In contrast, Natanam and Kadirvel (1990) reported significantly reduced body weight gain with incorporation of EKC in the diet of White Leghorn pullets at a 10 percent level (18–22 weeks) compared with the control. The broiler chicks on diets incorporating 1 percent karanj oil and 10 percent expeller-pressed karanj cake had a growth depression of 51 percent, while those fed 2 percent oil and 20 percent cake showed 82 percent depression, compared with those receiving the basal diet. At the same time, chicks fed on a diet containing 40 percent cake (EE = 14.4 percent) suffered 100 percent mortality (Natanam, Kadirvel and Ravi, 1989). The increase in mortality as the cake level increased from 10 to 40 percent could be due to the corresponding increase in karanj oil content going from 1.4 to 5.6 percent in different groups. Such an adverse effect could be attributed to the presence of toxic factors such as karanjin and pongamol in the oil or oil fraction of the cake. Inclusion of raw karanj kernels at 5 percent level in the diets of broiler chicks to 4 weeks of age depressed growth rate by 50 percent, and processing of kernels (autoclaving/water washing) did not improve their performance (Natanam, Kadirvel and Ravi, 1989; Natanam, Kadirvel and Chandrasekaran, 1989a). Similarly, inclusion of de-oiled karanj meal at a 5 percent level in the diet of male chicks up to six weeks of age did not support good growth (Chaudhury *et al.*, 1991). In an experiment with unsexed Japanese quail from 14 to 42 days old, Dhara *et al.* (1997) reported that de-oiled karanj cake could safely be included in the diet to a maximum level of 4.45 percent of feed replacing 20 percent red til cake protein without affecting growth, but a further increase (7 to 22.5 percent) adversely affected their daily gain. Dietary incorporation of alkali-treated SKC at 6.43 percent did not have any adverse effect on body weight gain or feed conversion efficiency during 0–4 weeks old. However, there

was growth retardation subsequently and the body weight gain during 0–6 weeks old age was significantly ($P < 0.05$) lowered in birds fed a diet incorporating alkali-processed SKC at 6.43 percent. Supplementation of methionine to the diet with alkali-processed SKC at 6.43 percent was found to be beneficial in alleviating the growth depression (Panda, Sastry and Mandal, 2005).

Egg production

Inclusion of 10 percent SKC in the diet of layers did not affect egg weight and egg production, whereas a 15 percent inclusion had an adverse effect, although egg quality was not affected by the incorporation of SKC at either a 10 or 15 percent level (Mandal and Banerjee, 1981). Verma, Gupta and Srivastva (1984) reported poor egg production and feed consumption in addition to leg weakness in birds given 10 percent SKC in the diet. In a 22-week trial with White Leghorn pullets from 18-week old age, Natanam, Kadirvel and Vishwanathan (1989c) found that inclusion of either expeller-pressed or solvent-extracted karanj cake in the diet at a 10 percent level did not result in any significant variation from the pullets fed the control diet with respect to weight of first egg, but birds fed on expeller-pressed cake had poor hen-day production and depressed feed efficiency. Egg production was significantly lowered in groups receiving karanj cake diets (at 31.2–43.2 percent) compared with those fed the control diet. The reduction in egg production and poor feed conversion efficiency observed in birds fed karanj cake diets was due to poor protein quality or presence of toxic flavonoid compounds, or both (Parmar, Sahrawat, and Mukherjee, 1976; Natanam, Kadirvel and Chandrasekaran, 1989).

Blood biochemical profile

Incorporation of SKC up to 6 percent level in cockrel rations had no adverse effect on the erythrocyte sedimentation rate, PCV, Fe or haemoglobin content of blood (Mandal and Banerjee, 1982a). The feeding of karanj cake at 10 percent level in the diet of broiler chicks significantly ($P < 0.05$) lowered the haemoglobin (7.75 vs 4.50 g/100 ml) and PCV (26.7 vs 18.2 percent) compared with the control. However, Natanam and Kadirvel (1990) found no adverse effect on haemoglobin level and PCV by dietary incorporation of 10 percent karanj cake fed to 18-week old White Leghorn pullets. It was attributed to the age of the birds, as adult birds are more tolerant than younger ones.

Liver is the primary site of detoxification and any material suspected of toxicity is frequently tested with reference to its potential to cause liver damage. Due to higher tissue turn over and synthesis of dispensable amino acids during stages of early life, the active level of serum transaminases (AST and ALT) is expected to be relatively high (Lehninger, 1984), but elevated levels are suggestive of damage to vital

organs (Oser, 1971). Mandal and Banerjee (1982b) found no adverse effect on SGOT activity in layers fed rations containing 6 percent SKC. These workers reported that incorporation of the extracted karanj cake replacing 30 percent N of black til cake is not detrimental to bird liver function. Samanta and Sasmal (1986) studied the effect of different solvent extracts of karanj seeds (neutral and acid fractions of petroleum ether extract, benzene extract, chloroform extract, phenolic and non-phenolic extracts of alcohol and water soluble part of alcoholic extract) in chicks. They observed that only the neutral fraction of petroleum ether extract significantly increased AST activity, while the other extracts had no adverse effect on its activity.

Carcass characteristics

There was no difference in organ weights (liver, heart, kidney and spleen) of cockerels due to dietary replacement of black til cake with de-oiled karanj cake at a 30 percent level (Mandal and Banerjee, 1982a). Similarly, Dhara *et al.* (1997) found no variation in weight of different organs (giblet, liver, heart and gizzard) and commercial cuts (neck, wing, thigh, shank, breast and trunk) due to incorporation of de-oiled karanj cake up to 22.4 percent in the diet of Japanese quail. However, dietary inclusion of 1 percent oil and 10 percent karanj cake in the diet of broiler chicks significantly increased the weight of liver and pancreas (Natanam, Kadirvel and Ravi, 1989), and the adverse effect of diet on organ weights relative to the body weight was attributed to the growth depression. None of the slaughtered and dressing characteristics differed significantly due to incorporation of SKC at 6.43 percent in the diet of broiler chickens (Panda *et al.*, 2006). No untoward and abnormal qualities with regard to appearance, odour, taste, texture, tenderness, juiciness and overall acceptability were found in meat of broilers fed processed karanj seed cake (Panda *et al.*, 2007).

Harmful effects on health

The neutral fraction of the petroleum ether extract of the karanj seed exhibited high toxicity, with 100 percent mortality when fed to Rhode Island Red chicks (Samanta and Sasmal, 1986). Grossly, the heart was dilated with accumulation of fluid in the pericardium, and the liver and kidney showed red infarction. On histological examination, the myocardium, especially the peripheral zone, showed moderate degenerative fatty changes. Liver sections showed dilation and congestion of central veins. Kidneys exhibited hypercellularity, atrophy, mesangial cell proliferation, capsular epithelial cell crescent formation and tubular degenerative changes with coagulative necrosis. These workers attributed the harmful effects of feeding karanj to the toxic compounds present in the neutral part of petroleum ether extract. Verma (1988) reported

histopathological changes in the liver at a higher level of inclusion (16.8 percent w/w) of SKC. Mild degenerative changes were noticed in the form of cloudy swelling in the liver of chicks fed a diet containing a mixture of agro-industrial co-products having 63 percent SKC replacing 29 percent of a standard diet on an isonitrogenous basis (Haque *et al.*, 1996). The liver and pancreas of chicks receiving 2 percent karanj oil and 20 percent EKC in the diet showed necrosis, fatty changes and disrupted structures (Natanam, Kadirvel and Ravi, 1989). Pathological studies showed no remarkable gross changes in vital organs at lower levels of inclusion (i.e. at 20 percent replacement of red til cake) of SKC (4.45 kg in 100 kg of feed) in the diet of Japanese quail, but higher levels of inclusion induced minor pathological changes in liver, heart, kidney and lungs (Dhara *et al.*, 1997). Dietary incorporation of either processed or unprocessed karanj cake beyond a 25 percent replacement (6.43 percent in diet) level, except for NaOH-treated SKC (12.86 percent in diet), resulted in histopathological abnormalities and the severity increased with increase in the level of replacement (Panda *et al.*, 2008). The severity of lesions was comparatively higher in the group fed a diet incorporating 25.72 percent NaOH (2 percent)-treated EKC. Livers showed hepatic degeneration, with distortion; kidneys showed tubular degeneration with necrotic lesions; spleen cells showed degeneration with necrotic foci and depletion of lymphocytes; and testes had degenerative changes of testicular follicles and vaculation. Feeding of SKC after treatment with either NaOH or Ca(OH)₂ was found to be beneficial instead of feeding SKC as such, since untrated SKC induced more severe histopathological lesions in the vital organs of broiler chickens. Treating SKC with 1.5 percent NaOH effectively minimized the toxic effects of karanj.

The results from different laboratories confirm that EKC as such is unsuitable for poultry feeding. However, after detoxification with alkali (2 percent NaOH, w/w) it can be incorporated, but only at a low level (3.24 percent in diet), replacing 6.25 percent of the N moiety of SBM for broiler diets without adversely affecting performance. However, SKC can be incorporated after alkali (1.5 percent NaOH, w/w) processing at an enhanced level of 6.43 percent in the diet, replacing 12.5 percent of SBM N, in broiler diets up to 4-weeks old, beyond which the observed growth depression on this diet could be alleviated by 0.2 percent methionine supplementation. Such a diet, by partially substituting for the costly and scarce conventional oil cake, can support optimum nutritional performance in broiler chickens. However, further research should be focused on developing improved methods for detoxification to reduce the bitterness and toxic factors in karanj cake, permitting its inclusion at a higher level, making poultry production more economic.

NEEM SEED CAKE

Neem oil and other products of the neem tree are used traditionally for making cosmetics (soaps, mild detergents, creams, teeth cleansers) and traditional Indian medicines (for skin infections, inflammations, fever, leprosy, malaria, tuberculosis, worm infestation, eczema, etc.), in addition to being a source of anti-bacterial and anti-fungal agents in bio-manure and plant protection. In 1995, the European Patent Office granted a patent on neem as an anti-fungal agent to the United States Department of Agriculture and multinational company W.R. Grace, to which the Government of India objected, as neem has been used as an anti-microbial agent for more than 2000 years. This was decided in favour of India in 2000, but when the multinational mounted an appeal, it took five more years before dismissal of the appeal, in March 2005.

Distribution of the neem tree

Neem or margosa (*Azadirachta indica*; syn. *Melia azadirachta* Linn.) is a fast growing evergreen perennial tree with a height up to 20 m, and belongs to the family Maliaceae. It is found widely in semi-arid to sub-humid areas of the tropics, but it can thrive well even in warm, dry arid regions having rainfall less than 500 mm annually. Though neem is native to India, it has spread to Pakistan, Bangladesh, Sri Lanka, Malaysia, Indonesia, Thailand and the Near East. In Africa, it was introduced by Indian settlers and is abundant in the whole tropical belt from East to West Africa. Neem is also reported to occur in the West Indies Islands and some countries of Central and South America (Anon., 1948). Neem can grow in a wide range of climatic conditions. Such a wide adaptation and tolerance to varied soil and climatic conditions confirms its high degree of heterozygosity and potential scope for increasing production through selection, if the nutritional worth of its co-products are proved and found safe for feeding. India has about 25 million neem trees, with an average annual production potential of 900 000 tonne of neem seed cake (NSC) as a residue after oil extraction (Singh, 1993).

Bitter and toxic neem compounds

Neem seed kernel cake, a protein rich (35–40 percent CP) agro-industrial co-product hitherto utilized as fertilizer-cum-pesticide, was found unsuitable for animal feeding due to presence of bitter and toxic triterpenoids (azadirachtin, salanin, nimbin, nimbidiol, etc.). The bitterness of neem is attributed to limonoids, which are the triterpenoids. The pioneer work of Siddiqui (1942) revealed that the bitter principles (1.2 percent of dry matter) comprised both water- and fat-soluble fractions. The main feature of these compounds is that they are mostly tri- or tetraterpenoids. The structure and chemistry of these compounds has recently been reviewed by Devakumar and Dev (1993),

TABLE 13
Chemical composition of various type neem cakes (percentage of DM basis)

Type of cake	Crude protein	Ether extract	Crude fibre	NFE	Total ash	Ca	P	Source
Neem seed cake (NSC)	12.4–19.6	1.8–3.3	17.9	52.5–64.3	13.9–14.3	1.5	0.4	Bedi, Vijan and Ranjihhan, 1975a; Nath, Vijjan and Ranjhan, 1978.
Deoiled NSC	17.9–18.4	0.4–3.6	25.9–30.1	35.0–46.2	5.5–16.2	0.7–1.0	0.2–0.6	Christopher, Ahmed and Sastry, 1976; Garg, 1989.
Neem seed kernel cake (NSKC)	33.5–40.8	7.9–10.4	11.4–23.0	19.6–26.6	12.3–15.0	–	–	Rajgopal and Nath, 1981; Nath, Rajagopal and Garg, 1983; Reddy, 1992.

Notes: NFE = nitrogen-free extract.

according to whom these can be classified into several groups: protomeliacins; limonoids with modified side chain (e.g. γ -hydroxybutenolides, azadirone and its derivatives); vilasinin-type compounds; and those belonging to 3c-seco-meliacins, namely nimbin, solanin and azadirachtin. The chemical structure of these compounds indicates the presence of polar and non-polar groups, a property that has been exploited in extraction of these compounds.

Chemical composition

The chemical composition of neem seed cake (NSC) and neem seed kernel cake (NSKC) varies greatly and depends on many factors. Crude protein and crude fibre contents of cake are inversely co-related and largely depend upon the type of seeds and method of oil extraction. When decorticated kernels are processed for oil, the cake obtained has high crude protein and low crude fibre, while the undecorticated cake is low in crude protein and high in crude fibre. Cakes obtained from partially de-pulped and decorticated seeds are intermediate depending upon the degree of de-pulping and/or decortication of the seeds (Table 13). The mineral composition of NSC, as well as of leaves, fruit and seed (Singhal and Mudgal, 1984) are summarized in Table 14, together with the amino acid composition reported by Singhal and Mudgal (1983) and Tewari (1992).

Feeding of neem seed cake to ruminants

Initially, Christopher (1970) showed the possibility of using of NSC as a protein source in cattle feed. Later, several feeding studies were conducted in the country to determine its palatability, nutritive value and possible use as animal feed. Studies with calves (Rao and Nath, 1979), buffalo bulls (Bedi, Vijan and Ranjihhan, 1975a), cross-bred bulls (Ananthasubramanian, Menacherry and Devasia, 1979) and sheep (Gupta and Bhaid, 1980) showed that NSC as such was unpalatable, although the water extracts of neem seed cake showed no adverse effect on the hydrolytic enzymes of the rumen (Agarwal *et al.*, 1991) when tested *in vitro*. Most of the later studies concentrated on improving the palatability of NSC by feeding it together it with highly palatable ingredients such as starch, molasses, maize or jaggery [crude sugar from palm sap] (Christopher, 1970).

TABLE 14
Mineral composition and amino acid profile of neem seed cake

Amino acid	Amino Acid Profile		Mineral Composition	
	g/16 g N		Mineral	Content
Aspartic	7.31–8.19		Ca %	0.96
Threonine	1.88–3.13		P %	0.30
Serine	2.88–3.63		Mg %	0.44
Glutamic	15.00–15.13		Na %	0.40
Proline	5.25		K %	0.98
Glycine	2.44–6.75		Cu, ppm	19
Alanine	2.88		Zn, ppm	19
Cystine	2.13–10.81		Fe, ppm	2705
Valine	3.00–4.75		Co, ppm	1.5
Methionine	0.88–4.38		Mn, ppm	70
Isoleucine	2.06–3.75		Cr, ppm	1
Tyrosine	1.63		Pb, ppm	10.5
Phenylalanine	3.88–5.00		Cd, ppm	–
Histidine	1.00–1.31			
Lysine	1.75			
Arginine	3.56–4.56			

Sources: Adapted from Singhal and Mudgal, 1983, 1984, and Tewari, 1992.

Urea-ammoniated NSKC was found to be quite palatable to buffalo calves (Reddy, 1992) and kids (Anandan, 1994).

Effect of neem seed cake on performance of ruminants

Neem seed cake, when fed as such, besides being unpalatable, is harmful to animals as it adversely affects growth, the male reproductive system, and has at times led to haematuria (Nath, Vijan and Ranjihhan, 1978; Rao and Nath, 1979). Various attempts have since been made to detoxify the cake, making it suitable for feeding ruminants with optimum growth and better nutrient utilization.

Bedi, Vijan and Ranjihhan (1975a) observed poor palatability, depressed growth rate and reduced nutrient digestibility (DM, CP, CF and NFE) in cross-bred calves fed concentrate mixtures containing 25 and 57 percent NSC. When NSC was substituted at rates of 25 and 50 percent digestible crude protein (DCP) for GNC in concentrate mixtures, loss of body weight with poor palatability was noted in buffalo calves, and there was significantly depressed nutrient digestibility, especially at the higher level of incorporation (Bedi, Vijan and Ranjihhan, 1975b), indicating that

untreated NSC was not suitable even for maintenance of animals. Adverse effects on protein utilization were also recorded in buffalo calves (Arora, Singhal and Ludri, 1975) when fed concentrate mixtures containing 50 or 27 percent NSC. The neem derivative nimbin did not adversely affect microbial protein synthesis in buffalo calves fed on rations containing 20 percent NSC, although nutrient intake and growth were significantly reduced (Ludri and Arora, 1977). Impaired protein metabolism, as indicated by presence of albumin and bile salts in the urine, was recorded in cattle receiving 10 and 20 percent NSC in the concentrate mixture (Anon., 1977-78).

Pyne, Moitra and Gangopadhyar (1979) noted no change in milk composition and general health of lactating buffaloes fed on 10, 15 or 20 percent NSC-supplemented concentrate mixture for a period of 60 days. However, RBC, WBC and haemoglobin levels were higher and serum protein was lower in experimental animals than controls. In view of the unaltered serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase activities and blood calcium and phosphorus levels, Gangopadhyar *et al.* (1981) suggested it was safe to incorporate NSC at up to 20 percent of the concentrate mixture for lactating buffaloes. Later, NSKC, a neem seed by-product rich in protein but low in crude fibre content in comparison with NSC, was tried by Rajagopal and Nath (1981). They observed significant ($P < 0.01$) depression in growth rate without affecting nutrient digestibility and DMI in cross-bred bull calves fed a ration containing 45 percent NSKC, showing that untreated NSKC is toxic to the animals. Even after solvent extraction, when de-oiled NSC was incorporated at 45 percent level in the concentrate mixture for cross-bred cow calves, it resulted in pronounced growth depression, higher DMI to compensate for 17 percent lower intake, along with reduced blood haemoglobin content, higher serum glutamate pyruvate transaminase (SGPT) and similar serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase and cholesterol when compared with calves on GNC-based control ration (Garg, 1989), and hence was considered unsuitable for animal feeding.

Soaking NSC in 1 percent NaOH overnight and then washing with water reduces the content of bitter principles and alkaloids (Anon., 1977-78). Nath, Vijjan and Ranjhan (1978) also reported that boiling of NSC with NaOH (8 g/kg cake) in 2.5 litre of water for 30 minutes, followed by water washing, resulted in a product palatable to cattle. However, feeding a concentrate mixture containing 50 percent NaOH-treated and water-washed NSC significantly depressed the DM digestibility and growth rate due to lower availability of energy, though the DMI was comparable to the control at the end of 50 days of feeding. But alkali treatment followed by water washing also has limitations, as along with the toxic compounds it removes part of CP,

most of the soluble sugars and sulphur. In contrast, Vijjan, Rao and Nath (1978) noticed no ill effects from such alkali-treated NSC on creatinine excretion in urine, but rather serum icteric index, blood inorganic phosphorus and serum alkaline phosphatase activity were unaffected in cross-bred calves. Calves fed 45 percent alkali-treated NSC (ATNSKC) in their ration showed similar DM intake, nutrient digestibility and calcium, phosphorus and sulphur balances, but significantly ($P < 0.05$) reduced growth rate (Rao and Nath, 1979). There was no difference in respect of serum icteric index, inorganic phosphorus and alkaline phosphatase activity between the groups fed control and experimental diets, but significantly reduced haemoglobin content in ATNSKC-fed calves, which suggests that treating the cake with lower levels of alkali followed by water washing was only partially effective in removing toxic compounds, even though the calves did not exhibit any palatability problem over the ATNSKC incorporation. Later, water washing of NSKC as an alternative to alkali treatment was tried by Nath, Rajagopal and Garg (1983). Such water-washed NSKC (WWNSKC), after sun-drying and grinding, when incorporated in concentrate mixture and fed to male cattle calves for a period of 273 days at a level of 45 percent showed statistically insignificant reduced growth rate in the group fed the experimental diet, but DMI, digestibility of nutrients, balance of calcium, phosphorus and nitrogen, and TDN intake were comparable to control. The blood haemoglobin content, serum acid phosphatase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase did not differ significantly, implying the removal of toxic bitter compounds to a greater extent by water washing. In another study, WWNSKC was incorporated in the concentrate mixture of buffalo calves at a 40 percent level in a feeding trial that continued for 179 days and involved a balance trial. It resulted in significantly ($P < 0.05$) lower digestibility of DM and total carbohydrates, but higher ($P < 0.05$) nitrogen balance in the experimental group. However, faster ($P < 0.05$) growth rate was observed in animals receiving WWNSKC compared with those on the control diet (Table 15).

A full lactation experiment (300 days) involving a balance trial on cross-bred milch cows (32) divided into two groups was carried out. The control group was given a concentrate mixture consisting of 40 percent GNC, 30 percent maize, 27 percent wheat bran, 2 percent mineral mixture and 1 percent common salt. In the experimental group, the GNC was replaced with WWNSKC. The results showed that there was no difference ($P < 0.05$) in the milk yield, butter fat content and organoleptic evaluation of milk (Table 16), DMI, digestibility of nutrients, haemoglobin content, SGOT, SGPT, acid phosphatase and alkaline phosphatase in blood, and reproductive ability of the cows in the two groups. The nitrogen balance was higher ($P < 0.05$) in the WWNSKC

TABLE 15
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on the performance of buffalo calves

Parameter	Treatment	
	Control	Experimental
Initial body weight (kg)	83.2	83.2
Growth rate (g/day)	507 a	606 b
Dry matter intake (kg/day)	2.63	2.87
Digestibility (%)		
Dry matter	61.3 b	55.5 a
Crude protein	71.4	68.7
Ether extract	56.4 a	69.9 b
Total carbohydrates	61.8 b	56.1 a
Nitrogen balance (g/day)	27.5	37.4

Source: Agrawal, Garg and Nath, 1987.

TABLE 16
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on milk yield and composition

Parameter	Treatments	
	DGNC	WWNSKC
Average daily milk yield (kg)	7.68	7.20
Average daily milk yield corrected for initial yield (kg)	7.21	7.55
Average daily fat-corrected milk yield (kg)	8.46	7.43
Fat (g/kg)	45.0	41.3
Crude protein (g/kg)	34.4	34.3
Total solids (g/kg)	122.4	115.6
Solids-not-fat (g/kg)	76.4	72.4
Ash (g/kg)	7.7	7.6
Calcium (g/kg)	1.52	1.55
Phosphorus (g/kg)	0.53	0.55

Notes: DGNC = de-oiled groundnut cake. Source: Nath et al., 1989.

TABLE 17
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on dry matter intake, digestibility of nutrients and nitrogen balance

Parameter	Treatments	
	DGNC	WWNSKC
DM intake (kg/day)	9.07	8.74
DM intake as % of body weight	2.56	2.61
Digestibility		
Dry matter	0.55	0.55
Crude protein	0.67	0.67
Organic matter	0.58	0.58
Ether extract	0.66	0.67
Crude fibre	0.43	0.48
Nitrogen-free extract	0.62	0.59
Total carbohydrate	0.56	0.55
Nitrogen intake (g/day)	244.0	245.0
Nitrogen excretion (g/day)		
In urine	101.67 a	75.0 b
In faeces	78.75	80.06
In milk	26.98	31.21
Nitrogen balance (g/day)	36.6 a	57.9 b

Notes: DGNC = de-oiled groundnut cake. a,b = means in a row with different suffixes are significantly different ($P < 0.05$). Source: Nath et al., 1989.

TABLE 18
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on blood measurements

Parameter	Treatments	
	DGNC	WWNSKC
Haemoglobin (g/L)	94.0	93.3
SGOT (IU/L)	36.0	34.70
SGPT (IU/L)	10.50	11.50
Acid phosphatase (IU/L)	5.99	7.01
Alkaline phosphatase (IU/L)	36.63	39.34
Urea (mg/L)	422.5 a	345.0 b

Notes: SGOT = serum glutamate oxaloacetate transaminase; SGPT = serum glutamate pyruvate transaminase; DGNC = de-oiled groundnut cake. a,b = Means in a row with different suffixes are significantly different ($P < 0.05$). Source: Nath et al., 1989.

group due to less excretion of urinary nitrogen and a concomitant decrease in blood urea nitrogen (Tables 17 and 18).

The water washing technique developed above for preparing WWNSKC can be adopted in all countries producing neem seed oilcake, so that the material, hitherto unused, can be used for animal feeding. Feeding NSKC (untreated) and WWNSKC to cross-bred calves had no adverse effect on rumen pH, TVFA concentration, holotrich protozoa count, activity of amylase, xylanase and CM-cellulose (Table 19), but there was significant depression in total-N, NH_3 -N, TCA-soluble N, total protozoal count and urease and protease activity. TCA-ppt.-N and medium-sized and total spirotrich protozoa were significantly reduced with feeding of NSKC, whereas these were comparable between WWNSKC and control (DGNC) groups (Mondal and Garg, 2002).

In another experiment, metabolic studies showed that feeding of NSKC and WWNSKC had no adverse effect on intake of DM, OM and TDN, and on digestibility of DM,

TABLE 19
Effect of feeding neem seed kernel cake (NSKC) and water-washed NSKC (WWNSKC) on rumen fermentation (units/dl SRL) and enzyme specific activity (U/mg) in rumen contents

Parameter	Ration group		
	DGNC	NSKC	WWNSKC
pH	6.33	6.57	6.53
TVFA (mmole)	8.46	7.51	7.12
Total-N (mg)	145.21 a	105.78 b	116.36 b
NH_3 -N (mg)	24.50 a	12.13 b	16.33 b
TCA soluble-N (mg)	55.13 a	46.85 b	45.11 b
Amylase	6.65	6.26	5.50
CMCellulase	1.35	1.55	1.26
Xylanase	5.07	5.22	4.47
Urease	24.71 a	14.14 b	14.19 b
Protease	13.77 a	8.38 b	9.55 b

Notes: TVFA = total volatile fatty acids; TCA = trichloroacetic acid. a,b = means with different suffixes in a row differ significantly ($P < 0.05$). One unit of enzymic activity = amount of enzyme needed to produce 1 μmole of glucose/xylose/ NH_3 (for CMCellulase/xylanase/urease) or for degradation of 1 mg of protein (for protease) per minute under assay conditions. Source: Mondal and Garg, 2002.

TABLE 20
Effect of feeding alkali treated neem seed kernel cake (ATNSKC) or urea-ammoniated neem seed kernel cake (UNSKC) on growth rate, feed intake, nutrient utilization and plane of nutrition in buffalo calves

Parameter	Control	ATNSKC	UNSKC
Initial body weight (kg)	235.5	203.0	221.3
Growth rate (g/day)	357.4	375.9	371.3
DM intake (g/kg W ^{0.75})	84.2	84.3	84.4
Concentrate:roughage ratio	51.49	46.54	54.46
Nutrient digestibility (%)			
Dry matter	55.1	52.5	52.9
Organic matter	57.6	54.6	56.2
Crude protein	63.9	61.9	63.7
Ether extract	60.7	64.9	66.5
Crude fibre	45.0	49.9	52.9
Nitrogen-free extract	61.6 b	54.1 a	55.0
Total carbohydrate	56.0	52.5	54.1
Nutrient balance			
N retention (g/day)	34.4	36.6	31.41
N retention (% of intake)	28.0	30.1	24.8
Ca retention (g/day)	19.4	15.6	16.8
P retention (g/day)	10.2	9.6	9.9
Nutrient value of ration (%)			
DCP	7.9	8.1	8.3
TDN	52.6	49.4	51.2
Plane of nutrition (g/day)			
CP intake	776.8	774.7	792.3
DCP intake	494.6	478.9	504.4
TDN intake	3300	3000	3200

Notes: DCP = digestible crude protein; TDN = total digestible nutrients; W^{0.75} = metabolic weight (size). a,b = means with different suffixes in a row differ significantly ($P < 0.05$).

Source: Sastry, Katiyar and Agrawal, 1999.

TABLE 21
Effect of feeding alkali-treated neem seed kernel cake (ATNSKC) or urea-ammoniated neem seed kernel cake (UNSKC) on blood-biochemical parameters

Parameter	Control	ATNSKC	UNSKC
Haemoglobin (g, %)	11.0	12.1	13.8
Alkaline phosphatase (IU/L)	17.3	17.8	20.8
SGOT (IU/L)	25.1	24.3	29.7
SGPT (IU/L)	29.2	30.2	34.4
Urea (mg/100ml)	32.8	39.2	35.3

Notes: SGOT = serum glutamate oxaloacetate transaminase; SGPT = serum glutamate pyruvate transaminase.

Source: Sastry, Katiyar and Agrawal, 1999.

OM, total carbohydrate, NDF and ADF. While DCP intake was significantly reduced in the NSKC group, it was comparable in control and WWNSKC groups (Mondal, 1994). Cross-bred milch cows fed WWNSC replacing the GNC moiety of concentrate mixture at 10, 20 and 30 percent levels showed no significant difference in DM intake per 100 kg body weight (3.06–3.13 kg) nor per unit metabolic body weight (130.6–133.5 g). Similarly, average daily milk yield (6.60–6.88 kg), 4 percent FCM yield (6.52–6.81 kg),

average fat (3.91–3.94 percent), non-fat solids (8.91–8.98 percent) and total solids (12.83–12.91 percent) did not differ significantly among different ration treatments (Kumar *et al.*, 1992). Though there was improvement in palatability of NSC and NSKC after laborious repeated water washing of the cakes, as soluble toxic bitters were removed to a great extent, still the process was not feasible for industrial application, besides being uneconomical due to loss of soluble nutrients. Therefore, WWNSC can only replace concentrate mixture fed to cross-bred cows up to 30 percent without undesirable effects.

Though water washing of cake converted it into a wholesome protein substitute (Nath *et al.*, 1989; Sastry and Agrawal, 1992), 22 percent DM was lost. To avoid such loss, processing the cake in alkaline medium without water washing was tried, either by soaking it in water (1:5 w/v) containing either NaOH (2 percent w/w) for 24 hours or by ensiling with 2.5 percent urea (w/w) for 5–6 days (Nagalakshmi *et al.*, 1996, 1999). The sun-dried and ground alkali-treated (ATNSKC) and urea-ammoniated (UNSKC) cakes were found suitable for feeding cattle and buffalo calves (Reddy, 1992; Sastry, Katiyar and Agrawal, 1999), growing lambs (Anandan *et al.*, 1999) and kids (Musalia *et al.*, 2000) without affecting their growth, nutrient utilization, blood profile, rumen fermentation pattern, physical and chemical carcass characteristics, including organoleptic sensory score and gross and histopathology of vital organs.

Alkali treatment or urea ammoniation therefore converts NSKC into a wholesome substitute for DGNC for feeding growing buffalo calves, and no discernable effect or clinical symptoms of ill health could be noticed due to feeding of processed NSKC (Tables 20 and 21) during the entire 270 days of the growth trial, thus confirming effective detoxification of NSKC by either of the processing methods.

Neem seed cake in poultry feeding

Feeding of de-oiled neem seed meal (DNSM) at or above 5 percent level for 8 weeks resulted in adverse effects on growth and feed efficiency in White Leghorn chicks (Subbarayudu and Reddy, 1975). Choudhary *et al.* (1981) also reported poor growth and nutrient utilization on feeding of either raw or water-soaked NSC at 30 percent level during 0 to 6 weeks of age in broiler chicks. Studies with Babcock cockerels fed DNSM for 4 months resulted in comparable feed intake, but the birds excreted reddish brown, fluidy faeces with gradual and progressive emaciation (Christopher, Ahmed and Sastry, 1976). Similarly, Sadagopan, Johri and Reddy (1981) observed significant reduction in weight gain of broiler chicks fed raw NSC at a 2.5 to 7.5 percent level. However, solvent extraction of the cake improved the growth rate. Undecorticated expeller neem cake at 10 percent of broiler chick diets depressed

($P < 0.05$) weight gain, while feed efficiency was reduced at the 20 percent inclusion level, and the growth inhibition was linearly correlated with increasing level of incorporation (Reddy and Rao, 1988a). In contrast, incorporation of undecorticated NSC improved its utilization (Reddy and Rao, 1988b). Furthermore, overnight acid (1 N HCl) followed by alkali (5 percent KOH w/v) soaking for 15 minutes, with water washing in between each treatment of solvent-extracted undecorticated neem cake, removed the bitterness, as indicated by comparable feed intake, growth and feed efficiency of chicks (Reddy and Rao, 1988c). Similarly, saponification of neem oil with 10 percent KOH completely detoxified the oil, as evident from comparable performance of broiler chicks fed saponified neem oil and groundnut oil (Reddy, Rao and Reedy, 1988).

Chand (1987) fed diets having either 10, 20 or 30 percent raw neem seed meal (NSM), or its equivalent as 0.9, 1.8 or 2.7 percent neem oil, to chicks and recorded poor growth and low feed efficiency. Inclusion of alcohol- and hexane-extracted NSM improved utilization, but inclusion beyond 30 percent decreased performance. Incorporation of alkali-treated and urea-ammoniated neem kernel meal (NKM) in broiler chick diets gave comparable performance at 50 percent replacement of groundnut meal, and no untoward effects were noted for carcass traits as well as sensory evaluation of meat (Nagalakshmi, 1993). Broiler chicks fed full fat NSM (FFNSM) at 2.5, 5, 7.5 or 10 percent of dietary levels showed a negative ($P < 0.05$) correlation between the level of inclusion and the gain and feed conversion efficiency during the starter phase (0–5 weeks), whereas birds in the finisher phase (6–10 weeks) exhibited comparable performance, probably due to colonization by counteracting gut microbiota (Salawu, Adedeji and Hassan, 1994), and gross pathology of visceral organs were normal. During 8-week trials with cockerels, Odunsi *et al.* (2009) reported that water-soaked neem seed cake added with charcoal (0.4 percent, w/w) can replace 20 percent of SBM in the diet with no adverse effect on growth, feed efficiency or carcass traits.

Egg production

When groundnut meal was replaced with NSC at 25, 50, 75 and 100 percent levels in layer diet it caused a significant reduction in egg production, but egg weight and egg quality were not affected (Sadagopan, Johri and Reddy, 1981). Similarly, Verma, Gowda and Elngaovan (1998) recorded no adverse effect of feeding raw or pre-treated (2 percent NaOH) NKM at 10 percent of dietary inclusion on feed consumption, egg production, egg weight, internal egg quality (yolk colour, Haugh unit), shell thickness and organoleptic evaluation of boiled eggs during a 12-week period of laying. However, raw NKM at levels of 15 and 20 percent dietary inclusion adversely affected performance in layers (Gowda *et al.*, 1998).

Blood biochemical profile

Feeding of 30 percent DNSM to Babcock cockerels resulted in significant depression in haemoglobin level and total erythrocyte count in the blood (Christopher, Ahmed and Sastry, 1976), with elevated total leucocyte count (TLC). Similarly, Chand (1987) reported a higher TLC when feeding 10–30 percent alkali-treated neem seed cake. However, no significant differences in the concentration of haemoglobin and total erythrocyte count (TEC) could be observed when feeding 2.5 percent urea-ammoniated neem kernel meal in broilers during a 6-week study. Similarly, no difference could be noticed in any blood parameters (haemoglobin, TEC, TLC) when feeding of water-soaked neem seed cake up to 3.6 percent in the diet of cockerels (Odunsi *et al.*, 2009).

Carcass characteristics

No difference in the dressed meat yield of broilers could be observed due to feeding of 2.5 percent urea-ammoniated neem seed kernel cake (UNSKC) as reported by Nagalakshmi (1993). No untoward effect on appearance, odour, taste, texture, tenderness or juiciness could be observed when pressure-cooked meat, with or without salt (1.5 percent, w/w), was subjected to sensory evaluation on a 7 point hedonic scale by a panel of semi-trained judges. At the same time, greater breast and thigh meat yield was observed in cockerels fed 3.6 percent WWNSKC compared with the UNSKC group.

Harmful effects on health

Detailed patho-anatomical examination of vital organs of birds fed 30 percent DNSM by Christopher, Ahmed and Sastry (1976) revealed pale and shrunken muscles, pale visceral organs, with slightly smaller, light brown liver, spleen, kidneys and pale intestine. Histologically, liver, spleen kidneys and lungs showed extensive fatty changes, with catarrhal enteritis of varying degrees. Similarly, Vijjan and Parihar (1983) recorded mild degenerative changes even at a 10 percent level of feeding alcohol-treated NSC. Mild to severe nephritis, hepatitis and enteritis were noticed in birds on diets with 5–50 percent NSM. Birds fed a diet with 20 percent NKM inclusion showed degenerative ovarian changes, with loss of ovarian follicles, and histological changes in liver, kidney and intestine (Gowda and Sastry, 2000). Similarly, water-washed NKM (WWNKM) in the diet of cockerels at 20 percent level for a period of 12 weeks adversely affected spermatogenesis and fertility (Tyagi, Tyagi and Verma, 1996.), confirming the anti-fertility effect of neem. However, alkali- (1 and 2 percent, w/w) and urea- (1.5 and 2.5 percent, w/w) treated NSKC when replacing either 50 or 100 percent of GNC and fed to the broilers, produced no gross or histopathological abnormalities.

RECOMMENDATIONS

Based on the knowledge available, it appears that both neem and karanj oil cakes are rich in protein, which can be very good supplements for ruminants fed on roughage-based diets. They could also be incorporated in poultry diets for increasing productivity, but neither oilcake is suitable for feeding without pre-treatment because of the presence of toxic compounds and poor palatability.

The anti-nutritional factors of karanj cake are soluble in oil. Therefore, complete removal of oil from cake may be a more effective method than other chemical treatment methods. The chemical structure of toxic and bitter compounds of neem cake are a mixture of polar and non-polar groups. Some of these compounds might be toxic for the animals, since they are either soluble in water or fat. This is perhaps the reason why water-washing of neem seed cake has shown promising results with minimal toxicity.

Water-washed or de-oiled karanj cake and water-washed neem seed cake may be incorporated at up to 50 percent of the nitrogen part of conventional protein supplements like soyabean meal or groundnut cake without adverse effects on nutrient metabolism, growth and health of lambs.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Numerous experiments have been conducted on the detoxification of these two oil cakes to render them suitable for feeding to cattle, buffalo, sheep, goat and poultry, but not much information is available on the selective removal of the compounds with the most toxic effects.

Once the pre-treatment process for detoxification of these oil cakes has been standardized, there will be the need to develop an industrial process for detoxification so that treatment cost is minimized and their use becomes economically feasible.

Long-term feeding trials to examine the effects of feeding the treated oil cakes on the quality of livestock products (milk, meat and eggs) are needed before these cakes can be recommended for practical application by farmers or for use in commercial compound feeds.

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Chapter 23

Co-products of the United States biofuels industry as alternative feed ingredients for aquaculture

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ABSTRACT

The tremendous growth of the biofuels industry has made large amounts of co-products (i.e. distillers grain and crude glycerin) available for use in aquafeeds. This chapter reviews the prospects and challenges associated with their use in aquafeeds. Properties of each product as it pertains to fish nutrition and available research are described for different fish species. Despite the apparent deficiency in lysine and the high fibre content in DDGS, considerable amounts of DDGS can be fed to omnivorous fish species without impact on growth or product quality. Nutrient variability is, however, an issue that needs to be considered when feeding DDGS to fish. The use of crude glycerin in fish is less clear, and further research is necessary before nutritional recommendations can be made.

INTRODUCTION

High energy prices and government policies that encourage the use of biofuels have spurred a tremendous growth in the ethanol and biodiesel industries, both in the United States and internationally over the last decade. In 2005, United States total ethanol production was estimated at 15.8 billion litres, and by early 2010, 51 billion litres of ethanol were produced (RFA, 2011). Similarly, biodiesel production has increased dramatically from 284 million litres in 2005 to 1.7 billion litres in 2007 (NBB, 2007). The surge in biofuel production has been simultaneously accompanied by a growing supply of co-products such as distillers grain and crude glycerin (i.e. glycerin or glycerol). Total supply of United States distillers grain was estimated at 32.9 million tonne in 2010, an increase of more than 13 fold compared with 2000 (Figure 1). The United States biodiesel industry is expected to produce an estimated 640 000 tonne of crude glycerin between 2006 and 2015 (Nilles, 2006). Excess glycerin in the market creates enormous marketing challenges and requires finding new uses for this co-product. Competitive pricing of low value crude glycerin has created opportunities for this co-product to be used in livestock feeding.

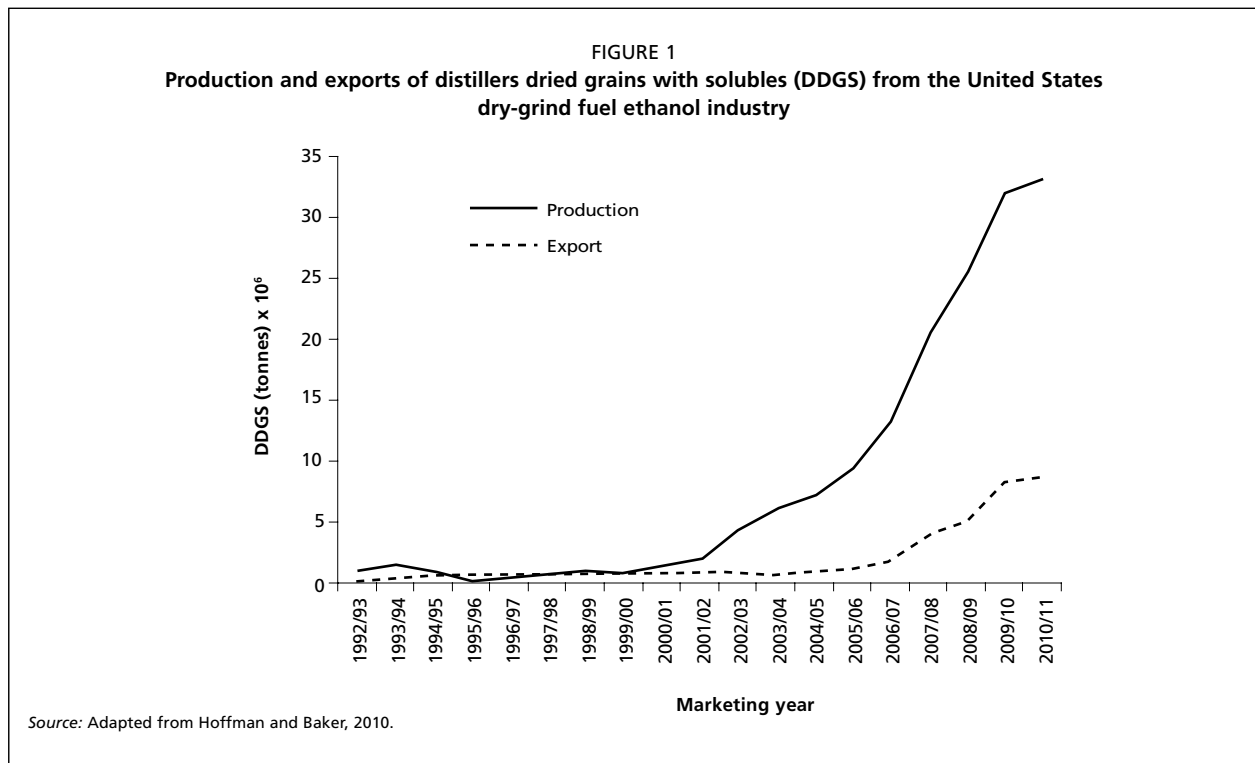
To date, distillers grain from the dry-grind ethanol industry have received considerable attention in animal feeds. In contrast, glycerol has been used more in industrial applications, although new research has shown that glycerol appears to be a promising energy source in animal diets. Distillers grain include traditional co-products, such as distillers wet grains, dried distillers grain with or without

solubles (DDG and DDGS), and condensed distillers solubles (CDS). DDGS is the co-product that is most extensively produced in the ethanol industry. Recently, fractionation technologies used in ethanol production have resulted in new feeds with unique chemical compositions. Also, it is important to note that a small fraction of distillers grain is produced from beverage distilleries. However, the contribution of distillers grain from the beverage distilleries represented less than 2.7 percent of all the distillers grain produced in 2010/11 in the United States (Hoffman and Baker, 2010). In addition, maize (corn) is the primary feedstock grain used to make ethanol, accounting for more than 98 percent of all DDGS produced (Hoffman and Baker, 2010). Hereafter, the term "distillers grain" will refer to distillers dried grains with solubles (DDGS) (from maize) unless otherwise noted. Currently, DDGS is fed primarily to beef and dairy cattle, swine and poultry (Figure 2). No estimates on the current use of DDGS in aquafeeds could be found, but it is expected to be very small.

Another high growth sector in recent years has been aquaculture. Aquaculture has been growing at a rapid pace of approximately 6.2 percent per annum, from 38.9 million tonne in 2003 to 52.5 million tonne in 2008 (FAO, 2008), and currently accounts for over 50 percent of all food of aquatic origin consumed by humans worldwide. The value of aquaculture production was estimated at US\$ 98.4 billion in 2008. However, concerns exist over the sustainability of aquaculture for a number of reasons, one of which is the increased pressure on feed ingredients, especially fishmeal

MAIN MESSAGES

- DDGS from fuel ethanol production can be an effective protein ingredient in aquafeeds.
- DDGS serves to replace SBM and maize in the diet, but not fishmeal.
- For most fish species, a level of 20% DDGS appears to be the maximum inclusion if supplemental lysine is not added.
- If supplemental lysine is used, maximum DDGS levels greater than 20% can be used.
- Crude glycerine from biodiesel production appears to be a potential energy source.
- Much work needs to be conducted on use of glycerin in fish diets.



and fish oil. Fishmeal used in aquaculture represented 68.2 percent of total global fishmeal production in 2006 (Tacon and Metian, 2008), but increased pressure due to exploiting marine resources and rising prices could ultimately decrease the use of fishmeal, as it will inevitably be replaced by less expensive alternative proteins.

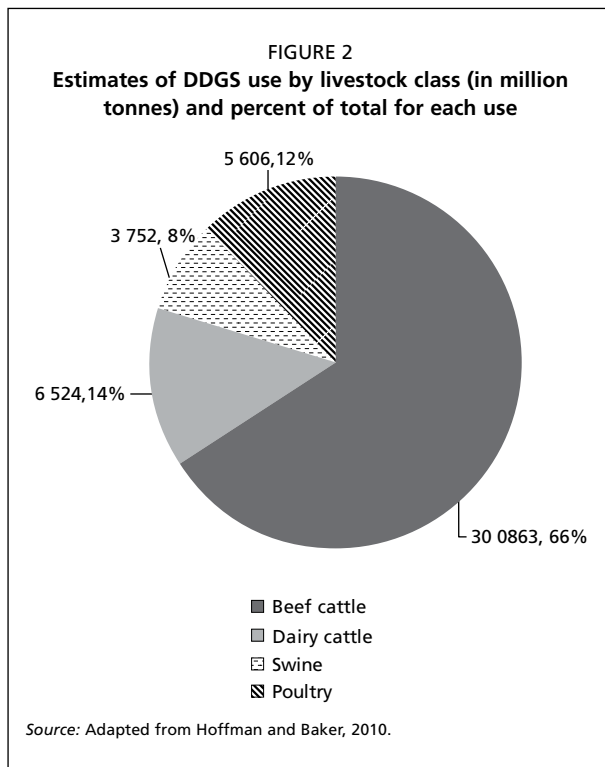
DDGS, a relatively cheap protein source (Figure 3) compared with fishmeal, is a candidate plant protein. During the last 10 years, DDGS market price has been generally between 5 percent and 20 percent that of fishmeal. While DDGS is not recommended as a direct, complete replacement for fishmeal, it can be used with, or in lieu of, other plant proteins (such as soybean meal – SBM) to reduce the use of fishmeal in aquafeeds. As shown in Figure 3, over the last decade the price of DDGS has ranged from approximately 20 percent to 70 percent that of SBM.

This chapter will review the nutrient composition of major biofuels (i.e. maize-based fuel ethanol and soy-based

biodiesel) co-products (i.e. distillers grain and crude glycerin), will provide summaries of available nutritional studies for different fish species, and will conclude with final remarks on challenges associated with these co-products and areas of needed research.

Before proceeding, however, it is important to note a few key issues. First, maize is the primary feedstock for fuel ethanol production in the United States. Other starch-rich materials can theoretically also be used to produce ethanol, including barley, cassava, field peas, millet, triticale, oats, rice, rye, sorghum, sweet potato and wheat. Unfortunately, most of these alternative starch sources have only been investigated on a laboratory- or pilot-scale and are not readily commercially available. Not surprisingly, fish feeding trials are essentially non-existent for co-products from these substrates, and thus will not be discussed in this chapter.

In contrast, biodiesel can be produced from a variety of oilseeds and lipid-containing materials, including canola



based biodiesel co-products. But, our discussion will be limited to glycerin, and will not cover SBM or various soy protein concentrates or isolates. These topics have been covered in depth elsewhere (Gatlin *et al.*, 2007; Hertrampf and Piedad-Pascual, 2000; U.S. Soybean Export Council, 2011). Furthermore, algae-based biofuels have much promise for the future of the biofuels industry, but, to date, post-extraction algal residues use in any fish feeding trials has not been reported.

PROPERTIES OF DISTILLERS GRAIN
Physical properties

Some of the physical properties that are important to aquafeeds include particle size, bulk density and flowability. Because of the small size (<1.4 mm, on average) and variable size distribution of particles (Bhadra, Muthukumarappan and Rosentrater, 2009), handling DDGS can pose some logistical problems. Bulk density determines capacity of transport vessels and storage facilities. As with other properties, bulk density varies among DDGS sources and averages ~480 kg/m³ (Rosentrater, 2006). For comparison, bulk densities of maize and SBM are 721 and 658 kg/m³, respectively (Letsche, Lammers and Honeyman, 2009.). The low bulk density of DDGS translates into higher transportation costs. Variations in bulk density may be due to differences in particle size and to the amount of condensed distillers solubles (CDS) added back to distillers grain during manufacturing. Several factors affect the

(rapeseed), castor beans, copra, cottonseed, flaxseed, jatropha, palm oil, poppy seed, safflower seed, sesame seed, shea nut, sunflower seed, animal rendering and others. The primary source for biodiesel in the United States, however, is soybean. Thus, our discussion will be focused on soy-

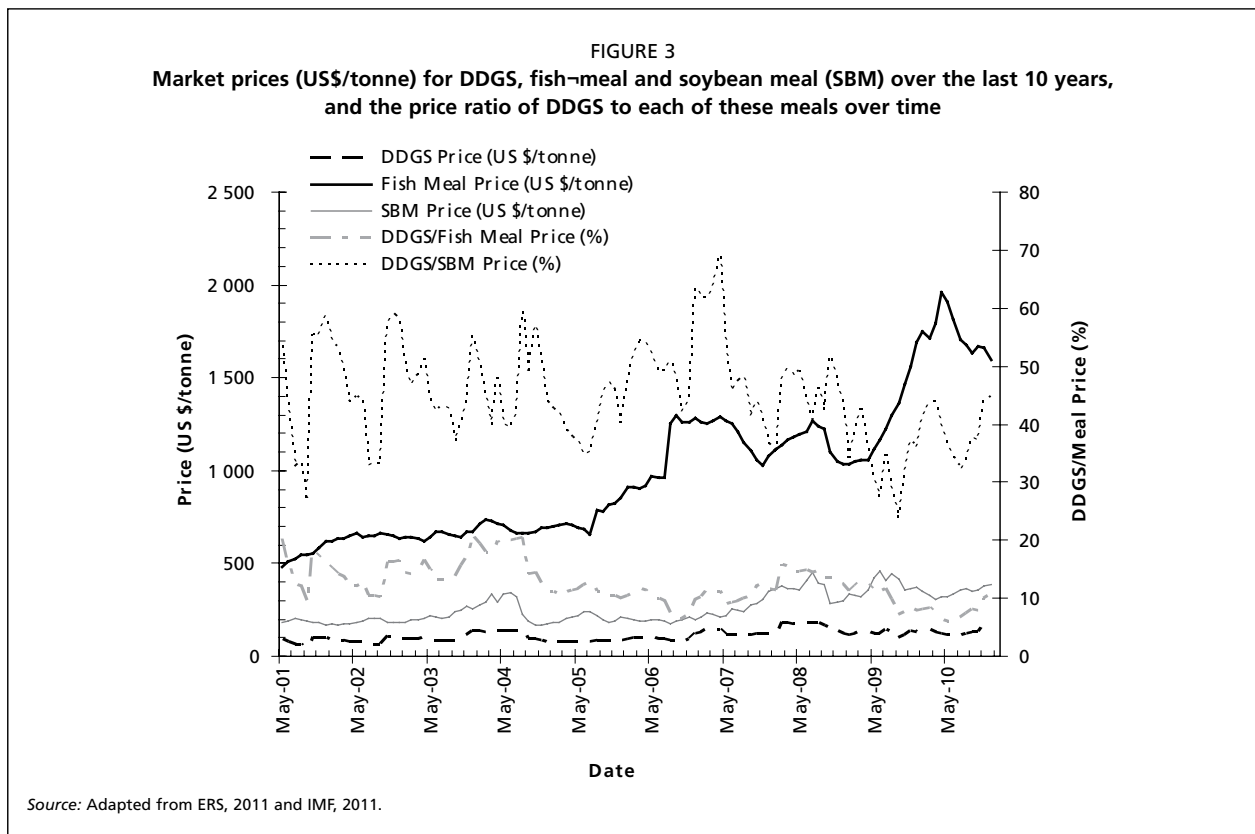


TABLE 1
Typical physical properties of distillers dried grains with solubles (DDGS)

Physical property	Mean (SD)	Range
Water activity (-)	0.53 (0.02)	
Bulk density (kg/m ³)	389.3 (24.1)	490–600
Angle of repose (°)	26.5 (1.8)	35.94–41.60
Colour Hunter L (-)	40.0 (1.6)	36.56–50.17
Colour Hunter a (-)	8.0 (0.4)	5.20–10.79
Colour Hunter b (-)	18.2 (0.9)	12.53–23.36

Notes: (-) denotes dimensionless quantities. Sources: Means (and Standard Deviations) from Rosentrater, 2006; Ranges from Bhadra, Muthukumarappan and Rosentrater, 2009, 2010.

flowability of DDGS, including particle size, soluble solid and fat contents (which are due to the CDS addition level), dryer temperature and moisture content at dryer exit (Ganesan, Muthukumarappan and Rosentrater, 2008). Manipulation of these properties and pelleting often improves the flowability of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Rosentrater, 2007).

The colour of DDGS can vary from golden yellow to dark brown. The amount of condensed distillers solubles (CDS) added to the distillers grain, the original colour of the feedstock grain and drying temperature are all factors that affect the colour of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Noll, Parsons and Walters, 2006). Colour can be a good indicator of heat damage (Maillard reaction) that occurs during the drying of distillers grain, especially to the most heat labile amino acid, lysine. In fact, the digestibility of lysine in swine has been shown to vary substantially among different DDGS sources (Stein *et al.*, 2006). In addition, a strong correlation has been determined between brightness/lightness (i.e. Hunter colour L) and digestible or bio-available lysine content of DDGS for poultry (Pahm *et al.*, 2009; Fastinger, Latshaw and Mahan, 2006) and swine (Pedersen, Pahm and Stein, 2005). Other physical characteristics of DDGS are summarized in Table 1.

Chemical properties

Nutrients in DDGS are concentrated nearly three times compared with those found in maize. This is because starch, which constitutes about two-thirds of the maize kernel, is removed during the fermentation process to produce ethanol. Predicting DDGS composition from that of maize, however, has to reflect multiple factors. Differences in processing within and among ethanol plants, especially drying conditions (temperature and time) and the amount of CDS added to the distillers grain and, to a lesser extent, the source and quality of maize, can create considerable variations in the nutrient composition of DDGS.

In fact, nutrient concentrations can vary substantially among DDGS sources (i.e. ethanol plants) (Table 2). Several papers on nutrient composition and influencing factors

are available (Spiehs, Whitney and Shurson, 2002; Belyea, Rausch and Tumbleson, 2004; Belyea *et al.*, 2010). It is also important to recognize that nutrient composition of DDGS, as found in older publications (such as NRC, 1993), may no longer be applicable because DDGS from that generation was predominately made from alcohol beverage distilleries, not the newer fuel ethanol plants of today. In general, DDGS is a good source of energy and protein for various livestock animals. Fuel-based DDGS contains, on average, 11.0 percent moisture, 30.8 percent crude protein, 7.4 percent crude fibre, 11.2 percent crude fat and 5.5 percent residual starch (UMN, 2011).

Specifically, fish have requirements for amino acids rather than crude protein, per se. The amino acid profile of DDGS reflects that of maize, with lysine being the most

TABLE 2
Typical nutrient composition of distillers dried grains with solubles (DDGS)

Item	UMN	Spiehs, Whitney and Shurson, 2002	NRC
Dry matter (% as is)	89.2 (1.6)	88.9 (1.7)	91.0
Crude fat	11.2 (14.3)	10.9 (7.8)	10.2
Crude fibre	7.4 (14.9)	8.8 (8.7)	10.0
Starch	5.5 (34.4)	-	-
Crude protein	30.8 (4.9)	30.2 (6.4)	29.7
Amino acids			
Arg	1.35 (9.6)	1.20 (9.1)	1.23
His	0.82 (9.1)	0.76 (7.8)	0.70
Ile	1.17 (7.1)	1.12 (8.7)	1.20
Leu	3.51 (8.8)	3.55 (6.4)	3.18
Lys	0.97 (12.6)	0.85 (17.3)	0.71
Met	0.60 (12.1)	0.55 (13.6)	0.55
Cys	0.61 (13.4)	-	0.51
Phe	1.49 (12.6)	1.47 (6.6)	1.53
Thr	1.12 (7.2)	1.13 (6.4)	1.08
Trp	0.23 (11.8)	0.25 (6.7)	0.11
Tyr	1.10 (12.6)	-	1.09
Val	1.55 (8.2)	1.50 (7.2)	1.65
Ash	5.7 (1.4)	5.8 (14.7)	7.0
Ca	0.05 (135.3)	0.06 (57.2)	0.15
P	0.79 (14.3)	0.89 (11.7)	0.73
K	1.02 (17.2)	0.94 (14.0)	0.44
Mg	0.31 (17.9)	0.33 (12.1)	0.18
S	0.69 (35.6)	0.47 (37.1)	0.38
Na	0.26 (188.8)	0.24 (70.5)	0.57
Cl	0.19 (25.5)	-	0.18
Zn (ppm)	58.80 (23.1)	97.5 (80.4)	87.91
Mn (ppm)	17.00 (26.1)	15.8 (32.7)	25.05
Cu (ppm)	6.00 (29.2)	5.9 (20.4)	58.02
Fe (ppm)	110.00 (39.1)	119.8 (41.1)	259.34
NFE	34.1	33.2	34.1

Notes: All nutrient values expressed as a percentage on a 100% dry matter basis (Coefficients of variation presented in parentheses when available); NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). Sources: UMN data are a compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n=62). Spiehs, Whitney and Shurson (2002) is a compilation of data from 1997 to 1999 (n=118). NRC data are from Nutrient Requirements of Fish (NRC, 1993).

TABLE 3
Typical nutrient composition of other distillers grain products

Item	Wheat DDGS ⁽¹⁾	Triticale DDGS ⁽²⁾	Sorghum DDGS ⁽³⁾	De-oiled DDGS ⁽⁴⁾	Maize HPDDG ⁽⁵⁾	Sorghum HPDDG ⁽⁶⁾
DM (% as is)	90.9	89.4	88.4	87.5	91.4	92.3
Crude fat	5.7	–	10.8	3.5	4.0	3.2
Crude fibre	8.1	–	8.0	–	–	–
Starch	2.1	–	–	5.6	8.3	–
Crude protein	40.3	32.4	34.2	34.0	43.6	48.2
Amino acids						
Arg	1.65	1.45	–	1.59	1.70	1.85
His	0.82	0.75	–	1.04	1.17	1.11
Ile	1.37	1.17	1.41	1.47	1.79	2.18
Leu	2.67	2.51	4.44	4.26	5.99	5.89
Lys	0.89	0.78	1.01	1.09	1.28	1.73
Met	0.64	0.55	0.61	0.68	0.91	0.85
Phe	1.83	1.50	–	1.61	2.35	2.47
Thr	1.21	1.07	1.20	0.95	1.58	1.79
Trp	0.39	0.14	0.22	0.18	0.28	0.39
Val	1.78	1.49	1.86	1.43	2.25	2.63
Ash	5.7	–	4.5	5.3	2.1	5.0
Ca	0.18	–	–	0.06	0.04	0.13
P	1.05	–	–	0.84	0.45	0.82
NFE	31.1	–	42.8	–	–	–

Notes: DDGS = distillers dried grains with solubles; HPDDG = high protein distillers dried grain. All nutrient values expressed as a percentage on a 100% dry matter basis. NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). Sources: (1) Avelara *et al.*, 2010; Cozannet *et al.*, 2010; Oryschak *et al.*, 2010a; Bandegan *et al.*, 2009. (2) Oba *et al.*, 2010; Oryschak *et al.*, 2010b. (3) Jones *et al.*, 2010; Urriola *et al.*, 2009. (4) Mjoun *et al.*, 2010. (5) Jacela *et al.*, 2010; Mjoun *et al.*, 2010; Applegate *et al.*, 2009; Widmer *et al.*, 2008. (6) Jacela *et al.*, 2010.

limiting. Compared with SBM and fishmeal, DDGS supplies (on a crude protein basis) higher amounts of Met and Leu, similar amounts of His, Phe, Thr, Trp and Val, but lower amounts of Arg, Ile and Lys. When comparing the amino acid profile of DDGS with the requirements of tilapia and rainbow trout, it can be concluded that DDGS is deficient in lysine for both tilapia and rainbow trout, and in tryptophan for tilapia (Table 4). The imbalance of amino acids in DDGS can limit its value for fish when used as a sole protein source, although, when economically viable, synthetic amino acids can be used to correct deficiencies. Combining DDGS with other protein meals is another option. In addition, low digestibility of amino acids in DDGS may further limit its nutritional value in fish diets. It is, however, important to note that improvements in the protein quality of DDGS in terms of concentration and digestibility of amino acids from DDGS produced in new generation ethanol plants may be an indication of improved and more controlled production processes.

DDGS is also a good source of the vitamins niacin, riboflavin and vitamin E, as well as various minerals. DDGS contains high levels of P (0.80%), with the majority of this P being inorganic, making DDGS a good source of digestible P in chicks (Martinez Amezcua, Parsons and Noll, 2004) and swine (Pedersen, Boersma and Stein, 2007). In contrast, DDGS contains low concentrations of Ca, Cl and other trace minerals. In addition, unlike most plant proteins, DDGS does not contain anti-nutritional factors,

which can prove to be very problematic for some proteins. Variability in nutrient composition is still, however, an issue when dealing with DDGS. For this reason, access to reliable nutrient composition information is necessary to minimize risks associated with nutrient variation when DDGS is used in fish feeds.

As mentioned, the majority of United States distillers grain currently comes from dry-grind processing of maize into fuel ethanol, with smaller amounts being derived from sorghum (milo) and wheat, as well as a small percentage from beverage distilleries. In Canada, wheat and triticale represent the major grains used in ethanol production. Barley is another grain that can be used for ethanol production; however, lower ethanol yield and higher costs of production limit the use of barley in ethanol production. Moreover, barley DDGS has limited value in aquaculture feeds due to its residual content of beta-glucans. Likewise, recent changes aimed at increasing the efficiency of maize-based fuel ethanol production have resulted in a variety of distillers grain of different compositions, which are becoming available to the marketplace. The compositions of these other co-products that may have potential in aquaculture feeds are presented in Table 3. Although the chemical composition of some of these co-products appears attractive for use in aquafeeds, their nutritive value is still unknown. Except for limited information for high protein distillers dried grains (HPDDG), none of these co-products has been evaluated in fish diets. Research in monogastric species

TABLE 4
Ratio of essential amino acid supplies from different ingredients to the dietary requirements of different fish species

Amino acid	Tilapia			Rainbow Trout		
	DDGS	SBM	Fishmeal	DDGS	SBM	Fishmeal
Arginine	1.19	2.05	1.61	1.11	1.92	1.50
Histidine	1.77	1.77	1.50	1.44	1.44	1.22
Isoleucine	1.40	1.67	1.52	1.60	1.91	1.74
Leucine	3.84	2.62	2.34	3.09	2.11	1.89
Lysine	0.70	1.42	1.64	0.66	1.34	1.54
Methionine/cystine	1.39	1.01	1.27	1.49	1.08	1.36
Phenylalanine/tyrosine	1.74	1.75	1.39	1.78	1.79	1.42
Threonine	1.11	1.21	1.18	1.73	1.89	1.84
Tryptophan	0.87	1.63	1.15	1.44	2.71	1.91
Valine	2.06	1.85	2.05	1.59	1.43	1.58

Notes: DDGS = dried distillers grain with solubles; SBM = soybean meal. Sources: amino acid composition of DDGS from compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n = 62); amino acid composition of SBM and fishmeal from Nutrient Requirements of Fish (NRC, 1993). Dietary requirements from NRC, 1993.

indicates lower amino acid digestibility of wheat and sorghum DDGS compared with the parent grain (Bandegan *et al.*, 2009) or maize DDGS (Urriola *et al.*, 2009; Jacela *et al.*, 2010; Oryschak *et al.*, 2010a).

Feeding value of distillers grain to fish

Fish performance

DDGS had been fed to fish for some time. In fact, the use of DDGS as component in aquafeeds can be traced back to the late 1940s (Phillips, 1949). Formal evaluations of DDGS began in earnest during the last two decades, and can be divided into two phases: prior to the ethanol boom (before 2000–2001), where most research primarily involved the use of co-products from the beverage alcohol/distillery industry (see, for example, Wu *et al.*, 1994, 1996a, b, 1997; Tidwell *et al.*, 2000); and post 2000–2001, where the majority of evaluated DDGS came from the fuel ethanol industry (see, for example, Shelby *et al.*, 2008; Abo-state *et al.*, 2009; Schaeffer, Brown and Rosentrater, 2009; Schaeffer *et al.*, 2010). The chemical composition of DDGS produced from these two processes reflects the composition of the feedstock grain used. The distillery process usually uses a mixture of grains, including barley, rye, wheat and maize, while the fuel ethanol process primarily uses maize as the substrate for fermentation. Also, protein quality from the two processes may differ. As discussed previously, protein quality of DDGS has improved over time, resulting in a feed ingredient that is relatively consistent and highly digestible compared with older generation DDGS. Several factors control the amount of DDGS that can be effectively included in diet formulations for cultured fish. Those factors are related to species requirements and limitations imposed by the nutrient composition of DDGS. High fibre and unbalanced profile of amino acids in DDGS are the main constraints to including greater amounts in aquafeeds.

A summary of available data on feeding DDGS to various freshwater species is presented in Tables 5 and 6. These

studies were essentially designed to test the incremental inclusion rate of DDGS, with the goal of establishing an optimal feeding rate. Most studies included a control diet where no DDGS was fed, allowing for direct assessment of the effect of DDGS on fish performance. To date, DDGS has been evaluated in 8 freshwater species, namely Nile tilapia (*Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), yellow perch (*Perca flavescens*), common carp (*Cyprinus carpio*), freshwater prawn (*Macrobrachium rosenbergii*), red claw crayfish (*Cherax quadricarinatus*) and sunshine bass (*Morone chrysops* × *M. saxatilis*), and two saltwater fish species: milk fish (*Chanos chanos*) and Pacific white shrimp (*Litopenaeus vannamei*). Tilapia and catfish have been the most studied species (Table 5). In many cases, DDGS was used as source of protein and energy, replacing maize meal and SBM at different proportions. DDGS also replaced other feedstuffs such as fishmeal, rice bran, wheat middlings, sorghum meal and meat and bone meal. It appears from the dataset that DDGS is generally accepted by the aforementioned species, with some differences. Tilapia and catfish have been shown to tolerate higher amounts of DDGS in their diets. In fact, feeding DDGS at levels as high as 60 and 70 percent DDGS, supplemented with lysine, resulted in optimal growth and feed efficiency of tilapia (Shelby *et al.*, 2008) and channel catfish (Webster, Tidwell and Yancey, 1991), respectively. In those species, DDGS can be fed at up to 30 percent of the diet without the need for supplemental lysine. For most species, an inclusion rate of 20 percent DDGS seems readily acceptable. Although, the inclusion of DDGS was restricted at 10 percent in studies involving Pacific white shrimp, red claw crayfish and sunshine bass, feeding more than 10 percent may be possible. Another way to improve the utilization of DDGS in fish diets may be achieved through taurine supplementation. It has been shown that taurine is conditionally indispensable in several fish species fed all-plant-protein diets. In fact, replacing fishmeal (which is a

rich source of taurine), with plant proteins (which are usually very low in taurine), can result in taurine-deficient diets. Taurine supplementation has improved weight gain in several fish species, probably through enhanced voluntary feed intake (Takeuchi *et al.*, 2001; Park *et al.*, 2002; Gaylord, Teague and Barrows, 2006; Takagi *et al.*, 2006; Lunger *et al.*, 2007). It is expected that taurine supplementation for diets based on DDGS might improve feed utilization and growth of fish as well.

Tilapia

Published studies evaluating the use of DDGS in tilapia have involved a wide range of fish sizes (initial weight 0.5–190 g; final weight 6.1–907 g). In most studies, DDGS (0–100 percent) replaced maize and SBM in diets containing 0 to 8 percent fishmeal. Overall, feeding DDGS at levels between 15 and 30 percent appeared to maximize weight gain and feed efficiency. The addition of lysine allowed DDGS to be included at even higher levels of 40 to 60 percent. In general, feeding DDGS did not affect the flesh composition of tilapia.

Early studies on the use of DDGS in tilapia were conducted by Wu and colleagues using distillery-derived DDGS. Wu *et al.* (1994) reported that feeding 29 percent DDGS in combination with 6 percent fishmeal, or 22 percent DDGS in an all-plant-protein diet, to juvenile tilapia resulted in similar weight gain and feed conversion ratio as a control diet. Results from that study led Wu, Rosati and Brown (1996) to test whether higher inclusion rates of DDGS would sustain similar growth of tilapia compared with traditional diets. Two diets containing either 35 or 49 percent DDGS at dietary protein concentrations of 40 and 36 percent, respectively, were evaluated in tilapia fry. They found that the 35 percent DDGS diet resulted in similar weight gain and feed efficiency compared with the control diet, which was a 36 percent protein diet. Protein efficiency ratio was, however, higher in the control diet. At 49 percent DDGS, both weight gain and feed efficiency were depressed, indicating a lysine deficiency in diets containing the higher amounts of DDGS. Because lysine is the most limiting amino acid in DDGS-based diets, the addition of supplemental lysine may allow for greater DDGS inclusion levels. This question was investigated by Wu, Rosati and Brown (1997), who fed tilapia fry diets containing from 63 to 82 percent DDGS with added lysine. Overall, they found that, regardless of lysine supplementation, growth was negatively affected by high DDGS concentrations. In contrast, feed and protein efficiencies were similar for the 67 percent DDGS diet and the control diets.

In another study, Tidwell *et al.* (2000) evaluated the growth of juvenile tilapia fed diets consisting of pelleted or unpelleted DDGS (100 percent) in pond polyculture with freshwater prawn. Feeding either form of DDGS resulted in

a 24 percent decrease in weight gain and 0.5 unit increase in feed conversion ratio compared with a commercial catfish diet. The economic efficiency (feed cost/weight gain), however, showed savings of US\$ 0.29 and 0.40 per kg of fish produced, respectively, for pelleted and unpelleted DDGS, compared with the control diet.

To improve the dietary amino acid supply to the fish, one strategy is to feed DDGS as a blend with other proteins that are particularly rich in lysine. In this regard, Coyle *et al.* (2004) evaluated different protein blends in diets for juvenile hybrid tilapia. DDGS was included at 30 percent, with a combination of different protein sources, including fishmeal (8 percent), meat and bone meal (26 percent) and SBM (46 percent). They concluded that feeding DDGS with SBM resulted in lower weight gain and higher feed efficiency compared with the other protein combinations.

Additional studies (Lim *et al.*, 2007; Shelby *et al.*, 2008) evaluated the utilization of high levels of DDGS and whether supplementation with lysine would mitigate the associated negative effects on growth. Lim *et al.* (2007) found that optimal performance of juvenile tilapia was obtained at 20 percent DDGS without added lysine, while the addition of lysine to diets containing 40 percent DDGS improved feed utilization but not weight gain. Shelby *et al.* (2008), however, successfully included up to 60 percent DDGS with added lysine to diets containing 8 percent fishmeal, resulting in similar weight gain and feed efficiency compared with a control diet based on maize and SBM. These observations were confirmed by Abo-state, Tahoun and Hammouda (2009), who found that including up to 55 percent DDGS with added lysine in a 10 percent fishmeal diet resulted in even better weight gain and protein utilization by tilapia fingerlings compared with an SBM-based diet.

Recently, Schaeffer, Brown and Rosentrater (2009) found that weight gain, feed efficiency and fillet yield were adversely affected when DDGS was fed in excess of 30 percent of the diet, but their diets included no supplements. To more closely define the optimum inclusion rate for DDGS, Schaeffer *et al.* (2010) evaluated growth performance of juvenile tilapia fed diets with amounts of DDGS varying from 17.5 to 27.5 percent. They reported poorer growth of tilapia fed DDGS-based diets, and that feeding 20 percent DDGS resulted in maximum growth among the DDGS diets, although this corresponded to only 70 percent of that obtained with the commercial diet. The commercial diet contained 15 percent fishmeal, while the DDGS diets had 5 percent fishmeal.

It is clear that tilapia can effectively utilize DDGS; however, the large variability in the response of tilapia to feeding DDGS-based diets may indicate issues of consistency and quality of DDGS from different sources. Moreover, amino acid supplementation may be one way to improve the resulting performance of DDGS-based diets.

TABLE 5
 Summary of studies evaluating the effects of feeding distillers grain products on growth performance, feed utilization and flesh composition in different fish species

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine ⁽¹⁾ (%)	Optimum ⁽²⁾ (%)	Flesh composition	Reference ⁽³⁾
Nile tilapia <i>Oreochromis niloticus</i>	34.9–67.7	0–27.5	Maize and SBM	55	5	no	17.5	–	Schaeffer <i>et al.</i> , 2010.
	6.7–11	0–40	Maize and SBM	42	5	no	20	–	Schaeffer, Brown and Rosentrater, 2009.
	2–23	0–55	Maize and SBM	70	10	0–0.4	28/55	–	Abo-state, Tahoun and Hammouda, 2009.
	6.7–68.6	0–60	Maize and SBM	84	8	0.9	up to 60	–	Shelby <i>et al.</i> , 2008.
	9.4–60.5	0–40	Maize and SBM	70	8	0–0.4	20/40	Whole body protein decreased at 40%	Lim <i>et al.</i> , 2007.
	2.7–68.5	0–30	FM and SBM	70	0–8	no	30	No effect	Coyle <i>et al.</i> , 2004.*
	26–120	0–100.00	–	84	0	no	–	No effect	Tidwell <i>et al.</i> , 2000.
	0.5–11.4	0–82	CGF and SBM	56	0	0.25–0.75	none	–	Wu, Rosati and Brown, 1997.*
	0.4–20.9	0–49	Maize	56	0	no	35	–	Wu, Rosati and Brown, 1996.*
	30–122.4	19–29	Maize and SBM	103	0–6	no	29	–	Wu <i>et al.</i> , 1994..
Hybrid tilapia <i>O. aureus</i> x <i>niloticus</i>	1.5–6.1	0–40	FM and wheat	90	3	0.4	Up to 40	–	US grains Council, 2007a.
	190–907	0–15	Maize and rice bran	120	0	no	Up to 15	No effect	US grains Council, 2006.
	9.1–80.4	0–30	Maize, SBM, wheat midds	56	5	0.3	30	Fillet protein decreased	Li, Oberle and Lucas, 2011.
	12.6–156.7	0–30	Maize and SBM	63	0	0.3–0.39	10/30 ⁽⁴⁾	Fillet fat increased, Protein decreased	Li <i>et al.</i> , 2010a.
	86–491	0–30	Maize, SBM, wheat middlings	150	0	0.1–0.2	Up to 30	No effect	Zhou <i>et al.</i> , 2010a.
	1.2–8.7	0–30	Maize, SBM, wheat middlings	56	0	0.2	30	–	Zhou <i>et al.</i> , 2010b.
	13.3–67.1	0–40	Maize and SBM	84	8	0.4	40	Whole body fat increased	Lim, Yildirim-Aksoy and Klesius, 2009.
	48–1227	0–40	SBM and wheat midds	330	1	0.80–0.28	30/40	Fillet fat increased	Robinson and Li, 2008.
	33–226	0–30	Maize and SBM	110	8	no	30	No effect	Webster <i>et al.</i> , 1993.*
	12.4–54.5	0–35 ⁽⁵⁾	FM and maize	84	0	0–0.4	35/35	–	Webster <i>et al.</i> , 1992.*
10–79.3	0–70	Maize and SBM	84	10	0–0.4	35/70	Whole body protein decreased and fat increased	Webster, Tidwell, and Yancey, 1991.*	

TABLE 5 (Cont'd)

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine ⁽¹⁾ (%)	Optimum ⁽²⁾ (%)	Flesh composition	Reference ⁽³⁾
Rainbow trout <i>Oncorhynchus mykiss</i>	36.8–186.5	0–4 ⁽⁶⁾	SBM	84	31–33	no	4	–	Thiessen, Campbell and Tyler, 2003.
	49.8–96.2	0–22.5	In combination with CGM, replaced FM and wheat flour	42	7.5–22.5	0–1.23	15/22.5	Whole body fat decreased at 22.5% without Lys but not when Lys was added	Cheng and Hardy, 2004a.
	21–158.4	0–30	In combination with CGM, replaced FM and wheat flour	84	0	no	30 ⁽⁷⁾	Whole body protein decreased and fat increased	Stone et al., 2005.
Yellow perch <i>Perca flavescens</i>	19.1–54.3	0–50	SBM and Celufil	126	24	no	40	No effect	Schaeffer, Brown and Rosentrater, 2011..
Milkfish <i>Chanos chanos</i>	17.8–93.2	0–40	SBM, FM and wheat	–	2	0.3	20	–	US grains Council, 2007a.
Common carp <i>Cyprinus carpio</i>	41–168	0–15	SBM and rice bran	120	5	no	Up to 15	No effect	US grains Council, 2007b.
Freshwater prawn <i>Macrobrachium rosenbergii</i>	0.5–41.4	0–40	Maize, SBM, FM	105	0–7.5	no	40	–	Tidwell et al., 1993.*
Pacific white shrimp, <i>Litopenaeus vannamei</i>	0.45–25	0–10	Sorghum and FM	63	0	no	Up to 10	–	Roy et al, 2009.
Red claw crayfish <i>Cherax quadricarinatus</i>	0.12–4.2 5.75–62.3	0–10 0–30	FM In combination with other plant proteins, DDGS replaced FM	56 97	0 0	no no	Up to 10 Up to 30	– Tail muscle protein increased	de Yta et al., 2012. Thompson et al., 2006.
Sunshine Bass <i>Morone chrysops</i> x <i>M. saxatilis</i>	15–69.7	0–10	Maize, SBM, MBM	56	0	no	Up to 10	No effect	Webster et al., 1999.*

Notes: DDGS = distillers dried grains with solubles; SBM = soybean meal; FM = fishmeal; CGM = maize gluten meal; MBM = meat and bone meal. (1) Lysine needed to achieve the optimal performance. (2) Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. When two optimum concentrations are given, the highest value corresponds to optimum concentration when lysine was added. (3) * Indicates DDGS from alcohol distilleries, not fuel-based DDGS. (4) 10% for distillers solubles or distillers solubles from maize endosperm; 30% for DDGS. (5) Included at fixed rate with varying SBM levels, both replacing fishmeal and maize. (6) Thin distillers solubles. (7) Pellets containing DDGS processed either by cold pelleting or extrusion were tested: 20% inclusion of DDGS with cold pelleting resulted in similar gain weight and lower feed efficiency as control, but the inclusion of DDGS at all levels resulted in inferior performances when the diets were extruded at 130 °C.

TABLE 6
Summary of studies evaluating further aspects of feeding DDGS in different fish species

Species	Key findings	Reference ^c
Nile tilapia <i>Oreochromis niloticus</i>	The addition of up to 150 mg/kg of phytase to a 28% DDGS diet increased weight gain and feed utilization at 75 mg/kg Dietary DDGS, at levels of 0, 10, 20 and 40% in diets, had no effect on haematology, immune responses, or resistance of Nile tilapia to <i>S. iniae</i> infection. DDGS had no effect on immune function or disease resistance.	Tahoun, Abo-State and Hammouda, 2009. Lim <i>et al.</i> , 2007. Shelby <i>et al.</i> , 2008.
Channel catfish <i>Ictalurus punctatus</i>	Fish fed 20–40% DDGS diets had increased total serum immunoglobulin, and those fed the 30% DDGS diet had significantly increased antibody titres 21 days following <i>E. ictaluri</i> challenge. Organoleptic evaluation of filets indicated higher intensity of fat complex flavour for fish fed graded amounts of DDGS.	Lim, Yildirim-Aksoy and Klesius, 2009. Webster <i>et al.</i> , 1993.
Rainbow trout <i>Oncorhynchus mykiss</i>	Fractionation of wheat DDGS using sieving increased digestibility of DM and energy nutrient content in rainbow trout. Phytase supplementation in diets containing 15% DDGS improved digestibility of dry matter, fat and some minerals. Replacing 50% of fishmeal with SBM and 1.65 g MHA/kg in a diet containing 18.5% of DDGS improved weight gain, FCR and apparent retention of crude protein and phosphorus.	Randall and Drew, 2010. Cheng and Hardy, 2004b. Cheng, Hardy and Blair, 2003.
Sunshine Bass <i>Morone chrysops</i> × <i>M. saxatilis</i>	Digestibility of dry matter and organic matter, but not protein and lipid, with DDGS diets were less than those with diets consisting of fish and SBM.	Thompson <i>et al.</i> , 2008.

Notes: MHA is a feed supplement which contains methionine; FCR = feed conversion ratio.

Channel catfish

In most channel catfish studies, DDGS was included in place of a combination of SBM and maize. These studies agreed that DDGS is highly acceptable for channel catfish at levels in excess of 30 percent. Also, supplementation with lysine or the presence of fishmeal, or a combination, further increased the potential for inclusion rate of DDGS up to 40 percent or even higher. Filets from fish fed DDGS appeared to be relatively low in protein and high in fat content, reflecting the composition of DDGS.

Early studies in catfish reared in recirculating systems and floating cages (Webster, Tidwell and Yancey, 1991; Webster *et al.*, 1992, 1993) showed successful feeding of DDGS up to 35 percent, which could be increased to 70 percent in a diet that contained 10 percent fishmeal and supplemental lysine. Webster, Tidwell and Yancey (1991) demonstrated that a blend of DDGS and SBM could be used to replace all of the fishmeal in the diet of juvenile channel catfish. The efficacy of feeding high amounts of DDGS in pond or recirculating systems was confirmed in recent studies (Li *et al.*, 2010a; Li, Oberle and Lucas, 2011; Zhou *et al.*, 2010a, b; Lim, Yildirim-Aksoy and Klesius, 2009; Robinson and Li, 2008). From these studies it can be concluded that feeding DDGS at levels up to 35 percent with supplemental lysine is feasible in an all-plant-protein diet.

New fractionation techniques being used in the ethanol industry offer the aquafeed industry new opportunities as well as challenges. Novel co-products often contain high crude protein concentration, which makes them more suitable for aquafeeds. Li *et al.* (2010a) showed that feeding HPDDG and distillers solubles at 20 and 10 percent, respectively, as part of an all-plant-protein diet resulted in improved weight gain and feed efficiency.

Rainbow trout

It is thought that DDGS has limited nutritional value for salmonids because of its high content of non-nutritive components, such as non-starch polysaccharides (NSP) and pigments. Conversely, the few available studies (Stone *et al.*, 2005; Cheng and Hardy, 2004a) showed some success in feeding DDGS to rainbow trout. These studies have demonstrated that DDGS can partially replace fishmeal when fed with maize gluten meal (CGM) and supplemental lysine. More specifically, Stone *et al.* (2005) evaluated the effects of feeding DDGS (0–30 percent) and pellet processing method on growth and feed efficiency of rainbow trout. They found that when cold pelleting was used, weight gain was maintained up to 30 percent DDGS, but feed efficiency was depressed at all DDGS inclusion levels. In contrast, feeding DDGS resulted in inferior performances when the diets were extruded at 130 °C.

In another study, Cheng and Hardy (2004a) reported that 50 percent of the fishmeal could be replaced by feeding 15 percent DDGS with appropriate amounts of CGM. The inclusion rate was increased to 30 percent when the diets were supplemented with lysine.

On a different tack, Thiessen, Campbell and Tyler (2003) investigated the use of thin distillers solubles as a palatability enhancer in rainbow trout fed different proteins. The inclusion of 4 percent thin distillers solubles did not promote any additional appetite or growth of rainbow trout.

Other species

The value of DDGS in other species cannot yet be firmly established, since for most species, only one study can be found in the literature. Furthermore, in most cases,

the use of DDGS was restricted to relatively low levels. In yellow perch, Schaeffer, Brown and Rosentrater (2011) reported that the inclusion of DDGS up 40 percent to partially replace SBM resulted in maximum growth and feed efficiency. Such performances were probably possible because of the high inclusion of fishmeal in those diets (24 percent). In sunshine bass, replacing maize meal with DDGS at 10 percent resulted in similar weight gain and feed efficiency (Webster *et al.*, 1999).

In its efforts to expand the use of DDGS in Asian aquaculture, the United States Grains Council led multiple experiments in major fish species grown in Asia, such as tilapia, common carp and milkfish. Farm studies have demonstrated that DDGS can be effectively fed at up to 15 and 20 percent, respectively, for common carp and for milkfish (U.S. Grains Council, 2006, 2007a, b).

Studies in crustacean species suggest that DDGS can be a viable source of protein to partially replace common protein sources such as fishmeal and SBM. Tidwell *et al.* (1993) evaluated the inclusion of 40 percent DDGS with SBM to partially or completely replace fishmeal for prawns grown in ponds. The fishmeal was reduced from 15 to 0 percent of the diet. Prawns fed DDGS diets had similar survival, yield per ha and feed conversion ratio compared with prawns fed the control diet with 15 percent fishmeal.

In Pacific white shrimp, Roy *et al.* (2009) reported similar weight gain, but lower biomass due to a tendency for higher mortalities for shrimps fed 10 percent DDGS compared with other feed alternatives, including poultry co-products, fishmeal and pea meal.

Two studies are available for red claw crayfish. Thompson *et al.* (2006) evaluated two levels of DDGS (18.3 and 30 percent) in diets with or without fishmeal. As DDGS increased in the diet, SBM increased and both sorghum and fishmeal decreased. They reported that feeding DDGS with SBM was equally effective in maintaining growth and feed efficiency as diets containing fishmeal. In another study, de Yta *et al.* (2012) fed the same dietary treatments previously evaluated for white Pacific shrimp by Roy *et al.* (2009) and found that similar to white shrimp, red crayfish can be fed a diet that contains 10 percent DDGS.

Flesh nutritional characteristics

Available data (Tables 5 and 6) suggest that feeding DDGS to various fish species is associated with alterations primarily in protein and fat contents of the final fish flesh. Feeding DDGS appears to increase fat content and decrease protein content, and these changes occurred either disjointedly or simultaneously (see, for example, Li, Oberle and Lucas, 2011; Li *et al.*, 2010a; Lim, Yildirim-Aksoy and Klesius, 2009.; Robinson and Li, 2008; Lim *et al.*, 2007; Stone *et al.*, 2005; Cheng and Hardy, 2004a; Wu *et al.*, 1996; Webster, Tidwell and Yancey, 1991). In other instances, the

flesh composition remained unchanged (Schaeffer, Brown and Rosentrater, 2011; Tidwell *et al.*, 2000; Webster *et al.*, 1993, 1999). High fat concentrations and unbalanced amino acid profiles in the DDGS have been reflected in the flesh of fish fed DDGS-based diets. Thus, to mitigate some of these effects, dietary adjustments are necessary when feeding DDGS to various fish.

Organoleptic evaluations of fish fed DDGS-based diets are limited. Wu *et al.* (1996) found no differences in flavour characteristics of cooked tilapia, except a decline in "sweet" intensity for fish receiving 29 percent DDGS in their diets. Similarly, Webster *et al.* (1993) concluded that feeding DDGS to channel catfish had no adverse taste effects.

One of the concerns of feeding high amounts of DDGS is the negative impact on fillet pigmentation. DDGS contains, on average, 37 ppm of the xanthophyll pigments lutein and zeaxanthin, and this concentration varies among sources due to differences in heat treatment during drying of distillers grain (Salim, Kruk and Lee, 2010). Yellow pigments in DDGS will transfer to muscle tissues, which may render the final product less marketable. Li, Oberle and Lucas (2011) demonstrated that these pigments can be completely removed following the extraction of DDGS with ethanol. Feeding such products resulted in fillets with similar colouration to those from fish fed a SBM-based diet.

Digestibility

Digestibility coefficients are important for estimating the energy value and optimizing the use of ingredients in feeds. These are particularly important for co-products of the fuel ethanol industry, given the large variability associated with these materials. Evaluation of nutrient digestibility from DDGS in monogastric animals (swine) showed that digestibility of dry matter, energy, protein and lysine are low compared with traditional feedstuffs such as maize and SBM (Shurson, 2006; Stein *et al.*, 2006).

Information on DDGS nutrient digestibility in fish is rare. Thompson *et al.* (2008) compared nutrient digestibility from different feeds in sunshine bass. They reported very low digestibility coefficients for dry matter and organic matter (<15 percent) compared with SBM and fishmeal, which exceeded 40 and 60 percent, respectively. Protein and lipid digestibility for DDGS were 65 and 69 percent, respectively. Protein digestibility exceeded 84 percent for fishmeal and SBM, while lipid digestibility averaged 92 percent for fishmeal and 57 percent for SBM. Low digestibility of nutrients can increase faecal output and may deteriorate fish culture water quality. To date, there are no reports on nutrient digestibility from DDGS in omnivorous fish.

Fractionation techniques recently employed in ethanol production have resulted in new co-products, generally with higher protein, lower fibre and lower fat contents

compared with traditional DDGS. Thus such products may have improved digestibility and nutritive values compared with conventional DDGS. For example, Randall and Drew (2010) evaluated the digestibility of nutrients from different fractions obtained by sieving of wheat DDGS. Sieving increased crude protein and decreased fibre concentrations. In addition, sieving improved digestibility of dry matter and gross energy, whereas digestibility of ether extract and protein were unaffected and high, exceeding 90 and 100 percent, respectively. The use of enzymes such as phytase can improve the nutritive value of the feed. Studies in poultry showed that in addition to improvement in phosphorus availability, supplementation with phytase improved protein and amino acid digestibility and availability in poultry diets (Rutherford *et al.*, 2004). Similarly, Cheng and Hardy (2004b) evaluated different doses of microbial phytase in the diets of rainbow trout that contained 30 percent DDGS. Improvement in dry matter, ether extract, Ca, Mg, phytate-P, total P, Mn, Cu and Zn were observed when adding phytase as low as 300 FTU (phytase units)/kg of diet. Protein digestibility from these diets was high and similar to the reference diet, averaging 90 percent. Recently, Tahoun, Abo-State and Hammouda (2009) showed that feed utilization was improved by the addition of 75 mg/kg of phytase to a 28 percent DDGS diet fed to Nile tilapia.

Immune function

DDGS contains approximately 3.9 percent yeast cell biomass (Ingledew, 1999). Yeast components such as beta-glucans, mannan-oligosaccharides, chitin, proteins, nucleotides, vitamins and trace minerals are important in modulating immune function. The potential of DDGS to stimulate immune function in fish is unclear. For example, feeding DDGS had no effect on immune function or resistance to bacterial infection in Nile tilapia (Shelby *et al.*, 2008; Lim *et al.*, 2007). In channel catfish subjected to *Edwardsiella ictaluri* challenge, feeding DDGS increased immunoglobulin, antibody titre and days to first mortality. Mortality was decreased, suggesting improved resistance to pathogen infection (Lim, Yildirim-Aksoy and Klesius, 2009). The authors suggested further investigation of the immunostimulatory effect of DDGS and the identification of potential active components that may be present in DDGS.

DISTILLERS GRAIN: ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Overall, it appears that DDGS can be an effective source of energy and protein for fish. DDGS is not, however, recommended to be a direct, complete substitute for fishmeal or SBM. It is most effective when it replaces a combination of SBM and maize. Furthermore, the inclusion of DDGS is facilitated by the use of fishmeal and synthetic amino acids

(primarily lysine) to improve the overall supply of amino acids to fish.

The use of DDGS in aquafeeds does present some challenges and limitations. Quality variation remains a major shortfall to using DDGS. Fish require high quality and dependable sources of nutrients to achieve high performance levels. DDGS can fill such requirements, provided the source is known, of consistent quality, and access to nutrient composition is available on a regular basis to nutritionists. DDGS also has some nutritional limitations when it is fed to fish. The high fibre content of DDGS, coupled with low digestibility of some nutrients, may limit its use in some fish species where nutrient-dense feeds are required. In addition, in recirculating aquaculture systems, DDGS use may also affect water quality because of potential increased faecal output.

When technical aspects are considered, handling of DDGS can pose some logistical problems because of the inherent physical properties of this granular bulk solid. Low bulk density and particle stickiness, which can lead to flowability problems, are the major challenges to the use of DDGS in animal feeds. These issues create transportation inefficiencies at all feed manufacturing levels, from transportation to feeding systems at the farm. These issues can be managed by implementing approaches such as manipulation of particle size and moisture content, or by the addition of flow agents. In aquaculture, feed is commonly manufactured using extrusion processing. Since DDGS contains high fibre and fat contents, coupled with a low starch level, extrusion of feeds containing DDGS can pose some difficulties. Once gelatinized (due to high processing temperatures), starch acts as a binder. Our research has shown that these limitations can be surmounted through the understanding of different interactions between process parameters and feed material. We have evaluated the extrusion of aquafeeds based on DDGS under a variety of processing conditions. Generally, as DDGS increases in the blend, decreases in pellet durability, expansion ratio, mass flow rate (throughput) and an increase in unit density and sinking velocity (i.e. no floatability) are observed. See, for example, Ayadi *et al.* (2011); Chevanan, Rosentrater and Muthukumarappan (2010); and Kannadhasan *et al.*, (2010). Overall, it can be concluded that optimum pellets in terms of bulk density, durability and water stability can be obtained when DDGS is included at about 20 percent of the diet, which coincide with the optimum feeding level for most fish species. Improvements in pellet quality at high levels of DDGS are possible by the addition of different starches and binders.

Other challenges include mycotoxins, antibiotics and pigmentation. DDGS may contain mycotoxins if the parent grain is contaminated, although this risk is

minimal in the Midwest United States, where most ethanol production plants are located. In addition, more ethanol plants have implemented stricter standards for grain selection. Again, knowing the source of DDGS and test results, especially during growth seasons with most risk of mycotoxin development, are important for safe utilization of DDGS. Recently, concerns about antimicrobial residues in DDGS have surfaced. Antimicrobials, such as penicillin, virginiamycin, erythromycin and others, are commonly added to the fermentors to control bacterial infections with the goal to optimize ethanol production by yeasts. These antibiotics can end up in the DDGS; however, it is believed that they will be completely deactivated under the extreme temperatures and pH conditions applied during ethanol production. In addition, heat treatments associated with extrusion cooking could further inhibit such substances. Thus, the issue of antimicrobials in DDGS, although serious, is more speculation rather than a reality in animal feeds. Pigmentation of tissues is also of concern when feeding DDGS, especially to salmonids. Feeding DDGS to salmonids is believed to alter flesh pigmentation from the typical pink colour to a less desirable yellowish colour, but to date there are no published studies evaluating the effect of DDGS on the pigmentation of fish tissues.

Finally, as the ethanol industry increases the efficiency of producing ethanol, different distillers co-products will become available, creating more challenges and opportunities for the aquafeeds industry. These products are expected to be more nutrient dense, as the fibre fraction can be further fermented and the fat extracted, leading to products composed mainly of protein and ash. Such products may be more compatible with fish requirements, but will need research to characterize them and assess their nutritional value and efficacy for different fish species.

PROPERTIES OF CRUDE GLYCERINE

The principal co-product of biodiesel production is crude glycerin. Common feedstocks used in the biodiesel industry include pure or waste vegetable oils, or a mixture, and rendered animal fats. Refining of crude glycerin is often limited to large scale biodiesel producers, which make high purity glycerol for applications in the food, pharmaceutical and cosmetic industries. Small-scale producers generally limit the purification process to the removal of excess alcohol to yield a low value co-product with limited uses (Thompson and He, 2006).

Physical and chemical properties

Crude glycerine contains impurities, including spent catalysts, residual methanol, methyl esters, oils and fats, soaps, free fatty acids and various minerals such as Ca, Na, Cl, K, Mg, P and S (Thompson and He, 2006; Dasari, 2007). Some of the physiochemical properties of crude glycerine are presented in Table 7. Considerable variation exists among crude glycerine sources, largely because of differences in the biodiesel production processes and the parent feedstock used. Mader (2011) showed that crude glycerine derived from animal fats contained less glycerol and more impurities than that derived from vegetable oil feedstocks. Common glycerol content is between 75 and 85 percent; however, glycerol content as low as 38.4 and as high as 96.5 percent of the total crude glycerine can be found on the market (Hansen *et al.*, 2009). Other major constituents are moisture, fat and a variety of minerals. Residual methanol is usually found at low concentration (<100 ppm); however, samples containing higher concentration (>15 percent) can be found, creating some health concerns when crude glycerine is fed to livestock (Hansen *et al.*, 2009). The USDA Food and Drug Administration (FDA) limits methanol content

TABLE 7
Physiochemical properties of crude glycerine

Item	n	Average	Min.	Max.	Reference
Pure glycerol (%)	39	78.58	38.4	96.5	1, 2, 3, 4, 5, 6, 7, 8, 9
Moisture (%)	27	8.20	0	24.37	4, 5, 6, 7, 8, 9, 10
Protein (%)	10	0.26	0.05	0.82	1, 3, 4, 5, 9
Fat (%)	11	5.54	0.12	15	1, 3, 4, 5, 10
Ash (%)	31	4.15	0	29.4	1, 3, 4, 5, 6, 7, 9
Na (%)	2	1.23	1.2	1.26	5, 9
Cl (%)	2	1.78	1.7	1.86	5, 9
GE (KJ/kg)	9	18340	15119	20510	1, 5, 10
pH	25	6.20	2	10.8	4, 5, 6, 7, 10
Methanol (%)	31	1.72	0.0009	14.99	2, 3, 4, 5, 6, 7, 8, 10
Density (g/cm ³)	11	1.20	1.07	1.26	6
Viscosity (4.45 °C, cSt)	6	60.00	82	38	10
Viscosity (40 °C, cSt)	2	8.60	8.8	8.46	1
Colour (c.u.)	2	7.25	3.5	11	10

Key to references: 1. Thompson and He, 2006; 2. Dasari, 2007; 3. Groesbeck *et al.*, 2008; 4. Lammers *et al.*, 2008a; 5. Lammers *et al.*, 2008b; 6. Hansen *et al.*, 2009; 7. Kerr *et al.*, 2009; 8. Mach, Bach and Devant, 2009; 9. Gunn *et al.*, 2010; 10. Mader, 2011.

TABLE 8
Summary of studies evaluating the effects of feeding glycerol to different fish species

Fish species	Glycerol inclusion	Ingredient replaced	Optimum (%) ^a	Flesh composition	Reference
Channel catfish (<i>Ictalurus punctatus</i>)	0-20	Maize	10	Fillet fat decreased	Li <i>et al.</i> , 2010b ⁽¹⁾
Rainbow trout (<i>Oncorhynchus mykiss</i>)	0-12	Wheat middlings	–	No difference	Menton, Slinger and Hilton, 1986 ⁽²⁾

Notes: Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. (1) Glycerol from biodiesel production. (2) Free glycerol included in low energy diets compared with a diet with similar energy density.

to 0.015 percent (150 ppm) in the final animal feed (FDA, 2006). Because the boiling point of methanol is 64.4 °C (Lide, 2001), it is believed that extrusion processing, commonly used in preparing fish feed, could eliminate any residual methanol found in crude glycerine. The colour of crude glycerine can range from clear to dark, reflecting pigments and compounds found in the parent feedstock.

Feeding value of crude glycerine to fish

There has apparently been only one published study (Table 8) that has evaluated the use of crude glycerine from the biodiesel industry in fish (Li *et al.*, 2010b). This study used crude glycerine as a source of energy to replace maize meal in the diet of channel catfish. They determined that a level of 10 percent was optimal for weight gain and feed efficiency; fillet fat content decreased at levels in excess of 5 percent. Pure glycerol was evaluated in another study in rainbow trout (Menton, Slinger and Hilton, 1986). Replacing wheat middlings by free glycerol up to 12 percent of the diet resulted in comparable weight gain, feed efficiency and carcass composition as fish fed a diet with similar energy density. The authors also found that glycerol can be an effective precursor for gluconeogenesis, but not for lipogenesis; however, rainbow trout cannot efficiently utilize glucose as a source of energy.

CRUDE GLYCERINE ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Studies in other monogastric species suggest that crude glycerol can be a viable energy source. However, considering the current level of research in fish nutrition, which is essentially non-existent, an optimum level can not be recommended at this time. More studies are required to determine the efficacy of crude glycerol in major species such as tilapia, channel catfish, rainbow trout and yellow perch. As with other co-products, variability is an issue that hinders the use of crude glycerol in aquafeeds. Residual methanol is a potential safety hazard that needs to be addressed as well. Considering the physical characteristics of crude glycerol, other issues that should be evaluated include extrusion processing behaviour, handling and storage characteristics, potential corrosive effects, and the effect of feeding glycerol on flesh quality and health of fish.

CONCLUSIONS

DDGS and glycerine, co-products from the fuel ethanol and biodiesel industries, respectively, appear to be viable alternative feed ingredients for aquafeeds. DDGS is best used to replace a portion of SBM and maize in the diet. Because of variability issues and inherent nutritional limitations of DDGS, an inclusion level of up to 20 percent appears to be safe for most omnivorous fish species, whereas 10–15 percent is recommended for carnivorous fish such as rainbow trout. Specifically, DDGS can effectively be included at concentrations of 20 to 40 percent for channel catfish, tilapia and yellow perch, but at lower concentrations (10–15 percent) for rainbow trout, bass and some crustacean species. Nonetheless, when economically viable, supplementation with lysine will allow for higher DDGS inclusion rates. It has also been shown that DDGS can be included at high concentrations (up to 40 to 60 percent) while maintaining feed quality in terms of water stability and pellet durability when DDGS is part of extruded aquafeeds. Interestingly, the optimal inclusion level of DDGS in aquafeeds for superior pellet quality appears to be around 20 percent, which coincides with optimal fish performance in most species. In some species, nutritional characteristics of the final products can be altered. Lower protein and higher fat contents are usually observed when feeding DDGS above optimal levels. The efficacy of crude glycerine in fish diets is less evident. Very limited information suggests that glycerine might be used as an energy source. However, comprehensive investigation is still needed to address the use of glycerol as a feed ingredient for major fish species. The effect of glycerol on feed processing, final product quality, metabolism and health of fish are some areas that need further research before glycerine can be efficiently and safely used in aquafeeds. Biofuels will clearly continue to play a key role in the global energy portfolio over the coming years, and co-products such as DDGS, glycerine, as well as other new co-products yet to be developed, will continue to grow in quantity. Aquafeeds may be a viable opportunity for their utilization.

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Chapter 24

Cultivation of micro-algae for lipids and hydrocarbons, and utilization of spent biomass for livestock feed and for bio-active constituents

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ABSTRACT

The demand for energy is ever increasing, and concurrently the depletion of fossil fuels has been so rapid that it could lead to an energy crisis in the near future. At the same time, reducing the carbon footprint to mitigate global warming has been a subject of immediate attention. Production of energy through photosynthetic organisms such as micro-algae by harnessing solar energy might be a viable solution to some of these issues. In pursuit of renewable energy sources, efforts worldwide focus on identifying those organisms that can accumulate high quantities of biomass and produce molecules that can be converted to combustible materials. Economically viable processes for large-scale cultivation and downstream processing of biofuel precursors, such as lipids and hydrocarbons, have been a challenge, requiring adoption of technologies needing reduced inputs of energy and chemicals. Prudent energy audits to assess the viability of bio-energy processes are a necessity. The utilization of micro-algae for bio-energy production would be viable only when the whole process has a net energy gain, with complete utilization of algal biomass for biofuel and the co-products thereof used to produce food, feed and chemicals. The spent algal biomass—which is rich in proteins, carbohydrates, minerals and bio-active compounds—is ideal for feed applications. The paper outlines biorefinery approaches to integrated utilization of algal biomass for bio-energy, with co-production of valuable metabolites and nutrients as feed, with full utilization of all the fractions for economic viability of the process. These aspects are dealt with in detail in the various sections to provide a comprehensive overview of micro-algal technology for biofuel programmes *vis-à-vis* feed applications.

INTRODUCTION

Phytoplankton are the most important and major biomass producers in global aquatic ecosystems. Algae are the primary producers, generating approximately 52 000 000 000 tonne of organic carbon per year—almost 50 percent of the total organic carbon produced annually (Field *et al.*, 1998)—through photosynthesis utilizing solar energy and converting CO₂ and other simple inorganic compounds to myriad molecules (Chisti, 2007). These organisms populate the top layers of the oceans and freshwater habitats where they receive sufficient solar radiation for photosynthesis (Hader *et al.*, 1998). Micro-algae are unicellular to filamentous in form, whereas macro-algae or seaweeds are plant-like organisms. Micro-algae lack roots, vascular systems, leaves and stems, and are autotrophic in nature and photosynthetic. They are generally eukaryotic organisms, although cyanobacteria—a prokaryotic assemblage—are included under algae due to

their similar photosynthetic and reproductive properties (Greenwell *et al.*, 2009). The thalli of algae display a wide range of organization, ranging from single cells (*Chlorella* spp.), through motile (*Chlamydomonas* spp., *Dunaliella* spp.), colonial (*Volvox* spp., *Botryococcus* spp.), filamentous (*Spirulina* spp., *Spirogyra* spp.) and plant like (*Chara* spp.) to giant seaweeds (*Postelsia* spp., *Fucus* spp.). (Fritsch, 1935).

Depleting fossil fuel reserves, with associated escalation in petroleum prices, has engendered huge demand for development of technology for renewable fuels from photosynthetic organisms with a smaller carbon foot print and an overall positive energy balance. In this context, micro-algae could be a possible solution. Their ubiquitous distribution and ecological adaptations give them certain advantage over other groups. The ability to cultivate algae under varied conditions, including using non-potable wastewater and operating in marginal areas, coupled with their photosynthetic efficiency and higher surface area

MAIN MESSAGES

- Depletion of fossil fuels is leading to an energy crisis and highlighting the need for development of renewable energy sources
- Micro-algae, being photosynthetic with a high ability to produce hydrocarbons and lipids, offer multiple advantages as a source of bio-energy through harnessing solar energy, with the additional advantages of CO₂ sequestration and providing an eco-friendly alternative to meet energy requirements.
- In addition to providing bio-energy molecules, algae are good source of nutrients and health promoting substances, as well as valuable metabolites that are unique and of high commercial value.
- The algal biomass after extraction of bio-combustible materials can be used as feed for fish, poultry and animals.
- Development of eco-friendly and easily adaptable economic process for mass cultivation of biomass leading to net energy gain and their utilization towards various applications is discussed.
- A biorefinery approach to utilizing various fractions of algal biomass has been proposed to make processes more economical, with a bias towards energy production, biochemicals and feed in an eco-friendly manner utilizing algal biodiversity and harnessing solar energy.

productivity than higher plants, make them a potential and economically viable resource for renewable fuel production (Gouveia and Oliveira, 2009). Large-scale algal cultivation has been developed for various purposes and the biomass can be harvested frequently as algae can have very short doubling times, ranging from 4 to several days. Further, some of the micro-algae accumulate lipids up to 50 percent of the dry weight in certain growth conditions. The lipid accumulation in micro-algae is influenced by light intensity, culture pH, availability of nutrients, dissolved oxygen concentrations and several other environmental factors. Apart from lipids, some micro-algae, such as *Botryococcus*, accumulate long-chain hydrocarbons that have properties similar to petroleum hydrocarbons (Metzger and Largeau, 2005; Dayananda *et al.*, 2007b). Also, certain micro-algae occur in extreme environmental conditions, like brackish and high saline waters, acidic or alkaline lakes, and at chilling temperatures. These extremophilic micro-algae can be exploited for the production of novel compounds of commercial and functional importance.

The preliminary step, and an important part of sustainable micro-algal technology, is extensive germplasm collection and biodiversity screening for the production of lipids and hydrocarbons. Furthermore certain parameters, like biomass productivity, lipid or hydro-carbon content and daily yield, and possibility of co-product generation must be considered for viable micro-algal technology applications (Subramaniam *et al.*, 2010). Micro-algae chosen after initial studies under controlled conditions must be evaluated for their performance in outdoor and scaled-up conditions. Large-scale cultivation methodologies involve optimization of media for high biomass and lipid yields, and adjusting physical parameters like light requirements, culture mixing, supply of CO₂, etc. (Pulz, 2001). Use of simple and inexpensive nutrient sources and re-usability of media should be

considered in assessing potential for sustainable mass cultivation. Apart from mass cultivation, development of simple downstream processing must be critically evaluated, including harvesting procedures, processing the biomass for lipid or hydrocarbon extraction, and converting crude extracts to combustible fuels. Existing technology using energy-intensive methods, such as centrifugation and ultrafiltration for harvesting, and extraction methods like oil expelling or French pressing, has proven unviable for renewable energy production (Brennan and Owende, 2010). Nevertheless, it is increasingly evident that algae can be exploited for nutritionally and nutraceutically important metabolites for food and feed applications owing to their content of vitamins, proteins, pigments, fatty acids, sterols and polysaccharides. The potential of micro-algae as a source of antiviral, anti-tumour, antibacterial, anti-HIV agents and as food additives have also been well established (Cardozo *et al.*, 2007).

Production of bio-energy through photosynthetic systems is gaining strength and has a great future since it is renewable and eco-friendly. However, for any type of bio-energy production, systems need to be developed that operate in an economical manner in terms of total energy gain per unit area. A careful analysis of the present day technologies for utilizing algal biomass for energy production indicate that they do not take into account the total energy audit. One needs to look at the whole process from the point of view of net energy gain, addressing cultivation to utilization of the biomass for the production of lipids, hydrocarbons and other useful constituents. In addition to the target molecules for fuel generation, co-products could be of utility for food and feed, as well as a source of chemicals of importance to humankind. Utilization of sea water would address the water requirements for cultivation of biomass, which otherwise would compete with agriculture, potentially leading to water conflicts. Therefore, utilization

of marine forms and sea water has been advocated as a requirement for utilizing algal biotechnology for biofuels. Utilization of wastewater would also go a long way in not only producing algal biomass for energy but also providing wastewater treatment and bioremediation. Such technologies have the potential to be sustainable and eco-friendly, and could significantly help mitigation of pollution.

This review focuses on identification of suitable organisms for specific ecosystems, with a focus on cultivation in an economically viable manner and utilizing biomass in an eco-friendly approach, for the production of fuel, feed and chemicals. These aspects are dealt with highlighting developments in the respective areas and projecting trends in application technology under five headings: (1) algal biodiversity for the production of lipids and hydrocarbons; (2) large-scale cultivation of micro-algae; (3) downstream processing and conversion to biofuels; (4) use of micro-algae for food, feed and bio-actives; and (5) techno-economic analysis and bio-refinery concepts.

ALGAL BIODIVERSITY FOR THE PRODUCTION OF LIPIDS AND HYDROCARBONS

The ubiquitous occurrence of algae in marine, freshwater and terrestrial habitats with broad chemical diversity is the basis for their industrial and biotechnological applications (Figure 1). Several groups have been working on development of feasible systems for the production of lipids and other precursor molecules from micro-algae (Sheehan *et al.*, 1998; Illman, Scragg and Shales, 2000; Dayananda *et al.*, 2007a; Rodolfi *et al.*, 2009). The United States Department of Energy has initially invested more than US\$ 20 million in an Aquatic Species Program to develop biofuels from micro-algae, with the project, mainly focusing on identification of oleaginous micro-algae and evaluation

of different cultivation methods for the production of renewable fuels (Sheehan *et al.*, 1998). The lipid content of micro-algae varies between 5 and 80 percent on a dry weight basis, depending on the species, strain, growth phase and other environmental factors (Spolaore *et al.*, 2006; Harwood and Guschina, 2009). Micro-algal distribution is influenced by various biotic and abiotic environmental factors, and so bio-prospecting for hyper-lipid-producing strains must recognize local climatic conditions. Some of the parameters important in screening biodiversity are fast growth and tolerance to environmental fluctuations (Mutanda *et al.*, 2011). In natural habitats, the four most important groups of algae in abundance are Green algae (Chlorophyceae), Diatoms (Bacillariophyceae), Blue-green algae (Cyanophyceae) and Golden algae (Chrysophyceae). Green algae and Diatoms are two important classes of micro-algae generally exhibiting high oil productivities on a culture volume basis (Table 1). Extensive review of the biodiversity of these important classes is beyond the scope of this chapter, but many reviews are available, such as Becker (2004) and Singh, Bhushan and Banerjee (2005).

Some of the extreme conditions under which algae thrive are highly acidic or alkaline, hypersaline, with high or freezing temperatures, in high radiation zones or in polluted environments. These extremophiles tolerate such conditions with the help of endogenously produced compounds called extremolytes. Some micro-algal forms known to exist in extreme conditions are *Dunaliella salina* in hypersaline waters (2–5 M NaCl) and *Spirulina* spp. in highly alkaline conditions (optimum around pH 10.5). *Cyanidium caldarium* and *Dunaliella acidophila* are acidophiles with an optimum pH of 2 to 3. Certain psychrotolerant micro-algae growing in polar glaciers have unique lipid compositions with commercial applications (Rajkumar *et al.*, 2010).

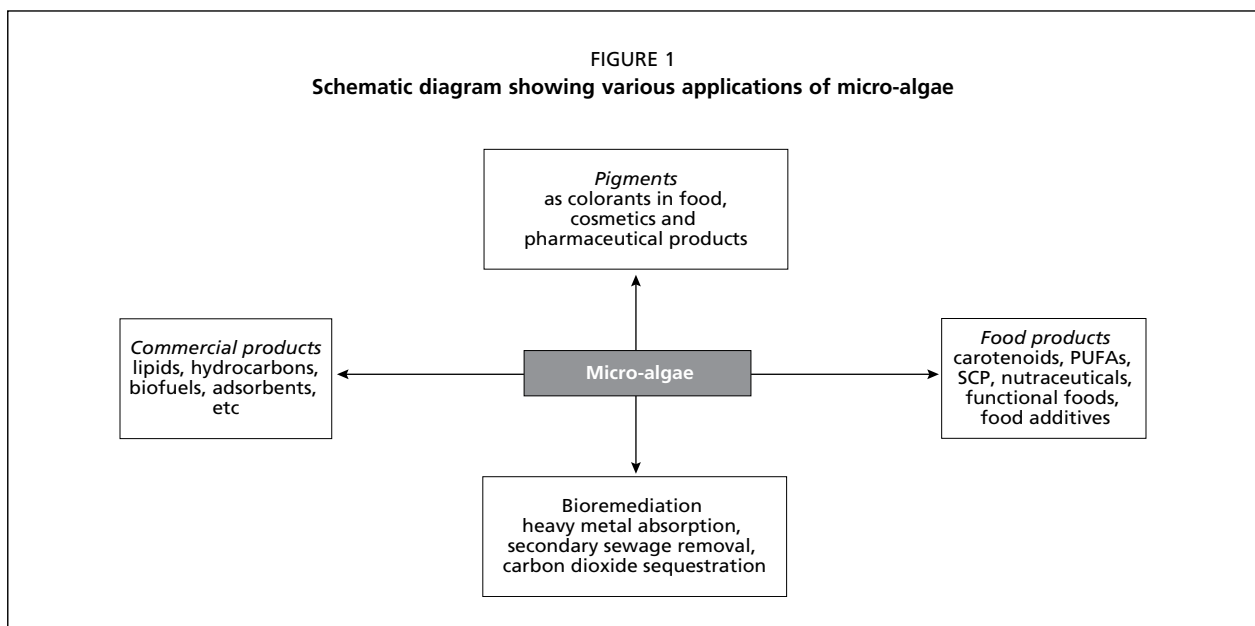


TABLE 1
Hyper-lipid-producing micro-algae

Micro-algae	Class	Lipid content (% dry weight)	Habitat
<i>Botryococcus braunii</i>	Chlorophyceae	25–75	Freshwater
<i>Chlorella vulgaris</i>	Chlorophyceae	30–35	Freshwater
<i>Chlorococcum</i>	Chlorophyceae	19.3	Freshwater
<i>Scenedesmus</i> spp.	Chlorophyceae	21.1	Freshwater
<i>Neochloris oleoabundans</i>	Chlorophyceae	35–54	Freshwater
<i>Cryptocodinium cohnii</i>	Dinoflagellate	20	Marine
<i>Tetraselmis suecia</i>	Chlorophyceae	36.4	Marine
<i>Dunaliella primolecta</i>	Chlorophyceae	23	Marine
<i>Isochrysis</i> spp.	Haptophytes	25–33	Marine
<i>Skeletonema costatum</i>	Bacillariophyceae	17.4	Marine
<i>Chaetoceros muelleri</i>	Bacillariophyceae	21.8	Marine
<i>Chaetoceros calcitrans</i>	Bacillariophyceae	17.6	Marine
<i>Nitzschia</i> spp.	Bacillariophyceae	45–47	Marine
<i>Nannochloropsis</i> spp.	Eumastigophytes	31–68	Marine
<i>Schizochytrium</i> spp.	Thraustochytriidae	50–77	Marine
<i>Monodus subterraneus</i>	Eumastigophytes	30.4	Marine
<i>Thalassiosira pseudonana</i>	Bacillariophyceae	17.4	Marine

Sources: Chisti, 2007; Rodolfi et al., 2009; Illman, Scragg and Shales, 2000; Mutanda et al., 2011.

Dunaliella bardawil has been reported to survive high salt and high radiation levels by accumulating β -carotene (Ben-Amotz and Avron, 1990). Certain cyanobacteria, such as *Chroococcidiopsis* spp., exhibit resistance against desiccation and also high irradiation, which is attributed to the accumulation of radio-responsive pigments and efficient DNA repair mechanisms (Billi et al., 2000). Extremophilic micro-algae offer certain advantages for large-scale cultivation as their growth requirements and conditions are unsuitable for other competing organisms and fight herbivore predation. In fact, the most commercially exploited micro-algae in outdoor cultivation are extremophiles such as *Spirulina* (tolerates pH 10–11) and *Dunaliella* (withstands salt concentrations several times that of sea water). Detailed studies on the use of algal extremophiles for sustainable energy production could help overcome some of the major hurdles in the mass cultivation of micro-algae, as discussed in later sections.

GREEN ALGAL LIPIDS AND HYDROCARBONS

Green algae have been extensively studied for their ability to accumulate lipids. Spoehr and Milner (1949) showed that *Chlorella pyrenoidosa* can accumulate lipid up to 85 percent in its biomass. Wood (1974) reviewed the lipid contents and fatty acid profiles of all the classes of green algae under different culture conditions. *Chlorella vulgaris*, *Chlorella sorokiana*, *Scenedesmus* sp., *Chlorococcum* sp. and *Tetraselmis suecia* have been reported as potential micro-algae for lipid production (Illman, Scragg and Shales, 2000; Chisti, 2007; Rodolfi et al., 2009; Huerlimann, De Nys and Heimann., 2010). Green algae have wide occurrence, grow faster than other groups and grow on simple nutrient media. Major fatty acids present in green algae are

palmitic acid (C16:0), oleic acid (C18:1) and alpha linolenic acid (ALA) (C18:3). The saturated fatty acids occur more in green algae, making them good sources for biodiesel production.

Botryococcus braunii, a colonial freshwater alga, has been extensively characterized for the production of hydrocarbons (Casadevall et al., 1985; Dayananda et al., 2007b). They accumulate long-chain hydrocarbons of >C30 chain length and are categorized into three races. Race A produces odd-numbered fatty acid-derived n-alkadiene type hydrocarbons ranging from C23 to C33. Race B produces unsaturated hydrocarbons called botryococcenes and methyl-branched squalenes. Race L produces tetra-terpenoid hydrocarbons known as lycopadiene (Metzger and Largeau 2005). Hydrocarbon production depends strongly upon the culture conditions, and it ranges from a minimum of 2 percent to a maximum of 86 percent (Dayananda et al., 2006). Apart from hydrocarbons, *B. braunii* is of interest for the production of exopolysaccharides (Bailliez, Largeau and Casadevall, 1985). Our studies have demonstrated anti-oxidant properties of *Botryococcus* biomass through production of lutein (Rao et al., 2006). Hydrocarbon obtained from *B. braunii* when hydrocracked produced a distillate with good fuel properties, comprising 67 percent gasoline fraction, 15 percent aviation fuel, 15 percent diesel fraction and remaining residual oil (Banerjee, Sharma and Chisti, 2002; Dayananda et al., 2006). These fuels are reported to be free from N and S oxides (NO_x and SO_x) after combustion.

Dunaliella spp. (*D. tertiolecta*, *D. salina* and *D. bardawil*) are marine chlorophytes and have been the most studied strain for industrial exploitation, such as for β -carotene production. They accumulate β -carotene up to 14 percent on a dry weight basis (Ben-Amotz, 1995) and glycerol for

osmoprotection in hypersaline to brackish water environments. *D. tertiolecta* can be exploited for lipid production as it accumulates neutral lipids up to 50 percent under stress conditions, and has high CO₂ absorption capacity and fast growth (Oilgae, 2010). The β -carotene obtained from *Dunaliella* spp. can be used as natural food colorant and also in enhancing fish flesh colour and egg yolk colour (Becker 2004). Due to the rich content of β -carotene and lipid accumulation they can even be positioned as nutritional supplements. The carotenoid-rich fraction is composed of all-*trans* and the 9-*cis* isomer of β -carotene, which have high antioxidant activity and are known to prevent some forms of cancer. Carotenoids with their quenching action on reactive oxygen species have an intrinsic anti-inflammatory property, hence *Dunaliella* spp. can replace synthetic carotenoids (Murthy 2005).

DIATOMS AS SOURCES OF LIPIDS

Diatoms are a class of unicellular micro-algae belonging to Bacillariophyceae, and dominant phytoplankton in oceans, contributing up to 25 percent of global primary productivity (Ramachandra, Mahapatra and Karthick, 2009). The diatoms are a rich source of lipids, especially polyunsaturated fatty acids (Tan and Johns, 1996; Lebeau and Robert, 2003). The lipids are accumulated as oil droplets in marine diatoms, which could be explained as a physiological and biochemical adaptation providing cell buoyancy compensating for the heavy siliceous cell wall, and also as storage material against unfavourable conditions (Ramachandra, Mahapatra and Karthick, 2009). Silicon limitation in media is the major trigger for lipid accumulation in diatoms. Mysristic acid, palmitic acid and palmito-oleic acid are the dominant fatty acids in diatoms. Mixotrophic and heterotrophic cultivation of diatoms are being exploited for polyunsaturated fatty acid (PUFA) production. Apart from PUFA production, many diatoms, such as *Chaetoceros muelleri*, *Skeletonema costatum* and *Thalssiosira pseudonana*, are used as a feed source in aquaculture in view of their good fatty acid profiles. The complete genome sequence for two diatoms, *Thalssiosira pseudonana* and *Phaeodactylum tricornutum*, are available, and transgenic systems for many diatoms are well established, like *Navicula* spp. and *Cyclotella* spp., pro-

viding opportunities to improve lipid productivity by genetic engineering (Dunahay, Jarvis and Roessler., 1995).

At present, the production of lipids in general and PUFA in particular by marine and freshwater micro-algae is the subject of intense research and commercial importance. Some of them are industrially exploited as potential sources of eicosapentaenoic acid (EPA), such as *Nitzschia laevis* and *Phaeodactylum tricornutum*. The annual worldwide demand for EPA is about 300 tonne, and fish oil is the major source of PUFAs (Singh, Bhushan and Banerjee, 2005.). However, the search for vegetarian sources of PUFAs and purified micro-algal PUFA as an alternative to fish oil, which is complex to purify and with intense odour, appears promising (Wen and Chen, 2003). Benefits of PUFA supplementation are well understood. One rare PUFA of micro-algal origin is gamma linolenic acid. Gamma linolenic acid (GLA; C18:3) is an isomer of ALA and is present in significant amounts in *Spirulina* spp. GLA has been identified as contributing to prevention of skin diseases, diabetes and reproductive disorders (Gunstone, 1992). PUFAs from micro-algae are incorporated as supplements in infant formula and nutritional supplements (Table 2).

LARGE-SCALE CULTIVATION OF MICRO-ALGAE

The commercial cultivation of micro-algae began with the cultivation of *Chlorella* in Japan in the 1960s, followed by cultivation of *Spirulina* in Mexico and United States in the 1970s. In the last four decades, the industrial biotechnology of photosynthetic micro-organisms has grown tremendously and diversified. Large-scale cultivation systems of micro-algae takes two main forms: open ponds and closed reactors, reflecting the nature of the organism, culture media composition and other parameters, including culture pH, salinity and cultivation conditions.

The main goal of mass cultivation is to achieve higher productivity in terms of biomass for production of a metabolite. The three important factors affecting the mass cultivation of micro-algae are culture depth and related light levels, mixing or turbulence, and biomass density (Grobelaar, 2009). The economics of large-scale cultivation are dictated by maximal yields and high rates of production. For industrial production systems, the micro-algae are generally grown

TABLE 2
Polyunsaturated fatty acids (PUFAs) produced from micro-algae

PUFA	Application	Micro-algal source
Gamma linolenic acid (GLA) C-18:3 – omega 3	Nutritional supplements and infant foods	<i>Spirulina</i> spp.
Arachidonic acid (AA) C-20:4 – omega 6	Nutritional supplements, immunomodulatory therapeutics	<i>Porphyridium creuntum</i> , <i>Parietochloris</i> spp.
Eicosapentaenoic acid (EPA) C-20:5 – omega 3	Nutritional supplements and aquaculture	<i>Nannochloropsis</i> spp., <i>Phaeodactylum</i> spp., <i>Isochrysis pavlova</i>
Docosahexanoic acid (DHA) C-20:6 – omega 3	Nutritional supplements and Infant foods	<i>Schizochytrium</i> spp., <i>Cryptocodinium</i> spp.

Sources: Spolaore et al., 2006; Harwood and Guschina, 2009.

in open outdoor shallow ponds for simple maintenance and relatively low input costs.

Open cultivation systems

Most algae for commercial use are grown in the open air. The two most common open cultivation systems are circular and raceway ponds, in use for more than five decades. These systems can be developed using natural water bodies such as lagoons and ponds or artificial ponds such as raceways. Raceway ponds are generally oval shaped, closed loop channels of 0.2–0.5 m in depth where mixing is generally provided using paddle wheels. Raceway ponds are usually constructed from cement, or sometimes compacted soil channels with plastic liner (Brennan and Owende, 2010). Their shallow nature and continuous mixing meets the light requirements of the culture and prevents sedimentation. The open cultivation pond is cheap and they do not compete for agricultural land as they can be built on non-productive (marginal) land, or coastal regions for marine algal cultivation. The open system requires minimal investment in terms of light source and operations (Borowitzka, 1997; Chisti, 2008; Brennan and Owende, 2010; Mutanda *et al.*, 2011).

Open systems such as coastal shallow brackish-water ponds are extensively used for feed production in aquaculture and for other industrial applications. *Dunaliella* spp. are widely grown in these systems. These natural ponds are economical in terms of their operations, but only a limited number of species can be grown. Other physico-chemical parameters that affect productivity in open systems are evaporation losses, temperature fluctuations, inefficient mixing and light limitation. The evaporation losses increase the ionic concentration in the media causing severe osmolarity changes (Becker, 2004; Pulz, 2001; Lee, 2001). CO₂ requirements are more than can be met from the natural environment and thus constrain productivity. Hence carbonates and bicarbonates are used as carbon sources. Flue gases and CO₂ can be directly used as inorganic carbon for growth of cells in autotrophic mode. Due to the abovementioned limitations, the productivity of open ponds is low, and hence developing closed bioreactor systems for biomass production is preferred. One of the possible solutions to prevent contamination and severe evaporation losses are to cover the ponds with a greenhouse, which limits pond size considerably but gives a quasi-controlled environment.

Pond management

Since open ponds are highly susceptible to environmental fluctuations and contamination, certain measures are needed to keep the cultures healthy and productive. Contamination by other algae is very common in open ponds; this can be effectively managed by maintaining a critical cell concentration, preventing competing species

growth. Contamination by rotifers can be controlled by reducing the culture pH, since they are sensitive to low pH, and later re-adjusting cultures to optimum pH. Mixing is essential as accumulation of biomass in one place leads to anaerobic decomposition and accumulation of bacteria. The most efficient way of maintaining cultures is through a batch system, with fresh unialgal inoculum as starting material for every batch. An initial optimal optical density of the culture is a key factor for health of culture and maintenance. Since the emphasis is on the production of biomass with the minimum of energy inputs, it is desirable to use windmills for agitation of cultures and also use marine forms to avoid or minimize competition from contaminating organisms (Borowitzka, 2005).

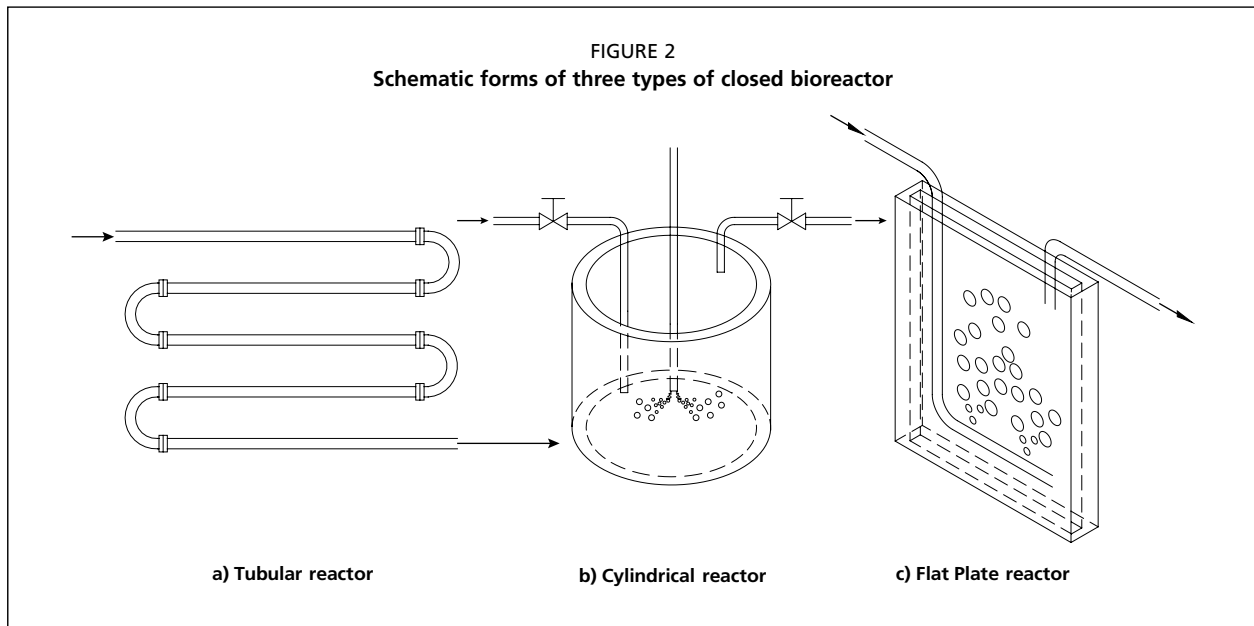
Closed cultivation systems

Maintenance of uni-algal culture in open ponds is very difficult, but can be achieved in closed bioreactor systems. In closed configurations, various culture parameters can be controlled and environment-sensitive strains growing in near-neutral pH conditions can be grown with higher productivities in closed photobioreactors. The closed reactor systems have high biomass productivity compared with open ponds since culture parameters such as illumination, turbulence and air exchange can be carefully regulated (Grobbeelaar, 2009, 2010).

Six parameters or subsystems for photobioreactors are, light source, optical transmission system, reaction area, gas exchange system, filtration system (removal of biomass) and sensing system. Light source is an important design consideration, which includes variables like type of light source, intensity of light source, effect of light source on cell development in the algal culture, and dark period requirement of the algae (Anderson, Anil and Schipull, 2002). Several of these parameters interact, such as the optical transmission system and gas exchange system via the mixing that takes place in the reaction area.

Three types of closed reactors are commonly employed for mass cultivation: tubular; cylindrical or columnar; and flat plate (Lehr and Posten, 2009).

Tubular bioreactors consist of an array of glass or plastic transparent tubes connected by U bends to capture more sunlight (Tredici and Materassi, 1992). They can be aligned in a flat plane or as a coil around a vertical cylindrical support framework (Borowitzka, 1999). The tubes are generally 5–10 cm in diameter. The algal cultures are circulated in these narrow tubes using mechanical pumps or airlift systems (Brennan and Owende, 2010). Tubular bioreactors have high surface to volume ratio, hence light capture is higher and gives high productivities. *Spirulina platensis* and *Chlorella* spp. have been successfully grown in these systems. Combined airlift-tubular systems have been used in production of *Porphyridium cruentum*, *Phaeodactylum*



tricornutum and *Haematococcus pluvialis* (Garcia-Malea Lopez *et al.*, 2006; Converti *et al.*, 2006). A combined airlift-tubular system has two parts: an airlift system and a solar receiver. The airlift system provides gas transfer and means to harvest biomass, while the tubular solar receiver provides high surface to volume ratio.

Cylindrical or column bioreactors are generally vertically aligned with aeration provided at the bottom and illuminated through transparent walls. They offer efficient mixing, facilitating cell growth. Bubble column or airlift column type mix by a static mixer or baffles, or by air spargers for controlled agitation. Mixing increases the frequency of cell exposure to light and reduces the dark volume of the reactor. It also enhances mass transfer between nutrients, facilitates dissipation of heat, and prevents oxygen build up (Brennan and Owende, 2010; Kunjapur and Eldridge, 2010). Efficient illumination can be achieved by internal light guides that spatially distribute light into cultures (Kunjapur and Eldridge, 2010). Duration of light and dark cycles influences photosynthetic efficiency. Alternatively, improvement of productivity is possible by flashing light with optimal pulsing, although this will incur additional overall maintenance costs (Posten, 2009). LED photodiodes are being used for illumination instead of fluorescent lights, and continuous illumination can be achieved by using solar light during day and solar power driven LED lights at night to improve productivity (Briassoulis *et al.*, 2010). Light transmission into dense cultures is very difficult and this can be overcome by using light guides that direct the incoming light into the culture, increasing the reactor area exposed to light. Anderson, Anil and Schipull (2002) extensively studied the use of light guides in improving light penetration in photobioreactors. The simplest large-scale outdoor closed-cultivation system is use of bag reactors, which are easy to construct.

Flat plate bioreactors are highly robust and very high biomass yields can be achieved due to their high ratio of light illumination surface area to volume. They have a small light path – from a few to 70 mm at most. Mixing is achieved by sparging with CO₂-enriched air. Their simple configuration supports construction of multiple plates placed close to each other and thereby efficiently utilizing land space (Posten, 2009).

Mixotrophic and heterotrophic production of biomass

Micro-algae being photosynthetic are hence predominantly cultivated in open ponds, exploiting their photoautotrophic nature. In heterotrophic growth, algae utilize organic substrates for metabolic process, while in mixotrophy both light and organic substrates are exploited. Under mixotrophic cultivation, the diurnal cycle can be efficiently utilized to prevent losses during aerobic dark respiration. *Spirulina platensis* and *Chlamydomonas reinhardtii* are efficiently adopted for mixotrophic growth (Chen, 1996; Andrade and Costa, 2007). In heterotrophic cultures, glucose or acetate are commonly used carbon sources, with glutamine or glutamate, or aspartate or asparagine, as common nitrogen sources.

Advanced fermentation technology and sophisticated reactors offer immense potential in growing micro-algae in heterotrophic conditions. Heterotrophic production has been studied extensively in *Chlorella protothecoides*, which can otherwise be grown photo-autotrophically (Miao and Wu, 2006; Chisti, 2007). Production of specialty chemicals like lutein from *C. protothecoides* or docosahexaenoic acid (DHA) from *Cryptocodinium cohnii* have been studied (Shi *et al.*, 1997; Shi and Chen, 2002; De Swaaf, Sijtsma and Pronk, 2003). The heterotrophic mode of growth can be

TABLE 3
Economic comparison of phototrophy and heterotrophy

Factors	Phototrophy	Heterotrophy
Energy source	Light	Glucose
Energy cost	US\$ 0.07 per kWh	US\$ 0.67/kg
Estimated cost/kg of dry weight	US\$ 11.22	US\$ 0.81
Actual cost/kg of dry biomass	Less than US\$ 11.22	US\$ 2.01
Productivity	0.4 g/L/day	5.8 g/L/day

Source: Adapted from Behrens, 2005.

a better option for cultivating strains that are susceptible to environmental fluctuations, such as *Haematococcus pluvialis*. An increase in biomass and astaxanthin content was observed when *H. pluvialis* was grown in heterotrophic media with acetate as carbon source (Tripathi *et al.*, 1999).

Utilizing heterotrophy for bio-energy production through micro-algae may not be a solution in the long term since it defeats the purpose of CO₂ sequestration. But it was observed that the specific growth rate of micro-algae grown in mixotrophic media supplemented with glucose or acetate was higher than in autotrophic cultivation (Lee, 2001).

Behrens (2005) compared the economics of algal biomass production in both phototrophic and heterotrophic modes of cultivation, with *Chlorella* spp. as the model organism. The major factors considered were construction costs for photobioreactors and fermenters, and associated energy costs: electricity in the case of photobioreactors and organic carbon for fermenters. Some of the assumptions were (1) cost of electricity was US\$ 0.07/kWh; (2) 20 percent of the energy of the electricity is converted into visible light (based on the efficiency of fluorescent lamps); (3) all of the light energy is absorbed by the phototroph; (4) the photosynthetic efficiency of converting absorbed light into ATP and NADPH is 20 percent (theoretical efficiency for red light conversion into chemical energy); (5) the energy content of the algal biomass is 6.41 kWh per dry kilogram of algal biomass; (6) the carbon content of algae is 50 percent; and (7) all of the carbon of glucose is converted into algal biomass.

Therefore the heterotrophic mode of cultivation could also be an alternative for biofuel production, taking into consideration the high biomass productivity and promising results based on the trials conducted by Li, Xu and Wu, 2007.

CO₂ sequestration

CO₂ is one of the main gases responsible for greenhouse effects accelerated by human activities. The mitigation of CO₂ by biological methods is a very important strategy as this can give rise to biomass-derived energy options through photosynthesis. Among phototrophic organisms,

micro-algae are the most efficient systems in absorbing CO₂ (Skjanes, Lindblad and Muller, 2007; Li *et al.*, 2008). Micro-algae can absorb CO₂ from a variety of sources, including atmospheric CO₂, emissions, flue gases from industries and CO₂ from soluble carbonates (Wang *et al.*, 2008). The rate of CO₂ removal is species dependent. Francisco *et al.* (2010) compared some of the micro-algal strains for CO₂ removal and observed that there is a wide range in the rates, ranging from 1.5 mg/L/minute in diatom *Phaeodactylum tricoratum* to 28.0 mg/L/minute in cyanobacterium *Aphanothece microscopica*, with higher ratios of CO₂ absorption and desorption rates indicating their greater efficiency. Based on their study, *Chorella vulgaris* with 11–13 hours of photoperiod and continuous cultivation in photobioreactors could achieve bioconversion of 3.07 kg CO₂/L/cycle. The CO₂ fixation rate (P) of the micro-algae can be calculated based on the biomass productivity, according to the equation $P \text{ CO}_2 = 1.88 \times \text{biomass productivity}$, which is derived from the approximate molecular formulae of micro-algal biomass, CO_{0.48}H_{1.83}N_{0.11}P_{0.01} (Chisti, 2007).

It is evident from Table 4 that chlorophycean micro-algae are efficient species in sequestering CO₂. Some strains of *Chlorella* spp. and *Scenedesmus* spp. can absorb up to 15 percent CO₂ (Huntley and Redalje, 2007; Francisco *et al.*, 2010). CO₂ abatement can be achieved by either directly passing the gaseous emission into the culture or by converting the gases chemically to soluble carbonates. Apart from use of flue gases as sources of CO₂, wastewater could also be used as a source of nutrients, especially for N and P. This combination of CO₂ uptake and wastewater treatment could render the large-scale cultivation process economically viable. *Botryococcus braunii* was shown to remove N and P from treated wastewaters; and *Chlorella vulgaris* was shown to remove ammonia from a steel manufacture plant's effluent and also to act as a sink for discharged flue gases. The CO₂ fixation and ammonia removal rates estimated for a *Chlorella vulgaris* strain was 26.0 g CO₂/m³/h (0.624 g/L/day) and 0.92 g NH₃/m³/h, respectively (Yun *et al.*, 1997). The main problems in direct absorption of gaseous emissions are high temperatures and the presence of

TABLE 4
Average CO₂ absorption and fixation rates of micro-algae

Micro-alga	CO ₂ (%)	Temperature (°C)	P CO ₂ (g/L/day)
<i>Chlorococcum littorale</i>	40	30	1.0
<i>Chlorella kessleri</i>	18	30	0.163
<i>Chlorella vulgaris</i>	up to 15	25	0.045–0.624
<i>Chlorella</i> sp.	15–40	up to 42	up to 1.0
<i>Dunaliella</i> sp.	3	27	0.313
<i>Haematococcus pluvialis</i>	16–34	20	0.143
<i>Scenedesmus obliquus</i>	Upto 18	30	0.016–0.26
<i>Botryococcus braunii</i>	—	25–30	>1
<i>Spirulina</i> sp.	12	30	0.413

Notes: P - CO₂ fixation rate. Source: Adapted from Wang *et al.*, 2008.

other contaminants like NO_x and SO_x . Cooling of the gases is a costly option, but conversion of these gases into soluble carbonates such as Na_2CO_3 and NaHCO_3 is an easy option as some of the micro-algae can survive in extreme conditions of high pH, thereby minimizing invasive species and other biological contaminants (Benemann 2003; Wang et al., 2008).

Wastewater utilization and cultivation of micro-algae

One of the economical means of cultivation of micro-algae for biofuel production is utilizing municipal wastewater and industrial effluent. The utilization of effluents provides a way for removal of chemical contaminants, such as heavy metals. *Scenedemus obliquus* has been extensively studied for utilization of wastewaters (Voltolina, Gomez-Villa and Correa, 2005; Hodaifa, Martinez and Sanchez, 2008). Sawayama, Minowa and Yokayama (1999) developed a cultivation strategy for uptake of nitrogen and phosphorous from sewage water and production of hydrocarbon rich biomass of *B. braunii*. Micro-algae are used in effluents from aquaculture, dairy farms and the food processing industry for removal of nutrients (nitrates, ammonia and phosphates) and odour, and to reduce acidity without chemicals. In oil drilling, micro-algae are used for reducing sludge and to remove metals and their precipitates.

Effective utilization of algal biomass for waste treatment would ensure a net positive energy balance and economically viable algal cultivation technology.

An integrated approach of growing micro-algae in wastewater and utilization of biomass for biofuel is shown schematically in Figure 3. The spent biomass obtained after biofuel extraction would be utilized further for bio-energy production through bio-methanation, unlike freshwater-grown micro-algal spent biomass utilized for feed and other bio-active molecules.

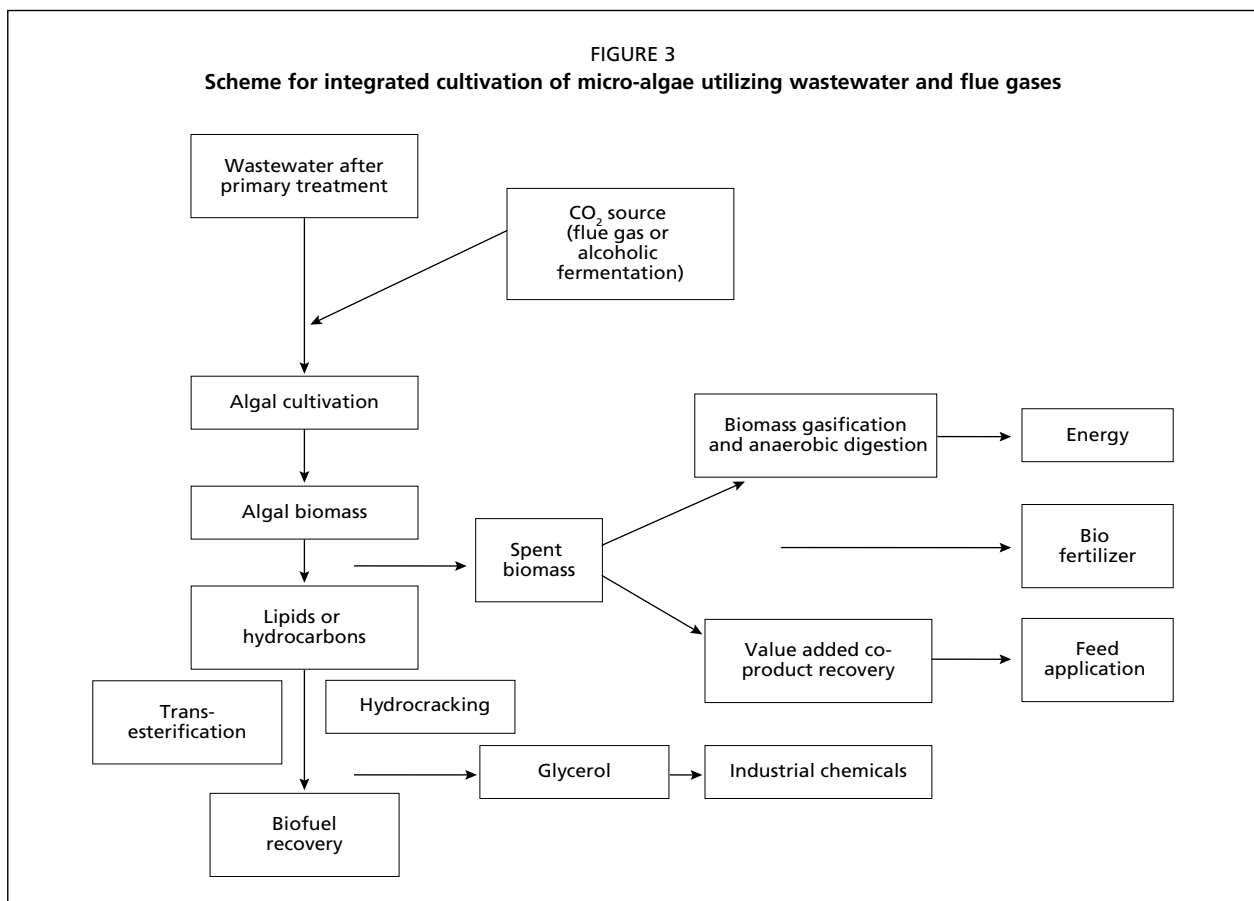
Alternately the spent biomass can be converted to fuel products through pyrolysis, chemical catalysis or hydrocracking, and used in diesel, jet fuel or gasoline.

DOWNSTREAM PROCESSING AND CONVERSION TO BIOFUELS

Harvesting and dewatering of micro-algae

Mass production of micro-algae for metabolites requires efficient harvesting and extraction methods. Harvesting of biomass requires one or more solid-liquid separation techniques, which depend on the nature of the alga, such as size, density and metabolite to be obtained. The common techniques applied in the harvesting of biomass are centrifugation, flotation, flocculation and gravity sedimentation.

Centrifugation recovery is the most preferred method of harvesting for high value metabolites. Laboratory experi-



ments conducted on harvesting of biomass indicated centrifugal recovery is rapid and about 80–90 percent of algal biomass can be recovered with 1000–5000 g for 2–3 minutes (Chen *et al.*, 2010). Centrifugation is a preferred method of harvesting algal biomass for producing extended shelf-life concentrates for aquaculture hatcheries and nurseries (Knuckey *et al.*, 2006). The only limiting factor is the high capital and operating costs for harvesting of large quantities of water and algae (Grima *et al.*, 2003).

Gravity sedimentation is a type of solid-liquid separation which is commonly applied for separating micro-algae in water and wastewater treatment. Particles with higher density can be separated easily by gravity sedimentation, whereas particles with a diameter of a few microns require flocculants to form larger aggregates. Lamella separators and sedimentation tanks are used for enhanced micro-algal separation through sedimentation (Uduman *et al.*, 2010). Biomass-sediment is collected in a sump and recovered by pumping. Gravity sedimentation in sedimentation tank is time consuming and very few reports are available on this method; it relies on the autoflocculation principle. Addition of flocculants increases the efficiency of gravity sedimentation, which could be an inexpensive process.

Filtration is used effectively for the harvesting of larger micro-algae like *Coelastrum* spp. or *Spirulina* spp., but fails to recover micro-algae with smaller cell dimensions (Mohn, 1980). Filtration is theoretically simple, but very expensive in practice, and its understanding involves several issues, like filter pore size, filter material and also the design of the filters. Larger filter apertures allows smaller cells to pass through, whereas decreased pore size results in binding or blocking of filter pores, and reduced filtration rates. Filter pore size is dependent on the size of the algal species and algal aggregation rate. Filtration materials and filtration design are of primary importance, which determine the cost economics and efficiency of the process (Oswald, 1991).

Flocculation can be a preliminary step in the bulk harvesting process, which helps to aggregate the micro-algal cells in order to increase the effective particle size. Flocculation can be enhanced by addition of flocculants that can reduce or neutralize the surface negative charge of the cells thereby increasing the effective particle size for gravity settling. An ideal flocculant should be inexpensive, non-toxic and effective at low concentrations, and also should not affect further downstream processing (Grima *et al.*, 2003; Murthy, 2005). Flocculants generally coagulate algal cells by neutralizing the surface negative charge, as in the case of polycationic inorganic or organic compounds such as polyvalent metal salts, which are iron- or aluminum-based coagulants, like ferric chloride, aluminum sulphate and ferric sulphate. Coagulation efficacy of metal ions increases with increasing ionic charge (Brennan and Owende, 2010). Multivalent salts like alum are used effectively to

harvest *Chlorella* and *Scenedesmus* in wastewater treatment processes (Grima *et al.*, 2003). Organic flocculants, apart from reducing or neutralizing the surface charge, can bring particles together by physical linkage through a process called bridging (Grima *et al.*, 2003). Chitosan is being used as a biodegradable organic flocculant that can be synthesized from natural sources (Divakaran and Pillai, 2002). Polymeric organic flocculants such as polyacrylamide, cationic starch, poly-ferric sulphate, etc., are commonly used for harvesting. Marine organisms cannot be effectively harvested through flocculation due to high ionic concentrations within cells (Bilanovic, Shelef and Sukenik, 1998). The main problems associated with flocculation are accumulation of the flocculant in the media and its effect on possible further recycling of media (Chen *et al.*, 2010).

Flotation relies on the attachment of air or gas bubbles to solid particles, which are then carried to the liquid surface and accumulate as float, which can be easily separated. Flotation is a more effective and beneficial harvesting method than flocculation since it obviates the use of chemicals. Solid particles can be separated by colliding air bubbles with the particles; for capturing particles smaller than 500 µm, flotation efficiency increases with decreasing particle size (Yoon and Luttrell, 1989; Uduman *et al.*, 2010). Although flotation could be a potential harvesting system, its efficacy has yet to be clearly evaluated (Brennan and Owende, 2010).

According to Uduman *et al.* (2010), any dewatering technology can be quantitatively evaluated by the following parameters: the rate of water removal of the dewatering technique; the solid content of the recovered micro-algae-water slurry (percent total suspended solids - TSS); and the yield of the processed micro-algae by the dewatering technique. They proposed a single-step simultaneous harvesting and dewatering process for micro-algae, from an initial 0.02 to 0.06 percent TSS to a primary harvest of 2 to 7 percent TSS, followed by dewatering to give 15 to 25 percent TSS.

The final step in harvesting is the complete dewatering and drying of the micro-algal slurry. This step is one of major economic importance. Selection of the drying method depends on the use for which the dried product is intended, and also the scale of operation. Some of the commonly available drying methods include sun drying, drum drying, freeze drying, air drying, oven drying and spray drying. Freeze drying and spray drying methods are found to be rapid and the most suitable drying method for algae, but is comparatively energy intensive. Spray drying is often used for algae, finding food applications such as with *Dunaliella* spp. and *Spirulina* spp. (Leach, Oliveira and Morais, 1998). Oven-type driers are also found to be effective and less energy intensive, but are not suitable for heat-sensitive metabolites (Mohn,

1978). Air drying and sun drying is the cheapest method for drying algal biomass, but it requires a large drying surface and has a long drying time (Prakash *et al.*, 1997). Lundquist *et al.* (2010) recommended online extraction of oil from wet biomass, avoiding the steps of drying and extraction, which could reduce operating costs by 20 to 25 percent.

Extraction of micro-algal lipids

Cell disruption is an important step in recovering intracellular products from micro-algae, and so properties of the cell wall play an important role in the extraction process. Some of the commonly used methods for cell disruption include mechanical disruption, like bead-beating, ultrasound and steam extraction (Mata, Martins and Caetano., 2010) and non-mechanical disruption, including application of organic solvents and addition of inorganic acids and alkali for pre-treatment processing. The most convenient method would be to efflux the metabolites or constituents of micro-algal cells using solvents, without disrupting cellular functions. Two-phase solvent mixtures, such as methanol-ethanol/hexane co-solvent systems, are advantageous, whereby more polar solvents are used to disrupt the membrane while the extracted lipids enter the non-polar solvent phase. This reduces the phase separation step during processing, thereby making solvent extraction more convenient and economical (Hejazi and Wijffels, 2004).

Green solvents such as ionic liquids and switchable polarity solvents can be exploited for extraction of lipids from micro-algae (Samori, Samori and Fabbri, 2010; Salvo *et al.*, 2011). Ionic liquids are non-aqueous solutions of relatively large asymmetric organic cations coupled with a small inorganic or organic anion salt that remain liquid at moderate to room temperatures. The ionic solvents have hydrophilic ionic liquid and polar covalent molecules for both extraction and partitioning of lipids from micro-algal cells. Salvo *et al.* (2011) used a hydrophilic ionic liquid, 1-butyl-3-methylimidazolium, for a single-step extraction process involving lysis of micro-algal cell walls and separation of cellular lipids. After the auto-phase separation, the lower hydrophilic ionic phase can be re-used for extraction of micro-algal cells.

Switchable solvents have physical properties, such as polarity, solubilizing capacity, viscosity and conductivity, that can be converted from one form to other. The main advantages of switchable solvents is that many processes, such as extraction, phase separation and purification, can be achieved with one single agent (Phan, 2008). Hydrocarbon yields were higher when a switchable polarity solvent system containing 1,8-diazobicyclo-[5.4.0]-undec-7-ene and alcohol was used for extraction in *B. braunii* compared with conventional solvent extraction by n-hexane (Samori, Samori and Fabbri, 2010).

CONVERSION OF ALGAL LIPIDS AND BIOMASS TO BIO-ENERGY

Trans-esterification

The extracted micro-algal oil can be converted to biodiesel by trans-esterification. The trans-esterification process involves reaction of an alcohol with the triglycerides, forming fatty acid alkyl esters, in the presence of a catalyst. Based on the type of catalyst used, the trans-esterification process can be acid or base catalysed, and involve enzymatic conversion. In acid-catalysed reactions, HCl, H₂SO₄ or H₃PO₄ is used for trans-esterification, while in base catalysis strong bases like KOH or NaOH are commonly used. Base catalysis has many advantages over the acid-catalysed reaction since it is conducted at low temperature and pressure, and it has a high conversion yield and provides direct conversion to biodiesel without intermediate compounds. Balancing the advantages are several drawbacks, including being energy intensive, with problems associated with removal and treatment of alkaline catalyst from the final product. These problems could be solved by the use of biocatalysts like lipases, but large-scale demonstration has not been reported (Svensson and Adlercreutz, 2008).

In situ acid-catalysed trans-esterification processes for biofuel production have been explored but the limiting factor is the high moisture content of algal biomass, affecting the conversion. The identification of lipid composition is an important criterion to assess the suitability of algal oil for high quality biodiesel production. Some of the important fuel properties considered for biodiesel include density, viscosity, flash point, ester value, cetane number and combustion heat (Mutanda *et al.*, 2011). In the study conducted by Francisco *et al.* (2010) on micro-algal strains of *Chlorella* spp., *Dunaliella* spp., *Phaeodactylum* spp., *Aphonotheca* spp., *Phormidium* spp. and *Scenedesmus* spp., it was found that the properties of biodiesel obtained from these strains were found to be similar to the American Society for Testing and Materials (ASTM) and European Union standards (Table 5).

Trans-esterification of algal lipids generates glycerol as the major co-product. Glycerol is an industrially important

TABLE 5
Characteristics of biodiesel

Properties	Biodiesel from micro-algal oil	Diesel fuel
Density (kg/L)	0.864	0.838
Viscosity (Pa/s)	5.2 × 10 ⁻⁴ (40°C)	1.9 – 4.1 × 10 ⁻⁴ (40°C)
Flash point (°C)	65–115	75
Solidifying point (°C)	-12	-50 – -10
Cold filter plugging point (°C)	-11	-3.0 (-6.7 max.)
Acid value (mg KOH/g)	0.374	0.5 max.
Heating value (MJ/kg)	41	40 – 45
HC (hydrogen to carbon) ratio	1.18	1.18

Source: Oilgae, 2010.

product that can be fermented to produce 1,3-propanediol, which is a precursor for many industrial products such as polymers, adhesives, paints, etc. (Yazdani and Gonzalez, 2007). The large-scale production of biodiesel by transesterification produces enormous quantities of glycerol, which poses a problem for its complete utilization and disposal. It has been estimated that for one billion gallons of biodiesel produced, approximately 400 000 tonne of glycerol will be generated (Fishman *et al.*, 2010). As the existing market for glycerol is currently saturated, a more efficient process for biodiesel production is required where there is a complete utilization of all the co-products generated in the large-scale production process.

Hydrocracking

Hydrocracking is another alternative for biofuel production from crude biomass extracts containing hydrocarbons. Although the acid and alkali catalysed reactions are faster and used extensively for biofuel production, these catalysts require additional treatment for their removal from the final product stream. A potential solution to this problem is the use of hydrocracking or catalytic cracking technology, where the conversion process involves two stages, combining catalytic cracking and hydrogenation. The hydrogenation step occurs at high temperatures (200–300 °C) and high pressures (35–200 bar), yielding low boiling, high quality hydrocarbons. The catalysts used for cracking are immobilized homogenous or heterogeneous metal-based catalysts derived from titanium or vanadate oxides, Al₂O₃, MgO and CaO. A wide range of feed stocks can be processed *via* hydrocracking, with removal of nitrogen and sulphur in the biomass feed stocks for gases yielding N- and S-free paraffinic hydrocarbons. Complex aromatic hydrocarbons and olefins are hydrogenated to simpler, lower boiling and lower molecular weight hydrocarbons. The challenges involved in this process are finding an ideal catalyst that operates at lower temperature and pressure, thereby reducing the energy inputs, and that also has resistance towards leaching by the active components of the feed stocks (Fishman *et al.*, 2010).

ETHANOL FROM ALGAL FEEDSTOCK

Ethanol is a clean burning fuel with high octane value. It is not used as 100 percent fuel but generally blended with gasoline in different proportions, thereby reducing dependence on gasoline and improve the octane rating of the blended fuel. Generally, ethanol is blended at 10 percent with gasoline—termed E-10—and is approved for use in United States. Another type of ethanol-based fuel is E-85, where the blend proportion is 85 percent ethanol and 15 percent unleaded gasoline, but used only in Flexible Fuel Vehicles (Oilgae, 2010). Algae could be the source of bioethanol due to their relatively high contents

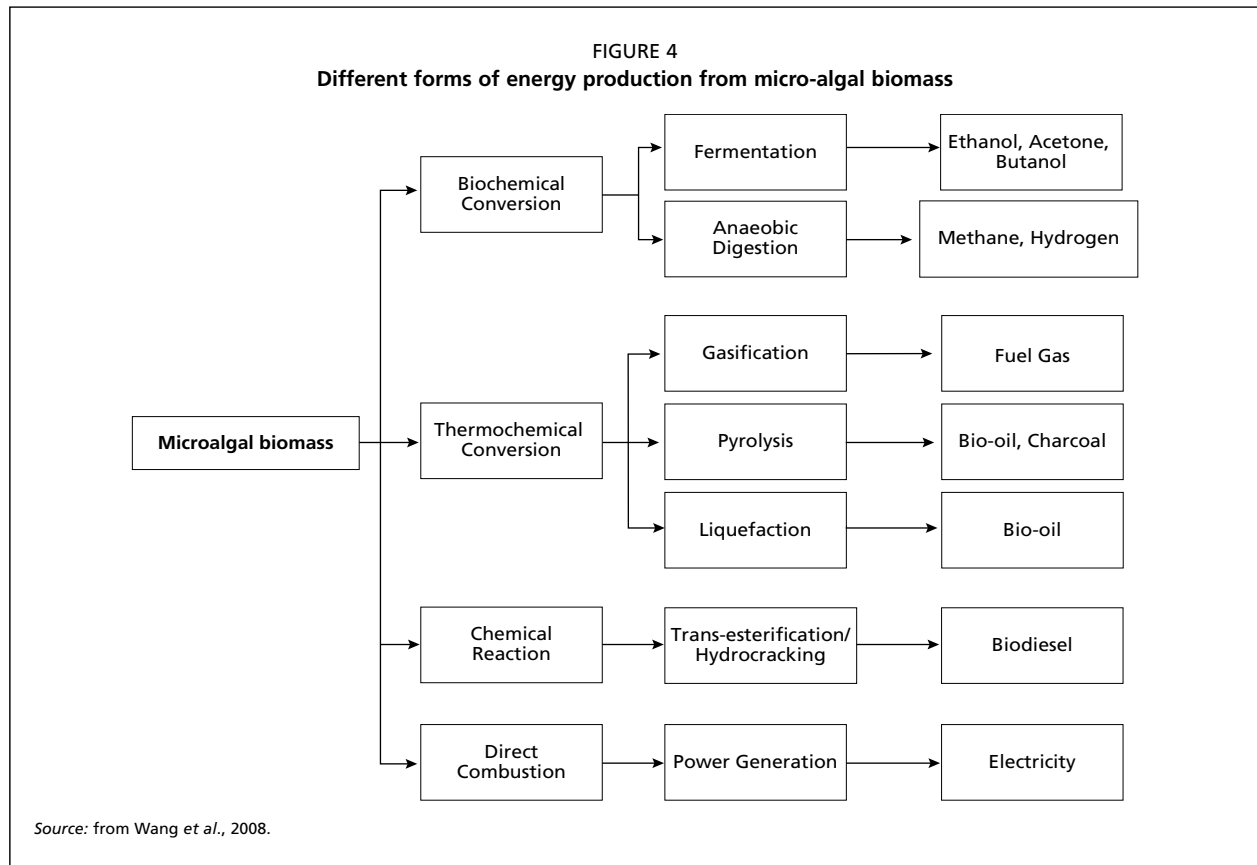
of carbohydrates in the form of polysaccharides. Ethanol can be produced either from algal biomass or from algal cake. Producing ethanol from algal cake is more economical than the use of biomass (Clarens *et al.*, 2010). Macroalgae are a better source for ethanol production because of their high polysaccharide content, such as *Sargassum* spp. (48%), *Gracilaria* spp. (45%), *Kappaphycus* spp. (35%) and *Eucheama* (45%). Cell wall components of algae are the major sources of carbohydrates. Green algae mostly contain cellulose and hemicellulose; red algae contain cellulose and polysaccharides like agar and carrageenan; and brown algae contain cellulose and alginic acids. The cellulosic content in micro-algae is relatively less for production of ethanol. Certain algae accumulate starch in stress conditions, and this can be exploited for ethanol production by fermentation. The ethanol generated can be distilled. The following section discusses some methods of biomass conversion technologies for energy production, including biomass gasification and fermentation.

Biomass conversion technologies for energy production

Biomass-derived energy can be obtained from the de-oiled algal cake or spent biomass using various conversion technologies. Various processes, such as combustion (heat energy), pyrolysis (pyrolytic gas), gasification (syngas), thermochemical liquefaction, and alcoholic fermentation (ethanol) are being explored to develop a sustainable technology for biofuel production from micro-algae (Figure 4).

Integrated biorefineries use residual biomass to produce biogas or other forms of energy to run the micro-algal production facility. Since micro-algal cultivation involves huge influxes of N & P, unutilized biomass would severely affect the environment and the economics of biodiesel production. Recycling the N & P from the residual biomass after oil extraction is important, and this can be achieved by anaerobic digestion of the algal waste to biogas-methane (Chisti, 2007; Sialve, Bernet and Bernard, 2009).

Thermal liquefaction of whole algal biomass by sub-critical water extraction can also be used for large-scale operations where the de-watering step can be eliminated. Sub-critical water extraction below critical temperatures and high pressure keeps water in the liquid phase, making the operation less polar and hence solubilising organic compounds from the cells. Cooling of water to room temperature creates phase immiscibility, leading to easy separation of metabolites. The operational advantage of sub-critical water extraction is shorter extraction time, environmental compatibility, and abundance and low cost of the extracting agent (Herrero, Cifuentes and Ibanez, 2006; Patil, Tran and Giselrod, 2008). The main product obtained after liquefaction is 'bio-crude', which can be further upgraded to combustibles (Fishman *et al.*, 2010). Biomass gasification of



the micro-algal concentrate can yield different liquid fuels; this process uses Fischer-Tropsch synthesis technology. The crude product obtained is called 'Syn-gas', which can be converted to various fuel derivatives by further processing, such as hydrogenation.

USE OF MICRO-ALGAE FOR FOOD, FEED AND BIO-ACTIVES

Food applications of micro-algae

The algal biomass remaining after extracting the hydrocarbons and lipids is a valuable co-product rich in nutritionally important metabolites for food and feed purposes. It is used for food applications, and also for feed applications in various industries, such as fish aquaculture and poultry, as well as in the nutraceutical market for both human and animal consumption. This section highlights the possibilities for utilization of algal biomass for feed and as a source of other valuable constituents. Such value addition would be of relevance not only to utilize all the co-products in a useful manner, but also as affording eco-friendly technology alternatives for the production of various nutrients and bio-actives apart from direct food applications.

Micro-algae with their immense chemical diversity provide an seemingly unlimited source for various applications in the food industry (Table 6). Algal products ranging from whole biomass to nutraceuticals like carotenoids and PUFAs are utilized in the food industry. The safety of these algae has been

evaluated and commercial use approved in several countries. Many reviews are available detailing the potential uses of micro-algae as food sources (Venkataraman *et al.*, 1980; Becker, 2004; Pulz and Gross, 2004; Spolaore *et al.*, 2006; Ravishankar *et al.*, 2008; Plaza *et al.*, 2009; Milledge, 2010).

Micro-algae as a source of vitamins

Micro-algae also represent a valuable source of nearly all essential vitamins (e.g. A, B₁, B₂, B₆, B₁₂, C, E, nicotinate, biotin, folic acid and pantothenic acid). Vitamin B₁₂ is mainly synthesized by certain bacteria that are associated with the gut flora of animals, contributing to the requirement of this vitamin. Since plants have no ability to synthesize vitamin B₁₂ because of the absence of cobalamin-dependent enzymes, strict vegetarians (vegans) are at risk of developing vitamin B₁₂ deficiency, and hence need to depend upon vitamin B₁₂-fortified foods or vitamin B₁₂-containing dietary supplements to meet the requirement. *Spirulina platensis* is one of the most widely consumed cyanophycean forms used as a food supplement and contains substantial amounts of vitamin B₁₂. Because the vegetarian diet does not contain vitamin B₁₂, *S. platensis*, along with other nutrients, helps in meeting the recommended daily requirement of vitamin B₁₂ in the vegetarian diet, and also of meeting the requirement of needy individuals with varied food habits or health status (Kumudha *et al.*, 2010). Other water-soluble vitamins, including Vitamin C, riboflavin and

TABLE 6
Micro-algae commercially exploited for food applications

Micro-algae	Biomass or metabolite and commercial applications	Cultivation system	Location	References
<i>Chlorella</i> spp.	Whole biomass for aquaculture and single-cell protein Astaxanthin as Pigment agent Ferredoxin for laboratory use	Circular ponds with rotating arms; heterotrophic fermenters	Japan, Taiwan, Thailand and USA	Carvalho, Meireles and Malcata, 2006.
<i>Dunaliella salina</i>	β -carotene as pigment agent	Extensive open ponds; raceway ponds	Australia, China, India, Israel and USA	Murthy, 2005; Ravishankar <i>et al.</i> , 2008; Plaza <i>et al.</i> , 2009.
<i>Haematococcus pluvialis</i>	Astaxanthin, colouring agent, nutraceutical	Two stage systems; tubular reactors	India and USA	Gouveia and Oliveria, 2009; Kamath 2007; Ravishankar <i>et al.</i> , 2008; Plaza <i>et al.</i> , 2009.
<i>Spirulina platensis</i>	Whole biomass as food or feed; phycocyanin as colouring agent	Circular ponds and open raceway ponds	China and India	Eriksen, 2008.
<i>Isochrysis galbana</i>	DHA - Food supplement and pharmaceuticals	Closed bioreactors		Day, Benson and Fleck, 1999.
<i>Phaeodactylum tricornutum</i>	EPA as food supplement and pharmaceuticals	Closed bioreactors		Spolaore <i>et al.</i> , 2006.
<i>Cryptocodinium cohnii</i>	DHA as food supplement and pharmaceuticals	Closed bioreactors (heterotrophic)	USA	Spolaore <i>et al.</i> , 2006.
<i>Schizochytrium</i> spp.	DHA as food supplement pharmaceuticals	Heterotrophic fermenters	USA	Sijtsma and Swaaf, 2004; Pyle, Garcia and Wen, 2008.

Notes: DHA = docosahexaenoic acid

TABLE 7
Comparison of vitamin content (mg/kg DM) of a few micro-algae with common foods and RDI

Source	Vitamin										
	A	B ₁	B ₂	B ₆	B ₁₂	C	E	Nicotinate	Biotin	Folic acid	Pantothenic acid
RDI (mg/day)	1.7	1.5	2.0	2.5	0.005	50.0	30.0	18.0	na	0.6	8.0
Liver	60.0	3.0	29.0	7.0	0.65	310.0	10.0	136.0	1.0	2.9	73.0
Spinach	130.0	0.9	1.8	1.8	na	470.0	Na	5.5	0.007	0.7	2.8
Baker's yeast	Trace	7.1	16.5	21.0	na	Trace	112.0	4.0	5.0	53.0	na
<i>Spirulina platensis</i>	840.0	44.0	37.0	3.0	7.0	80.0	120.0	na	0.3	0.4	13.0
<i>Aphanizomenon flos-aquae</i>	na	4.8	57.3	11.1	8.0	0.7	na	0.1	0.3	1.0	6.8
<i>Chlorella pyrenoidosa</i>	480.0	10.0	36.0	23.0	na	na	na	240.0	0.15	na	20.0
<i>Scenedesmus quadricauda</i>	554	11.5	27.0	na	1.1	396.0	na	108.0	na	na	46.0

Notes: na = not available; RDI = Recommended Daily Intake for an adult. Source: Adapted from Becker, 2004.

thiamine, are also present in certain marine micro-algae and in *Spirulina* spp. (Qasim and Barkati, 1985). Some marine algae, especially brown algae, contain α -tocopherol in significant quantities (Solibami and Kamat, 1985). Some of the lipid-soluble vitamins may be lost during oil extraction, but the spent biomass would be retained, containing proteins and other water-soluble vitamins that can be exploited for feed. Table 7 compares the vitamin composition of a few micro-algae with some common food stuffs and the recommended dietary intakes of vitamins.

MICRO-ALGAE AS SOURCES OF FEED

Micro-algae are commonly used in diets in the aquaculture industry, either as individual diets or as components of mixed diets. Some micro-algae rich in PUFAs, such as *Isochrysis galbana*, *Pavlova lutheri*, *Chaetoceros calcitrans* and *Thalassiosira pseudonana*, are used in culture of bivalve molluscs, crustacean larvae, and zooplanktons for crusta-

cean and fish larvae. Many reviews are available on application of micro-algae in aquaculture (Borowitzka, 1997; Renaud, Thin and Parry, 1999; Brown, 2002; Spolaore *et al.*, 2006).

One of the important applications of micro-algae in aquaculture is associated with its use as fish meal for colouring the flesh of salmonids and for inducing other biological activities. Several investigations have been carried out on the use of algae as additives, alongside fish meal. *Spirulina platensis* has been extensively used in rearing some fish species, including Red sea bream (*Pagrus major*), Cherry salmon (*Oncorhynchus masou*), Nibbler (*Girella punctata*), Striped jack (*Pseudocaranx dentex*), Yellow tail (*Seriola quinqueradiata*) and Mozambique tilapia, to improve weight gain, muscle protein deposition, raw meat quality, flesh texture and taste (FAO, 2009). Supplementation of *Spirogyra* spp. for Carp (*Catla catla*) improved growth, muscle protein and fat content (Kumar,

Gajaria and Radha, 2004). The predominant source of carotenoids for salmonids has been synthetic carotenoids like astaxanthin, which has been used for pigmentation for the last 20 years (Ravishankar *et al.*, 2008).

Natural sources of astaxanthin for commercially raised salmonids include processed crustacean waste from krill, shrimp, crab and crawfish. However, crustacean waste products contain large amounts of moisture, ash and chitin, which limit their use in salmon feed. The efficiency of dietary astaxanthin using micro-algae for flesh pigmentation of Atlantic salmon and rainbow trout has been demonstrated by Torrissen, Hardy and Shearer (1989) and Storebakken (1988). Astaxanthin is even considered as a vitamin for salmon, as it is essential for the proper development and survival of juveniles. Choubert and Heinrich (1993) showed that feeding rainbow trout with algae up to 6 percent of the diet had no major effect on growth or mortality. Thus, the algae were concluded to be a safe and effective source of pigment. Astaxanthin has been used to enhance the immune response of fish and shrimp for maximum survival and growth. Natural micro-algal astaxanthin has shown superior bio-efficacy over the synthetic form. The skin colour of ornamental koi carp fish increased considerably when fed with diet containing astaxanthin enriched *Haematococcus pluvialis* cells at 25 mg/kg in the feed (Kamath, 2007).

Algae can be used in integrated livestock management in manure ponds for growing fish and removing of nutrients, thereby serving the feed requirement and avoiding use of fish meal. The good nutrient profiles of algae, carotenoids and PUFAs can improve fish quality considerably (Phang, 1992).

Micro-algae, with their good nutritional properties, can be exploited as poultry feed. Pigments must be supplemented in diets to enhance pigmentation in poultry meat and eggs (Bortolotti *et al.*, 2003), and *Spirulina*, with its high protein content and carotenoid levels, can be used to enrich yolk colour. Another micro-alga widely used for enriching yolk colour is *Haematococcus pluvialis*. Astaxanthin, with its broad nutraceutical properties, can replace artificial colorants and also improve poultry health and other egg properties. In poultry, astaxanthin has been shown to reduce chick mortality by 50 percent, and to reduce *Vibrio* spp. infections in eggs, thereby improving the nutritional value of eggs (Ravishankar *et al.*, 2008). *Chlorella vulgaris* is rich in lutein and can also be used a feed supplement to improve yolk properties.

The exact composition of algal meal depends on the algal species and the cultivation conditions, and also on the amount of oil that has been extracted. The approximate NPK value (by weight) for algae meal is 8 percent N, 4 percent P and 3 percent K (Oilgae, 2010). The algae meal can be directly used as agricultural fertilizer, as with many seaweeds that have been used as natural fertilizers, but the protein content in the micro-algae is thereby not utilized. Table 8 gives the biomass composition of few micro-algae commonly used in industries. Relatively high protein content of the algal biomass gives an indication of their utility as protein-rich animal feed. Subsequent to the oil extraction, the algae meal can be used as source of proteinaceous feed. For example, protein digestibility of *Chlorella* was quite high in animals, up to 56 percent in the case of pigs (Oilgae, 2010). The protein quality is indicated by the amino acid profile and certain indices like chemical score and

TABLE 8
Nutrient composition of some micro-algae (% dry weight)

	Protein	Carbohydrate	Lipids	Nucleic acid
<i>Anabaena cylindrical</i>	43–56	25–30	4–7	na
<i>Aphanizomenon flos-aquae</i>	62	23	3	na
<i>Scenedesmus obliquus</i>	50–56	10–17	12–14	3–6
<i>Scenedesmus quadricauda</i>	47	na	1.9	na
<i>Scenedesmus dimorphus</i>	8–18	21–52	16–40	na
<i>Chlamydomonas rheinhardtii</i>	48	17	21	na
<i>Chlorella vulgaris</i>	51–58	12–17	14–22	4–5
<i>Chlorella pyrenoidosa</i>	57	26	2	na
<i>Spirogyra</i> sp.	6–20	33–64	11–21	na
<i>Dunaliella bioculata</i>	49	4	8	na
<i>Dunaliella salina</i>	57	32	6	na
<i>Euglena gracilis</i>	39–61	14–18	14–20	na
<i>Prymnesium parvum</i>	28–45	25–33	22–38	1–2
<i>Tetraselmis maculata</i>	52	15	3	na
<i>Porphyridium cruentum</i>	28–39	40–57	9–14	na
<i>Spirulina platensis</i>	46–63	8–14	4–9	2–5
<i>Spirulina maxima</i>	60–71	13–16	6–7	3–4.5
<i>Synechococcus</i> sp.	63	15	11	5
<i>Euglena gracilis</i>	39–61	14–18	14–20	na

Notes: na = Not available. Source: Adapted from Becker, 1994.

TABLE 9
Amino acid profile of a few algae compared with some conventional protein sources (g/100 g protein)

Source	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser
Egg	6.6	8.8	7.2	5.3	5.8	4.2	3.2	2.3	1.7	5.0	na	6.2	11.0	12.6	4.2	2.4	4.2	6.9
Soybean	5.3	7.7	5.3	6.4	5.0	3.7	1.3	1.9	1.4	4.0	5.0	7.4	1.3	19	4.5	2.6	5.3	5.8
<i>Chlorella vulgaris</i>	3.2	9.5	7.0	6.4	5.5	2.8	1.3	na	na	5.3	9.4	6.9	9.3	13.7	6.3	2.0	5.0	5.8
<i>Dunaliella bardawil</i>	4.2	11.0	5.8	7.0	5.8	3.7	2.3	1.2	0.7	5.4	7.3	7.3	10.4	12.7	5.5	1.8	3.3	4.6
<i>Spirulina platensis</i>	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	10.3	5.7	2.2	4.2	5.1
<i>Aphanizomenon flos-aquae</i>	2.9	5.2	3.2	3.2	2.5	na	0.7	0.2	0.7	3.3	4.7	3.8	4.7	7.8	2.9	0.9	2.9	2.9

Notes: na = not available. Source: Adapted from Becker, 2004.

TABLE 10
Major constituents of four important micro-algae

Component	Algal biomass (% w/w)			
	<i>B. braunii</i>	<i>Chlorella</i> sp.	<i>Scenedesmus</i> sp.	<i>Spirulina</i> sp.
Moisture	5–6	5–8	5–7	5–8
Ash	10–35	8–10	6–8	10–12
Fat	6.9–15	8–12	8–14	2–3
Carbohydrates	9.75	12–16	10–15	15–20
Protein	20.8	40–50	50–55	50–60
Hydrocarbon	5–15	nd	nd	nd
Total chlorophyll	2.6	nd	nd	nd
Total carotenoids	0.7	nd	nd	nd
Phenolics	0.77	nd	nd	nd
Nucleic acids	nd	6–8	4–6	5–7
Fibre	nd	6–8	10–12	5–8

Notes: nd = not determined. Sources: Data for *B. braunii* from Sarada, 2007[unpublished], and for *Chlorella* sp., *Scenedesmus* sp. and *Spirulina* sp. from Ravishankar *et al.*, 2008

essential amino acid (EAA) index. The amino acid profile of the micro-algae is comparable to that of standard protein sources, like milk or eggs. Table 9 gives the amino acid profile of few micro-algae. The amino acid profile is almost comparable with that of conventional protein sources, with some minor deficiencies in sulphur-containing amino acids such as methionine and cysteine.

Furthermore, micro-algae are good sources of carbohydrates, found in the form of starch, cellulose, sugars and other polysaccharides. The extractable micro-algal polysaccharides can be used as emulsifiers in the food industry. The available carbohydrates have good overall digestibility and are therefore suitable for feed applications. The spent biomass is rich in cellulosic polysaccharides and can also be utilized as a diet ingredient in ruminant feed mix as they have good cellulose digestibility. Addition of algae to the diet of cows resulted in a lower natural breakdown of unsaturated fatty acids and a higher concentration of beneficial compounds in meat and milk. It was observed that sewage-grown algae (supplemented at 5 percent) could replace 25 percent soybean meal used in broiler mash (Becker, 2004). In addition the crude fibre content of the spent algae could be used for therapeutic purposes. Many experiments with supplementation of whole algae biomass, from species such as *Spirulina*, *Scenedesmus* and

Chlorella, showed hypo-cholesterolemic effect. In *Chlorella* species, an important compound of therapeutic value is β -1,3-glucan, which is immunostimulatory, with blood lipid reducing effects. Efficacy of this compound against gastric ulcers and hypercholesterolemia has also been reported, and there is some antitumour effect (Spolaore *et al.*, 2006; Lee, Park and Kims, 2008).

Unconventional food and feed sources contain certain compounds, like nucleic acids, that are sources of purines, when consumed increase plasma uric acid concentrations, which are considered in humans to contribute to gout and uric acid stones in the kidney. The nucleic acid content of micro-algae varies normally between 4 and 6 percent (w/w), while other single-cell protein sources, like yeast and bacteria, are 8–12.5 and 20 percent, respectively (Becker, 2004). Compared with other sources, micro-algae as a source of feed is relatively safe, but it is recommended that intake of nucleic acids should not exceed 2.0 g from unconventional sources, indicating maximum intake of algal biomass not beyond 20 g/day or 0.3 g of algae per kg body weight. Extracts of the hydrocarbon-rich alga *B. braunii* showed significant antioxidant activity, and was non-toxic when whole biomass was supplemented as part of the diet for experimental animals. The antioxidant activity was attributed to carotenoids, especially lutein, which

constitutes 75 percent of the total carotenoid composition (Rao *et al.*, 2006; Dayananda, 2010).

MICRO-ALGAE AS SOURCES OF BIO-ACTIVE MOLECULES

The compounds of micro-algal origin that have found commercial application include fatty acids, steroids, carotenoids, phycocolloids, lectins, mycosporine-like amino acids, halogenated compounds, polyketides and toxins. Algal metabolites have exhibited a wide spectrum of activity, with the majority of them evaluated for properties such as herbicidal activity and cytotoxicity, and antibiotic, antitumour, antiviral, multi-drug resistance reversal and immunosuppressive effects (Burja *et al.*, 2001). Micro-algae have a cholesterol-lowering effect in animals and humans. *Aphanizomenon flos-aquae* also show a hypocholesterolemic effect that stimulates liver function and decreases blood cholesterol level (Vlad *et al.*, 1995).

Phycobiliproteins are one of most abundant proteins in many algae and cyanobacteria. It is used as a natural protein dye in the food and cosmetic industries. The major phycobiliproteins include phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin. Phycobiliprotein derived from *Spirulina* sp. is used as a natural pigment in foods such as chewing gum, dairy products and jellies (Santos *et al.*, 2004). Phycobiliproteins serve as labels for antibodies, receptors and other biological molecules in fluorescence-activated cell sorters, and are used in immunolabelling experiments and fluorescence microscopy for diagnostics (Roman *et al.*, 2002). Pharmacological properties attributed to phycocyanin include antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, antiviral and antitumor activity, treatment in atherosclerosis, lipase activity inhibition, and serum lipid reduction.

Polysaccharides from micro-algae include carbohydrates found in the form of starch, glucose and sugars, with good overall digestibility, making the dried whole micro-algal mass a source for foods or feeds (Becker, 2004). Micro-algal (cyanobacteria and diatoms) extracellular polymeric substances that are polysaccharidic in nature present unique biochemical properties that make them interesting biotechnologically (Singh, Bhushan and Banerjee, 2005). The hydrocarbon-rich micro-alga *Botryococcus* sp. has been exploited for exopolysaccharide production (Bailliez, Largeau and Casadevall, 1985; Dayananda *et al.*, 2007b). Some of these exopolysaccharides have biological activity attributes, such as cytotoxic and anti-tumour properties (Li *et al.*, 2011).

TECHNO-ECONOMIC ANALYSIS OF MICRO-ALGAL BIOMASS PRODUCTION FOR BIOFUELS, AND CO-PRODUCTS

For successful realization and utilization of algal biomass for fuel and feed purpose, it is important to produce algal

biomass at costs lower than US\$ 1/kg, with a high content of oil for producing biocombustibles, and subsequently utilize the spent biomass for feed purposes. The production cost of micro-algal biomass of *Spirulina* sp. or *Chlorella* sp. is around US\$ 4/kg. Strain selection to improve quality for bio-energy and feed use is a crucial determining factor in the economics, with US\$ 1/kg as the cost of biomass, and with lipids, carotenoids and other valuable as co-products, it would be economical to utilize the biomass for adoption for bio-refinery purposes.

In large-scale production, availability of water sources and their usage are the major factors determining the production costs, which reach the proportions of large-scale agriculture. Supply of nutrients like N, P and K, and use of commercial fertilizers at the large-scale industry level have potential negative impacts on energy balances. Therefore, use of agricultural and municipal waste streams is one possible option for reducing operational costs and also for achieving positive balance and reducing the carbon footprint. Freshwater-based cultivation is a costly process, so re-use of water and an integrated approach utilizing wastewater or industrial effluents could significantly reduce the cost of production. Further, the economic yield can be improved if the photosynthetic efficiencies of micro-algae can be pushed to achieve the theoretical limit, which is about 11 percent. However, under natural conditions (during summer), the photosynthetic efficiencies are about 2–3 percent, with an average biomass yield of 3.97 g DM/m²/day (Grobbelaar, 2009; Larkum, 2010). Various options to improve photosynthetic efficiencies are being considered, such as adjusting the frequency of light and dark cycles, development of short-light-path reactors with high turbulence to achieve up to 8 percent photosynthetic efficiencies and biomass yield of about 200 g DM/m²/day (Grobbelaar, 2009).

Commercialization needs thorough techno-economic modelling and analysis, life-cycle analysis (LCA) and resource assessment (Fishman *et al.*, 2010). LCA is an approach to assess the resource use and environmental impacts of industrial processes, mainly the green house gas emissions and carbon footprint. Yang *et al.* (2011) examined the LCA of biofuel production from micro-algae with respect to water footprint and nutrient balance. They reiterated the necessity of recycling water or using of marine or wastewater for making micro-algae-based biofuel production an economically competitive technology. According to them, to generate 1 kg of biodiesel, about 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate are required if freshwater is used without recycling. Recycling of water after the harvest of biomass, or use of sea or wastewater, decrease water requirement by 90 percent and eliminates nutrient requirements, except for phosphates. Gerbens-Leenes, Hoekstra and Van der Meer (2009) compared the

water footprint of bio-energy from some of the agricultural crops and concluded that the water footprint is high and not competitive enough.

Norsker *et al.* (2011) calculated the micro-algal biomass production costs for three different production systems operating at commercial scale: open ponds, horizontal tubular photobioreactors and flat-panel photobioreactors. The resulting biomass production costs for these systems were € 4.95, € 4.15 and € 5.96 per kilogram, respectively. The parameters included for the costing were irradiation conditions, mixing, photosynthetic efficiency of systems, medium, carbon dioxide costs and dewatering. Optimizing production with respect to these factors resulted in a price of € 0.68/kg. They conclude that at this cost, micro-algal-based biofuel production is promising and competitive. Lundquist *et al.* (2010) thoroughly assessed the technical and engineering systems involved in biofuel production from micro-algae. The biomass production systems considered are those for biofuel production or for wastewater treatment, with outputs as biogas, or biogas and oil, and a farm size of 100 or 400 ha. The major technical assumptions were 25 percent recoverable lipid content, biomass yield of 22 g DM/m²/day (80 t/ha/year) and a resulting oil yield of 20 000 L/ha/year. CO₂ was supplied from a flue gas source. According to their report, the overall production costs for biofuel production as a co-product of wastewater treatment was of high economic feasibility, at US\$ 28/barrel of oil or US\$ 0.17/kwh electricity produced through biogas generation. However, in the case of biofuel or biogas production as the major objective based on wastewater treatment, the system was very costly, at US\$ 332/barrel of oil and US\$ 0.72/kwh electricity generated through biogas. Their recommendations include use of bio-flocculation for harvesting biomass, online extraction of oil from wet biomass, and emulsification of oil, which together would reduce operating costs by 20–25 percent, avoiding the steps of drying and extraction.

BIOREFINERY APPROACH IN MICRO-ALGAL UTILIZATION

A bio refinery is an integrated approach to biomass conversion processes to produce fuels, power and value-added chemicals from biomass. The biorefinery is analogous to today's petroleum refinery, which produces multiple fuels and products. The first step in the biorefinery concept is cultivation of micro-algae, with limited inputs and avoiding use of nutrient chemicals like fertilizers. If the production systems are intended for biofuel production, nutrient-rich sources of wastewater can be utilized. This system is advantageous in terms of the natural treatment of the wastewater, which could be recycled for algal cultivation or used for irrigation. Industrial effluents like distillery wastes

or sewage ponds are good nutrient sources. Since micro-algae are better CO₂ sinks than higher plants, flue gases from industry can be used as a source of CO₂. Alternatively, if biomass production systems involve generation of valuable co-products, then cultivation systems utilizing marine ecosystems like coastal and estuarine areas are more economical in operational terms. Solar generated power can be effectively utilized in operating raceway ponds and pumping the culture for further downstream processing. The spent media can be utilized as a source of exopolysaccharides that have many potential bio-active properties. Lipid-rich diatoms like *Cylindrotheca closterium*, *Thalassiosira pseudonana* and *Skeletonema costatum* produce extracellular polysaccharides that can be harvested for further applications (Urbani *et al.*, 2005; Li *et al.*, 2011), and also recycled for algal cultivation.

There are different ways of realizing economic value from micro-algal spent biomass after oil extraction. The best option would be to achieve complete utilization of the biomass for maximum energy recovery by various conversion technologies, such as biomass gasification and thermochemical processes that generate syngas, which can be combusted or can be converted to chemicals like alcohols, ethers, etc. Pyrolysis can be employed where an oil-like liquid is produced that can be processed to fuels. Anaerobic fermentation of spent biomass yields methane, and power generation from these co-processes enhances the sustainability of micro-algal derived biofuels.

The second option is utilization of glycerol generated from the trans-esterification process of conversion of crude algal lipids to biodiesel. Glycerol can be used as feed for generation of biomass. Pyle, Garcia and Wen (2008) used a biodiesel-derived crude glycerol as a source of carbon for heterotrophic production of DHA by *Schizochytrium limacinum*, with comparable yields to other feeds. Glycerol is a highly reduced carbon source that can be converted to many industrially important compounds, including 1,3-propanediol, dihydroxyacetone, succinic acid, propionic acid, ethanol, citric acid, pigments, polyhydroxyalconate, squalene and biosurfactants by use of bacterial genera like *Klebsiella*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Propionibacterium*, *Anaerobiospirillum* and *Escherichia*. (Yazdani and Gonzalez, 2007; Silva, Mack and Contiero, 2009).

Another option is recovery of polysaccharides and proteins from mono-algal cultures grown in clean environments for use as animal feeds. Animal feeds can use spent biomass with low lipids but with high content of micronutrients such as minerals, anti-oxidants, proteins and vitamins, in supplementation of rations for fish, poultry and other livestock. Some enhancement of the spent biomass might be required for greater efficacy as feed supplement. The residual biomass could be used as soil

fertilizer and conditioner. Thus production and utilization of algal biomass with net energy gain in the process without waste generation would be desirable for the sustainable exploitation of the technology for energy needs.

Several companies worldwide are working on algae-based biofuels. Though cost effective, viable technology has not yet been developed, the approaches suggested in this review for utilizing spent biomass for feed and all other different fractions in a biorefinery manner would go a long way in making the process economic.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

- There is a continuing need to screen the vast biodiversity of micro-organisms to identify high yielding strains.
- Further genetic improvement of potential strains for achieving higher biomass and metabolite yield and subsequent scale-up..
- It is important to devise culture methodologies based on the nature of the alga.
- Efficient utilization of seawater, wastewater, flue gases and various carbon dioxide sources for enhanced production of biomass.
- Utilization of wind and solar power for culture agitation and harvesting.
- Drying of biomass employing solar drying systems.
- Achieving the least energy losses for net energy gain.
- Developing bio-refinery approaches with minimal energy inputs and effective utilization of all by-products and co-products.
- To develop integrated systems of feed and food production for use of biomass in a meaningful manner.
- Algal biomass derived feeds and feed supplements need to be developed for augmenting animal products, aquaculture and the poultry industry, thus adding value to existing technologies.
- Utilization of marginal land for algal biomass cultivation.
- Exploring setting up of production plants at the sea surface or in coastal areas.

Table 11 lists some of the potential process developments for utilization coupled to integration of technology with renewable energy inputs for net energy gain.

CONCLUSIONS

The energy demands of the world coupled with deteriorating environmental conditions have highlighted the need for eco-friendly measures for sustainable solutions. In this regard, algal biotechnology utilizing the vast biodiversity available for production of bio-energy molecules is already recognized as a promising area for meeting the dual demands for energy and respect for the environment. Having realized the importance of photosynthetic carbon fixation for the production of energy-rich molecules, the development of technologies to produce biomass on a massive scale would need research and developmental inputs for evaluating viable technology alternatives. The identification of algal forms, their cultivation and utilization would go a long way to realize the desired objectives. Approaches to achieving net energy gain in a sustainable and eco-friendly manner need to be developed and adopted. The current trends and future prospects touched on in this review could provide directions for advances in effective utilization of algal biotechnology for fuel, food, feed and chemicals.

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TABLE 11
Process innovation and renewable energy utilization for net energy gain and utilization of co-products

Process steps	Adoption of innovative measures and use of renewable energy
Employing organisms with high growth rate in addition to high product or metabolite production	Responsiveness to environmental conditions such as stress factors for increase yields needs to be explored.
Adoption of open bioreactors	Media optimization, culture conditions.
Mixing of culture	Windmill driven.
Harvesting of the culture	Through sedimentation. Adoption of proper gradient for separation of the biomass.
Recycling of the water back to the inoculum ponds.	Windmill driven.
Adoption of closed bioreactors	Use of solar radiation for providing the light source and mixing of culture through windmill-driven peristaltic pumps,
Drying of algal biomass	Solar drying system.
Extraction of constituents (lipids and hydrocarbons) and utilization of spent biomass	Biorefinery approach.

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Chapter 25

Land use in Australia for biofuels and bio-energy: opportunities and challenges for livestock industries

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ABSTRACT

Current biofuel production in Australia and the opportunities that the co-products offer for Australia's intensive and grazing livestock production systems are described. Based on the use of grain sorghum and co-products of grain and sugar processing, the current biofuels industry in Australia is small. At present it is not a significant challenge to the availability of feedstocks for the intensive livestock industries and only provides relatively small amounts of co-product for livestock utilization. However, new non-food biomass production systems for biofuel and bio-energy are being researched and developed. These include the use of lignocellulosic feedstocks from agricultural residues and on-farm plantings of short-rotation coppicing eucalypts, as well as new bio-oil feedstocks such as the low-rainfall oilseed crop *Brassica juncea*, the oilseed tree *Pongamia pinnata* and algae. This move towards the production of bio-energy and biofuels from non-food feedstocks raises the question: What will be the likely challenges and opportunities for the Australian livestock industries with land-use change for the production of these feedstocks? To answer this question, those developments that will affect livestock have been considered through an examination of Australian and other research. Factors examined include the diversion of feedstocks currently used by livestock (cereal stubble or straw), the production of co-products potentially useful to livestock (juncea and pongamia meals) and the development of a biomass production system that could be integrated with the livestock production systems in Australia (short-rotation coppicing (SRC) eucalypts). The process as discussed here includes research into the use of these crops. The systems of production of the new lignocellulosic feedstocks are of particular relevance for grazing livestock, both sheep and cattle. Research carried out over many years has been combined to identify the opportunities and challenges for grazing livestock as the new production systems for these feedstocks develop in Australia's agricultural lands.

INTRODUCTION

Australia has a large land area of 7.69 million km², a relatively small population of 22.4 million, and an advanced agricultural industry. It is therefore seen by many around the world as potentially a large bio-energy and biofuel provider.

Australia currently has only a small biofuel and bio-energy industry, based on first-generation technologies, as outlined in the next section. Australian ethanol is produced from three feedstocks: grain sorghum; waste wheat starch, a co-product of the extraction of gluten from wheat flour; and C-molasses, a co-product of the sugar industry. Australian biodiesel is produced from tallow and used cooking oil, with some production from juncea mustard seed (*Brassica juncea*), which is a low-rainfall *Brassica* under development as an alternative to canola.

Any major increases in the biofuel industry in Australia will most likely be predicated on new-generation process-

ing technologies and some new types of feedstocks. This is in recognition of the global issues raised by large-scale diversion of starches, sugars, fats and oils from the human and intensive livestock food chains into biofuels. The focus is therefore on non-food feedstocks such as lignocellulose from sources such as cereal stubbles, short-rotation coppicing (SRC) eucalypts and commercial forest residues, and oils from micro-algae or oilseed trees (Farine *et al.*, 2012). Australia has significant amounts of lignocellulose from existing production systems in agriculture and forestry, and a strong capacity to produce more (Farine *et al.*, 2012). In contrast, the current production base for plant-based oils is very small, and any scaling up of production would rely on new production systems, such as use of brassica, pongamia and algae (Farine *et al.*, 2012).

Unlike current processing technologies based on sugar, starch and food-based oilseeds, the new-generation technologies and feedstocks do not necessarily produce

MAIN MESSAGES

- The current small biofuels industry in Australia, based largely on the use of co-products of grain and sugar industry, is not a significant challenge to the availability of feedstocks for the intensive livestock industries and only provides a relatively small amount of co-product for livestock feed. An expansion of the current first generation biofuels industry would increase direct competition for grain, but would also increase the availability of protein feedstuffs – DDGS and oilseed meals, which could provide a useful source of supplementary protein for livestock grazing low-protein, dry summer pastures. DDGS is particularly suitable for this role in ruminants.
- New, non-food biomass production systems for biofuel and bio-energy are being researched and developed in Australia. These include the use of lignocellulosic feedstocks from agricultural residues and on-farm plantings of short-rotation coppicing eucalypts; and new bio-oil feedstocks, such as the low-rainfall oilseed crop *Brassica juncea*, the oilseed tree *Pongamia pinnata*, and algae. Much work remains yet to be done to fully design, test and implement the production systems.
- The harvesting of stubble for bio-energy should have little impact on grazing livestock in mixed grazing-cropping farming systems. There is little of nutritional value in stubble for grazing livestock. When modelled as part of a whole farm system, the value for livestock of grazing stubble is variable, often marginal or negative. The use of long-phase perennial pasture rotations in the cropping-livestock system is the most beneficial practice in the long-term maintenance of cropping soils and will always provide the major opportunity for livestock within the system, whether stubble is harvested for bio-energy or grazed.
- The re-introduction of trees for bio-energy and bio-fuels into cleared agricultural lands in Australia, will provide direct benefits in livestock productivity and animal welfare through the provision of shade and shelter as well as long-term benefits through land conservation for the grazing livestock industries. The integration of biomass production in the form of SRC eucalypts with pasture and livestock grazing may provide a benefit in improved resilience and land conservation while maintaining economic productivity of the land.
- Integration of cropping, grazing and bio-energy production presents a complex set of biophysical, social and economic interactions that will need to be well understood to ensure sustainable development of such land use. While some recent research at landscape scale has been reported here, there is need to continue this at a range of scales, including sociological, to better understand the likely land use changes in Australia associated with developing bio-energy industries.

processing co-products that can be used as livestock feed. However, the biomass production systems themselves can either compete with, or be complementary to, animal production systems. In this sense, animal production can be viewed as a legitimate co-product of biofuel production, albeit in a different part of the value chain than usually assumed.

The co-products produced from the current biofuels industry and the value of these for the Australian livestock industries are outlined as they are available and are being utilized now. However, the potential move towards the production of bio-energy and biofuels from non-food feedstocks raises the question: "What will be the likely challenges and opportunities for the Australian livestock industries associated with land use change for the production of these feedstocks for bio-energy and biofuels?"

CURRENT BIOFUEL PRODUCTION IN AUSTRALIA

The amount of biofuels currently being produced in Australia is small in comparison with global activities. In 2009,

as a percentage of the world's total, Australia's ethanol production was 0.15 percent, biodiesel was 0.4 percent (F.O. Licht, 2009), and, over all, biofuels represented only about 0.5 percent of Australia's transport fuel consumption. Over the past decade there have been numerous proposals for the development of first-generation biofuel production facilities in Australia, not all of which have proceeded. Of those that have, some are not currently in production due to changes in feedstock costs and other economic issues. In 2008–09 actual production of biofuels was approximately 50 percent of the stated production capacity (ABARE, 2010a; Geoscience Australia and ABARE, 2010). As a consequence the amount of co-product available for livestock is relatively small.

An estimate has been made of the amount of co-products, i.e. wet or dried distillers grain and protein meals, based on the stated capacities of the small number of bio-ethanol and biodiesel plants currently in production (Table 1). These are potentially available to the Australian livestock industries if the plants are operating at full capacity, and in the absence of imported biofuel co-products.

TABLE 1
Fuel ethanol production facilities in Australia, 2009

Facility	Capacity ($\times 10^6$ L/yr)	Feedstock	Co-products
Manildra Group, Nowra, NSW	180	Waste wheat starch, low-grade wheat and sorghum grain	175 000 t/yr DDGS
Dalby Biorefinery, Dalby, Qld	90	Grain sorghum	134 000 t/yr WDG 38 500 t/yr syrup
CSR Distilleries, Sarina, Qld	60	C-molasses	225 000 t/yr C-molasses converted to ethanol
Total fuel ethanol capacity	330		

Notes: NSW = New South Wales; Qld = Queensland.

Grain ethanol co-products

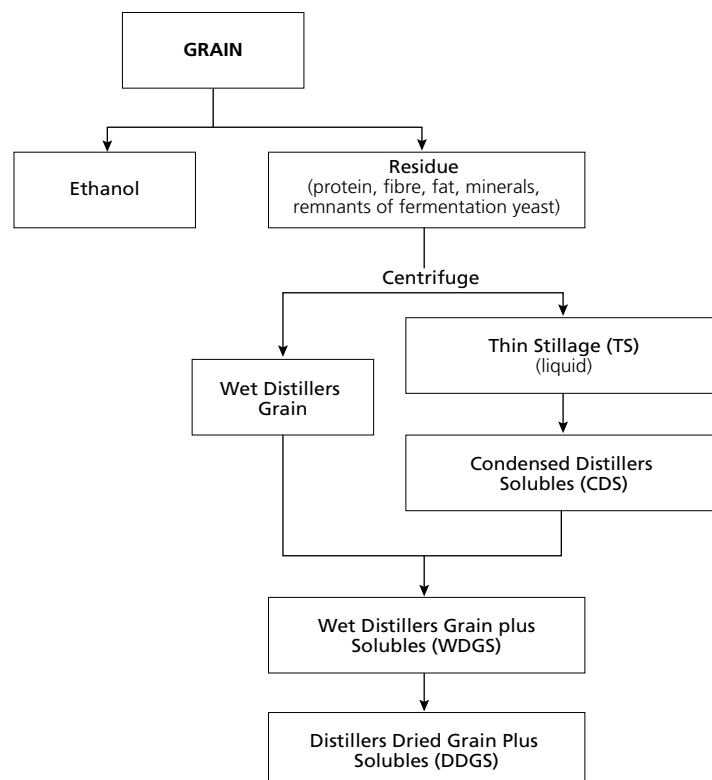
The process for the production of ethanol from grain and the associated co-products – whole stillage, thin stillage, condensed distillers solubles (CDS), wet distillers grain (WDG), wet distillers grain with solubles (WDGS) and dried distillers grain with solubles (DDGS) – is set out in Figure 1 and has been described, together with the composition of the co-products (Braid, 2007).

Research papers are available on the use of cereal ethanol co-products in the diets of a range of intensively farmed animals: beef and dairy cattle, pigs, poultry (broilers, laying hens and turkeys) and fish, and cover all the stages of production from weaning to finishing (Al-Suwaiegh *et al.*, 2002; Anderson *et al.*, 2006; Cheng and Hardy, 2004;

Lumpkins, Batal and Dale, 2005; Lumpkins, Batal and Dale, 2004; Whitney and Shurson, 2004; Whitney *et al.*, 2006). In general, the research findings are positive about the value and use of cereal ethanol co-products to replace a portion of grain or protein meal, or both, in intensive livestock diets.

The wet cereal ethanol co-product, WDGS, has a limited storage time of 3–5 days at 22 °C (Walker, 2004). On a dry matter basis, WDGS (30 percent DM) is expensive to transport and must also be handled according to any wet-waste transport requirements set by the local environmental protection agency. Drying WDGS to form DDGS uses 30–40 percent of the total energy requirements of a cereal ethanol plant (Ham *et al.*, 1994). However, DDGS can be readily transported, stored and added to pelleted feeds,

FIGURE 1
Co-products of a dry-grind cereal ethanol plant



Source: Braid, 2007.

making it more accessible to livestock industries and more marketable. DDGS can be used in diets without affecting production or reducing the quality of animal products – meat, milk, eggs, etc. – at rates of up to 20–40 percent for cattle; 10–25 percent for pigs; 9–15 percent for poultry and 15–22.5 percent for fish (Braid, 2007). DDGS is particularly useful for ruminants, providing a combination of rumen by-pass protein, digestible fibre and energy.

The information below is culled principally from ABARE (2010a).

In Australia, the Dalby Biorefinery produces ethanol from grain sorghum and, unlike many grain ethanol plants, does not produce DDGS, whereby thin stillage is condensed to form condensed distillers solubles (CDS) then added back to the wet distillers grain prior to drying. Instead, Dalby Biorefinery relies on the separate sale of wet distillers grain (WDG) and CDS for disposal of its ethanol co-products.

At full capacity, the Dalby plant produces 134 000 t/yr of WDG with a moisture content of 65 percent, equivalent to some 47 000 t of dried distillers grain. All of the WDG from the Dalby plant is sold direct to a beef feedlot in southern Queensland. The CDS, high in protein, fats, minerals and digestible fibre, is sold on to a livestock feed processor to be mixed with cane sugar molasses to form a highly nutritious feed supplement for horses and ruminants.

The Manildra Group at Nowra, NSW, uses a waste-starch stream from their flour-to-gluten plant, together with some low-grade wheat and grain sorghum, to produce ethanol and DDGS. At full capacity, the Manildra Group's current plant can produce 175 000 t/yr of DDGS. Some goes to beef feedlots, but the primary market is the NSW south coast dairy industry, which uses the DDGS either as inclusion in the grain supplement fed during milking or as a drought supplement (Mark Honey, 'Riversdale', pers. comm.).

Potentially, the current total annual amount of WDG and DDGS from ethanol production in Australia is equivalent to 225 000 t of dried distillers grain. To put this into perspective, this represents just 4.8 percent of the estimated 4 642 000 t of grain used annually in Australia for beef cattle in feedlots and for dairy cows (Hafi and Connell, 2003). It is difficult to accurately estimate the effect on availability and price of cereal grain for livestock use in Australia due to the current diversion of grain to ethanol production. Almost half of Australia's ethanol production comes from the Manildra Group's use of waste starch from food processing, i.e. from grain external to the livestock feed market. In addition, with approximately 60 percent of Australia's grain production going to export, international grain prices are a major influence in setting local prices.

There has been some concern from livestock producers that the diversion of cane sugar molasses to the production of ethanol would affect the availability and price of molasses, which is used as an energy supplement and car-

rier for minerals such as phosphorous for grazing livestock, particularly in northern Australia. On average, Australia produces 1 025 000 t of molasses annually (Anon., 1996–2007; ASMC, 1996–2007). At full capacity, CSR Distillers in Queensland would use approximately 225 000 t, or 22 percent of annual production, to produce 60×10^6 L ethanol per year. This increase in demand may affect availability and price, particularly in drought years. However, in part, the diversion of this molasses to the production of biofuel is offset by the addition of the 38 000 t/year of CDS from the Dalby Biorefinery into the molasses market for livestock energy supplementation.

Biodiesel co-products

There are two co-products from the production of biodiesel that can be used as feed for livestock: oilseed meal following the extraction of the bio-oil from the oilseed prior to its conversion to biodiesel, and crude glycerol, a co-product of the transesterification process. The majority of the biodiesel producers listed in Table 2 rely on a combination of tallow and used cooking oils as the feedstock for their plants and consequently do not produce an oilseed meal co-product.

Canola, a cultivar of rapeseed (*Brassica napus*), a common European feedstock for the production of biodiesel, is grown in Australia, which in 2009 produced 1 920 000 t of canola oilseed (ABARE, 2010b), of which 65 percent was exported as whole oilseed and the balance crushed in Australia for the production of canola oil for human use. The canola meal derived from this production of canola oil is used in the intensive livestock industries: poultry, pigs and dairy cows. Canola meal is not a co-product of Australia's biofuels industry as canola is not used for the production of biofuels in Australia.

However, there is a *Brassica* sp. that is increasingly being used in biodiesel production, *Brassica juncea*. As a non-food feedstock, this is described in the section on new production systems.

National Biodiesel Pty Ltd at Port Kembla, NSW, are in the process of developing a new facility for the production of soy biodiesel that will have a significant impact on the availability of Australian-produced biofuel co-products once it reaches its stated capacity. Based on projections, it will deliver more than 800 000 t of soybean meal per annum, initially from imported soybean. In 2009–10, Australia imported 512 000 t of soybean meal to meet the feedstock demand of the pig and poultry industries as the total Australian production of soybean was only 59 600 t. As this facility is not in production it has not been included in Table 2.

Glycerol

Glycerol occurs naturally in animal and vegetable fats where it is about 10 percent of the lipids. Crude glycerol is a co-product of the production of biodiesel and must

TABLE 2
Biodiesel production facilities in Australia, 2009

Facility	Capacity ($\times 10^6$ L/yr)	Feedstock	Co-products
Biodiesel Industries Australia, Maitland, NSW	15	Used cooking oil, vegetable oil	Crude glycerol
Biodiesel Producers Ltd., Wodonga, Vic	60	Tallow, used cooking oil	Crude glycerol
Smorgon Fuels, Melbourne, Vic	100	Juncea oilseed, tallow, used cooking oil, vegetable oil	10 000–15 000 t/yr juncea mustard meal; crude glycerol
Various small producers	5	Used cooking oil, tallow, industrial waste, oilseeds	Juncea meal; crude glycerol
Total biodiesel capacity	280		

Notes: NSW = New South Wales; Vic = Victoria. Source: ABARE, 2010a.

be refined to 95–99 percent purity for use as food grade glycerol. Under current transesterification biodiesel refining processes, 79 g of crude glycerol is produced for every litre of biodiesel (University of Idaho, 2006). Based on the total Australian plant capacity of 180×10^6 L of biodiesel (Table 2), this represents potentially >14 000 t/yr of crude glycerol produced in Australia. With large increases of biodiesel production around the world, there is considerable interest in utilizing crude glycerol in novel ways, including as a dietary energy source for livestock.

In Australia, the Pork Co-operative Research Centre, in association with Murdoch University, Western Australia, have carried out a two-part study in which the chemical compositions of crude glycerol samples from seven Australian biodiesel producers were analysed and the effects of feeding crude glycerol to growing-finishing pigs were assessed (Hansen *et al.*, 2009). The chemical composition of the crude glycerol varied greatly between samples. The pH ranged from 2.0 to 10.8, moisture from 0 percent to 16.1 percent, ash from 0 percent to 29.4 percent and methanol from <0.01 percent to 13.94 percent. One of the test samples, with a pH of 2.0, 76.1 percent glycerol and 1.83 percent ash, was selected for the feeding trial.

In this trial, groups of 12 Large White \times Landrace female pigs of 50.9 ± 5.55 kg live weight were fed mash diets containing 0, 4, 8, 12 or 16 percent glycerol for approximately 10 weeks prior to slaughter. All diets were formulated with a digestible energy of 13.5 MJ/kg with crude glycerol replacing grain (wheat and barley) as the energy source. In addition to recording daily feed intake, weight gain and meat quality at slaughter, the pigs were blood tested each week for plasma glycerol to assess the metabolism of the glycerol. When ingested, glycerol absorbed from the intestinal tract, is converted to glucose in the liver. If the gluconeogenic capacity of the liver is exceeded, excess glycerol remains in the plasma, to be excreted in the urine (Kijora *et al.*, 1995). Identifying the limits of glycerol conversion should contribute to understanding the effective levels of glycerol that can be fed to replace other energy sources.

In this study, the plasma glycerol levels increased markedly when dietary glycerol exceeded 4 percent, which suggests that the limit on glycerol conversion had been reached and potentially the energy supply to the pigs was lower. Even so, once the pigs had adapted to the diets by the end of the second week, the daily feed intake, weight gain, feed conversion ration, P2 backfat and meat quality were unaffected ($P > 0.05$) by the inclusion of up to 16 percent crude glycerol in the diet.

There are issues with the feeding of crude glycerol.

- The large variation between crude glycerols derived from biodiesel production is of concern when contemplating its use in livestock feeds. It would appear that monitoring of the chemical composition is vital when formulating diets containing crude glycerol from biodiesel production.
- High ash content may be associated with the use of sodium or potassium salts as catalysts during the process (Hansen *et al.*, 2009), or the use of used cooking oils, or both.
- Methanol is a known toxin in humans, and countries have established maximum permitted levels for methanol in crude glycerol for animal feed: 0.015 percent in USA, 0.1 percent in Canada, 0.2 percent in Germany and 0.5 percent in the European Union as a whole (Hansen *et al.*, 2009); Parsons, 2010).
- There can be feed handling problems. The mash diets in the study described containing >8 percent glycerol formed firm aggregates within 24 hours after mixing. It has been reported that up to 12 percent of crude glycerol can be added to feed prior to pelleting without affecting pellet quality.
- Crude glycerol derived from the use of tallow for the production of biodiesel should not be used in ruminant feedstocks due to the possibility of transmission of bovine spongiform encephalopathy (BSE).

In summary, the small biofuels industry in Australia, based on the use of residues, is currently not a significant challenge to the availability of cereal grains for the intensive livestock industries and only provides relatively small amounts of co-

product for livestock feed. Individual biofuel plants do provide an opportunity for local livestock producers to include co-products in livestock rations or for use as supplementary feed, an opportunity that has been embraced. An expansion of the current first-generation biofuels industry would increase the availability of protein feedstuffs – DDGS and oilseed meals – which could provide a useful source of supplementary protein for livestock grazing low-protein, dry summer pastures. DDGS is particularly suitable for this role in ruminants, as unlike whole grain, DDGS is low in fermentable carbohydrate and will not lead to the ruminal acidosis associated with high starch loads in some grain, making it a safe supplement that can be fed ad libitum.

NEW PRODUCTION SYSTEMS FOR BIOFUELS AND BIO-ENERGY IN AUSTRALIA

In many countries throughout the world, there is continuing development of new technologies and production systems for biofuels and bio-energy. The Australian government actively supports the development of non-food biofuel production systems through research programmes such as the Second Generation Biofuels Research and Development (Gen 2) Program, which currently funds research into biofuels from micro-algae, sugar cane bagasse and short rotation coppicing (SRC) eucalypts (DRET, 2009). Some Australian States also support initiatives, such as the use of municipal waste for biofuels (Invest Victoria, 2010). This section will only deal with those developments that will affect livestock:

- through the utilization of a feedstock currently used by livestock;
- the production of a co-product potentially useful to livestock; or
- through a biomass production system that might be integrated with the livestock production systems in Australia and therefore livestock and biomass can be considered as co-products.

The new production systems that will be considered are:

- Oil-based biofuels from *Brassica juncea*, algae and *Pongamia pinnata*.
- Lignocellulosic-based biofuels from two types of feedstocks: stubble (the stalk residue from cereal grain) and SRC eucalypts. SRC eucalypts, commonly known as oil mallees, characteristically have many stems that emerge from an underground lignotuber. When harvested close to the ground, the lignotuber remains intact, enabling the tree to survive and the multiple stems to re-sprout, i.e. coppicing.

Oil-based biofuels

Brassica juncea

Brassica species are recognized for their ability, when used as break crops, to reduce diseases in cereals and to improve the production of the subsequent crops. The biofumiga-

tion effect of brassica species reduces crown rot (*Fusarium pseudograminearum*), root lesion nematode (*Pratylenchus thornei*) (Trethowan *et al.*, 2009) and take-all, a soil-borne disease of wheat in south eastern Australia caused by *Gaeumannomyces graminis* (Sacc.) Arx & Oliv. var *tritici* (Kirkegaard *et al.*, 2000), while the broad-leaf cover of brassica crops reduces weed infestation. Canola (*Brassica napus*) is grown in the higher rainfall areas of Australia as a break-crop and for the value of its oilseed, but its distribution is limited by its rainfall requirement. Consequently, some Australian State government agricultural research agencies, universities and private companies have been involved in the breeding and development of *Brassica juncea* varieties for use as a break crop in the drier and hotter areas of the Australian wheat belt, where the mean annual rainfall is <425 mm, for the production of biofuels and the feeding of livestock.

Some of the development is in the juncea varieties high in the “hot and spicy” glucosinolates for condiment mustard. However, the main varieties of interest are in the juncea canola group that retain the low-rainfall growth ability but have oil fatty-acid profiles and levels, and types of glucosinolates in the meal, similar to canola. Glucosinolates are found in all brassicas and produce a range of active secondary metabolites that are responsible for the biological effects associated with feeding brassica meals. These effects are relative to the concentration of glucosinolates in the diet, but vary with the type of glucosinolates and secondary metabolites in the meal.

Amongst the glucosinolates, sinigrin and progoitrin and the glucosinolate metabolite isothiocyanates are associated with the bitter taste of some brassica meals that lead to reduced feed intake, while other glucosinolate metabolites affect thyroid function or cause goitrogenicity, hepatotoxicity, nephrotoxicity or endocrine disturbance due to non-specific antinutritional factors. In general, ruminants are more tolerant to glucosinolates than monogastric animals such as pigs and poultry. Tripathi and Mishra (2007) have published a comprehensive review of glucosinolates in animal nutrition. The content and type of glucosinolates vary between brassicas that have originated in the hot, dry conditions of the Indian sub-continent or the more temperate conditions of Europe. The availability of high-glucosinolate (HG) Indian mustards (glucosinolate content of 125 to >200 µmol/g) and low-glucosinolate (LG) European canola *Brassica* species (glucosinolate content of <10 to 30 µmol/g) has, through genetic manipulation, enabled plant breeders to retain the drought tolerance qualities of Indian mustards (*Brassica juncea*) while significantly reducing the glucosinolate content levels.

Smorgon Fuels Pty Ltd, Melbourne, in conjunction with the South Australian Research and Development Institute (SARDI), have developed a juncea variety, BioMaxDLJ200

for biofuel production and capable of growing in areas with average annual rainfall of less than 375 mm (SARDI, 2011). The Pork Cooperative Research Centre, in association with Rivalea Australia and Smorgon Fuels, have carried out a trial to evaluate juncea meal in growing pigs (Collins *et al.*, 2011). Groups of 14-week-old entire male Large White × Landrace pigs (live weight 40.4 ± 0.41 kg) were fed formulated diets as ad libitum pellets for 35 days, in which juncea meal replaced canola meal to make up diets containing 0, 6, 12, 18 or 24 percent juncea meal.

Juncea oilseed was sourced from crops grown in Victoria, New South Wales and South Australia, which was crushed using an expeller press and the resultant meal analysed for chemical composition and gross energy content, amino acid profile and glucosinolate concentration. There was very little difference between the canola meal and the juncea meal in amino acid profile, with the juncea meal higher in fat content and lower in fibre. The glucosinolate concentration, based on ten samples of the juncea meal, was 13–19 $\mu\text{mol/g}$, average 15.9 $\mu\text{mol/g}$. The glucosinolate concentration of the canola meal was not assessed but could be assumed to be in the 4–5 $\mu\text{mol/g}$ range of the meal from canola cultivars grown in southern Australia, which has been shown to not produce adverse effects in pig weaner diets when included at up to 25 percent of the diet.

While there was a linear decline in feed intake associated with increasing juncea meal concentration ($P < 0.001$), resulting in a reduction in growth rate over the whole test period, the conclusion of the trial was that the juncea meal could be fed at up to 18 percent of the formulated diet without affecting growth performance over the 35 days of the trial. At this level, the glucosinolate concentration was 2.85 $\mu\text{mol/g}$ diet. At 24 percent of the diet, there was reduced feed intake and slower growth rate in the pigs. Feed wastage throughout the study period was assessed as not significant. These results are in line with the findings of others (Opalka *et al.*, 2001; Roth-Mailer, Bohmer and Roth, 2004) where glucosinolate concentrations in diets of 2.2 $\mu\text{mol/g}$ or less did not affect growth performance in pigs.

It is estimated that somewhere between 13 000 and 19 000 ha of *Brassica juncea* was grown in Australia in 2010, providing 10 000–15 000 t of meal (Nelun Fernando, Smorgen Fuels Pty Ltd, pers. comm.). Currently this has little impact on the protein meal market in Australia, where some 400 000 t of Australian grown canola meal and 500 000 t of imported soybean meal is used annually in the intensive livestock industries (ABARE, 2011).

Micro-algae

There is currently no commercial algal biofuel production in Australia. Algal production systems based on 400 ha raceways co-located with the major CO₂ production sites in

Australia could produce up to 10.7×10^6 t of algal biomass each year (Farine *et al.*, 2012). Following oil extraction, the remainder algal biomass could be used for the production of bio-energy, for other co-products or, if suitable, be available as a protein feed or supplement for livestock. Micro-algae can be used in animal nutrition, primarily in aquaculture, but also as a vitamin and mineral supplement for farm animals and pets (Spolaore, 2006). However, the majority of the Australian sites noted in Farine *et al.* (2012), would rely on CO₂ flue-gas from coal-fired power stations, which can contain heavy metals and other toxins that are likely to be taken up by the algae, rendering the algal biomass co-product unsuitable as animal feed (DOE, 2010).

Pongamia

The oil-seed tree *Pongamia pinnata* is native to the Indian sub-continent and South-East Asia but has become naturalized in small areas along the coastal fringe and associated rivers in the tropical north of Australia. In India and Asia, it has traditionally been used as a fuel for cooking and lighting. More recently it has been recognized as a candidate for biofuel production in Australia, which has led to *Pongamia pinnata* (L.) Pierre, becoming a focus of academic and commercial research and development. At the time of writing, there are only small areas of trial plots with no commercial production of oil or associated oil-seed meal. The potential of the tree has, however, been recognized (Kazakoff, Gresshoff and Scott, 2011; Scott *et al.*, 2008) and a clear R&D strategy has been proposed (Murphy *et al.*, in review).

There is interest in developing large plantations in northern Australia to produce feedstock for aviation biofuel, local transport, and for GHG mitigation, with the pongamia co-products to be combusted for regional power generation and biochar, a carbon-rich co-product used for soil amendment (CleanStar Ventures, 2011), or for use as a protein supplement for grazing cattle, although 80–90 percent of the seed storage protein is now known to be similar to soybean 7S beta conglycinin, known to be a nutritionally poor source of protein (Scott *et al.*, 2008).

There has been considerable research, mainly in India since the early 1970s, on utilization of this protein meal as animal feed. The meal contains karanjin (a flurano-flavinoid) and pongamol in the residual oil that make it unpalatable. It also contains anti-nutritional factors such as phytates, tannins and protease inhibitors that affect rumen metabolites and the digestibility of protein and carbohydrates (Vinay and Sindhu Kanya, 2008).

Oil extraction carried out by the usual method of expeller pressing leaves 15–20 percent oil in the cake (expeller-pressed karanj cake – EKC). Solvent extraction removes more oil, and should increase the palatability of the meal and reduce toxicity, but research results indicate that inclusion of solvent-extract pongamia meal (solvent-

extracted karanj cake – SKC) in mixed diets still reduces both feed intake and growth rates.

Researchers have sought additional ways of detoxifying the meal, aimed at reducing the anti-nutritional factors through water leaching and the addition of mild acid or alkali. Vinay and Sindhu Kanya in a laboratory study (Vinay and Sindhu Kanya, 2008) used a 2 percent HCL treatment for 1 hour to reduce anti-nutritional factors: phytate (81 percent), tannin (69 percent) and protease inhibitors (84 percent).

A review of recent studies gives a good indication of the problems associated with using the pongamia meal derived from the production of biofuels as an animal feed. A long-term (34-week) performance trial of lambs was undertaken using diets containing either 24 percent EKC or 20 percent SKC pongamia meal, replacing half of the usual de-oiled groundnut cake as the source of protein. In this trial there were no further treatments of the meal to reduce anti-nutritional factors. The outcome of this long-term trial was that dry matter intake; digestibility of protein and carbohydrates; growth rate; and wool production were all reduced in the lambs receiving the diets containing either EKC or SKC. The authors identify other research with similar outcomes. In addition, by the end of the trial, the lambs had reduced bone density (osteoporosis), testicular degeneration, and liver and spleen lesions (Singh *et al.*, 2006).

In a study of growth performance in chickens, in which SKC was subject to one of three different treatments for anti-nutritional factors (untreated SKC, 1.5 percent NaOH SKC, 3 percent Ca(OH)₂ SKC) and EKC to one treatment (2 percent NaOH EKC), the pongamia meal was used to replace 12.5, 25 or 50 percent of soybean meal in the diet. The results showed depression of growth as well as severe pathological changes occurring in the chickens once the replacement level exceeded 25 percent, irrespective of the method of oil extraction or the anti-toxicity treatment. The pathological changes included lymphoid cell degeneration, and liver, kidney and spleen pathology (Panda *et al.*, 2008).

These growth performance trials in lambs and broiler chickens, despite efforts to reduce residual oil and toxicity factors in the meal, demonstrate that *Pongamia pinnata* meal is only useful and safe as an animal feed at low levels of inclusion. Other trials mentioned in the literature indicate that similar results have been found with cattle and goats (Konwar, Banerjee and Marshall, 1987; Srivastava *et al.*, 1990).

Finally, it should be noted that there is a benefit from pongamia containing the unpalatable karanjin and pongamol, as it allows the integration of grazing livestock in *Pongamia pinnata* plantations with minimal risk of the animals grazing and damaging the trees. At a trial plot in southern Queensland where the trees are 3–4-years old, sheep are grazed in the plantation to control grass and weed growth and to provide some additional income from the land (George Muirhead, pers. comm.).

LIGNOCELLULOSIC-BASED BIOFUELS

The technologies to use lignocellulosics such as cereal and forest residues for the production of biofuels are rapidly developing (Mohan, Pittman and Steele, 2006). In Germany, Choren Industries, Daimler AG, use a Fischer-Tropsch process to manufacture SunDiesel®, a biodiesel, from cereal stubble (straw) and forestry residues (Daimler AG Communications, 2011). Abengoa Bioenergía has pilot plants in Salamanca, Spain, and Nebraska, United States, using fermentation processes for the production of cellulosic ethanol from stubble, and is building a commercial-scale plant in Kansas, United States (Abengoa Bio-energy, 2011).

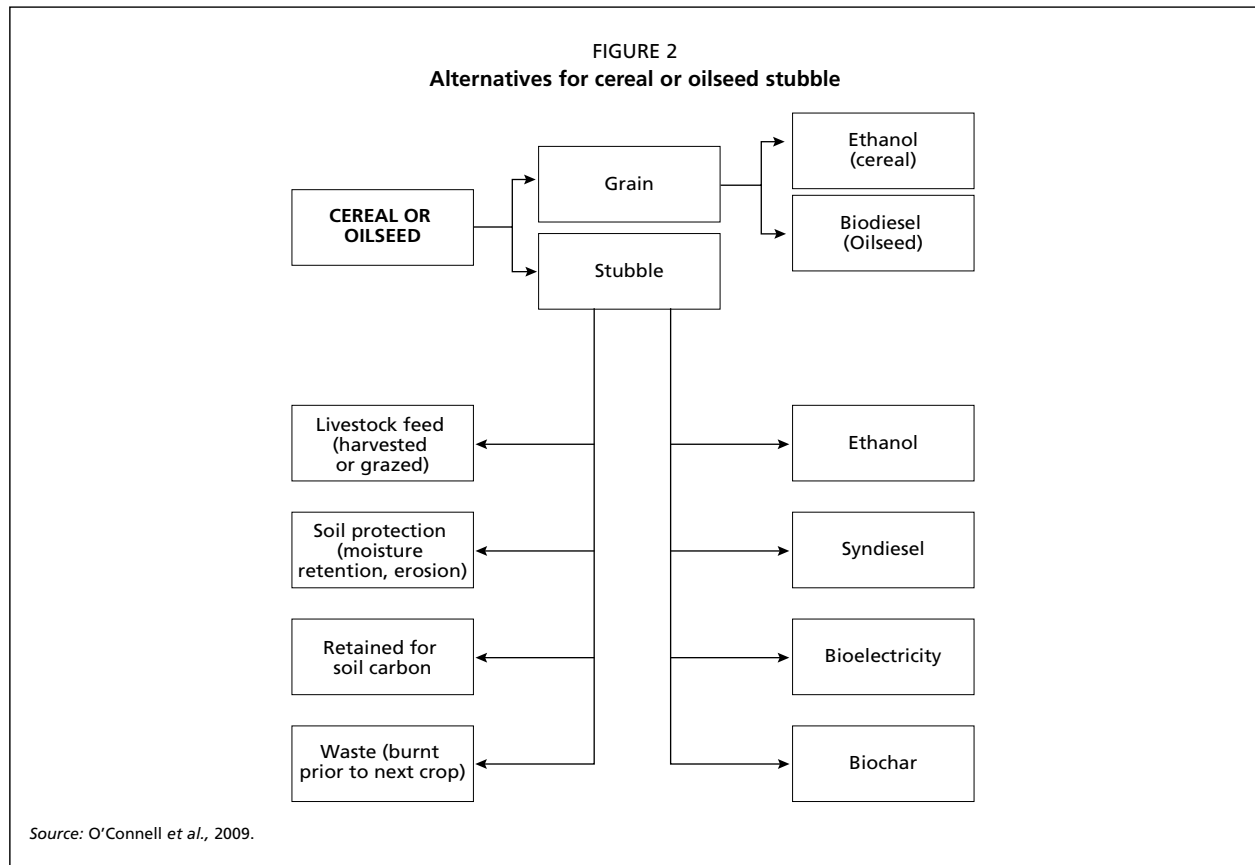
There are no obvious co-products suitable as animal feed from these processes. There may be a potential co-product from the fermentation process for cellulosic ethanol where the C₆ sugars from cellulose and hemicellulose are converted, but the lignin and C₅ (pentose) sugars remain in combination with the yeast remnants. Currently, all remnants from this process are being combusted for energy and not being promoted as an animal feed (Dr Andrew Warden, pers. comm.). There are, however, other opportunities and challenges for the livestock industries in the production and use of these lignocellulosic biomasses for second-generation biofuels in Australia, which will be discussed.

Stubble

There are a number of possible alternative uses for stubbles, including its use as livestock feed and the production of biofuels, as shown in Figure 2.

In Australia, while there are no commercial-scale plants, there is interest in the potential of cereal crop stubble for biofuel production. CSIRO has estimated the amount of cereal residues produced and available in Australia using a methodology based on harvest index combined with land-use maps and national statistics. Having allowed for the amount that can be physically harvested and that must be retained for soil protection, moisture conservation, retention of organic matter and carbon build-up, CSIRO has calculated that the straw available nationally, on average, is 21×10⁶ t/year. There is considerable variation due to climate, with the highest year since 2000 being 39×10⁶ t and the lowest 4×10⁶ t. If converted to ethanol, this is potentially equivalent to 25–50 percent by volume of Australia's petrol consumption (Herr *et al.*, 2010; O'Connell *et al.*, 2008).

Many farmers in Australia's grain growing areas practise mixed farming, combining livestock and cropping in their enterprise mix to reduce variability in income and financial risk (Fisher, Tozer and Abrecht, 2010). Since the 1980s, minimum-till and no-till cropping has revolutionized cropping systems through improving soil structure, better erosion control, the retention of soil moisture and timeliness of planting (D'Emden, Llewellyn and Flower, 2009; Flower,



Crabtree and Butler, 2008) which has allowed the expansion of cropping in the mixed farming regions (Fisher, Tozer and Abrecht, 2010). Ideally, no-till cropping systems include full stubble retention and this has brought into question the role of livestock grazing stubble in such systems. However, when stubble loads are high, retained stubble can impede the sowing of the following year's crop, and farmers are faced with reducing the stubble through various means, including grazing, harvesting or burning.

There are specific tradeoffs between harvesting of stubble for bio-energy and the current use of stubble by grazing livestock, that require further consideration. The nutritional benefits of stubble for livestock and the impacts of livestock grazing compared with stubble retention or stubble harvesting on soil, water, nutrient cycles and pest management in a no-till cropping system requires quantification in order for the terms of the tradeoffs to be defined more clearly.

The benefits of grazing stubble include the feedstock values, i.e. digestibility, metabolizable energy (ME) and protein of the cereal straw, leaf, chaff, spilt grain and weeds that makes up stubble, and other variables, including pasture growth elsewhere on the farm during the period livestock graze stubble, and the related effects of rain events and stocking rates.

While there is some information from stubble grazing trials on the uptake of the various components of grazed stubble and the effect on livestock production indicators,

current research is directed towards modelling the whole farm system (Moore and Lilley, 2006; Thomas *et al.*, 2010).

In integrated grazing-cropping systems, both the grazing of cereal crops early in their winter growth phase and the post-harvest summer grazing of stubble may be used to fill feed gaps in the south east winter-rainfall area of Australia (Moore, Bell and Revell 2009). Long-season cultivar wheats (e.g. cv. Mackellar), developed for dual-purpose winter grazing and grain production, tend to leave heavier stubble loads that need to be reduced prior to re-sowing the growing area. Moore and Lilley (2006), modelled the use of grazing to manage these high stubble loads in a project that looked at the effect on sheep of grazing to removed stubble or the harvesting of the stubble and later use of stubble as a supplementary winter feed. Using the APSIM cropping systems model (Keating *et al.*, 2003) and GRAZPLAN, a grazing systems model (Freer, Moore and Donnelly, 1997), Moore and Lilley (2006) found that the sheep grazing stubble would lose weight and have reduced wool production compared with sheep grazing dry pasture. In addition, the daily intake of conserved stubble used as a winter feed supplement for pregnant ewes was dependent upon the availability of alternative green pasture and was only beneficial at very low levels of green pasture. These findings are in line with those of Rowe *et al.*, (1998), who, in a trial of supplementary feeding of Merino sheep grazing stubble, found that once spilt grain and any germinated

grain and weeds were consumed, in the absence of supplementation, particularly with a protein source such as lupin grain, the sheep lost weight (Rowe *et al.*, 1998).

Thomas and co-workers, using similar modelling methodology, concluded “that the value of grazing crop stubbles cannot be predicted well using energy intake from stubble grazing”, finding that the estimated increase in farm gross margin was less than half the predicted value of the stubble energy content (Thomas *et al.*, 2010). The modelling also demonstrated the complex effects of the many variables and consequent difficulties in assessing the value of stubble. Overall, the model predicted a negative effect on lamb birth weight, survival and liveweight at sale when pregnant ewes are grazed on stubble.

The no-till, full stubble retention cropping system was developed in Australia to improve soil composition, reduce topsoil erosion by wind and water and to retain moisture in the system. Fisher, Tozer and Abrecht (2010) and Herr *et al.*, (2010) examined the effects on the soil, water, nutrient cycles and pest management in a no-till cropping system due to livestock grazing or the harvesting of stubble for bio-energy, and provide the basis for the discussion here.

The role of stubble in the protection of post-harvest soils from wind and water erosion is dependent upon the amount of biomass left in the paddock. Herr *et al.* (2010) identify a technical limit to harvesting stubble, with a minimum aboveground cutting height of 12.5 cm. They calculate that at this height, in a 2 t/ha grain crop, 30 percent of the aboveground biomass is left *in situ*, equivalent to 0.9 t/ha. In order to avoid wind and water erosion, the authors recommend this should be increased to 1–1.5 t/ha. Similarly, Fisher, Tozer and Abrecht (2010) quote guidelines for managing erosion (Carter, 2002) as recommending grazing management should be such that 1 t/ha of cereal stubble should be retained primarily to avoid loss of topsoil through wind erosion following loosening through the passage of livestock.

The recognition that conventional cultivation combined with stubble burning has led to significant losses of soil organic carbon (SOC) in Australian crop lands (Luo, Wang and Sun, 2010) has been one of the drivers for the development of no-till, full stubble retention cropping systems. Consequently, a proposal to remove stubble from the system for the production of bio-energy and the effect of this on SOC is of concern, and has been examined by Herr *et al.* (2010). Having considered all the current information, including simulation models, they conclude that the effect on SOC by retaining stubble is limited, as much of the standing stubble is not incorporated into the soil and is lost to the system through decomposition and photo-degradation, and that partial removal of stubble may not have a significant impact on SOC levels, although the research to quantify this in a reliable manner has yet to be conducted.

Both reports identify the greatest potential for retaining or improving SOC is the use of long-phase (4–6-year) rotations with perennial pastures in the cropping system. Fisher, Tozer and Abrecht (2010) identify research that has demonstrated that wheat yields were greater with long pasture phases compared with 2-year pasture-wheat or continuous wheat rotations, due to improved soil structure, increased SOC and decreased incidence of root diseases.

Both harvesting stubble for bio-energy or removal through grazing affect the nutrient cycle in the system. Herr *et al.* (2010) identify the amounts of nutrients – nitrogen, phosphorous, potassium and sulphur – removed in harvested stubble and provide information for farmers on replacement amounts and costs. By their calculation, for a 2 t/ha wheat crop, the harvesting of straw will remove 7 kg/ha N, 0.7 kg/ha P, 14 kg/ha K and 0.7 kg/ha S. Fisher, Tozer and Abrecht (2010) also identify the loss of potassium with the removal of biomass, i.e. lucerne, from a cropping-pasture system, but primarily they consider nutrient cycling in terms of the redistribution of the nutrients during grazing, and the re-introduction of some nutrients, particularly nitrogen, from leguminous pasture phases. Most of the nutrients removed by livestock during stubble grazing are excreted back into the system, with some concentration in stock camps, and loss of nitrogen due to urine volatilization, although Fisher, Tozer and Abrecht (2010) consider these impacts have been overstated as they are based on trials undertaken in small grazing plots. The direct loss of nutrients exported from the paddock as meat and wool when stubble is grazed may not be significant due to the poor growth rates associated with grazing stubble.

One of the recognized benefits of grazing stubble is the option it provides for the management of weeds, particularly the developing herbicide-resistant strains of ryegrass (*Lolium rigidum*) and wild radish (*Raphanus raphanistrum*). However, the efficacy of this is limited by the need to time grazing relative to the germination of the weeds and the choice of livestock. A recent option devised to reduce weed problems aims to collect all crop residues, including weed seeds, direct from the grain harvester and bale it for removal from the crop area (see <http://www.glenvarbaledirect.com.au/>). Such a system would fit well with the harvesting of stubble for bio-energy.

In summary, once spilt or germinated grain and weeds have been consumed, there is little of nutritional value for livestock grazing on stubble. Even when modelled as part of a whole farm system, the value for livestock of grazing stubble is variable, often marginal or negative. In terms of the effect of grazing stubble compared with the harvesting of stubble in no-till systems, careful management of grazing livestock or harvest practices can mitigate many of the potential problems. It is apparent that the most beneficial practice in long-term soil maintenance is the use of long-

phase perennial pasture rotations in the cropping-livestock system. This will provide the major opportunity for livestock within the system, whether stubble is harvested for bio-energy or grazed.

Trees for bio-energy and biofuels – SRC eucalypts

Much has been written on the impact on the Australian ecosystem from 200 years of European settlement due to a combination of land clearing for human habitation and agriculture, overgrazing with introduced livestock species, and forestry (Hobbs and Yates, 2000; Saunders, Hopkins and How, 1990). In parts of the agricultural lands, the long-term effects of the replacement of deep-rooted perennial vegetation with shallow-rooted annual crops and pasture species have been rising water tables, increased groundwater flows, water and soil erosion, and expanding areas of dryland salinity (Stizaker, Vertessy and Sarre, 2002). It is estimated that “cleared land”, defined as land with <5 percent tree cover, occupies some 70 million hectares of the wheat-sheep and high rainfall zones (Reid and Landsberg, 2000).

The recognition of the role of trees in the conservation of biodiversity, the reduction of land degradation due to wind erosion, dryland salinity and water logging, together with private and government initiatives, has led to some significant on-farm re-vegetation through plantings of native trees and shrubs. The recent interest in the additional use of tree plantings on agricultural land for bio-energy, for lignocellulosic biofuels and for carbon sequestration has added a potential new land use and income stream for farmers, which could be integrated with their usual agricultural production activities from the land that they manage (Abel *et al.*, 1997; GHD Hassall, 2010).

One such system is the on-farm plantings of SRC eucalypts or oil mallees, of which *Eucalyptus loxophleba* subsp. *lissophloia* and *E. polybractea* are the most commonly grown. Originally planted in an effort to control dryland salinity, SRC eucalypts have undergone 25 years of research and development in Western Australia and are now seen primarily as energy plantings for low to medium rainfall areas (300–600 mm annual rainfall) that can be integrated into existing agricultural cropping and grazing systems, with the associated benefits of reducing the risk of dryland salinity, restoration of biodiversity and provision of shade and shelter for livestock. The design of the planting systems, referred to as alley farming, consist of belts of SRC eucalypts with alleys of crops or pastures 70–80 m in width between the rows. Plantings are generally along the contour, with the area occupied by the trees approximately 8 percent of the paddock area (Bartle and Abadi, 2010; Smith, 2009; Wu *et al.*, 2005).

This new, integrated agricultural and bio-energy land use has potential benefits for all grazing livestock in the

provision of shade and shelter. This is especially so for lambing ewes, when trees act as windbreaks such as occurs with the addition of alleys of SRC eucalypts as energy plantings as described.

The windbreak effect: shelter from wind and cold

Since the 1960s, Australian researchers have been examining the benefits of various types of windbreak shelter in reducing lamb mortalities, as these have been of particular concern in the cold, over-cleared sheep grazing areas of Victoria and New South Wales.

In the absence of established tree shelter belts, researchers set up lambing studies in the western district of Victoria and at Armidale, NSW, using sheet iron (Lynch and Donnelly, 1980), Sarlon garden mesh (Lynch and Alexander, 1977), cypress (*Cupressus macrocarpa*) hedges (Egan *et al.*, 1972; McLaughlin *et al.*, 1970), patches of un-grazed, rank *Phalaris tuberosa* (Egan, Thompson and McIntyre, 1976) and strips of an unpalatable hybrid *Phalaris* (*P. tuberosa* × *P. arundinaceae*) (Alexander and Lynch, 1976) to provide windbreaks. Table 3 collates the outcomes of these studies.

Overall, the provision of shelter under the full range of weather conditions at the sites during mid- to late-winter lambing (July–August) on average halved the mortality rate for both single-born lambs (13.9 percent to 7.5 percent) and multiple-born lambs (49.1 percent to 27.6 percent) (Table 3).

Revegetation and its capacity as shelter for ewes and lambs

An understanding of the capacity of revegetation to provide shelter for livestock, crops and pasture has been established through research undertaken under the National Windbreaks Program by Cleugh and co-workers (Cleugh *et al.*, 2002). This research used a combination of field and wind tunnel studies to accurately establish the spatial and scalar effects of windbreaks on wind speed and near surface air temperature, factors important to the survival of new-born lambs.

The spatial effects of windbreaks are described in terms of H, where H is the height of the windbreak. The known effects of a windbreak on near-surface wind speed and air temperature are described as follows:

- **Wind speed** The sheltered zone of reduced near-surface wind speed extends 5H upwind and over 30H downwind of a windbreak, with the maximum shelter near the surface occurring at around 6H downwind. Windbreak porosity (β) determines the reduction in wind speed. As a rough guide, wind speed reduction is similar to windbreak density ($1 - \beta$), i.e. a porosity of 30 percent equates roughly to a 70 percent reduction in wind speed at the most sheltered location, around 6H.
- **Air temperature** The spatial trend in near-surface air temperature mirrors that for near surface wind speed up

TABLE 3
Effect of shelter on lamb mortality during their first 48 hours – all weather conditions

Type of shelter	Location	Duration	Lamb mortality (%)				Significance	Reference
			Single born		Multiple births			
			Shelter	No shelter	Shelter	No shelter		
<i>Phalaris</i> hybrid strips	Armidale, NSW	14 days of lambing	10.2	13.9	n/a	n/a	Alexander and Lynch, 1976.	
<i>Phalaris</i> hybrid strips	Armidale, NSW	5 year's pooled results	9.0	17.5	35.8	51.3	$P < 0.005$ Alexander <i>et al.</i> , 1980.	
<i>Phalaris</i> patches	Western Victoria	4 year's pooled results	9.3	12.0	18.5	32.7	$P < 0.01$ (multiples) Egan, Thompson and McIntyre, 1976.	
Cypress hedges	Hamilton, Victoria	18 days of lambing	6.3	18.9	n/a	n/a	$P < 0.01$ Egan <i>et al.</i> , 1972.	
<i>Phalaris</i> hybrid strips	Armidale, NSW	15 days of lambing	5	11	41	68	$P < 0.01$ (multiples) Lynch and Alexander, 1977.	
Sarlon garden mesh	Armidale, NSW	15 days of lambing	6	11	31	68	$P < 0.01$ (multiples) Lynch and Alexander, 1977.	
Cypress hedges	Hamilton, Victoria	2 year's pooled results	6.9	13.4	11.7	25.5	$P < 0.01$ McLaughlin <i>et al.</i> , 1970.	
Average mortality rates (all weather conditions)			7.5	13.9	27.6	49.1		

Notes: n/a = not applicable. NSW = New South Wales.

to 15H, where near-surface air temperature returns to upwind value. The magnitude of rise in near surface air temperature increases with increasing shelter. The peak near surface air temperature rise from medium (40 percent) and low (30 percent) porosity windbreaks is about 0.7 °C and 1.4 °C respectively

This knowledge on the reduction in near-surface wind speed and the increase in near surface air temperature due to windbreaks can be combined with much earlier studies to provide a theoretical basis to the field trials on shelter and lamb mortalities.

Donnelly, from studies of mortalities of lambs born in the Canberra region in late winter/early spring, established the probability of single and multiple born lambs dying within three days birth related to maternal body weight and the Chill Index (Nixon-Smith, 1972) which is an index based on wind speed, rainfall and temperature (Donnelly, 1984).

The results of Cleugh *et al.* (2002), Donnelly (1984) and Nixon-Smith (1972) can be used to estimate the probability of mortality of lambs born with or without the benefit of a windbreak. Taking wind speed and temperature of 6.2 m/second and 8 °C from (Egan *et al.*, 1972) then applying a 40 percent reduction in wind speed and a 0.7 °C increase in near-ground temperature due to the effect of a windbreak, the calculated Chill Index is reduced from 1102 to 1034 kJ/m²/h. When this reduction in Chill Index is applied to Donnelly's probability of mortality for lambs born to 55 kg Merino ewes, the probability of mortality of multiple born lambs is reduced from approximately 0.5 to 0.3 and for single born lambs from 0.2 to 0.1. This finding complements the research studies described, where the provision of various types of windbreak shelter has been found, on average, to halve mortalities of both single and multiple born lambs. The starvation-mismothering-exposure (SME)

complex has been identified as the primary cause of lamb mortalities (Jordan and Lefevre, 1989). With the work of Cleugh *et al.* (2002) and Donnelly (1984) providing theoretical backing to other research on the effect of shelter on lamb mortalities, it can be concluded that the provision of shelter reduces the number of SME lambs leading to more lambs surviving and lower mortalities.

Sheep and pasture production and the benefits of shelter

A series of trials on the effects of shelter has also shown that it can improve pasture growth and sheep production. Bird *et al.* (2002) carried out two trials to assess the effect of windbreaks on pasture growth in south-western Victoria using single lines of established two-row tree windbreaks. The only clear differences over the four years of the trial were in the competition zone (0.5–1.0H) along the margins of the windbreaks, where competition from the trees reduced pasture production, and there was no significant effect in the sheltered zone. However, the windbreaks only provided shelter for 28 percent to 42 percent of the time and the authors concluded that, in that region, no single windbreak was capable of offering adequate protection. A second trial (Bird, Jackson and Williams, 2002) was designed to test this conclusion by providing more complete shelter through the use of a synthetic mesh windbreak of 50 percent porosity, surrounding small, uniform areas of land. The outcome of this trial was a small but significant increase in temperature of 0.1 ° to 0.9 °C in the sheltered plots compared with the open plots, and a consistent increase in pasture growth of about 9 percent ($P < 0.01$).

Lynch and Donnelly also used synthetic windbreaks to study the effects of shelter on pasture production, live-weight change and wool production in sheep grazed at high stocking rates at Armidale (Lynch and Donnelly, 1980).

At the highest stocking rate (37.5 sheep/ha) wool production in the sheltered paddocks was increased by 31 percent over the 5 years of the trial, and the live weights of the sheep in the sheltered paddocks at 15 and 30 sheep/ha were significantly higher than those in the unsheltered paddocks, attributed to a combination of increased pasture production and a saving in metabolizable energy of the sheep in the sheltered paddocks.

Following Lynch and Donnelly's findings with artificial windbreaks and high stocking rates on high-input pastures, Reid and Thompson set up a project to look at the effect of natural windbreaks, consisting of a combination of native trees and shrubs, on sheep grazing low-input modified native pastures (Reid and Thompson, 1999). Sheep in the windbreak paddocks finished the year 13 percent heavier ($P = 0.067$) and cut 13 percent more wool per head (3.4 vs 3.0 kg, $P < 0.05$) than those in the paddocks without windbreaks. In the second year, stocking rates were varied based on calculations from pasture cuts in the paddocks the previous spring, resulting in 34 percent more sheep being carried in the windbreak paddocks (5.1 vs 3.8 sheep/ha) than on the paddocks without windbreaks, with the sheep maintaining higher body weights throughout the year.

Alexander, in a project to assess the effect of hybrid *Phalaris* strip windbreaks on lamb mortalities, also measured the effect on the subsequent growth rate of lambs (Alexander and Lynch, 1976). The mean growth rate for lambs up to 21 days of age from the sheltered paddocks was 6.6 percent greater ($P < 0.05$) than for those from the unsheltered, despite the lambs only being sheltered for a few days before being moved to an unsheltered lucerne pasture 1–3 days after being born.

Shade: shelter from heat

While shade and the shelter from heat are recognized as important factors in animal production, land cleared for cropping usually has very little shade for livestock to utilize. The provision of shade reduces radiant heat and the use of shade by livestock during hot, sunny weather is well recognized (Blackshaw, Blackshaw and Kusano, 1987; Daly, 1984). The type of shade is also important, with trees seen as providing the most beneficial shade through protection from the radiant heat of sunlight combined with the cooling effect of evapotranspiration from the leaves (Blackshaw and Blackshaw, 1994).

Heat, such as that found in north-western Queensland, where there are consistently high daily temperatures, affects ewe fertility, causes hyperthermia, particularly in non-adapted ewes, with a decline in uterine blood flow, retarded foetal growth, lower birth-weight and less viable lambs (Hopkins, Nolan and Pepper, 1980; McCrabb, McDonald and Hennoste, 1993; Parker, 2006). Shade assists neonate lambs in reducing heat stress, panting,

exhaustion, failure to suck and subsequent death due to starvation. Modelling from meteorological data indicates that the most severe heat effects are limited to northern Australia (Parker, 2006), although reproductive wastage in sheep due to the effects of heat on ram fertility and ewe fecundity may be seen at other sites further south. In a South African trial, it was found that the provision of artificial shade for autumn lambing ewes showed a significant improvement in lamb weaning weight and first-year lamb growth (Cloete, Muller and Durand, 2000).

For cattle, shade has been shown to improve milk yield, milk fat yield and reduce mastitis scores in dairy cows (Ingraham, Stanley and Wagner, 1979). Concern about the effects of radiant heat on the welfare of feedlot cattle has led to research on heat stress in beef cattle. Blackshaw and Blackshaw, in a review of heat stress in cattle and the effects of shade on production and behaviour (Blackshaw and Blackshaw, 1994), found the breed of cattle to be the most significant factor, with *Bos indicus* breeds having a much greater ability to adapt to heat than *Bos taurus* breeds. However, all cattle showed a reduction in feed intake and an increase in water intake in response to heat, with one study showing that the provision of cooled water to *Bos taurus* breeds improved all production parameters. Cattle grazing in tropical Queensland were observed to spend 9–11 hours in shade in the summer and to continue to ruminate during the middle of the day if in shade but not so in the sun. Trials on the effect of artificial shade on production of cattle in feedlots were variable, often confounded by breed differences, although the benefits for shade to pure *Bos taurus* breeds such as Herefords were significant.

The foregoing discussion indicates that for the grazing livestock industries, the re-introduction of some trees for biofuels and bio-energy into cleared agricultural lands will provide direct benefits for the livestock in terms of shade, shelter and animal welfare, thus enhancing industry productivity, while for agriculture more generally there would be longer-term benefits through land conservation. At the same time, the development of new, second-generation biofuels based on lignocellulosic feedstocks production systems, as described earlier, may have an impact on the availability of grain to the intensive livestock industries, were some current grain-producing land to be planted with SRC eucalypts as feedstock for biofuel or bio-energy. If this is combined with a loss of cropping productivity associated with climate change, as Bryan, King and Wang (2010b) predict in their models, this could be significant.

EXPANDING LAND USE FOR BIO-ENERGY AND BIOFUEL – THE EFFECT ON LIVESTOCK INDUSTRIES

Although the development of bio-energy and biofuel industries has been slow in Australia, the drivers of fuel

security and regional development are as strong as ever and the industries continue to enjoy support through State Government mandates and Federal Government excise relief for biofuels and the Renewable Energy Target scheme (Department of Climate Change and Energy Efficiency, 2011) for bio-energy. In addition, at this time it is the Australian government's declared intention to introduce a pricing mechanism for carbon, which would provide further incentive for the development of alternative energy sources, including biofuels and bio-energy.

In this section we consider how further development of first- or second-generation biofuels and bio-energy affect the livestock portion of mixed cropping-grazing farming in Australia.

Bryan, King and Wang (2010b) have considered this question at a landscape scale. Using a mixed farming area of South Australia and adjoining regions in Victoria, they modelled four-year rotations for cropping (wheat-wheat-lupins-wheat), mixed cropping-grazing (wheat-grazing-lupins-grazing), continuous grazing (grazing-grazing-grazing-grazing) and biofuels (continuous wheat-canola rotations for the production of ethanol and biodiesel). The aim was to assess the impact of establishing a first-generation biofuels industry in the area and to quantify the trade-offs between biofuel, food (grain, meat) and fibre (wool) production (Bryan, King and Wang, 2010).

To do this they used APSIM (Keating *et al.*, 2003) to spatially model production of food and biofuel under baseline, mild, moderate and severe climate change scenarios. The effect of introducing farm subsidies tied to the net greenhouse gas (GHG) emissions abatement achieved by a switch to biofuels was calculated based on the GHG emissions and energy cycle of the biofuels and food agriculture systems. Finally, they calculated economic returns with or without subsidy, then applied a rational economic model of adoption where farmers switch to biofuels agriculture where it is more profitable than food agriculture and continue with food agriculture in all other areas.

The modelling predicted that at baseline climate and no carbon subsidy, the take up of biofuels agriculture on the economically viable areas would use 44 percent of the arable land in the modelled area, reducing sheep meat production by almost 60 percent and wool production by 78 percent. As would be expected, with a subsidy of AUD 30/tonne CO₂-eq, the model predicted the use of arable land for biofuels agriculture rising to 54 percent, further reducing sheep meat and wool production. However, under the severe climate change scenario with no carbon subsidy, the economically viable area for biofuels agriculture was predicted to be just 10 percent of the arable land. While all productivity decreased at each climate change scenario, the percentage decrease in canola for biodiesel was almost double that of sheep.

The approach is a useful one, but the model had several fundamental problems:

- The model was based on the growing of biomass feedstocks for first-generation biofuels only and did not examine the case for second-generation biofuels.
- The carbon payments were made to farmers when no reduction in carbon emissions were achieved at the farm level, as the same high input crops were grown. Instead, the reduction in carbon emissions is achieved further along the value chain at the point where biofuels replace fossil fuels. This lacks logic. As such, it is unlikely to be a policy action in Australia under the current government.
- Rational economics are applied for the adoption of farming systems, which does not include risk or farmers' perception of risk. As stated earlier, the primary reason for mixed cropping-grazing systems is the reduction of risk through a balance of enterprises. The predicted relative productivity decreases from the modelling could be interpreted to suggest that farmers may continue to combine grazing with cropping for food or biofuels to reduce the risks associated with seasonal variations and climate change, and to utilize the grazing phase of cropping rotations so necessary for re-building soil carbon and subsequent crop productivity.

Modifying the approach to address these problems, and include feedstocks relevant to new-generation technologies, would be a very useful next step.

In a similar piece of research, Bryan, King and Wang, (2010a) modelled, at a landscape scale, the planting of woody biomass (SRC eucalypt) over the same area of South Australia and Victoria, again with spatial modelling of agricultural production and woody biomass plantings under climate change scenarios. A drawback of this model is the use of plantations rather than integrating SRC eucalypt alleys into agricultural lands, which has the potential to provide greater benefits in production and conservation. Economic returns were calculated based on three biomass prices (AUD 30, AUD 40 and AUD 50/t), biomass planting and maintenance costs, and average agricultural prices and costs, including those for sheep and wool. In addition, the effects on dryland salinization, wind erosion and carbon emissions were estimated. Although the economic model included sheep, results were given as agricultural production without identifying changes in production from sheep.

The relative value of biomass and agricultural production varied with the price assigned to biomass, climate change scenario and area within the study region, so in some areas, even under moderate climate change, biomass became more profitable than agriculture. However, pasture, a predictor of sheep production, was found to be the least sensitive to climate change.

Overall, biomass tended to be more viable than agriculture in marginal agricultural areas. At the landscape scale, it

was found that, as well as the economic benefits, biomass production can provide benefits by controlling dryland salinity, wind erosion and carbon emissions reduction.

These results suggest that the integration of biomass production in the form of SRC eucalypts with pasture and livestock grazing may provide a good outcome in resilience and land conservation while maintaining economic productivity of the land.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Integration of cropping, grazing and bio-energy production presents a complex set of biophysical, social and economic interactions that will need to be well understood to ensure sustainable development of such land use. While some recent research at landscape scale has been reported here, there is need to continue this at a range of scales, including sociological, to better understand likely land use changes in Australia associated with developing bio-energy industries.

Knowledge from this research will be needed in the continuing development of certification of sustainable biofuel production. Current certification, such as the Roundtable on Sustainable Biofuels (RSB) Certification Scheme, and the sustainability standard upon which it is based (Roundtable on Sustainable Biofuels, 2011), have been developed from certified sustainable forest management and as such tend to address sustainability issues as applying to single land use energy crops. Assessment of the sustainability of the combined production of food, fibre and bio-energy biomass from integrated land use will require re-examination of the criteria and indicators within biofuel sustainability standards.

CONCLUSIONS

The current small biofuels industry in Australia, based largely on the use of co-products of grain and sugar industry, is not a significant challenge to the availability of feedstocks for the intensive livestock industries, and only provides a relatively small amount of co-product for livestock feed. An expansion of the current first-generation biofuels industry would increase direct competition for grain, but would also increase the availability of protein feedstuffs – DDGS and oilseed meals – which could provide a useful source of supplementary protein for livestock grazing low-protein, dry, summer pastures. DDGS is particularly suitable for this role in ruminants.

New non-food biomass production systems for bio-fuel and bio-energy are being researched and developed in Australia. These include the use of lignocellulosic feedstocks from agricultural residues and on-farm plantings of SRC eucalypts; and new bio-oil feedstocks such as the low-rainfall oilseed crop *Brassica juncea*, the oilseed tree

Pongamia pinnata and algae. Much work remains yet to be done to fully design, test and implement suitable production systems.

Research has been undertaken in Australia into the use of biodiesel co-products in pigs. Both juncea meal following oil extraction from *Brassica juncea*, and crude glycerol from the transesterification process to convert bio-oils to biodiesel have been trialled.

Algal biofuel production has yet to be commercialized anywhere in the world. The algal meal remaining after the extraction of bio-oil may not be suitable for livestock feed due to the use of CO₂ flue-gas from coal-fired power stations, which may contain heavy metals and other toxins that are likely to be taken up by the algae.

Pongamia pinnata plantations are being developed in Australia for the production of biofuel, which could result in the availability of pongamia meal for livestock feed. However, despite considerable research and effort to reduce residual oil and toxicity factors in pongamia meal, studies have shown that *Pongamia pinnata* meal is only useful and safe as an animal feed at low inclusion levels.

There is a benefit from pongamia containing the unpalatable karanjin and pongamol, as it allows the integration of grazing livestock in *Pongamia pinnata* plantations with minimal risk of the animals grazing and damaging the trees.

The harvesting of stubble for bio-energy should have little impact on grazing livestock in mixed grazing-cropping farming systems. There is little of nutritional value in stubble for grazing livestock. When modelled as part of a whole farm system, the value for livestock of grazing stubble is variable, often marginal or negative. The use of long-phase perennial pasture rotations in the cropping-livestock system is the most beneficial practice in the long-term maintenance of cropping soils and will always provide the major opportunity for livestock within the system, whether stubble is harvested for bio-energy or grazed.

The re-introduction of trees for bio-energy and biofuels into cleared agricultural lands in Australia will provide direct benefits in livestock productivity and animal welfare through the provision of shade and shelter, as well as long-term benefits through land conservation for the grazing livestock industries. The integration of biomass production in the form of SRC eucalypts with pasture and livestock grazing may provide a benefit in improved resilience and land conservation while maintaining economic productivity of the land.

The development of new, second-generation biofuels may have an impact on the availability of grain to the intensive livestock industries, as some current grain-producing land is planted with SRC eucalypts as feedstock for biofuel or bio-energy. Combined with a loss of cropping productivity associated with climate change, this could be significant.

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Chapter 26

An assessment of the potential demand for DDGS in Western Canada: institutional and market considerations

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ABSTRACT

The rise of the ethanol industry in Western Canada during the start of the twenty-first century has precipitated the development of market opportunities for co-products from the ethanol industry. Previously, dried distillers grain with solubles (DDGS) was imported from the United States for use in the beef feedlot industry, but the potential for more regionalized, if not localized, production now exists. As with the development of any new market, there are challenges and opportunities. This chapter provides an overview of the development, potential and challenges facing the DDGS market in Western Canada.

INTRODUCTION

The Canadian grain-based ethanol industry has been growing consistently over the past decade (Coyle, 2007). The driver for this growth comes largely from provincial and federal government subsidies for the development of new biofuels, and since the ethanol plants are based on the use of grain feedstocks, they are located in areas of high wheat production. A major consequence of this expansion is the production of dried distillers grain with solubles (DDGS)—a feed ingredient that can be incorporated into livestock feed rations as supplemental protein or an energy source. For livestock producers in Western Canada, the availability of distillers grain presents enormous opportunity. The region's high livestock numbers and abundance of grain offer significant potential for the production of ethanol and the marketing of distillers grain. Already, seven out of the fifteen grain-based ethanol producers in Canada are located in the region. With two more proposed plants to be located in Alberta, the total regional ethanol production capacity could increase to 704 million litres/year from the current 514 million litres/year (CRFA, 2010a). This implies an increased supply of domestically produced distillers grain.

Under the present circumstance, an understanding of the DDGS market in Western Canada is critical for both suppliers and consumers (primarily beef feedlots). For the latter, an in-depth understanding of market trends and structure would enhance the potential to reap full benefits from the availability of the feed ingredient. The former might reap even greater benefits as information on market structure and trends could, in the short term, enhance cur-

rent marketing efforts, and the overall competitiveness and viability of the enterprise in the long term.

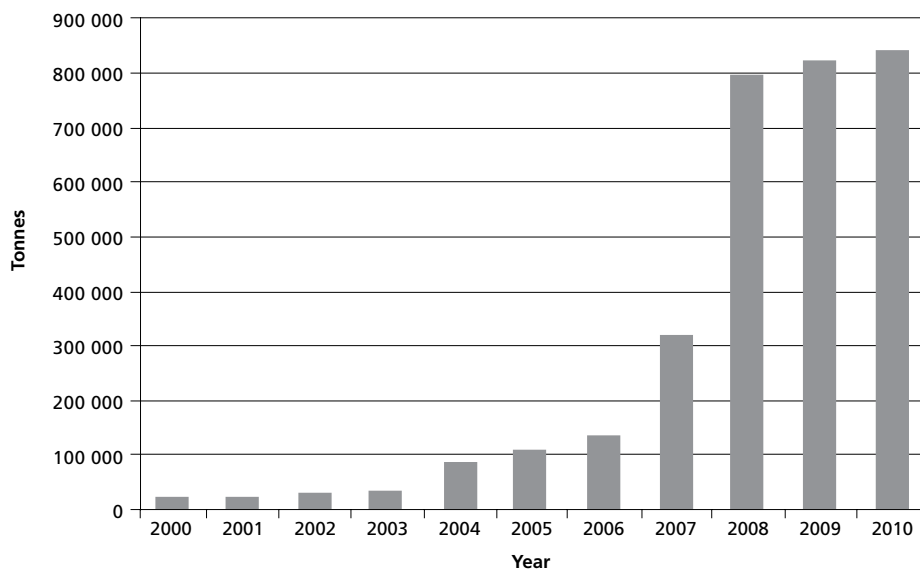
For livestock producers in Western Canada, the proximity to the supply of distillers grain from the United States could make the feed ingredient a critical component of the feed market. The United States is the world's largest producer of distillers grain. The production of the ethanol co-product has increased dramatically over the last decade, from 2.7 million tonne in 2000 to 30.5 million tonne in 2009 (CRFA, 2010a). It is projected to reach 88 million tonne by 2016, based on assumptions of aggressive industry expansion (Tokgoz *et al.*, 2007). This high level of production has resulted in the situation where the international feed market is gradually gaining prominence as an important market for the use of DDGS as a feed ingredient. In 2009, over 5 million tonne of distillers grain were exported, accounting for approximately 15 percent of total production (USDA-FAS, 2011). Canada and Mexico are the main markets for the product.

Over time, Canada has emerged as an importer of maize distillers grain. A livestock production system that mimics that of the United States, the absence of tariffs under the North American Free Trade Agreement (NAFTA) and the option to ship by rail has facilitated the movement of the commodity from the United States to Canada (Fox, 2008). This is aside from market factors such as the recent high prices of traditional feed grains. In 2008, imports of United States distillers grain were nearly 800 000 tonne, up over 475 000 tonne from 2007 (USDA-FAS, 2011). Figure 1 shows the trend in Canadian distillers grain imports. Imports

MAIN MESSAGES

- There is a potential demand from the beef feedlot industry of 1.4 million tonne of DDGS products in Western Canada, of which 40 percent can be supplied domestically.
- When the exchange rate between the Canadian dollar and the United States dollar exceeds \$CAN 0.80, Canadian ethanol firms will import United States maize to use as feedstock.
- Standardization of DDGS product quality will be an important component in the development of a domestic DDGS industry in Canada.
- The successful development of a domestic DDGS industry will require a strong and committed champion to drive the development and structure of the market.
- Animal nutrition research has identified the biological impact of DDGS, and therefore use of this ingredient can be fully made based on economic indicators.
- Additional research on the use of DDGS or fractions of DDGS in monogastric diets is necessary prior to being able to make purely economic decisions on its use in their diets.

FIGURE 1
Canadian DDGS imports from the United States



Source: USDA-FAS Database, 2011.

of DDGS from the United States has slowly increased over time. There was a doubling of imports between 2007 and 2008, reflecting the availability of the product due to growth of the United States ethanol industry and increasing utilization by Canadian livestock producers.

The importing of maize-based DDGS into Western Canada is a recent phenomenon, due to the abundance and price of this product in the United States following the growth of their ethanol industry. Locally produced barley has, and continues to be, the major feed ingredient, but due to the competitive pricing practices of United States DDGS exporters, maize-based DDGS has recently increased its market share. One major factor that affects this is the currency parity between the Canadian and United States currencies. With the Canadian dollar at present in a

strong position relative to the US dollar, feed companies in Western Canada are now able to economically include maize-based DDGS as an ingredient. With the rate of United States maize-based DDGS imports strongly correlated to the currency exchange rate, the continuation of this trend in imports is uncertain.

Competition from United States maize-based DDGS will be a challenge for the development of a Western Canadian wheat-based DDGS industry. Many of these challenges extend beyond actual product attributes and enter the realms of regulation and economics. As mentioned above, the Canada-United States border can no longer be viewed as a barrier to market development, not to mention the proximity of supply, so competition from international production sources will be an integral component of DDGS

industry development. The size of the United States ethanol industry is many times that of the industry in Western Canada, which creates economies of scale for the United States production of maize-based DDGS. As with the development of markets for new products, niches exist and can be exploited for economic advantage.

There has been minimal research in terms of market analyses for DDGS in Western Canada, although some studies on the United States market do exist (e.g. Dooley, 2008; Dhuyvetter *et al.* 2005). This chapter addresses this research gap by estimating a potential market for distillers grain in Western Canada. The following section provides an overview of the scale and scope of the agriculture industry in Western Canada. The subsequent section discusses the economic challenges in creating markets for new products. This is followed by an assessment of the potential of a new DDGS market in Western Canada. The conclusions follow a concise discussion of information gaps, and knowledge and research needs.

CHANGES AND TRENDS IN WESTERN CANADIAN AGRICULTURE

Size, concentration and location of the beef feedlot industry

In Western Canada, the co-products from the ethanol industry are primarily fed to beef cattle. Beef management systems include cow-and-calf operations, operations that feed for background growth of cattle, and feedlots where animals are fed until they are finished to a desired slaughter weight. Cow-and-calf and backgrounding operations involve pasture grazing, where some DDGS may be fed to supplement forages. The use of DDGS in pasture management systems represents a minor component of DDGS use. The majority of DDGS use in Western Canada is in beef feedlot operations.

In Western Canada, the most common grain in beef rations is barley. In terms of energy and protein requirements, barley has consistently been the commodity of choice for livestock feed. Other grains that may enter the ration include wheat and oats. The use of DDGS in beef rations has increased with the expansion of ethanol production, the common incorporation rate being 20 percent of the ration.

The beef livestock finishing management system is very intensive. Animals are brought into a feedlot and will be

fed differently depending on the weight of the animal. Steers and heifers that are brought in as weaned cattle are typically fed so that they gain approximately 1 kg/day. They are started on a ration made up primarily of forages, and progressively more grains are added to the ration until the ration is approximately 90 percent grain. Steers coming in at heavier weights, such as 350 kg, are moved to the high-grain diet more quickly than weaned calves. At the end of the programme, a typical rate of gain is 1.3 kg/day on the high-grain ration. Cattle typically spend 100–150 days in a feedlot prior to being sold to a beef processing facility. Steers are normally processed at a weight of 600–650 kg and heifers are normally processed at 525–575 kg.

There are currently 12.9 million head of cattle in Canada (Statistics Canada, 2011a). The beef inventory has slowly decreased over the past decade. Table 1 presents the beef inventory by province and region. There has been a decline of 16 percent in the number of cattle in Canada in the past decade. In part this was precipitated by the Bovine spongiform encephalopathy (BSE) case in Western Canada in the early part of the last decade. The detection of this one animal closed the United States border to Canadian beef, depressing beef prices.

There are more than 4000 feedlots in Alberta (ABP, 2011), with a wide range in size. There are approximately 100 feedlots with more than 1000 head, which produce more than 75 percent of the cattle in Alberta. Saskatchewan feedlots are much fewer: approximately 250 feedlots finish more than 400 000 cattle, with the top 30 feedlots finishing nearly 80 percent of the cattle (SCFA, 2011). Manitoba has approximately 225 feedlots, ranging in size from 80 to 6500 head (Government of Manitoba, 2011). The size of the beef industry in British Columbia is considerably smaller, with less than 50 feedlots in operation.

The majority of beef feedlots in Western Canada are located in south or south-central Alberta, due to proximity to beef processing facilities. There are several processing facilities located throughout Western Canada, but beef processing is largely concentrated in southern Alberta. It is unlikely that the changes in beef feedlot location that have occurred in the United States with the expansion of the domestic ethanol industry will be mirrored in Western Canada.

TABLE 1
Canadian beef inventory

Year	Canadian beef cattle herd ('000s)							Total
	BC	AB	SK	MB	ON	QC	Atlantic	
2001	815	6 500	2 900	1 425	2 130	1 360	295	15 425
2005	710	5 930	3 040	1 490	2 189	1 415	289	15 063
2011	519	5 190	2 645	1 220	1 765	1 310	255	12 900

Notes: BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC = Quebec; Atlantic = Atlantic provinces.
Sources: Statistics Canada, 2002, 2006a, 2011a.

Development of the ethanol industry

Although significantly smaller than that of the United States, the Canadian ethanol industry has not been exempt from the recent enthusiasm for renewable fuel production. The Canadian industry comprises 15 operational plants, with a total operating capacity of about 1.82 billion litres per year (CRFA, 2010b). Not unlike other major ethanol producers, grains are the main feedstock used in the production of biofuel in Canada. Geographically, Canadian wheat-based ethanol production is predominant in the west and maize-based production is mostly in eastern Canada.

In 2011, seven ethanol plants were operating in Western Canada, all of which were producing DDGS (Table 2). The ethanol production plants range in capacity from 475 000 to 400 million litres per year, using various feedstocks. In Western Canada, the main feedstock used has been wheat, producing DDGS with more protein and less fat than DDGS from maize, although it is not uncommon for the plants to import maize from the United States to be used as feedstock. Decisions are largely based on the current price differential between United States maize and Canadian feed wheat. Some ethanol plants in Western Canada do have contract requirements that dictate that the feedstock must be local or regional feed wheat. Most have the freedom or flexibility to use the cheapest feedstock available for the production of ethanol.

Seven ethanol plants produce the DDGS supplying livestock operations in Western Canada. There is approximately 460 000 tonne of wheat DDGS produced in Western Canada each year (Table 2), with most sold into beef feedlot operations, especially those concentrated in southern Alberta. The second-highest usage of DDGS is for dairy markets, the exception being Terra Grain Fuels, which sells mainly into the dairy market. There is some DDGS utilized by swine operations, but this market comprises only a small percentage of the total livestock use. The high fibre content of DDGS makes DDGS a less attractive ingredient for monogastric animals. The majority of production is as DDGS. However, Permolex produces a modified distillers' grains with the gluten removed and Poundmaker Ag Ventures Ltd feeds the thin stillage and wet distillers' grains directly to the feedlot adjacent to the facility.

DDGS USE IN RATIONS

A variety of products result from the ethanol manufacturing process and which can be utilized in the beef industry. Figure 2 illustrates the process for producing ethanol from grain and identifies the various co-products. The co-products that are utilized in cattle rations are a product of the distillation process. Whole stillage is produced when the fermented beer slurry is pumped through the distillation system. Ninety-five percent pure ethanol is removed from the top of the system and whole stillage is removed from the bottom of the distillation system. Whole stillage consists of grain residue, the unfermented grain particles, yeast cells and fibre, oil and protein liberated from grain cells, and water. Following centrifugation, thin stillage and wet distillers grain are produced. Although the main co-product utilized in Western Canada is DDGS, all ethanol companies have the capacity to produce and sell wet distillers grain and thin stillage or condensed distillers solubles (CDS). DDGS is the primary product, as drying the co-products increases the storage life, eases handling and is cheaper to transport as water has been evaporated with drying. Variation in co-product production occurs because of variations in the feedstock material used in a facility, facility modifications of the process described in Figure 2, degree of drying, and protein damage due to drying.

A study by Walter *et al.* (2010) examined the use of wheat or maize DDGS in a small pen trial at the University of Saskatchewan, Canada. Their intent was to determine the relative feed value of the two sources of DDGS, which differ in terms of fat and protein content. Maize or wheat DDGS were fed at 20 or 40 percent of the diet. The DDGS ingredients replaced dry-rolled barley in the ration; the control ration contained dry-rolled barley, the most commonly fed base ingredient in Western Canadian feedlot rations. Once animals met a target weight of 645 kg they were shipped to slaughter. The performance data for the trial is presented in Table 3. Both DDGS groups were similar to the dry-rolled barley control group. There was no significant difference in average daily gain for any of the treatment groups, except at the 40 percent inclusion rate of maize DDGS. At this level the dry matter intake and

TABLE 2
Ethanol plants operating in Western Canada in 2011

Plant	Feedstock	Feedstock use (t/yr)	Ethanol production (litres)	DDGS production (t)
Permolex International Inc., AB	wheat	110 000	42 000	modified
Husky – Lloydminster, AB	wheat ⁽¹⁾	353 600	130 000	130 000
Husky – Minnedosa, MB	wheat ⁽¹⁾	353 600	130 000	130 000
Poundmaker Ag Ventures Ltd., SK	wheat	32 640	12 000	feedlot
Norwest BioEnergy Inc., SK	wheat	68 000	25 000	25 000
NorAmara BioEnergy Corp., SK	wheat	68 000	25 000	25 000
Terra Grain Fuels Inc., SK	wheat	408 000	150 000	150 000
Total		1 393 840	514 000	460 000

Notes: (1) = may manufacture using a wheat+maize mix. AB = Alberta; MB = Manitoba; SK = Saskatchewan.

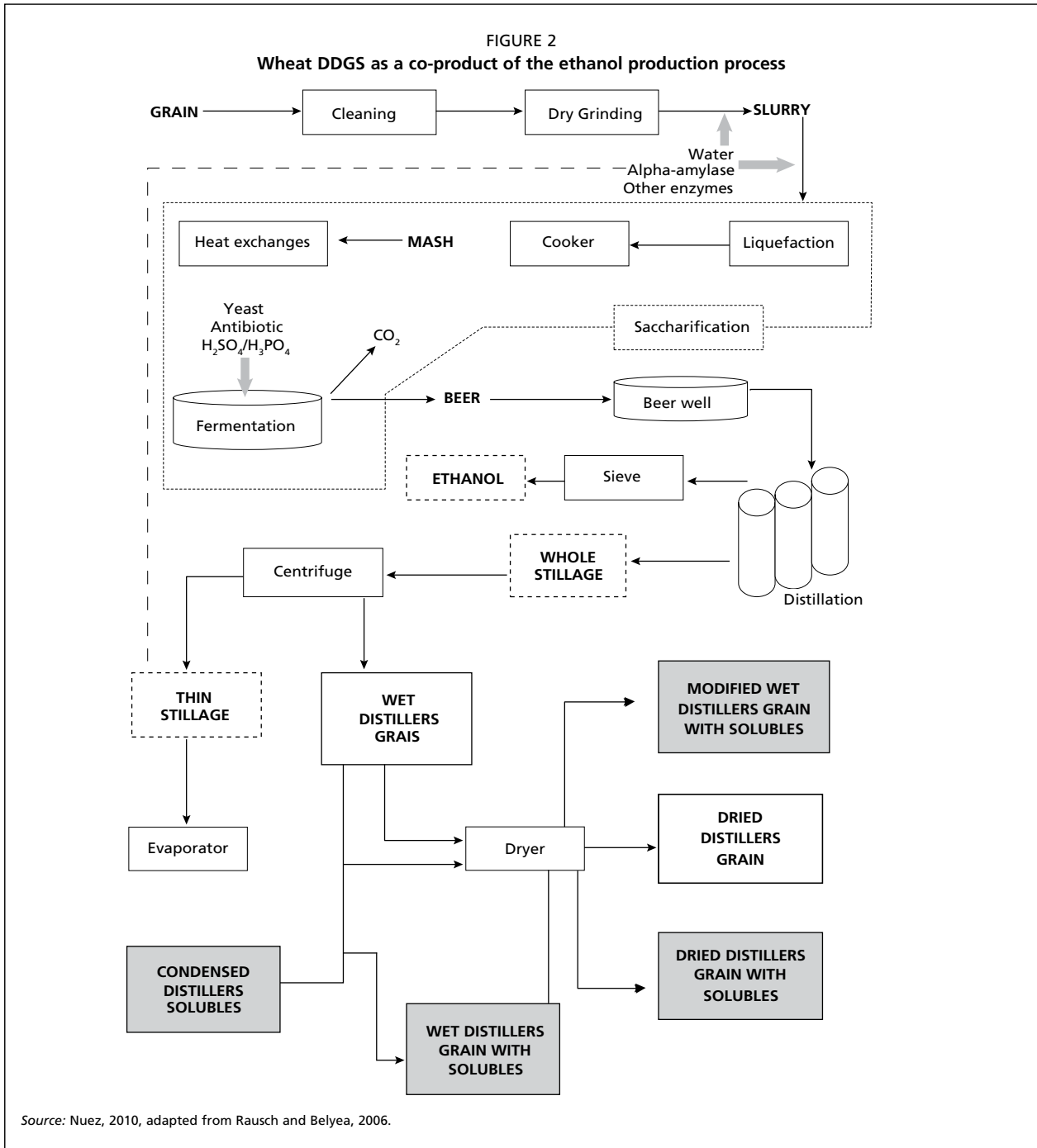


TABLE 3
Baseline and performance data summary of a DDGS feeding trial

	Control	20% wheat DDGS	40% wheat DDGS	20% maize DDGS	40% maize DDGS	PSEM	P value
Initial weight (kg)	375	376	377	376	376	0.8	0.7
Slaughter weight (kg)	654	649	648	652	653	2.28	0.34
Average daily gain (kg)	1.62	1.63	1.73	1.66	1.68	0.03	0.13
Dry matter intake (kg/day)	10.4	10.2	10.9	10.2	8.8	0.11	<0.01
Gain:feed ratio	0.156	0.159	0.158	0.163	0.192	0.002	<0.01
Hot carcass weight (kg)	371.9	370.8	374.8	375.3	375.6	5.34	0.54
Dressing percentage	58	58.6	59.2	59.4	59	0.27	0.01
Grade fat	7.8	8.2	9	8.2	9	0.41	0.18
Estimated lean yield (%)	61.2	60.6	59.8	60.6	60	0.45	0.23

Notes: PSEM = pooled standard error of the mean; P value = probability value. Source: Walter et al., 2010.

gain feed ratio improved over the control and other treatment groups. There were no differences in carcass quality in the DDGS-fed animals relative to the control diet. This trial illustrates that both maize and wheat distillers grain can be used in beef rations at up to 40 percent inclusion rate with no deleterious effects on animal performance or carcass characteristics relative to the commonly fed barley control ration.

Poundmaker Agventures Inc. is the only integrated ethanol and feedlot facility in Canada and is the only feedlot using wet mash and thin stillage as their feed input. Poundmaker Agventures is located in Lanigan, Saskatchewan, and operates a small 12 million litre facility, which produces two streams of products – thin stillage and wet distillers grain – that are fed to the beef in the feedlot on site. Poundmaker produces approximately 250 000 litres of thin stillage per day. Thin stillage is the water recovered from centrifugation of the whole stillage. It has low levels of solids, approximately 8.5 percent; the thin stillage is piped to the feedlot 1000 m away and distributed through an additional 1000 m to the water bowls in the feedlot. The approximately 8 000–16 000 animals receiving thin stillage need no supplemental water. Poundmaker also produces 50 tonne of wet distillers grain daily, with 23–24 percent solids. The wet distillers grain are picked up by a feed truck and directly mixed with barley silage at 10–20 percent of the ration.

OPPORTUNITIES FOR DEVELOPMENT OF THE DDGS MARKET IN WESTERN CANADA

Studies such as Walter *et al.* (2010) have illustrated the impact of maize or wheat DDGS on both animal performance and meat carcass quality. With the understanding of the biological indicators, livestock owners can begin to make decisions regarding the inclusion of DDGS in livestock rations based on the economics of the DDGS ingredient. Least-cost formulation is used to determine when specific ingredients are brought in or taken out of a ration. Cost of the ingredient, plus cost of transportation and ability to store ingredients, are included in the economic decisions.

Transportation logistics influence strongly the potential range of distribution of DDGS. Most imported maize-based DDGS from the United States is transported by railway. Transshipment sites for transfer of DDGS from rail to truck are needed as feedlots do not have the ability to receive ingredients by rail. In Western Canada, several transshipment sites are located in southern Alberta. These sites reduce transportation costs compared with trucking in DDGS. As a result, more maize DDGS is used in southern Alberta than in central Alberta or Saskatchewan.

CHALLENGES OF CREATING NEW MARKETS

In a competitive marketplace made up of many informed buyers and sellers, a market exchange is an institution that

very effectively governs the production and consumption of products. The prices generated in a market system create Adam Smith's 'invisible hand' to match the marginal cost of providing a product to the marginal value of that product to industry. In a great many instances in the market place, a simple exchange of products at an agreed upon price is a low-cost transaction that provides the correct incentives for the buyer and sellers. When the marketplace fails to operate in a manner such that the marginal benefit is not equal to the marginal cost of the action, then a market failure is said to exist. Market failures can be addressed through government, collective or private actions.

A market failure that has attracted attention in the investment literature is referred to as the hold-up problem. The hold-up problem, according to Milgrom and Roberts (1992), is "the general business problem in which each party to a contract worries about being forced to accept disadvantageous terms later, after it has sunk an investment, or worries that its investment may be devalued by the actions of others." The hold-up problem may be induced by other forms of market failure, but deals more specifically with the investment decision. Because the hold-up problem often prevents otherwise advantageous investment it can create market failures that are real obstacles to industry development, such as the development of the new feed markets for DDGS in Western Canada.

There is a relationship between the presence of transaction-specific and asset-specific investments and the potential for *ex post* hold-up (Williamson, 1983; Grossman and Hart, 1986; Tirole, 1988; Choate and Maser, 1992). With asset-specific (specialized) investments, the value of the asset in its specific use is far greater than its value in the next-best use. In order for the initial specific investment to be undertaken, the real rents to each party (returns in excess of *ex ante* investment) must not be negative. However, when one party's *ex post* opportunity cost is reduced to the initial investment, its bargaining power is also reduced, and it is less likely for this party to cover the initial investment. This party will recognize the potential for *ex post* hold-up and will therefore be unwilling to incur the *ex ante* investment cost. Hence, if the initial investment is high enough relative to the respective *ex post* opportunity cost, the initial investment will not be undertaken by that party and market failure will occur since the specific transaction is Pareto superior to all alternative transactions.

Addressing market failures through institutions

Institutions encompass a set of rules, both formal (e.g. statutes) and informal (e.g. norms), that constrain the behavioural relationship among individuals or groups (North, 1990). Institutions are effective rules, not nominal rules, with an emphasis on enforcement (Eggertsson, 1994). They can be established, enforced and policed, either by

an external authority or by voluntarily acceptance. They are predictable, stable and applicable in situations that are repetitive. Institutions define the decision-makers' utility choice set and their structure of incentives.

The establishment and enforcement of property rights allows attributes to be traded within a market system. In many cases, if property rights can be effectively assigned, then a market for the attribute will develop and the market failure will be addressed. In some cases, the assignment of property rights is not sufficient to address a market failure. In these cases, other private, collective or public actions may be lower-cost alternatives.

There are several forms of private action that can address market failures. In particular, Williamson (1983) suggested common ownership (e.g. vertical integration) as a response to site specificity. Additionally, Klein and Crawford (1978) concluded that

"the lower the appropriable specialized quasi-rent the more likely that transaction will rely on a contractual relationship rather than common ownership. Conversely, integration by common or joint ownership is more likely the higher the appropriable specialized quasi-rents of the assets involved."

Klein and Crawford (1978) defined the quasi-rent as "value of the asset is the excess of its value over its salvage value, that is, its value in its next best use to another renter."

Williamson (1983) argues that the potentially opportunistic party making an *ex ante* credible commitment to the exchange can support transactions that are (potentially) subject to hold up. *Ex ante* credible commitment usually takes the form of partial redistribution of specific investment costs to the potentially opportunistic party.

Long-term contracting can be another solution to some market failures. Specifically, Joskow (1987) states that, with many types of asset-specific investments, long-term explicit contracts can reduce the potential for *ex post* hold-up. However, with this solution it may be very costly to identify all the contingencies of the investment. Hence, appropriate institutional arrangements may be a solution to the threat of a hold-up.

Institutional responses

Particular institutions tend to be better suited than others to govern particular types of transactions. Picciotto (1995) classifies institutions into three general types and then describes what type of attributes these institutions best govern. One type of institution is represented by the hierarchy or government sector. This institutional structure's stakeholders are all the citizens of the state. The incentive in this sector is the re-election of the politicians so as to maintain power. Hence, they pursue goals for the best interest of the whole society. A second set of institutions is represented by the participation sector. This sector has

stakeholders who voluntarily join because they believe that benefits can be obtained by collective action. The members of the participating sector represent a group in society with a common interest. The last sector is the private sector. The individuals who own property rights are the stakeholders of this sector. The main incentive here is to maximize their return to asset investment (profit). Hence, each sector represents different individuals and has different incentives.

Each institutional structure tends to be more effective than others at producing particular types of goods. The government sector is best at producing public goods (e.g. justice) that are consumed by all citizens, and where the voice of interest groups is not important. Public goods are characterized by low excludability and low subtractability (rivalry). In this case, the low excludability makes privatization infeasible and the broad common interest in provisions is best represented at the government level where free riding can be eliminated.

The participation sector is best at governing common-pool goods (e.g. marketing services) or public goods where voice is important. These goods have the problem of excludability, which prevents them from becoming private goods. In addition, the benefits of common-pool goods are often restricted to a group of individuals or firms that are in the position to use the goods. In this case, it is in the common interest of the group to manage the good to their mutual benefit. It is also often the case that some group has greater interest in providing the good than the public at large and has more of the information required to manage the resource, making voice important.

The private sector tends to dominate whenever property rights can be assigned to make the goods excludable and the goods produced are subtractable. The property of exclusion allows private firms to charge for the use of the good. This allows the producers of the good to sell at the marginal cost of production. Where hold-up problems exist, transactions take place within larger private institutions or between institutions with long-term contractual arrangements. Excludability is not a sufficient condition for a good belonging in the private sector. If a good has low subtractability then there are economies of size in its provision, resulting in the failure of a natural monopoly and creating the potential need for government intervention.

Applications to credence goods

Introducing new products is difficult under almost any circumstance, but especially so when the product offers new or different quality traits. Given that the quality of DDGS is dependent on the quality of the feedstock entering the biofuel plant, the quality of the DDGS is going to be considerably variable. There has been an increasing volume of research on the theoretical and practical challenge of introducing new products.

TABLE 4
Product attributes and public and private responses

	Search attributes	Experiential attributes	Credence attributes
Public role in setting rules for the transaction	Consumer labelling laws to prevent fraud	Regulations ensuring consistent quality	Health, safety and environmental regulations Product liability and tort laws
Private mechanisms for managing the transaction	Voluntary labelling	Patents and trademarks backed up by identity-preserving production and marketing systems	Patented products offering private or brand warranties, or both, backed up by identity-preserving production and marketing systems

TABLE 5
Relationships between regulations, standards and private brands

Regulations for public-good purposes	Regulations based on standards	Commercial and private standards	Private brands become standards	Private brands and warranties
Driver: Public-good market failures without regulation		Driver: Common-pool goods requiring voice; collective rather than firm-based or regulatory based		Driver: Private, firm-based profit maximization

In the production system, the public sector has tended to establish the general environment for private actors to effect transactions (Table 4). Laws and regulations usually set the base rules for health and safety (e.g. the Canadian Feeds Act sets rules for animal feed usage). The private sector frequently establishes common-property or private mechanisms to manage the transactional elements to the attributes. Companies employ trademarks, brands and warranties to assure customers of the value of their product. Experience has shown, however, that the costs of developing private standards are high; for many agriculture products there are efficiencies that can be gained through collective action (e.g. the Canola Council of Canada story described in Gray *et al.* 1999).

In essence, both public regulation and commercial product standards can only really be understood in the context of all mechanisms used to manage markets (Table 5). At one extreme, governments or agents for governments set regulations to achieve public goals, such as health and safety, or environmental objectives. At the other extreme, private companies develop brands and provide private warranties to assure consumers of the quality of their products. In the middle, an array of public, private and collective actors may be critical. The long-term achievement of consistent quality in credence goods markets will require action on the part of all three types of actors (Smyth and Phillips, 2001, examine the canola industry to illustrate this point).

The challenge for the emerging DDGS market in Western Canada is going to be that of consistency of quality, as quality will vary greatly depending on the quality of the seed grain that enters the ethanol plant and the specific processing conditions of a biofuel plant. Federal regulations exist that ensure that at least a minimal description of DDGS is included with each shipment.

However, if a robust quality control testing regime is not in place, out-of-specification variation may not be identified. Feedlot firms will be extremely hesitant to enter into supply contracts (either long or short term) if the consistency of the feed quality is not guaranteed.

Two options exist for the DDGS industry: they can rely either on federal regulators to establish rigid standards for DDGS feed quality, or on the biofuel plants, in cooperation with the feedlots, developing industry standards to which both parties agree. The former option will include industry consultation, but the end result will be that the standards will be forced upon the industry and the industry input will be rather minimal. The latter option provides the DDGS industry with great flexibility in the development of standards, with the remaining challenge for the industry being to find a means of enforcing the standards and to develop response protocols in the event of specific products failing to meet expectations.

EMERGING DDGS MARKET

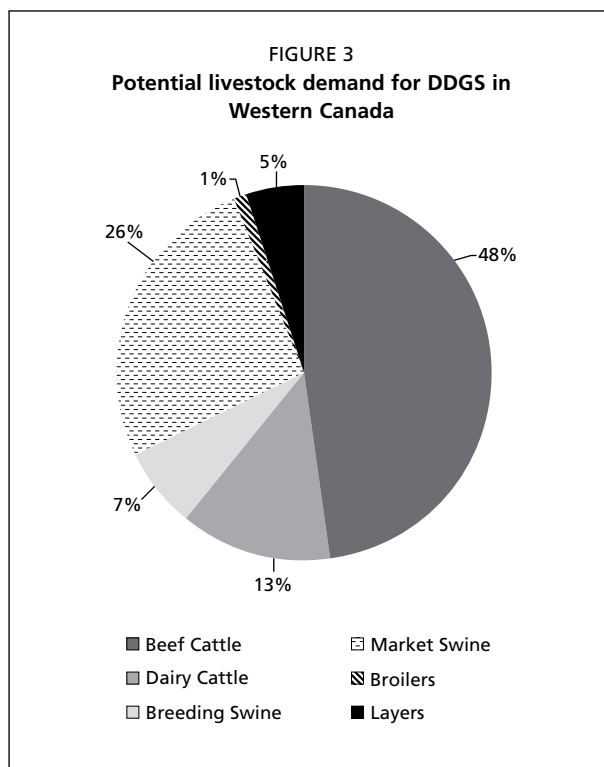
Based on current capacity of ethanol plants and grain-to-distillers grain conversion factors, the potential supply of distillers grain in Western Canada has been estimated (Table 2), based on a grain-to-ethanol conversion rate of 365 L/tonne of feedstock and a distillers grain yield rate of 290 kg/tonne of wheat (Racz, 2007). Consistent with the ethanol production capacity distribution across Western Canada, Saskatchewan is the leading supplier of distillers grain, with an annual estimated volume of 272 600 tonne (65 percent), with Alberta (10 percent) and Manitoba (25 percent) cumulatively accounting for the remainder of the region's total supply of distillers grain.

Based on livestock inventory, inclusion and adoption rates, it is possible to estimate the potential demand for DDGS in Western Canada (Table 6 and Figure 3).

TABLE 6
Calculation of potential DDGS feed ingredient usage in livestock in Western Canada

	Western Canada population	Daily Intake as fed (kg DDGS/day)	Days fed per year	DDGS consumed (kg/head/year)	Total DDGS ('000 tonne/yr)
Beef Cattle	1 933 700	2.80	120	336	649 700
Dairy Cattle	233 260	2.05	365	746.59	173 800
Breeding Swine	586 940	0.55	310	169.09	99 000
Market Swine	4 333 360	0.23	365	82.95	358 700
Broilers	29 803 780	0.0091	56	0.51	15 100
Layers	12 971 685	0.014	365	4.98	64 400
Total					1 360 700

Sources: Cattle numbers by class from Statistics Canada, 2011a; Hog numbers from Statistics Canada, 2011b; Poultry numbers from Statistics Canada, 2006b.



The total potential DDGS consumption is calculated by determining the number of animals from Statistics Canada sources, the daily intake and the total days on feed. Feed inclusion rates are largely representative of feeding practices in Western Canada, although some producers could feed in excess of the rates used. For example, a 20 percent inclusion rate of DDGS is used in the beef cattle estimate, even though research (Walter *et al.*, 2010) has indicated that up to an inclusion rate of 40 percent can be used in the rations.

It is estimated that the cattle sector market demand for DDGS would be about 823 000 t. Of this, the beef cattle sub-sector remains dominant. In the monogastric sector, hogs represent a potential key market, with demand mainly driven by the feed requirements for market hogs.

Among the various livestock species analysed, the demand for poultry seems to be the lowest. This result is

not unexpected considering that inclusion rates are lowest for this livestock category.

Overall, the current estimate for the potential DDGS demand for Western Canada is approximately 1.4 million tonne per year. However, this estimate is sensitive to the underlying assumptions of inclusion and adoption rates, intake values and days on feed. For example, Dooley (2008) noted that large-size operations are more likely to feed the co-product relative to their small-size counterparts due to scale advantages. This notwithstanding, the use of a 100 percent adoption rate is a critical assumption in estimating an upper market boundary for the co-product.

Available market estimate

This section estimates the potential demand for the various provinces under similar assumptions. Given this demand and local DDGS supply, surpluses or deficits are estimated for the different provincial markets. This is to give an overview of the available market for imports and future increases in domestic supply. The present analysis implicitly assumes the domestic utilization of all distillers grain produced and the absence of inter-provincial trade.

It is observed that the overall available market for DDGS is about 70 percent of the total market demand (Table 6). With the exception of Saskatchewan, which is likely to export the commodity, all the other provinces have substantial supply deficits. Of the three provinces, however, it is posited that Alberta is likely to be the main market for DDGS in Western Canada as demand is mainly driven by the beef cattle sector (40 percent). The available market for DDGS in Manitoba and British Columbia in contrast is mainly driven by the hog (50 percent) and poultry (80 percent) sectors respectively. Traditionally, adoption and inclusion rates have been lowest for these sectors, and hence it remains unlikely that these provinces would be important markets for the co-product. Although, of the two provinces, Manitoba would more likely be the larger market because of the relative higher inclusion rate for hogs.

Evidence from available DDGS import data (Table 8) supports the analysis of the previous section. Alberta is

TABLE 7
Estimate of the available market for DDGS in Western Canada

Province	Potential DDGS demand	Demand as % of total potential market demand	Domestic DDGS production	Supply Surplus or (Deficit)	Potential available market (%)
Manitoba	435 000	31	104 000	(331 000)	+76
Saskatchewan	299 000	21	272 000	(27 000)	+9
Alberta	517 000	37	40 000	(477 000)	+92
British Columbia	136 000	10	0	(136 000)	+100
Total	1 360 000	100	416 000	(971 000)	69

Notes: Potential available market indicates proportion of market potentially available to imported DDGS.

TABLE 8
Annual maize DDGS imports from the United States (2000–2009)

Province	Average annual value	Share
Manitoba	\$CAN 8 382 909	29%
Saskatchewan	\$CAN 1 365 665	5%
Alberta	\$CAN 17 411 275	60%
British Columbia	\$CAN 2 015 858	7%
Western Canada	\$CAN 29 175 706	100%

Source: Industry Canada, 2011.

the main market for imports of United States maize-based DDGS, followed by Manitoba. The large beef cattle herd in southern Alberta accounts for this high demand. Imports for Saskatchewan and British Columbia are less than 10 percent of total potential demand.

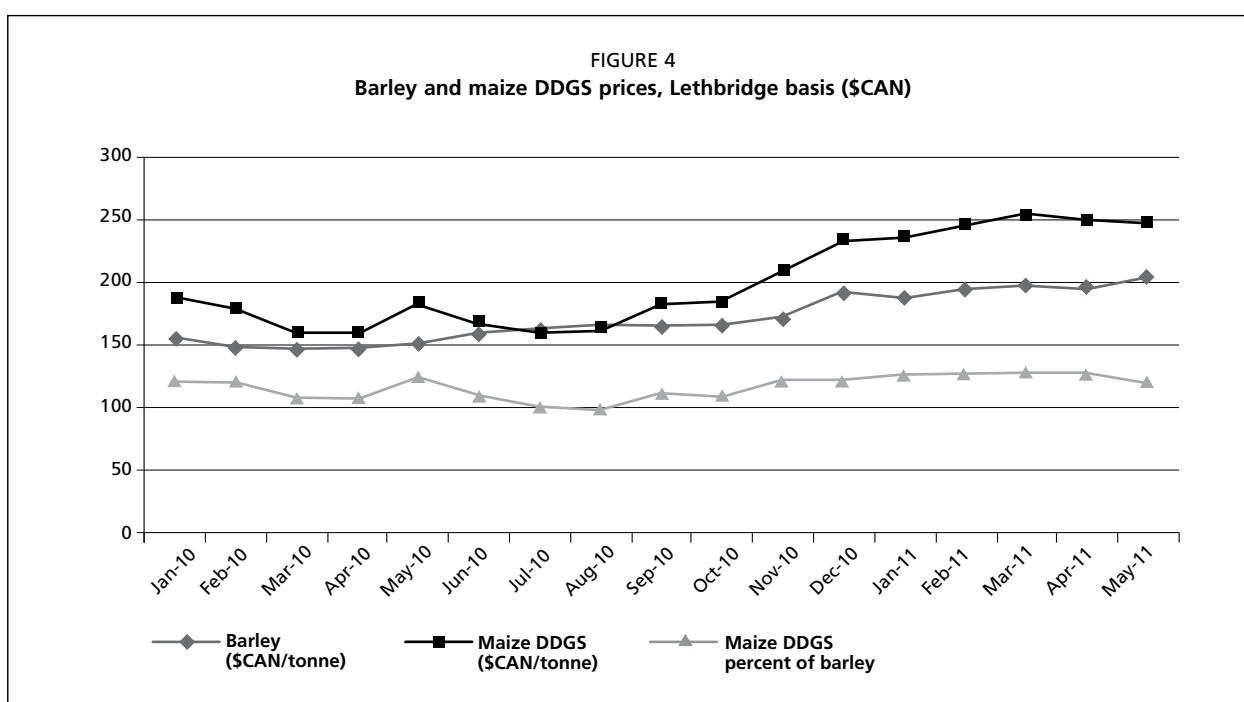
Substitute feed ingredient price

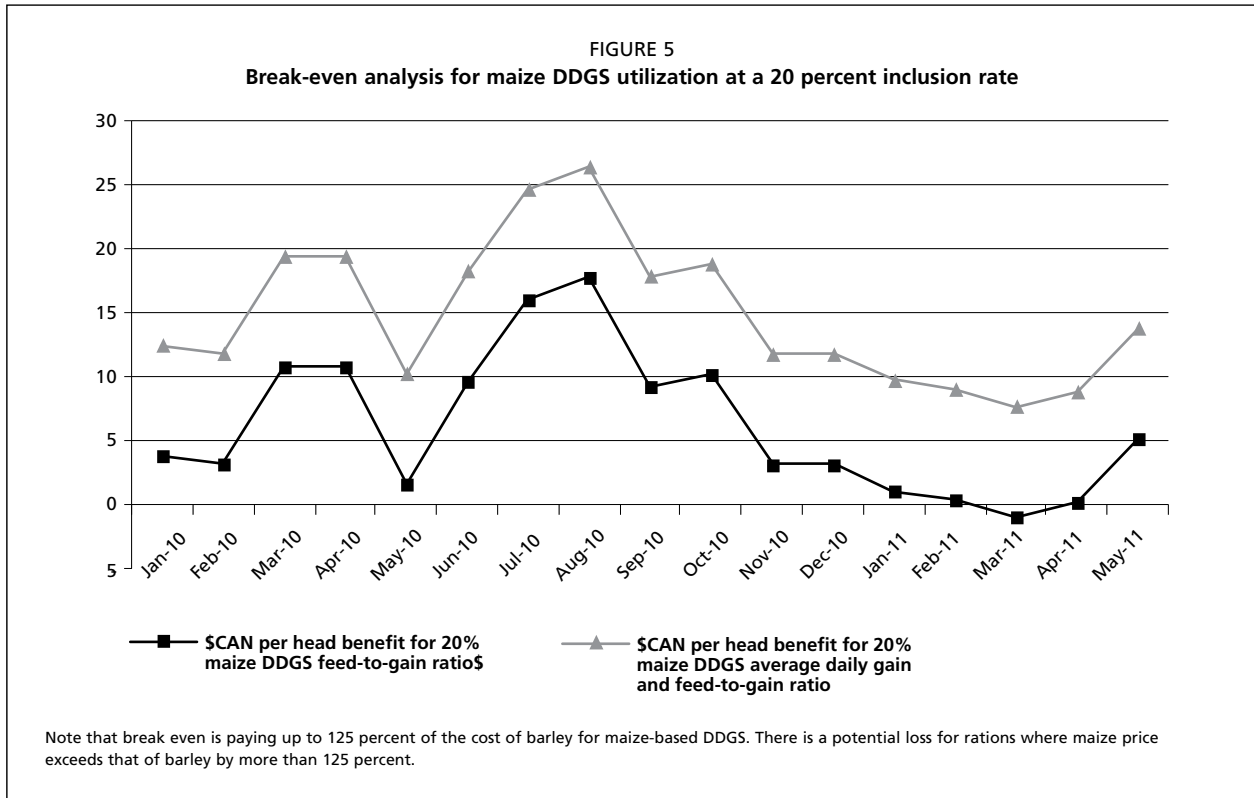
Feed rations are calculated using the least-cost scenario for all feed ingredients. The work of Walter *et al.* (2010) indicates how beef cattle would perform on maize-based DDGS. Robinson (2011) used the animal performance data

obtained by Walter and co-workers to identify the price of maize-based DDGS at which feedlot operators would benefit from using the maize-based DDGS. Robinson obtained prices for barley and maize for a 16-month period (Figure 4). Based on common feedlot operational costs and the work of Walter *et al.* (2010), Robinson calculated the break-even point for the 16-month period (Figure 5).

Given the feed-to-gain ratio determined by Walter *et al.* (2010) and common feedlot operational costs, feedlot operators would obtain a \$CAN 1/head advantage or better if the ratio of the cost of maize-based DDGS was less than 125 percent of that of barley. Walter *et al.* (2010) also determined that, on average, animals on maize DDGS were in the feedlot three days fewer than control animals. Figure 8 includes the cost savings to the feedlot operator when average daily gain, as well as feed-to-gain ratio for maize DDGS inclusion at 20 percent of the ration, is calculated.

A key factor that affects the demand and usage of a feed ingredient is the price of substitute feeds. Livestock producers usually substitute among feed ingredients in order to take advantage of price variations. A major con-





sideration regarding the competitiveness of DDGS as an ingredient is its energy and protein value *vis-à-vis* other feed ingredients. If formulation models are rigid, wheat-based DDGS tends to be a substitute for protein-based feeds and maize-based DDGS tends to be a substitute for energy-based feeds. Therefore, it can be deduced that wheat-based DDGS attains a higher value as other protein-based feed prices increase. Given other protein-based feed prices staying constant, the value of maize-based DDGS increases and replaces wheat-based DDGS as the price of energy-based feeds increase (Boaitey, 2010). Discussions with livestock producers in Western Canada revealed that rations formulated without limits on protein are common (McKinnon, Univ. Saskatchewan, pers. comm.). When rations are formulated without an upper limit restriction on protein, wheat-based DDGS, with its higher protein content, becomes more prominent.

Supply chain logistics and economic impacts

Given the proximity of the Canadian and United States markets, especially regarding the supply of feedstocks for ethanol plants and consequent DDGS production, an important market factor will be the exchange rate between the Canadian and United States currencies. Dessureault (2009) estimated that in 2010, 75 percent of Canadian ethanol was derived from maize, 23 percent from wheat and 2 percent from other feedstock. Most of the maize feedstock is used in Eastern Canada, while wheat feedstock is used in Western Canada. With the wheat-based ethanol plants in

Western Canada, there is little competition with livestock feedlots, given the reliance of feedlots on barley as the major ingredient for their feed supplies. However, when it is cheaper for ethanol plants in Western Canada to import United States maize for use as feedstock, rather than buy wheat produced in Western Canada, ethanol firms will use maize. When this occurs, the ethanol subsidies received by the Canadian ethanol firms are essentially used to support United States maize growers in the American Midwest, as opposed to grain farmers in Western Canada. This raises a host of interesting policy issues that are beyond the scope of this chapter.

As noted above, the United States ethanol and co-product industry is over 60 times the size of the Canadian industry, producing over 30 million tonne of DDGS in 2009, compared with 0.5 million tonne in Western Canada. The 800 000 tonne currently exported to Western Canada account for less than 3 percent of total United States DDGS supply. Some projections have the United States ethanol industry tripling capacity over the next five years, which would also increase the supply of DDGS. One would expect that, with the potential increase of supply, there would be a corresponding decrease in the price in Canada of imported maize-based DDGS.

As the Canada-United States dollar exchange rate fluctuates, the price of maize-based DDGS changes for the livestock feed industry in Canada, and the competitiveness of wheat-based DDGS is affected. Given the current strength of the Canadian dollar *vis-à-vis* the United States

dollar, United States maize-based DDGS is much more competitive than Canadian wheat-based DDGS, to the point where making ethanol out of imported United States maize may be more profitable than using wheat in Western Canada. Boaithey and Brown (2011) have estimated that when the Canadian dollar is above US\$ 0.80, it will be cheaper for the livestock industry in Western Canada to import United States-produced, maize-based DDGS. The Canadian dollar was last below US\$ 0.80 in the early months of 2009, so since then it has been cheaper for the Canadian livestock industry to import United States DDGS. Given that the Canadian dollar is currently on par with the United States dollar and has been so for virtually all of 2011, a decline in the Canadian currency is not anticipated in the near future. Indeed, Boaithey (2010) observed that the vast majority of livestock rations in the southern Alberta feedlots are based on imported maize-based DDGS from the United States.

The cost of transportation can change over time and this can affect the competitiveness of wheat-based DDGS and maize-based DDGS. Maize-based DDGS usually has to be transported longer distances, from ethanol plants in the United States, but wheat-based DDGS is less dense and therefore fewer tonnes can be loaded into the same size of vehicle, thereby raising the cost of transportation (McKinnon, Univ. Saskatchewan, pers. comm.) Taken in tandem, the greater density of maize-based DDGS and the price sensitivity of a high Canadian dollar mean that the economics for Canadian wheat-based DDGS supplies are poor. Western Canada imported about 800 000 tonne of maize-based DDGS each year in 2008–2010, which amounts to approximately two-thirds of the total DDGS demand, giving the maize-based DDGS firms a sizeable market share. Given that American Midwest ethanol plants are able to export maize-based DDGS into southern Alberta – the feedlot market nearest to the source of supply – implies that if maize-based DDGS suppliers can serve this market, they will be able also to economically serve all other feedlot markets in Western Canada. The combination of quantity of supply and the ability to economically export DDGS from the American Midwest to southern Alberta implies that the United States DDGS suppliers have considerable market power and might be able to use pricing strategies to disadvantage Canadian wheat-based DDGS production.

Overall, the market for DDGS in Western Canada would most likely be determined by the interplay of local supply, the supply of traditional feeds, United States ethanol expansion and market factors such as freight rates and currency exchange rates. However, producers and marketers of the product can facilitate its utilization by promoting increased inclusion rates amongst livestock producers in Western Canada.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The development of new markets is a process filled with opportunities, challenges and pitfalls. While the development of a new market for Canadian wheat-based DDGS is not as complicated as the development of the market for a new food product, we have shown that it is neither as simple nor easy as one might first think.

There are roles for both the public and private sectors in the development of this market. To address the feedlot operators' concerns about consistency of quality, it is possible that the sector might turn to the federal government to regulate the quality of wheat-based DDGS products. This could be done through an update of the Canadian *Feeds Act*. Given that the quality of the final product is so heavily dependent on quality of the wheat entering the biofuel plant, it is unlikely that the biofuel or the feedlot industry would engage in this option.

A more likely outcome to control for issues of product consistency would be for the biofuel industry to begin to brand their DDGS products in an attempt to create value for their specific DDGS products as being of higher quality or consistency than those of their competitors. This may, or may not, include some form of a product warranty if the feedlot tests a batch of DDGS products and finds them not meeting some set quality parameter. The biggest challenge for the emerging DDGS market in Western Canada is going to be that created by the competition that does, and will, exist from cheaper United States maize-based DDGS. Without a doubt, there is a considerable degree of United States produced maize entering Western Canada to be used in the production of ethanol, resulting in local and regionally based competition for wheat-based DDGS production. One must also bear in mind the competition that already exists with the United States production of DDGS from their own domestic biofuel production and their ability to export maize-based DDGS products into Western Canada at competitive prices.

One of the potential hold-up problems that might affect the development of a DDGS industry in Western Canada is the nature of feedlot industry contract preferences. Feedlots have a preference for short-term contracts of two or three months duration. While a series of contracts might be with the same supplier, the length of the contracts is always of a short-term nature. The inability to secure longer-term contracts would be a barrier to ethanol plants trying to enter the feed industry. The longer the supply contract, the lower the risk of entering into the market, but with the feedlot industry preference for short-term contracts, the risk of entering the DDGS market might be too great for ethanol plants.

A major limitation of Boaithey (2010) was the lack of adequate price data on wheat DDGS. Aside from the

industry in Western Canada being relatively young, most of the ethanol producers contacted were unwilling to provide Boatey with such data. As a result, approximation techniques were used to derive the price of wheat DDGS. This might affect the validity of some of the conclusions made from the time-series analysis, especially regarding the inter-relatedness between wheat DDGS and prices of barley and canola meals. The effect of approximation pricing on the least-cost ration results may not be as significant.

Secondly, Boatey does not incorporate nutrient management costs. It indirectly isolates feed costs from other costs incurred as a result of certain feeding practices. Future studies could incorporate these costs to ascertain how conclusions may differ. Any incorporation of nutrient management costs in addition to improving the price data for key feed ingredients, such as wheat DDGS, in future studies would provide a better understanding of the economic value of distillers grain. Furthermore, future research could consider the effect of nutrient variability on the conclusions of the present study.

As Table 6 has indicated, the demand for DDGS, if consistently used in livestock rations, is greater than the production of DDGS by Western Canadian companies. The mandate of the Feed Opportunities from the Biofuels Industries (FOBI) research network (www.ddgs.usask.ca) was to investigate the use of DDGS by all livestock sectors to determine both the biological parameters affected by DDGS and the economics of DDGS use. For the beef sector, research was conducted on inclusion limits and biological performance (Walter *et al.*, 2010). Biological performance parameters such as average daily gain and feed-to-gain ratio, as well as potential negative health impacts such as liver abscesses, were investigated. No negative health impacts were observed at any level of DDGS inclusion. With this data, it becomes possible to fully calculate cost of production, including cost of feed with operational costs. The impact is that higher inclusion rates of DDGS may be accepted into the diet, even if it increases the length of stay in the feedlot, given a lower, favourable cost of the ingredient.

Given that the biological implications of the use of DDGS are known for the feedlot industry, more research on market indicators are required to fully understand how the beef feedlot industry might utilize domestic wheat DDGS or maize DDGS imported from the United States. Existing supplier relationships tend to be very strong, with feedlots continually purchasing feed supplies from the same firm. The ability of ethanol plants, be they in Canada or the United States, to break this strong bond will need to be examined to determine the full market potential for suppliers of DDGS-based feed ingredients. While United States maize-based DDGS products can be cheaply transported by rail to the feedlot industry in southern Alberta, the requirement

for a transshipment capacity is fundamental, and the farther away that a feedlot is from a transshipment point, the greater the propensity to continue to utilize existing supply relationships that are predominantly based on barley grain.

Use of DDGS by monogastrics such as poultry and swine was also investigated in the FOBI research network. The research was not as focused on commercial parameters as the network's ruminant research because the use of DDGS as a feed ingredient is not as widespread in monogastrics. Yet, if the quantities of DDGS produced is going to continue to increase with the expansion of the ethanol industry, assessments of impacts on nutrition, health and biological performance will be needed. The FOBI research network investigated the potential to fractionate DDGS. Removal of the fibre from DDGS to produce a high-protein concentrate would increase the acceptance of DDGS in monogastric diets. Although preliminary trials were promising, additional research is necessary to develop a cost-effective method of separating fibre from DDGS.

The preferred form of co-products for sale by ethanol companies is predominantly as wet DGS. However, transportation costs and storage issues for the co-product in this form mean that sales of wet DGS only occur within a limited radius around ethanol facilities. A 50-mile [80 km] radius is generally accepted in North America as the maximum distance it is economically feasible to transport wet distillers grain (Konecny and Jenkins, 2008). However, a study from Australia (Bonnardeaux, 2007) suggests that a 125-mile [200 km] radius is economically viable. Transporting products greater distances requires drying the distillers grain; dryers imply expensive capital and operational costs. Research programmes such as FOBI have explored additional fractionation technologies, which could potentially diversify bio-ethanol facility product lines. However, the costs of purchasing and developing these new product lines may be prohibitive.

Further research must be done by individual buyers regarding the variability of the DDGS that they purchase. Nuez (2010) and Nuez and Yu (2010) indicate that there is variability both between batches and between plants in the quality (protein content and digestibility) of DDGS. Until individual plants develop standardized processing parameters and quality assurance programmes, quality must be addressed by the buyer.

CONCLUSIONS

We have shown that the potential annual supply of DDGS feed ingredients from ethanol plants in Western Canada could be close to 500 000 tonne, while demand for the same products could be more than 800 000 tonne more, with a possible demand of 1.4 million tonne of DDGS products. The shortfall in supply will have to be filled from somewhere, and the logical source would be imported

United States maize-based DDGS products. The development of the DDGS industry in Western Canada, regardless of whether it derives from domestic wheat-based ethanol or United States maize-based ethanol, has three crucial parameters.

First, the development of a Canadian-based DDGS industry is directly connected to the exchange rate between the United States and Canadian currencies. Ethanol plants will use the cheapest available input, which is often going to be United States maize-based DDGS. The combination of the availability of United States maize and the commodity price means that when the Canadian dollar is above an exchange rate of US\$ 0.80, it will be more economical for Canadian livestock firms to import United States maize-based DDGS to use as a feed ingredient. This means that the potential for the development of a Canadian wheat-based DDGS industry is completely price sensitive, and given the current exchange rate between the two currencies, the further development of a Canadian wheat-based DDGS industry should not be expected.

Second, the geographical disconnect between the supply and the demand is going to be an economic barrier to the use of DDGS by feedlots. Most supplies of DDGS feed inputs are going to come from the ethanol plants, which are predominantly located in Saskatchewan. The greatest percentage of the demand for the product will come from the highly concentrated beef feedlot industry in southern Alberta. The disconnect between the two end points of the potential supply chain could reach 1000 km. The additional transportation costs for the feedlot industry will directly affect the profit margins of the feedlots, and the local supply and price of feed barley is likely to mandate barley as the preferred feed ingredient. The lower volume of wheat-based DDGS that can be transported per transport unit (railroad car or lorry) compared with maize-based DDGS is a further barrier, not to mention that, at present, feedlot firms in southern Alberta are able to import United States maize-based DDGS more economically than purchasing wheat-based DDGS from Saskatchewan.

Third, the high degree of quality variability in DDGS products for factors such as protein and fat content will have to be addressed before the beef feedlots will begin to contemplate a shift in feed ingredients. With the feedlot preference for short-term contracts already in existence, the quality variability of DDGS will probably only reinforce this preference, and the length of contracts for DDGS inputs may be even shorter in the absence of any form of standardization from DDGS suppliers.

The ultimate success of a developed DDGS market in Western Canada will require a champion that is willing to drive the process. The few ethanol plants currently operating in Western Canada do not have the economies of scale to likely be the driver, compared with the United States,

where the higher number of ethanol plants has resulted in a market surplus of DDGS products. With an economic efficiency radius of 50 miles from an ethanol plant, it may be that the market will develop more rapidly for wet distillers grain. Regardless of the product or the location, the development of a market for any form of distillers grain is going to require a strategic plan, capital, and some dedicated human resources to ensure there is a sustained momentum to develop and maintain the market for this new feed product. While the opportunities are quite apparent, the challenges in developing this market will, without a doubt, be numerous.

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Chapter 27

Biofuels: their co-products and water impacts in the context of life-cycle analysis

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ABSTRACT

Life-cycle analysis (LCA) of biofuels, including maize ethanol, sugar cane ethanol, cellulosic ethanol and biodiesel, must incorporate the impact of co-products. Distillers grain with solubles, an animal feed co-produced with maize ethanol, is one such co-product. Electricity, a significant co-product of cellulosic ethanol production, can provide significant greenhouse gas credits over the life cycle of a biofuel. This chapter examines biofuel production technologies and biofuel co-products, and methods for allocating energy and water consumption and environmental burdens among the biofuel and its co-products. Allocation methodologies include displacement, mass-based, energy-based, market-value-based and process purpose. It is also possible to combine these approaches in a hybrid methodology. We present LCA results (energy consumption and GHG emissions) for maize and cellulosic ethanol, and examine the effect of co-product allocation methodologies on these results. We also discuss water consumption in the life cycle of maize and cellulosic ethanol. As biofuel production technology matures, it is likely that the portfolio of biofuel co-products will evolve, requiring LCA practitioners to re-assess their effect on the life-cycle impacts of biofuels.

INTRODUCTION

Life-cycle analysis (LCA) is a tool to systematically examine the energy and environmental impacts of products, processes and systems (Allen and Shonnard, 2002; ISO, 2006). Its application to biofuel production has expanded rapidly in recent years, but not without controversy. Applying LCA to biofuels raises issues such as accounting for greenhouse gas (GHG) emissions from land-use change (LUC), allocating the environmental impacts of biofuel production among co-products, including animal feed, and assessing the impact of biofuel production on water quality and consumption. In this chapter we present recent advances in the application of LCA to biofuels, including the impact of technology developments, improved estimates of LUC impacts, advancements in the understanding of animal feed as a co-product of ethanol plants, and advances in quantifying water consumption impacts of biofuel production.

BIOFUEL PRODUCTION TECHNOLOGIES

Production of biofuels in the United States has escalated since the United States began its fuel ethanol programme in 1980. United States production of maize ethanol was 76 million litres in 2000. In 2010, it had increased to 49 billion litres (RFA, 2011). Production of bio-ethanol is increasing worldwide. In the European Union (EU), for example, 3.7 billion litres of ethanol were produced in 2009, up

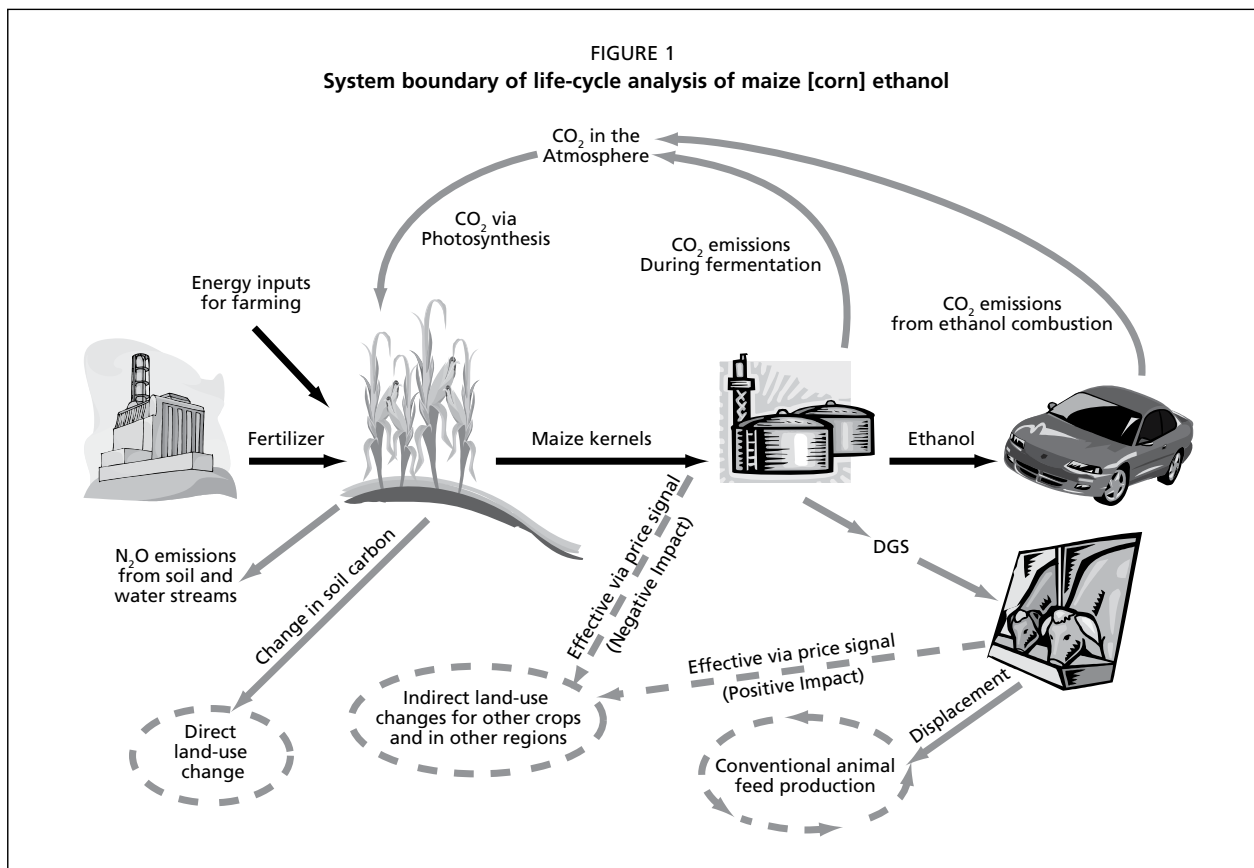
six-fold from 2002 (ePure, 2010). In Brazil, which is the second-largest ethanol producer in the world, ethanol accounts for 40 percent of the gasoline market (Wang *et al.*, 2008). Brazil's 2008/2009 ethanol production was 28 billion litres, more than double production in 1990-1991 (UNICA, 2011).

Biofuels can be classified as first, second or third generation. First-generation biofuels derive from cereal, oil and sugar crops, which are converted to fuels with mature technology. Of the first-generation fuels, maize ethanol has received the most attention in the LCA arena. Figure 1 depicts the life cycle of this biofuel, which is the most widespread fuel alternative to gasoline in the United States.

Ethanol plants use dry- or wet-milling technologies. In wet-milling plants, maize kernels are soaked in SO₂-containing water. De-germing of the kernels and oil extraction from the germs follows. The remaining kernel material is ground, producing starch and gluten. The former is fermented to ethanol. In dry-milling plants, starch in milled maize kernels is fermented into ethanol. Residual materials are generated that have value as commercial animal feed, called distillers grain with solubles (DGS), which can be sold in wet (WDGS) or dried form (DDGS). Integration of maize fractionation in the dry-milling process permits production of germ and fibre co-product streams from whole maize kernels prior to fermentation. Front-end fractionation has

MAIN MESSAGES

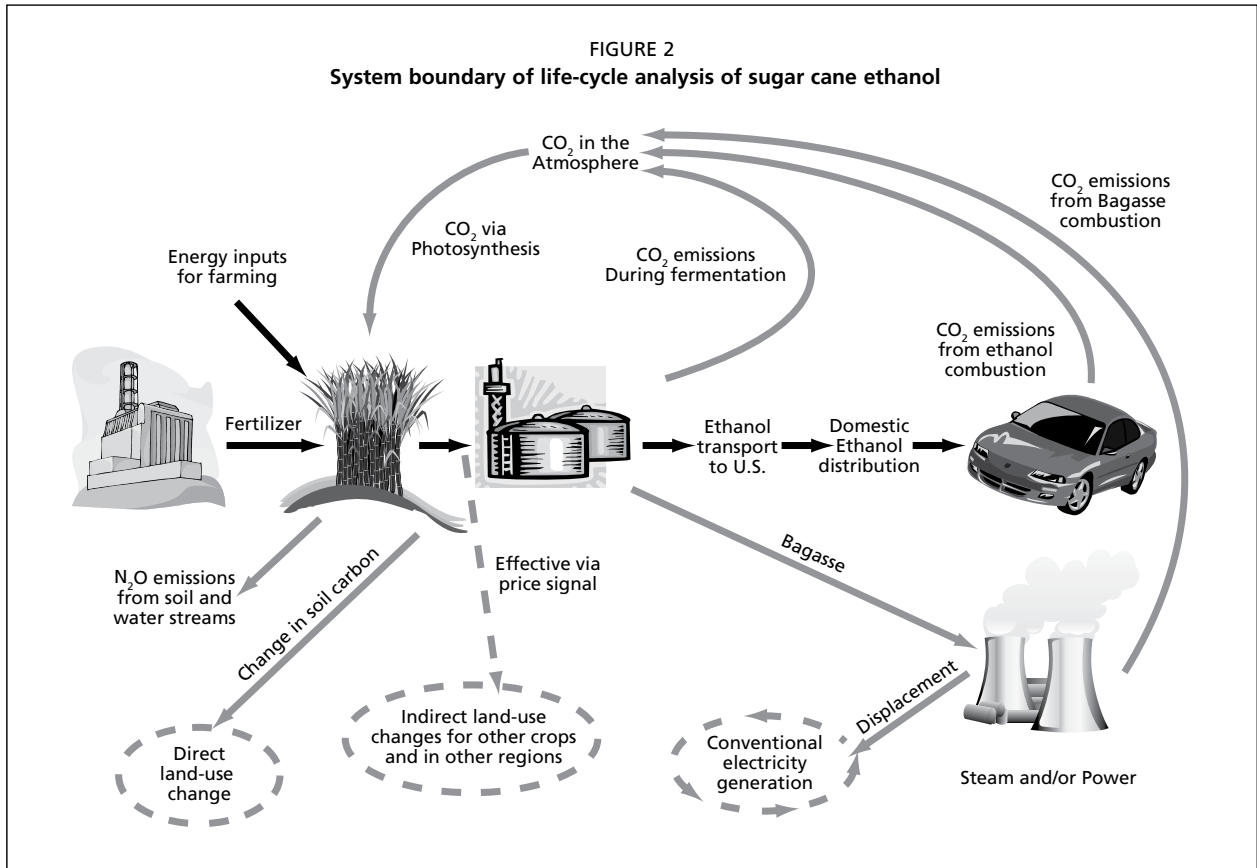
- Maize, cellulosic, and sugar cane ethanol have been the subject of life-cycle analysis with the GREET model, as has been biodiesel produced from soybean.
- Co-products of biofuels, including animal feeds such as distiller grain with solubles, have significant effects on life-cycle energy consumption and greenhouse gas emissions associated with biofuels.
- In the past decade, production of maize ethanol has become more energy efficient, both on the farm and at the factory.
- Land-use change greenhouse gas emissions can significantly affect life-cycle impacts of biofuels, and these remain a subject of active research and debate.
- Biofuels offer life-cycle energy consumption and greenhouse gas emission advantages compared with conventional petroleum-derived fuels. Co-products influence these life-cycle impacts. The allocation methodology selected to divide well-to-pump life-cycle burdens among co-products influences life-cycle results, at times considerably.
- Water consumption impacts for biofuels are dependent upon the growing location and associated irrigation practices. Cellulosic ethanol has the potential to have a lower water consumption impact than gasoline.



emerged as a promising technology to reduce energy use, increase ethanol yield and produce valuable co-products. Dry mills can also adopt a maize-oil extraction step, in which maize oil is removed from the stillage, or distillation column output stream, and used as animal feed or as a biofuel. Dry mills have eclipsed wet mills as the dominant maize ethanol production technology and currently account for nearly 90 percent of the total United States capacity (Wang *et al.*, 2011).

In Brazil, ethanol is produced from sugar cane, as Figure 2 illustrates. Sugar cane mills extract sugar juice from the cane. The juice is fermented to produce ethanol and possibly sugar. Combustion of solid residues (bagasse) from juice extraction produces steam and electricity, which mills integrate into the plant to improve energy efficiency. Brazilian mills have exported surplus electricity beyond the plant gate since 2000.

Second-generation biofuels are produced from lignocellulosic feedstocks such as maize stover, forest



residue and dedicated energy crops (switchgrass, miscanthus and various other plants). Figure 3 sketches the life cycle of ethanol from switchgrass. Conversion technologies for these feedstocks are at pilot-plant scale now, and research and development activities abound in China, the EU and the United States (e.g. Feng *et al.*, 2011; Scordia *et al.*, 2011). Because commercial-scale lignocellulosic facilities are in development, techno-economic analyses and LCAs of this technology are based on process models, such as those produced by the National Renewable Energy Laboratory (Humbird *et al.*, 2011). In general, prior to fermentation, cellulosic feedstocks must undergo a chemical, thermal or biological pre-treatment step to release sugars from biomass and separate lignin. The subsequent fermentation step converts the sugars to ethanol. Combustion of lignin can fuel on-site steam and power generation. As with sugar cane ethanol plants, this on-site power can be used at the plant and possibly exported to the grid. This ability of second-generation biofuels to produce power as a co-product is an attractive characteristic. Further, feedstocks such as maize stover and forest residues do not compete directly with food production. Feedstocks such as dedicated energy crops pose less competition with food production than do grains and oilseeds as biofuel feedstocks.

Third-generation biofuels include biodiesel and renewable diesel from algae, and other hydrocarbon fuels similar

to gasoline and diesel (sometimes called drop-in fuels) from cellulosic biomass via gasification, pyrolysis and hydro-liquefaction. Significant research and development efforts are underway to develop technologies for these third-generation biofuels. Besides biofuels from algal oil, algal feedstocks can provide significant amounts of biomass for methane production via anaerobic digesters. The bio-methane can be further used for electricity production. Production of hydrocarbon fuels from biomass can co-produce other energy products such as electricity and fuel gas.

MARKET POTENTIAL OF BIOFUEL CO-PRODUCTS

As noted above, the production of starch and lignocellulosic ethanol results in the generation of several co-products. This section discusses co-products from these pathways, as well as co-products generated from soybean and rapeseed-derived biodiesel. Table 1 catalogues co-products yields in various selected biofuels pathways analysed by Argonne National Laboratory (2010).

ANIMAL FEED BY-PRODUCTS OF MAIZE STARCH ETHANOL MANUFACTURING

As discussed above, DGS, used as animal feed, is a co-product at dry-mill ethanol plants. A plant’s decision to produce WDGS or DDGS must weigh the competing costs of the energy to dry DGS to make DDGS against the shorter shelf life and increased transportation costs of heavier WDGS,

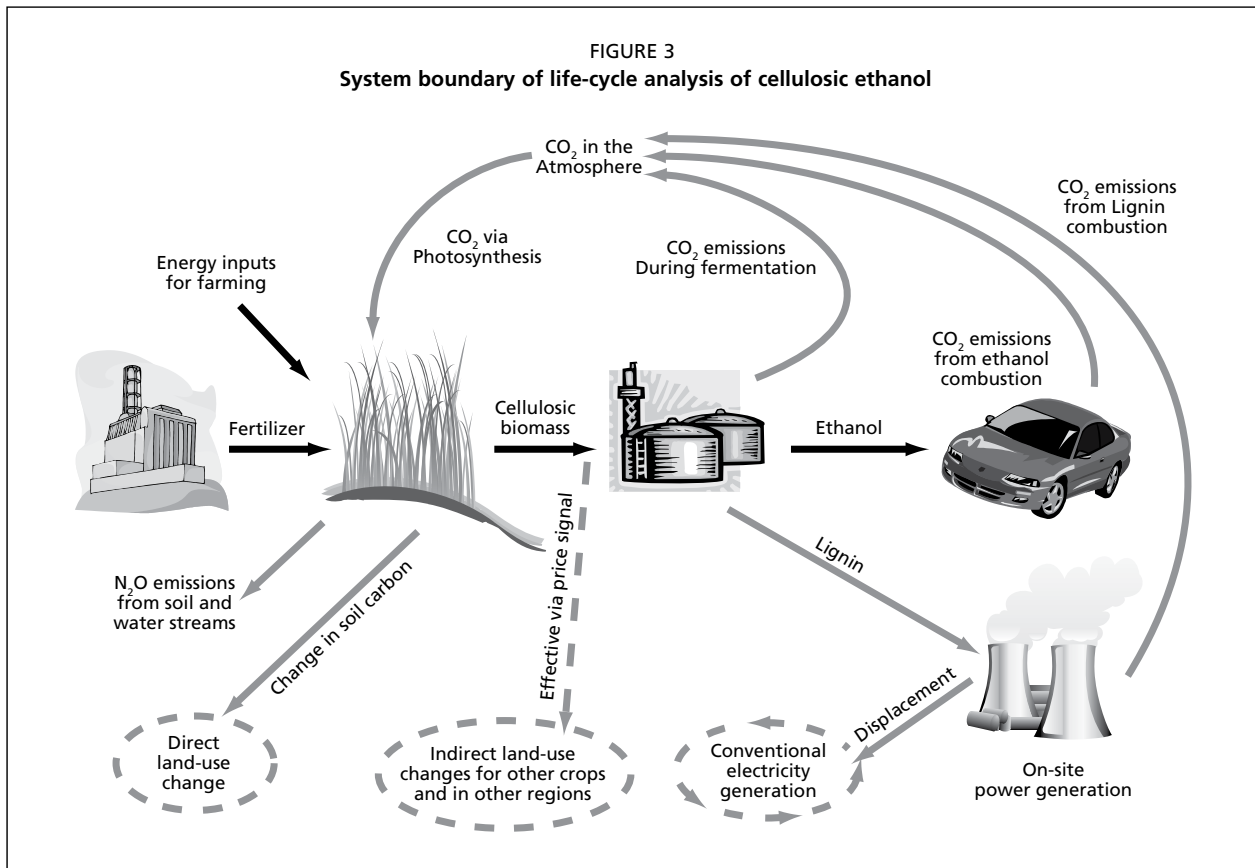


TABLE 1
Product yields of different biofuel production pathways

Product	Yield
Maize to ethanol: per litre of maize input	
Ethanol: undenatured litres ⁽¹⁾	0.28
DGS: kg (dry matter)	0.19
Switchgrass to ethanol: per dry tonne of switchgrass input	
Ethanol: undenatured litres	374
Electricity credit: kWh	226
Soybean crushing: per litre of soybean input	
Soy oil: kg	0.14
Soy meal: kg (dry matter)	0.53
Soy oil to biodiesel: per kg of soy oil input	
Biodiesel: kg	0.96
Glycerin: kg	0.21
Soy oil to renewable diesel: per kg of soy oil input	
Renewable diesel: kg	0.66
Fuel gas: kg	0.17
Heavy oils: kg	0.12

Notes: (1) Ethanol yield for average of wet and dry mills.

Source: Argonne National Laboratory, 2010.

which limits its customer base to a roughly 100-mile radius. In 2007, approximately one-third of dry-ethanol mills reported selling WDGS rather than DDGS.

Production of DGS continues to increase in the United States, as Figure 4 depicts. DGS provides between 10–20 percent of dry-mill ethanol plant revenues (Arora,

Wu and Wang, 2010). Table 2 outlines the United States DGS market size on the basis of grain-consuming animal units (GCAU). With 100 percent market penetration of given DGS inclusion rates for different animals, the market for DGS across all animal types would exceed the amount of DGS produced if the United States produces 56 billion litres of ethanol in 2015, as Congress has legislated. Assuming 100 percent market penetration and using the 2010 market price for DGS (US\$ 136 per tonne) (ERS/USDA, 2011), the total value of DGS produced would be US\$ 5.1 billion. Approximately 19.6 percent of the US production of DGS could be exported (Arora, Wu and Wang, 2010).

Table 3 compares maize ethanol co-product properties to those of conventional animal feeds. Experience with feeding DGS to livestock has revealed some benefits to replacing traditional feed with DGS. For example, beef cattle fed with DGS gain weight faster and can be brought to market sooner than conventionally-fed animals, which also affects ethanol life-cycle GHG emissions, as will be discussed later. In short-term studies, dairy cattle produced more milk when their diet included up to 30 percent co-products and energy and protein sources were also replaced at equal levels with maize grain and soybean meal. Long-term studies did not find a detrimental or beneficial effect to including DGS at this level. Because of its availability, price and effect on performance, consumption of DGS has expanded beyond the traditional feeding of ruminants (beef and dairy cattle) to

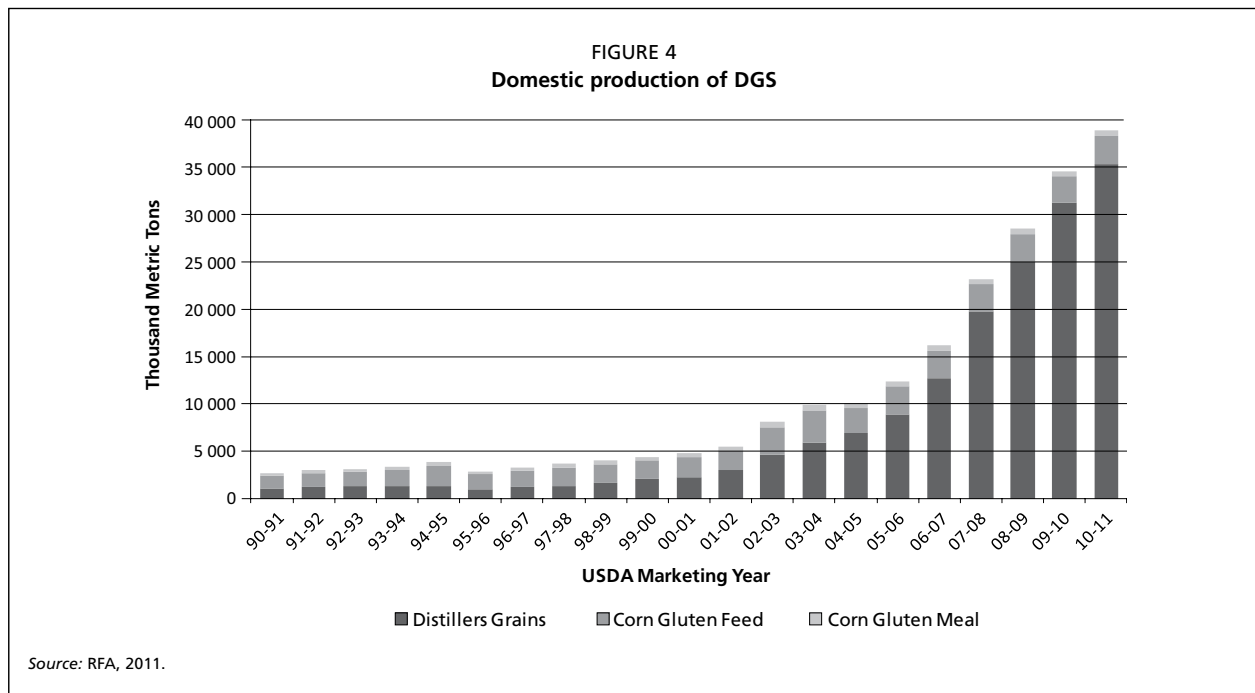


TABLE 2
United States distillers grain market size as DDGS on an as fed or sold basis (Arora, Wu and Wang, 2010)

Animal type	GCAU (10 ⁶ units)	Feed per GCAU ⁽¹⁾ (tonne/unit)	DGS inclusion (%)	Potential DGS usage at different market penetration levels (×10 ³ tonne)	
				50%	100%
Dairy	10	4.0 ⁽²⁾	20	4 020	8 041
Beef	20	2.2	20	4 236	8 472
			40	8 472	16 943
Swine	26	2.2	10	2 821	5 642
Poultry	31	2.2	10	3 278	6 556
Total market size ⁽³⁾				18 591	37 181

Notes: GCAU = grain consuming animal units. (1) Includes energy feeds (i.e. grains), oilseed meals, animal-protein feeds, grain-protein feeds and other by-product feeds. Excludes feeding of distillers grain because of data unavailability. No roughage (i.e. alfalfa hay) is included. (2) Corrected on the basis of the feed consumption report by Anderson *et al.*, 2006, assuming an annual feeding period of 300 days and a feed DM content of 85.5%. Represents the maize and soybean meal portion of the diet. Total feed per dairy-GCAU is 8.21 tonne/year. (3) 40% inclusion for beef.

TABLE 3
Properties of maize ethanol co-products and conventional animal feeds on a dry matter basis

Animal feed and other co-products	Dry matter (%)	Crude protein (%)	Fat (%)	Low heating values (MJ/kg)
Maize	85.5	8.3	3.9	18.7
Soybean meal	87.8	50.1	1.4	18.5
DDGS	89.2	30.8	11.2	20.2
WDGS	30.0	36.0	15.0	20.2 ⁽³⁾
d-DGS ⁽¹⁾	92.3	34.0	2.7	20.2 ⁽³⁾
HP-DDG ⁽²⁾	87.5	48.6	3.4	20.2 ⁽³⁾
Maize gluten feed	89.4	23.8	3.5	18.5
Maize germ	90.6	17.2	19.1	NA
Maize oil	—	—	—	17 ⁽⁴⁾

Notes: (1) De-oiled DGS. (2) High-protein dried distillers grain. (3) Assuming low heating values equal to DDGS on a DM basis. (4) Assuming low heating value equal to soybean oil.

monogastric animals (swine, poultry). DGS-fed monogastric animals have not exhibited superior performance.

The incorporation of technologies such as maize fractionation and maize oil extraction have enabled the

production of new, higher-value co-products that may enter the market and change the co-product mix. These co-products include high-protein dried distillers grain (HP-DDG), maize gluten feed, maize germ, de-oiled DGS

and maize oil. As these co-products displace significant amounts of conventional feed, LCA practitioners must monitor their market penetration and effect on the environmental impacts of ethanol.

Electricity generation with cellulosic ethanol

Cellulosic ethanol plants have the potential to produce electricity from the combustion of lignin. The National Renewable Energy Laboratory (NREL) calculates a net export of electricity of 0.61 kWh per litre of cellulosic ethanol produced from maize stover and switchgrass (Wang, Huo and Arora, 2011). Using the 2010 rate for industrial electricity in the United States (6.54 cents per kWh) (EIA/DOE, 2011), the electricity generated during the production of cellulosic ethanol could be worth US\$ 0.04 per litre, or US\$ 9 million annually for a 227 million L/year capacity cellulosic ethanol plant (Humbird *et al.*, 2011).

Electricity generation with sugar cane ethanol

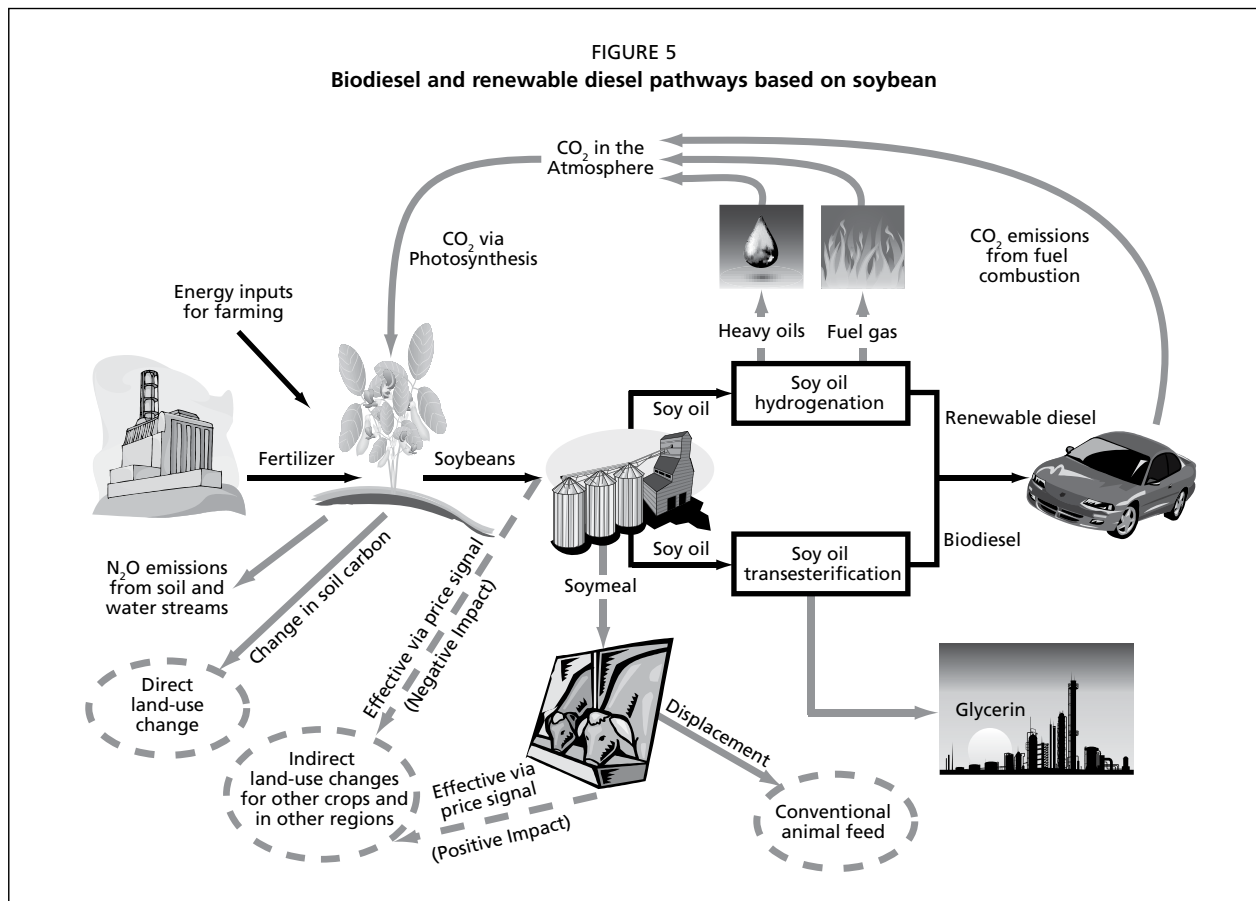
During the production of sugar cane ethanol in Brazil, 0.25 kWh/litre ethanol of surplus electricity is generated. In 2009, the rate for industrial electricity in Brazil was US\$ 0.159 per kWh (IEA, 2011). The electricity co-produced at a sugar cane ethanol plant could therefore be worth the same amount per litre in Brazil as in a cellulosic ethanol plant in the United States, or US\$ 0.04/L.

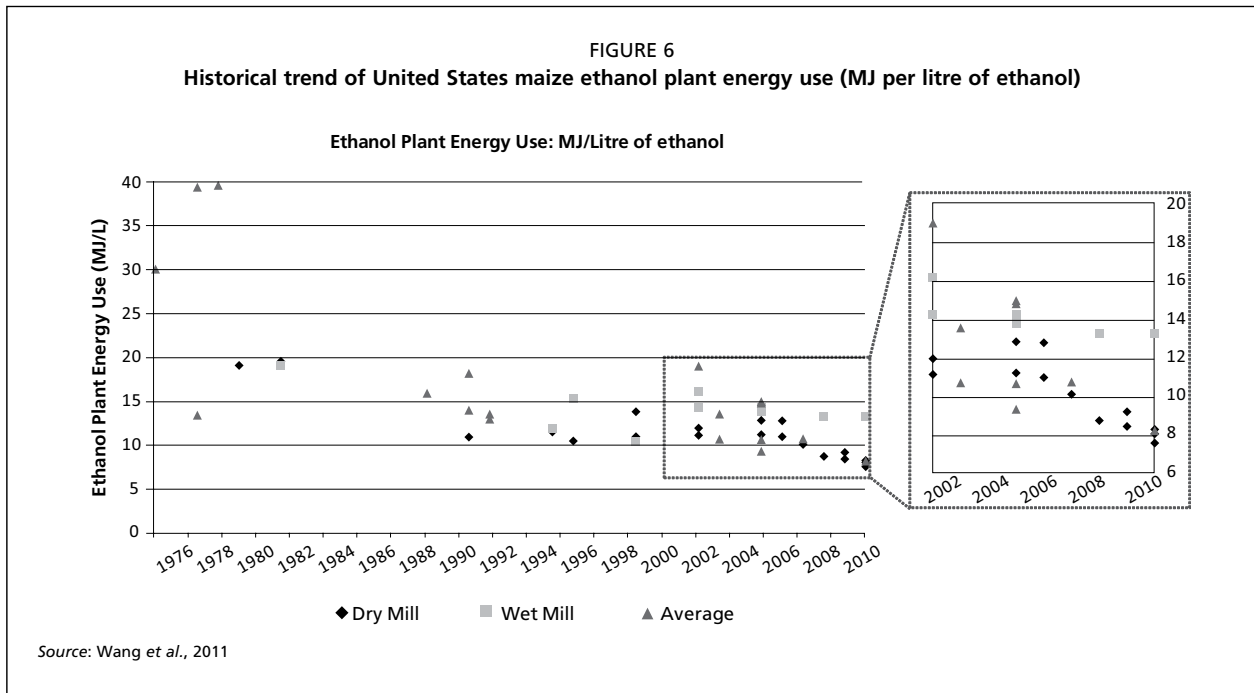
Sugar is also a by-product of sugar cane ethanol manufacturing. Market demand determines the split between sugar and ethanol produced at sugar cane ethanol plants.

Co-products with biodiesel

Biodiesel can be made from several feedstocks, depending on the region of production: soybeans (North America), rapeseed oil (Europe) and palm oil (Southeast Asia). Palm oil by-products with market potential include palm kernel oil, which can replace coconut oil; palm kernel extract (an animal feed); and glycerin (a feedstock for specialty chemicals) (Bauen *et al.*, 2010). Rapeseed oil by-products include rapeseed meal (an animal feed) and glycerin (Bauen *et al.*, 2010). Animal fat and waste cooking oils can also serve as biodiesel feedstocks, but co-products of animal origin are not permitted to enter the animal food chain.

Figure 5 shows the pathways and co-products for biodiesel and renewable diesel from soybeans. Renewable diesel, with properties very similar to petroleum diesel, is produced via hydrogenation or hydrotreating. In the biodiesel pathway, soybean meal, an animal feed, is an output of the soybean crushing operation, which also produces soybean oil. Subsequent transesterification of soybean oil produces biodiesel and glycerin.





LCA OF BIOFUELS

Improvements in energy efficiency of maize ethanol plants

Recently, Argonne National Laboratory updated the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET) model's simulation of ethanol life-cycle impacts (Wang et al., 2011). One enhancement in this analysis is Argonne's accounting for the shifts in the predominant ethanol production technology and enhancements in energy efficiency in ethanol production over the previous eight years. As discussed earlier, energy-efficient dry maize ethanol mills have become the dominant technology in maize ethanol production. Figure 6 illustrates the increasing energy efficiency of maize ethanol plants as a result of this trend. In this figure, average values are for dry- and wet-mill ethanol plants combined. For a single year, the values for dry mills, wet mills and combined dry and wet mills are sometimes from different studies. As a result, average values are sometimes outside the range of the individual values for dry mills and wet mills.

Reduction in fertilizer use and enhanced energy efficiency on maize farms

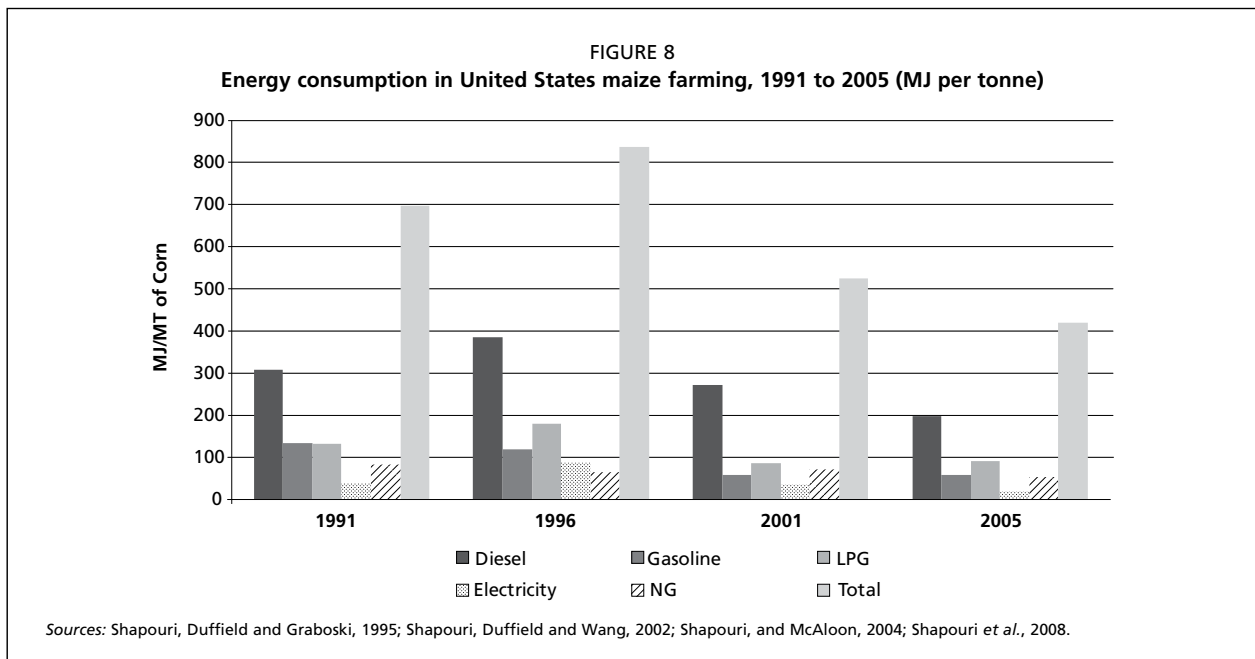
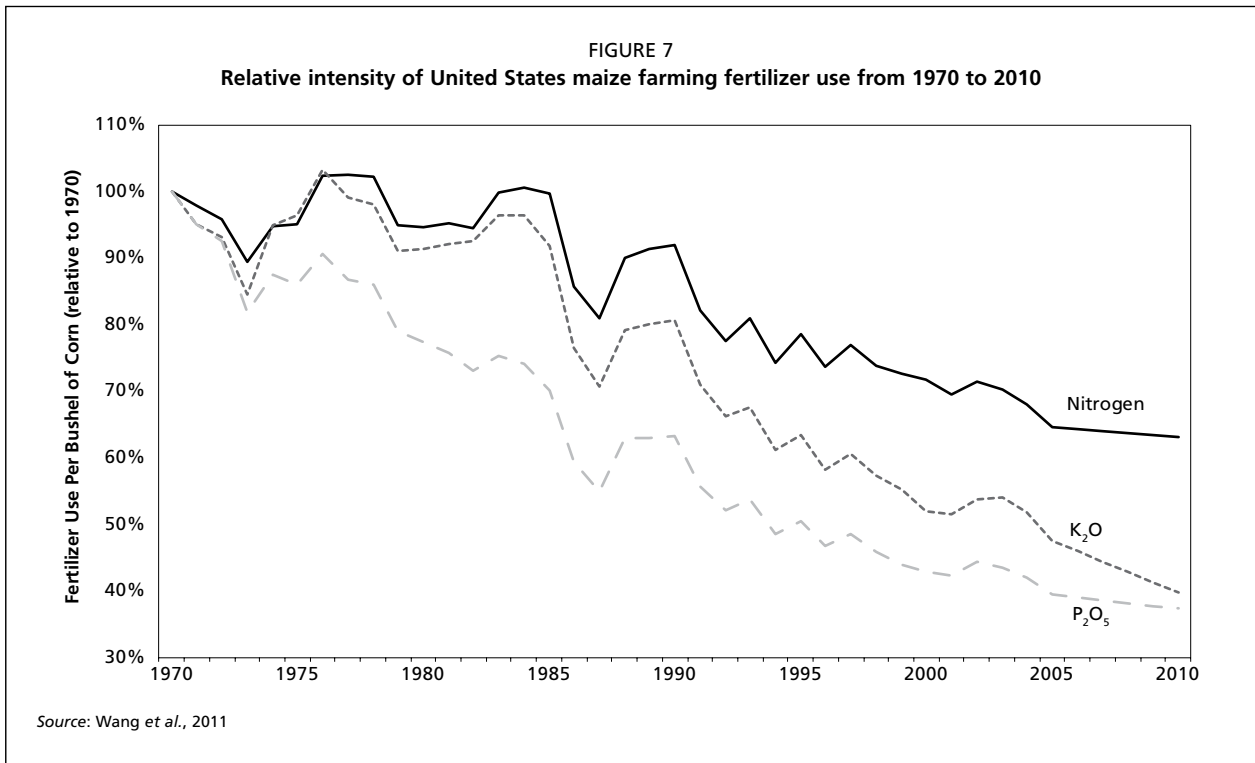
Agricultural practices have become more efficient in the past several decades, consuming less fuel and chemicals per litre of maize harvested. United States Department of Agriculture (USDA) data indicate that fertilizer intensity (kg fertilizer/litre of maize harvested) is decreasing. This decrease, depicted in Figure 7, reduces the environmental impact of ethanol production (Wang, Wu and Huo, 2007). For example, from 1975 to 2010, United States farms decreased nitrogen fertilizer application by 37 percent.

In addition to cutting upstream impacts from fertilizer manufacturing, less N fertilizer application reduces life-cycle GHG emissions in a second way because nitrogen fertilizer releases nitrous oxide (N₂O), a potent GHG, when it undergoes nitrification and denitrification on farm fields. Further reducing maize ethanol's life-cycle energy consumption, farming operations in the United States have become more energy efficient, consuming less diesel fuel, natural gas, propane and electricity as Figure 8 illustrates. Note that in 1996, wet weather in the US Midwest caused abnormally high energy use during harvest.

CO-PRODUCTS

Displacement effects of animal feed by-product

As discussed previously, animal feeds co-produced with maize ethanol can offset the need for conventional livestock feeds, including maize, soybean meal and urea, and in fact may offer improved animal performance when included in animal diets. Sales of ethanol co-produced animal feeds in the animal feed market reduce the energy and environmental impacts of producing conventional animal feeds. Incorporating the displacement of conventional feeds by DGS and other animal feed into the LCA of ethanol therefore provides GHG "credits" for the biofuel. These credits are considered direct credits that are simulated in GREET. Argonne has updated GREET parameters, called displacement ratios, that reflect the displacement of conventional animal feeds by the by-products of ethanol production (Arora, Wu and Wang, 2010). Table 4 contains these ratios at the feedlot level, where feedlot is defined as an animal feeding operation used in factory farming for finishing livestock (e.g. beef and dairy cattle, swine, turkeys and chickens).



Analysis using these updated ratios shows that DDGS and WDGS could displace 27.9 million tonne of maize, which is 20 percent of the maize projected to be required for ethanol production in 2015 according to the United States Environmental Protection Agency (EPA) renewable fuel standard. With a maize yield of 14 797 litres per hectare by 2015 in the United States, the DDGS and WDGS production levels equate to maize yields from 2.6 million hectare of maize fields. DGS could also displace significant amounts of soybean. The reduced demand for both maize and soybean

could produce LUC credits in computable general equilibrium (CGE) modelling for maize ethanol production.

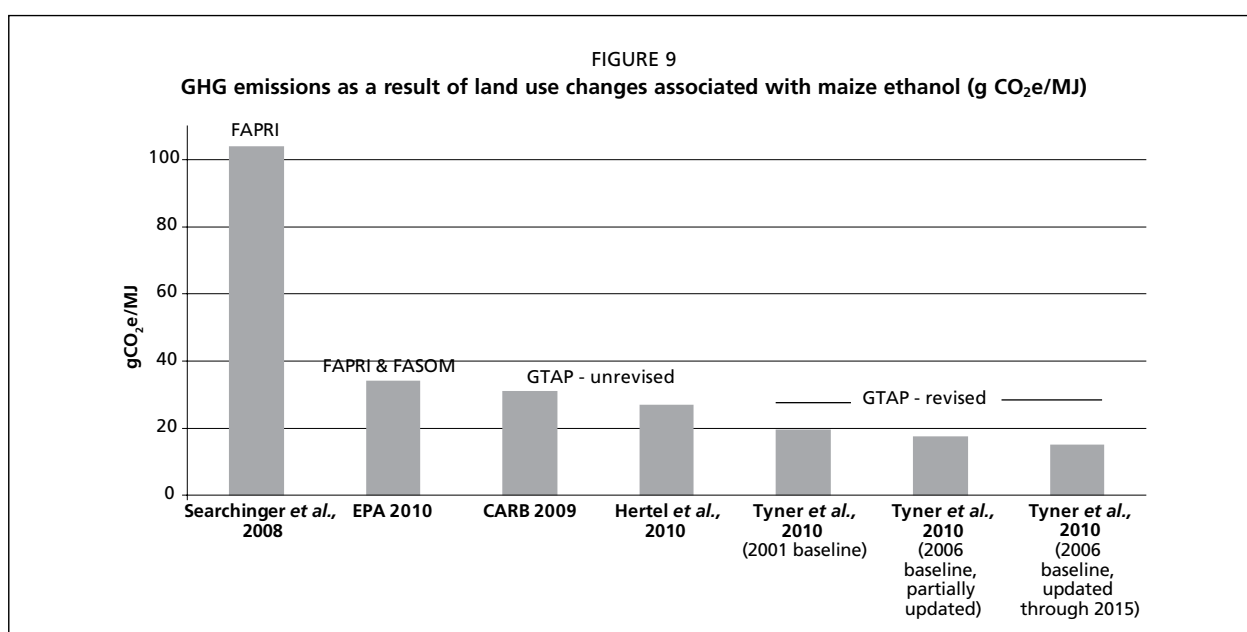
Land-use change

Since early 2008, several studies using economic models simulated direct and indirect LUC associated with the production of maize ethanol and other biofuels in the United States. At first, these economic models did not address several key issues, including crop yield increases in response to increased commodity price, future grain supply and

TABLE 4
Distillers grain with solubles displacement ratios at the feedlot level

Livestock	Displacement ratio between DGS and conventional feed (kg/kg of DGS on a DM basis)					
	Dry DGS			Wet DGS		
	Maize	Soybean Meal	Urea	Maize	Soybean Meal	Urea
Beef Cattle	1.203	0.000	0.068	1.276	0.000	0.037
Dairy Cattle	0.445	0.545	0.000	0.445	0.545	0.000
Swine	0.577	0.419	0.000			
Poultry	0.552	0.483	0.000			
Average	0.751	0.320	0.024			
				Dry and Wet DGS Combined		
				0.788	0.304	0.022

Source: Arora, Wu and Wang, 2010.



demand trends without ethanol production (the so-called reference case for global food supply and demand), and accurate modelling of the substitution of conventional animal feed with DGS. One model that permits calculation of LUC is Purdue University's Global Trade Analysis Project (GTAP) model, which has been developed primarily to evaluate global agricultural commodity trade linkages.

GTAP has recently been modified to model maize ethanol production. Figure 9 compares the revised GTAP model predictions of GHG emissions resulting from LUC with previous studies for maize ethanol programmes. The previous studies either used other models (Iowa State University's Food and Agricultural Policy Research Institute (FAPRI) model, Texas A&M's Forest and Agricultural Sector Optimization Model (FASOM)) or older GTAP versions.

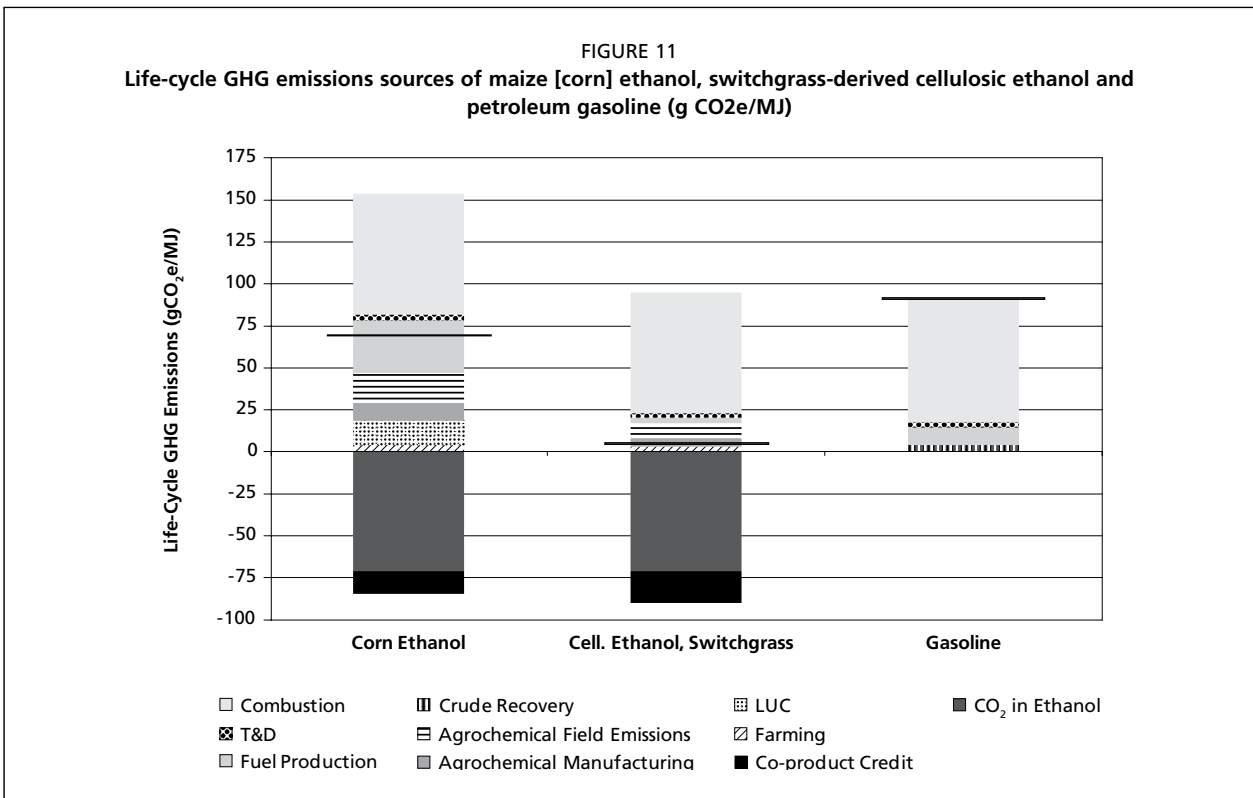
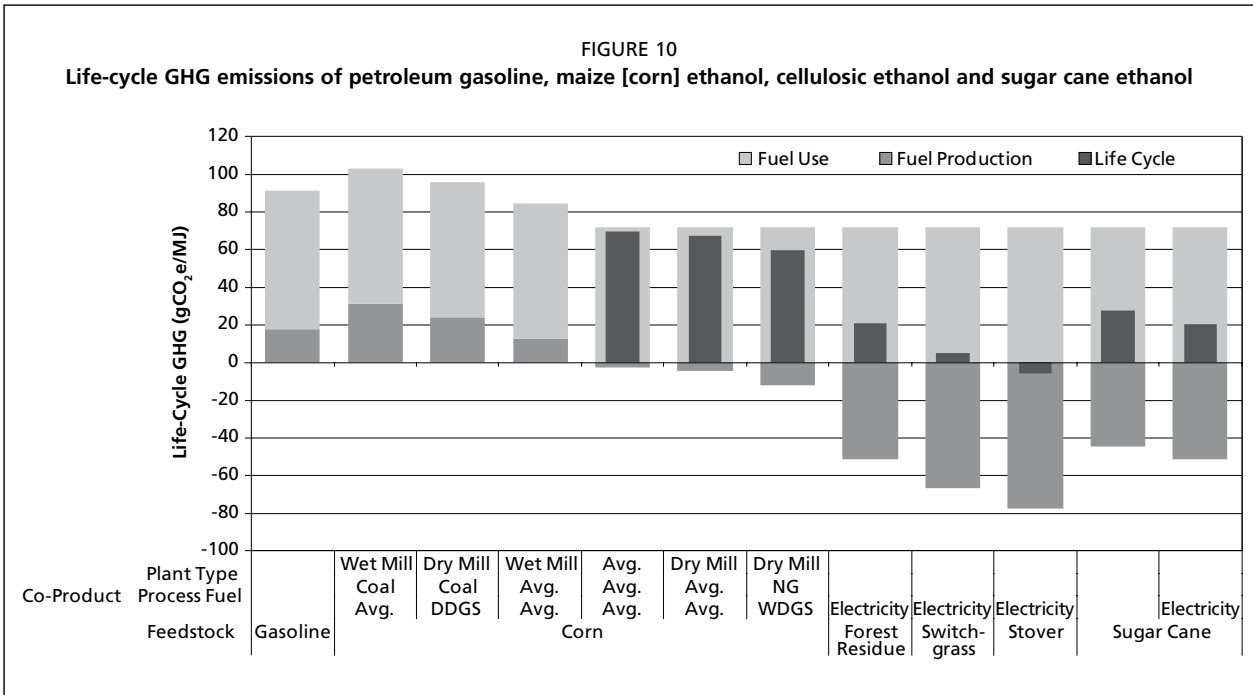
The most recent GTAP model version predicts significantly lower LUC and resulting GHG emissions than previous studies. For example, Searchinger et al. (2008), who used the FAPRI model, predicted GHG emissions (in gCO₂e/MJ of ethanol) that were 70 percent higher than calcula-

tions by California Air Resources Board (CARB, 2009) and Hertel et al. (2010), who used an earlier version of GTAP. Revisions to GTAP resulted in an estimate of GHG emissions 85 percent below that of Searchinger et al. (2008), as reflected in the results of Tyner et al. (2010).

Although the advances made in this work are significant, it should be noted that research is ongoing to further reduce uncertainties in incorporating LUC into economic models. In particular, uncertainties still exist in CGE models, including (1) modelling of DGS and other co-produced animal feeds; (2) global growth in food supply and demand; (3) global available land types and their potential grain production yields; and (4) below- and above-ground carbon stocks for different land cover types.

BIOFUEL LCA RESULTS

Figure 10 displays life-cycle carbon dioxide equivalent (CO₂e) emissions for petroleum gasoline, six types of maize ethanol, three types of cellulosic ethanol, and sugar cane ethanol. Maize ethanol produced at coal-powered plants



does not offer GHG reductions compared with gasoline. Maize ethanol produced at a dry-milling plant using an average fuel mix (i.e. a mix of natural gas and coal for the ethanol industry), however, does offer a GHG emissions reduction compared with gasoline.

Cellulosic ethanol, regardless of feedstock type, offers significant reductions in GHG emissions compared with gasoline, in part because cellulosic feedstock production

requires less energy and fertilizer inputs, and because of the benefits of generating electricity as a co-product. Similarly, sugar cane ethanol has lower life-cycle GHG emissions than gasoline. The benefit is more pronounced when considering electricity as a co-product.

Figure 11 presents GHG emission sources for three fuel types. It is clear that CO₂ uptake during crop growth and co-product benefits result in the reduced GHG emissions

advantages of bio-ethanol. Emissions during the use phase constitute the bulk of GHG emissions for both maize and cellulosic ethanol (based on switchgrass).

CO-PRODUCT ALLOCATION METHODOLOGIES AND IMPACTS ON LCA RESULTS

Biofuel co-products introduce complexity into biofuel LCA. Wang, Huo and Arora (2011) explore six methods of allocating energy and emissions impacts among biofuel co-products. Table 5 conveys the differences in methodology among these approaches and the advantages and drawbacks of each. Wang, Huo and Arora (2011) considered the pathways and the displaced products listed in Table 6. The life-cycle impacts of gasoline and diesel were included in the analysis as baseline fuels, and Wang and co-workers allocated impacts among petroleum refinery co-products by their energy contents. Not every pathway was analysed with all co-product allocation methods. For example, electricity is

massless, so evaluation of the switchgrass-to-ethanol pathway did not include a mass-based allocation analysis.

We present well-to-wheel (WTW) energy consumption and GHG emissions results for the biofuel pathways in this analysis. Figure 12 illustrates total energy use for the production of the two petroleum-fuel-based cases and for four biofuel pathways. The feedstock for biodiesel and renewable diesel is soybeans (Figure 5). All biofuel pathways consume more energy than the petroleum-based fuels because, when biomass feedstocks undergo conversion to biofuels, a larger amount of energy is lost. When considering fossil energy use in Figure 13, however, biofuels consume less fossil energy in their life cycles than do petroleum-based fuels. This is because while energy in a petroleum feedstock is fossil energy, energy in biomass is not fossil energy. Sometimes, energy debates on biofuels vs petroleum fuels centre on total energy. But the renewable energy in biofuels is not relevant to energy issues such as energy resource deple-

TABLE 5
Co-product allocation methodologies in LCA

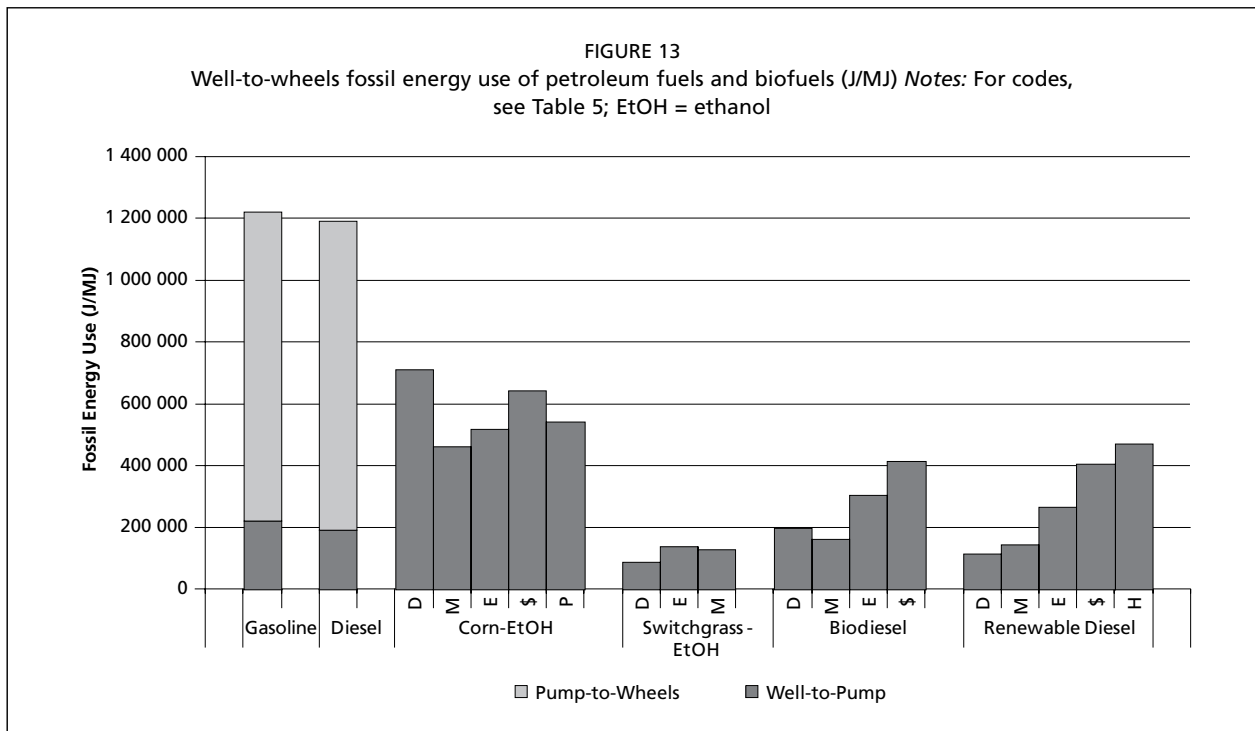
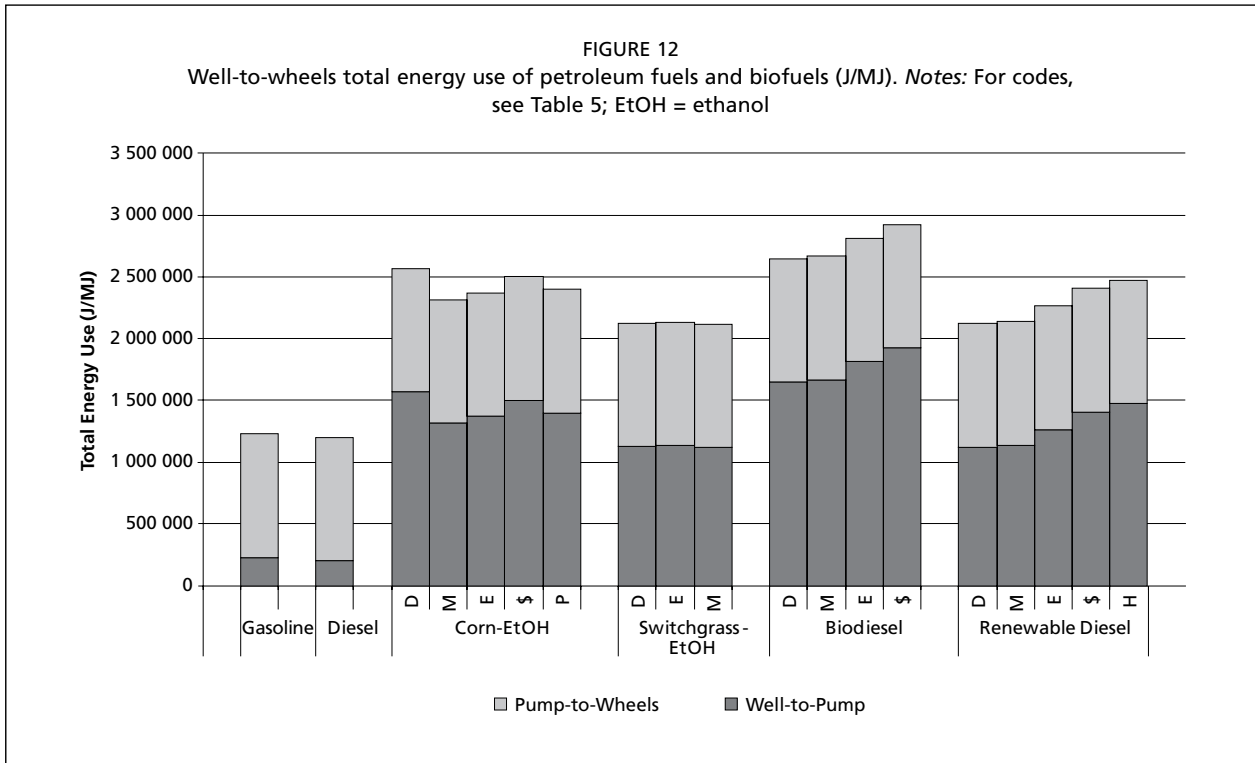
Code	Method	Description	Benefits	Drawbacks
D	Displacement	Determine life-cycle impacts of conventional products to be displaced by biofuel co-products. Account for the displacement of these impacts by the biofuel co-product in the biofuel LCA.	Tends to represent actual effects of creating multiple products.	Must conduct LCAs for conventional, displaced products. May produce distorted results when a significant amount of biofuel co-product is produced.
M	Mass-Based	Allocate energy use and emissions burdens by mass output shares.	Straightforward assumptions. Typically used in consumer product LCAs.	Problematic when co-products have different uses (e.g. electricity vs fertilizer) or no mass (electricity).
E	Energy-Based	Allocate energy use and emissions burdens by energy output shares.	Applicable when majority of products are energy (e.g. fuels or electricity).	Problematic when co-products have different uses (such as nutrition for animal feed).
\$	Market Value	Allocate energy use and emissions burdens by economic revenue shares of individual products.	Normalizes all products to a common basis regardless of use.	Subject to price fluctuations, including those in the future. Does not reflect physical processes consuming energy and generating emissions.
P	Process-Purpose	Estimate energy use and emissions burdens of individual processes in a facility, and assign to products.	Straightforward when unit processes produce a single product.	Many processes have multiple product outputs. Requires detailed energy and emission data at process level for a facility. Energy and emissions upstream of the facility still require use of other allocation methods.
H	Hybrid Allocation	Combine one or more of above methods.	Obtain more precise allocation of impacts.	Increases complexity of analysis. Creates inconsistency of allocation methods.

Notes: The code column refers to horizontal axis labels in Figures 12 to 14, q.v.

TABLE 6
Conventional products to be displaced by biofuel co-products for the displacement method

Biofuel pathway	Co-products	Displaced products	GHG Credit (g CO ₂ e/MJ biofuel)
Maize to ethanol	DGS	Maize, soybean meal, urea	12
Switchgrass to ethanol	Electricity	United States average electricity	19
Soybeans to biodiesel	Soybean meal	Soybeans	24
	Glycerin	Petroleum glycerin	0.46
Soybeans to renewable diesel	Soybean meal	Soybeans	34 ⁽¹⁾
	Fuel gas	Natural gas	0.38
	Heavy oil	Residual oil	0.26

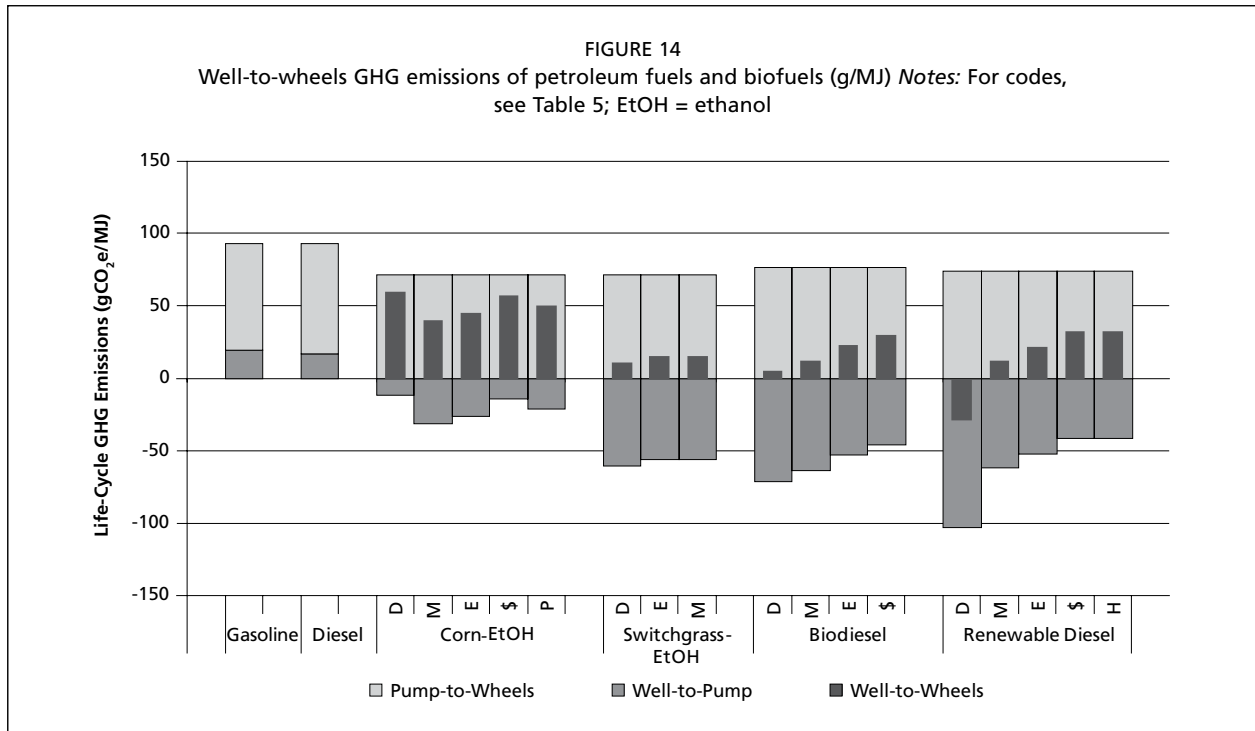
Notes: (1) The GHG credit in grams per MJ of fuel of soybean meal for renewable diesel is larger than that for biodiesel because fuel yield in MJ per unit of soybean is smaller for renewable diesel than for biodiesel. Thus, normalization of soybean credit to fuel production results in larger credits for renewable diesel than for biofuels.



tion and national energy security. More meaningful energy debates should focus on fossil energy or imported energy (such as petroleum energy in the United States context). Figure 14 depicts WTW GHG emissions for each of the pathways analysed. The horizontal axis labels in Figures 12 to 14 refer to the code column in Table 5.

In Figures 13 and 14, the effect of co-product allocation methodologies is strongest for biodiesel and renewable

diesel. This result stems from the high mass of a non-fuel product (soybean meal) that is produced as a by-product of soybean crushing and oil extraction in the pathways of these two fuels. In the biodiesel pathway, for example, crushing one litre of soybeans yields 0.14 kg and 0.53 (dry) kg of soy oil and soy meal, respectively. Four times more animal feed than oil is therefore produced, strongly affecting model outputs as the allocation methodology changes.



The biofuels community has not standardized its approach to allocation of environmental impacts among co-products in biofuel LCA. Based on the results of this analysis, however, Wang, Huo and Arora (2011) recommend that LCA practitioners apply the following convention:

- When an energy product is the main product, non-energy products can be called by-products. Other energy products can be called co-products.
- When energy and non-energy products are produced equally (according to mass, energy or revenue allocation), both products can be called co-products.

In the former case, the displacement method can be used for energy product LCA. In the latter case, the displacement method may not be appropriate for an LCA of the energy product. To cite an example considered herein, in a dry-mill maize ethanol plant, ethanol and DGS are produced at rates of 0.22 and 0.19 kg/L, respectively, and thus may be treated as co-products with the displacement allocation methodology.

Most importantly, LCA practitioners must maintain transparency when delivering biofuel LCA results, clearly explaining their allocation methodology in dealing with joint products and conducting sensitivity analyses of different allocation methods. For detailed discussions, see Wang, Huo and Arora (2011).

WATER CONSUMPTION ALLOCATION BETWEEN ETHANOL AND CO-PRODUCTS

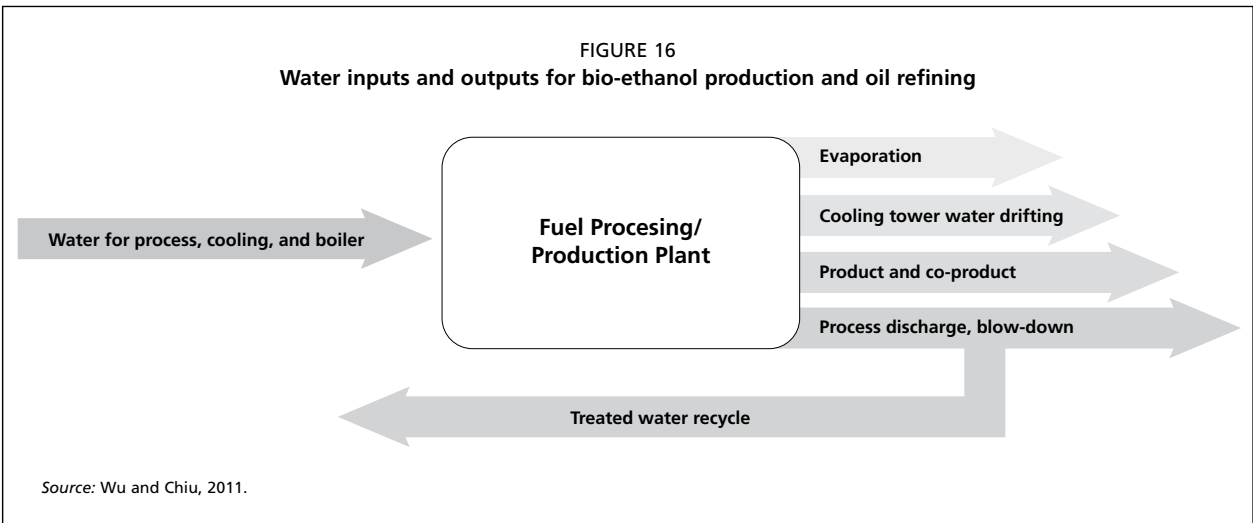
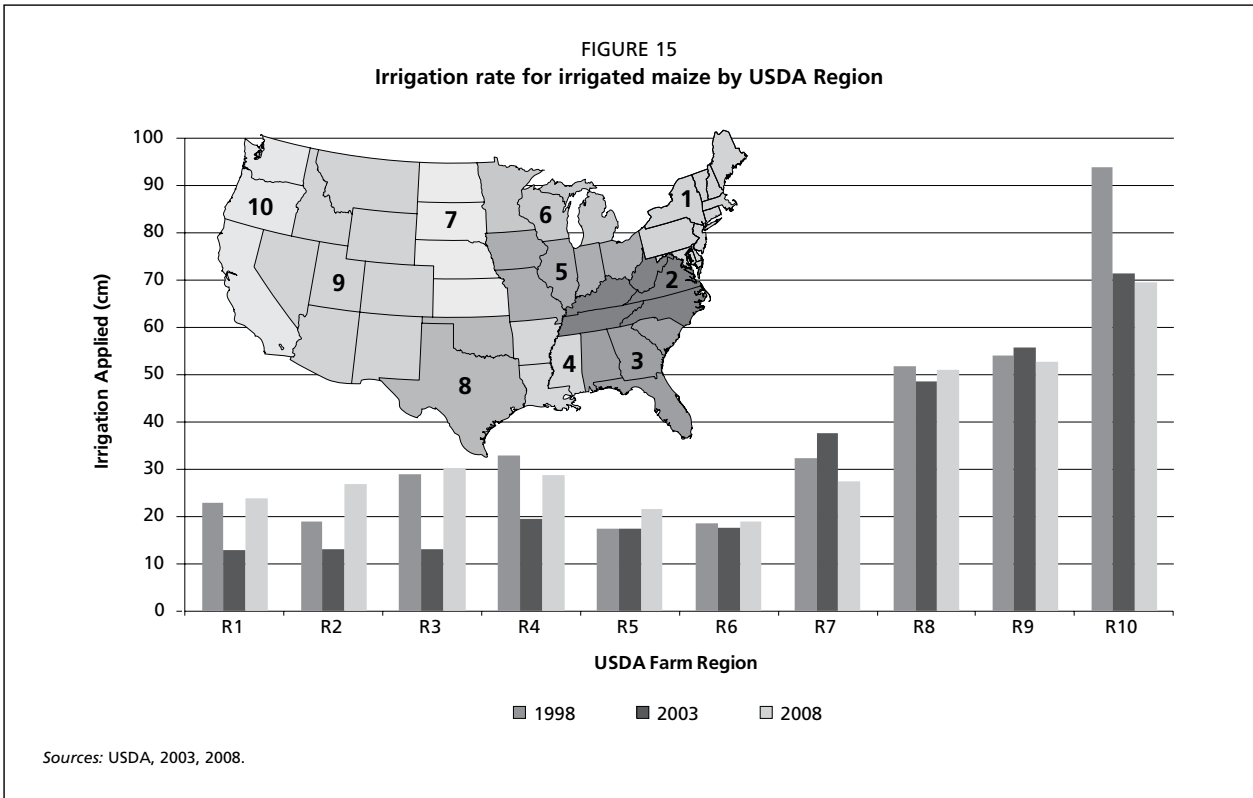
Co-products of starch and cellulosic ethanol production affect not only the allocation of energy consumption and GHG emissions, but of water consumption as well. A recent

study (Wu and Chiu, 2011) considered water consumption in the production of first- and second-generation biofuels, comparing water consumption in these fuel pathways to water consumption during the production of traditional gasoline from United States conventional crude, Saudi Arabian crude and Canadian oil sands. The authors also allocated water use among biofuels and their co-products (DDGS and electricity). The study included in its scope the feedstock production (growth and harvesting) and fuel production steps of the fuels’ lifecycles. It defined water consumption as the difference between freshwater input during both feedstock and fuel production and used water that is recycled or returned to water bodies. Irrigation water, process water and make-up water for fuel processing were considered water inputs. Consumed and recycled water were considered total water output. Finally, water loss includes evaporation, discharge, disposal and uptake into products.

The study included USDA Regions Five, Six and Seven (Figure 15) because the bulk of the nation’s biofuel feedstock and ethanol derives from these regions. The authors estimated consumptive irrigation water use for each region and determined ethanol plant water consumption use in the regions.

For cellulosic ethanol, the authors assumed the switchgrass feedstock to be grown without irrigation. Water consumption during fuel processing was determined from a NREL process model (Humbird *et al.*, 2011) because technology for converting lignocellulosic feedstocks to biofuels is not yet fully commercialized.

Ethanol manufacturing from maize uses water during grinding, liquefaction, fermentation, separation and drying.



The process also consumes water as a source of cooling and heating. Figure 16 depicts the division among water sinks during ethanol production, the most significant of which are the cooling tower and the dryer.

Water management practices in maize farming and ethanol production are favourably affecting ethanol's water consumption footprint. Although the feedstock production phase is generally the most water-intensive phase in a biofuels' life cycle, water management practices in the agricultural sector are improving such that the volume of irrigation water declined 27 percent over the last 20 years while maize yields consistently increased. Data from different sources (Figure 17) illustrate that water use during

ethanol production is also decreasing. Water stewardship practices in ethanol production include increasing process water recycling and steam integration. Plant siting at a location where the facility will not unduly affect groundwater levels is also critical to reducing the water impacts of ethanol production.

Table 7 outlines water consumption during growth, harvesting and conversion of maize to ethanol for USDA Regions Five, Six and Seven. In this table, consumptive water during crop production (irrigation) and conversion is divided between maize ethanol and its co-product, DDGS, based upon a heuristic that in dry-mill plants, one-third of the carbon in the maize kernel is converted to each

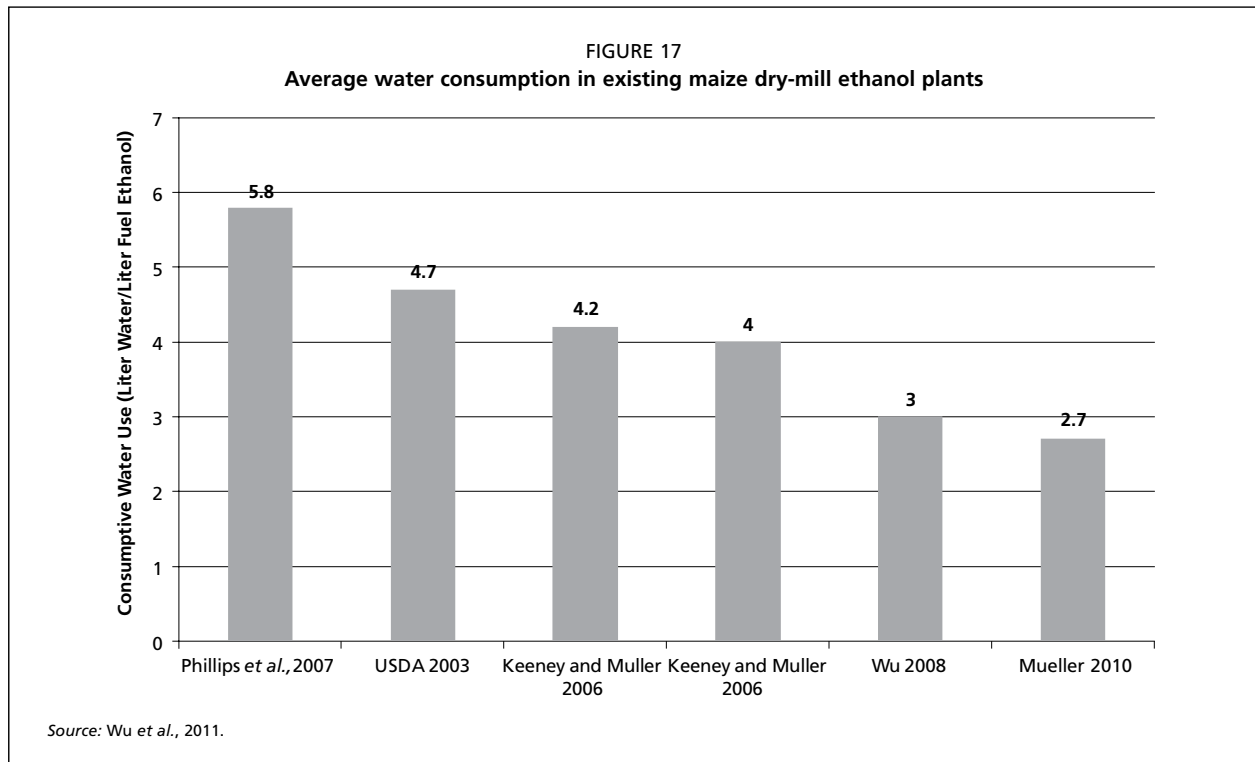


TABLE 7
Consumptive water use from maize farming to ethanol production in USDA Regions 5, 6 and 7 (litre water per litre denatured ethanol produced)

USDA Region	Region 5	Region 6	Region 7
Share of United States ethanol production capacity (%) ⁽¹⁾	50	15	23
Share of United States maize production (%) ⁽²⁾	50	16	23
Maize irrigation, groundwater ⁽³⁾	12	19	224
Maize irrigation, surface water ⁽³⁾	2	3	12
Ethanol production ⁽⁴⁾	3	3	3
Total (maize irrigation and ethanol production) without co-product allocation	17	25	239
Total water consumption with mass-based co-product allocation ⁽⁵⁾	11	17	160

Notes: (1) Based on 2008 ethanol production capacity in operation (RFA, 2011). (2) Based on 2008 maize production (USDA-NASS, 2011). (3) USDA, 2008. (4) Production-weighted average (Wu, 2008). (5) Mass-based and carbon displacement-based allocation according to the heuristic that one-third of biomass in maize kernel goes to ethanol, one-third goes to CO₂ and one-third goes to DDGS.

TABLE 8
Water consumption for cellulosic ethanol production

Process	Average water consumption (litre/litre biofuel)	Electricity export (kWh/litre biofuel)	Average water consumption after co-product allocation (litre/litre biofuel)
Biochemical (Humbird <i>et al.</i> , 2011)	5.4 ⁽¹⁾	0.47–0.55 ⁽³⁾	4.5–4.6
Gasification (Phillips <i>et al.</i> , 2007)	1.9 ⁽¹⁾	0	1.9
Pyrolysis (Jones <i>et al.</i> , 2009)	2.3 ⁽²⁾	0	2.3

Notes: (1) Cellulosic ethanol produced from switchgrass. (2) Forest residue as feedstocks. (3) Maize stover 1.77 kWh/gal and Switchgrass 2.07 kWh/gal, both from a 2000-dry-ton/day ethanol plant. Source: Wu and Chiu, 2011.

of ethanol, DDGS and CO₂ emissions during conversion. Irrigation water is allocated with the same ratio (one-third assigned to maize, one-third assigned to ethanol).

Table 8 compiles water consumption during production of cellulosic ethanol from maize stover, switchgrass, and forest residue (Wu and Chiu, 2011). No irrigation water is included because, in contrast to maize, these feedstocks may not require irrigation. The electricity generated during

cellulosic ethanol production can displace conventionally-produced electricity, the production of which consumes on average 1.6 litres per kWh in the United States (Wu and Chiu, 2011). As a result, 0.75 to 0.89 litres of water per litre of cellulosic ethanol are conserved when ethanol is produced via biochemical technology. The consumptive water use attributed to each litre of cellulosic ethanol produced is therefore 4.5 to 4.6 litres.

TABLE 9
Water consumption for ethanol and petroleum gasoline production

Fuel (feedstock)	Net water consumed	Major factors affecting water use
Maize ethanol ⁽¹⁾	11–160 L/L ethanol ⁽²⁾	Irrigation requirements vary regionally because of different climate and soil types
Cellulosic ethanol ⁽¹⁾	0.47–0.55 L/L ethanol	Production technology
Gasoline (USA conventional crude) ⁽³⁾	3.4–6.6 L/L gasoline	Age of oil well, production technology and degree of produced water recycle
Gasoline (Saudi conventional crude)	2.8–5.8 L/L gasoline	Age of oil well, production technology and degree of produced water recycle
Gasoline (Canadian oil sands) ⁽⁴⁾	2.6–6.2 L/L gasoline	Geological formation and production technology

Notes: (1) Water consumption allocated between co-products. (2) USDA regions 5, 6 and 7 combined for maize. Irrigation water included for maize. (3) Petroleum Administration for Defense Districts II, III and V combined. (4) Includes thermal recovery, upgrading, and refining.
Source: Wu and Chiu, 2011.

This study also developed estimates of water consumption during production of petroleum-based fuels. Table 9 compares the net water consumed among bio- and petroleum-based fuels. Maize ethanol has the most significant water footprint of the fuels examined, although if maize is produced with little irrigation, the water consumption during its production will be closer to the lower end of the range reported in Table 9. It is also important to note that data for oil production has more gaps than data for biofuel production, leading to greater uncertainties in the figures reported for petroleum-based fuels.

Irrigation can have a significant negative impact on water consumption in biofuel production. Growing cellulosic crops like switchgrass in their native habitat without irrigation is critical to maintaining a low level of water consumption. At the same time, water consumption during fuel production in general is decreasing as water management practices in farming, oil recovery and fuel production improve.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The evolution of biofuel production technology and co-product types and uses reveal knowledge gaps and future research needs in the LCA of biofuels and their co-products. Second- and third-generation biofuels are a special case in that broad-scale commercialization is still on the horizon. Actual plant energy efficiencies and co-product generation are still in the conceptual domain. As these technologies become mainstream, biofuel LCAs must be adapted to reflect real-world conditions. The agrochemical and energy intensity of feedstock growth and harvesting are also subject to uncertainty, given that many candidate feedstocks are under consideration and their production and harvest have yet to be optimized. Additionally, as co-product quantities and their end uses become clearer, LCAs must incorporate data that reflects their entry into the market and displacement of conventional products.

CONCLUSIONS

Biofuel production technology is rapidly advancing, especially in the case of second- and third-generation biofuels.

LCAs conducted with current life-cycle inventory data for biofuels, however, indicate that biofuels probably offer significant environmental and energy consumption benefits in comparison with their traditional, fossil-fuel-based counterparts. All biofuel production pathways jointly produce fuels and other products. Biofuel LCA results can be influenced significantly by the methodologies used to deal with co-products. On the one hand, failure to address biofuel co-products in LCAs generates incorrect LCA results for biofuels, since co-products are often a critical factor for pathway selection and economics of biofuels. On the other hand, the choice of certain co-product methodologies can heavily influence biofuel LCA results. While a co-product methodology may not be universally accepted for different biofuel pathways and for different analysis purposes, transparency of methodology selection and consequent LCA implications need to be clearly presented in any given biofuel analysis.

Water consumption in biofuel production is influenced heavily by biofuel feedstock production. Regional variation in biofuel water consumption is pronounced because of potential irrigation need for biomass growth. Avoidance of irrigation for feedstock growth can help reduce a biofuel's water footprint dramatically. Furthermore, maize ethanol plants have experienced significant water use reductions over the past 20 years. In the future, water use will probably be limited in second- and third-generation biofuel plants.

ACKNOWLEDGEMENTS

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Chapter 28

Utilization of co-products of the biofuel industry as livestock feeds – a synthesis

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INTRODUCTION

This book has explored the history of the biofuels industry, and the current state of knowledge with particular reference to co-products and their uses. Furthermore, the perceived gaps in knowledge that could possibly increase the efficiency of use of what is available have been addressed, and predictions made as to how the industry is likely to develop over the next ten to twenty years. The information is summarized in seven sections: (1) Introduction; (2) Background; (3) Ethanol production, co-products and their nutritive value; (4) Biodiesel feedstocks, co-products and their nutritive value; (5) Micro-algae; (6) Economics and socio-economic aspects; and (7) Summary of perceived knowledge gaps and future research needs. The sources of information presented in this book are used as an indication of the major centres of activity for the industry, although there is little information on China, with its approximately 1200 beverage alcohol plants and an ethanol production industry contributing significant amounts of distillers grain to the livestock feed industry (Table 1). This table also presents the primary biofuel product (ethanol or biodiesel) and their co-products and the animal species to which they are likely to be fed.

Geographically, current interest is centred in North and Central America (13 contributions), Europe (5), India (5) and the rest of the world (5). In this book, 19 papers focus on the co-products of ethanol production and 16 on those with those resulting from biodiesel production, with several contributions dealing with more than one co-product. Ruminant nutrition (cattle, buffalo and small ruminants) was a subject in 19 papers, non-ruminants (pigs and poultry) in 14, and fish in 4. The original interest in North America and Europe was in first-generation feedstocks in the form of cereal-based ethanol production and soya- or rapeseed-based production of biodiesel. This generated a continually expanding range of co-products for livestock: ruminants, non-ruminants, poultry and in aquaculture. However, there is now increasing interest in the development of second-generation feedstocks such as cellulosic sources, trees, shrubs and arable crop residues. Ethanol from these materials is produced from cellulose rather than the sugar and starch in first-generation feedstocks. Micro-algae are also of considerable interest and capable of producing co-products, some of which need detoxification before feeding to livestock.

TABLE 1
Country of origin and major topics covered in each chapter of this publication*

Topic	Ethanol	Biodiesel/ Bio-oil	Micro-algae	Ruminants	Non-ruminants	Aquaculture	Humans	Environment issues
Country								
Australia	25	25		25	25			
Brazil	15	14		15	14			14
Canada	26			26	26			
France	9				9			
Germany	7, 11	11, 21		7, 11	11	21		
India	12, 16, 20, 24	20, 22	24	12, 16, 20 22, 24	12, 22, 24	24	24	12, 16, 20, 22, 24
Israel		18		18				18
Malaysia		13		13	13	13		13
Switzerland		19		19	19			
UK	2							2
USA	1, 3, 4, 5, 6, 10, 23, 27	1, 3, 8, 10, 17, 23, 27		1, 5, 6, 8, 27	1, 10, 14, 27	23		1,4, 5, 6, 8,17, 23, 27
TOTALS	19	16	1	19	14	4	1	17

* Numbers in the body of the table denote chapter number in book (see Appendix 1).

Scene setter

Distillers grain co-products have been fed to livestock for more than a century. Currently production far exceeds that of glycerol. Emerging new markets include aquaculture, horses, companion animals and human foods, but these market applications need research support.

Shurson, Tilstra and Kerr

Economics

Today, grain, sugar and oilseeds are the major agricultural commodities for biofuels. This could lead to modest increases in livestock and poultry production costs, but substitution of co-products for traditional feedstuffs could mitigate these increases.

Cooper and Weber

Jatropha

Jatropha is a drought tolerant shrub or tree growing wild on degraded land in Central and Southern America, Africa and large tracts of Asia. Seeds are rich in oil and the kernel meal rich in crude protein. After treatment, both *Jatropha curcas* and *J. platyphylla* residues can replace over half the protein in diets of fish. Non-toxic *Jatropha* species could be valuable feed resources for the future.

Makkar, Kumar and Becker

Small-scale approaches

In India, decentralized crushing and syrup-making units are based on sweet sorghum, providing food, feed, fodder and fuel. The system encompasses small-scale farmers and complements the centralized approach applicable to larger farmers.

Rao et al.

Micro-algae

Production of energy through photosynthetic organisms, like micro-algae, harnessing solar energy might be a viable solution avoiding competition for land, or land-based resources such as fresh water. Residues have potential as chemicals, foods and feeds, but prudent energy audits are needed.

Ravishankar et al.

Oil Palm

Oil palm residues come from the field and processing mills. Their diversity allows complete diets from oil palm products for various livestock species, including in aquaculture. Malaysia needs to increase ruminant production and there is huge potential to integrate this with the oil palm industry.

Wan Zahari, Alimon and Wong

BACKGROUND

Distillers grain (DG), originally a by-product of the alcoholic drink and beverages production industry, has been fed to livestock for many years, initially to pigs and dairy cows. The upsurge in the use of DG was itself a by-product of the search for transport fuel other than from fossil fuels, which in recent years has been supported by a large increase in research funding into the use of co-products (Shurson, Tilstra and Kerr, 3). Currently, co-products are an important feed resource in over 50 countries, for ruminants, non-ruminants and aquaculture (Table 1). The co-products are the residues after extraction of the biofuel, whether ethanol or biodiesel. Biofuels contribute to the twin objectives of increasing fuel security and reducing emissions of greenhouse gases (GHG) (Cooper and Weber, 1). In Europe, the use of fossil fuels for transport contributes an estimated 18 percent of all GHG emissions, a figure that has the potential to be reduced by half through increased efficiencies in use and a projected four-fold increase in the production of biofuels by 2020 (Hippenstiel *et al.*, 11). If achieved, this rate of increase would result in 6 percent of global fuel needs coming from biofuels. As the majority of currently used feedstocks to produce biofuels are crops grown on existing agricultural land, the requirements for food, feed and fuel must be balanced so that the quest for biofuels does

not result in an inflationary rise in the cost, or shortage, of food or feed. This raises the question of second-generation feedstocks from cellulosic sources, the use of crop residues and stubbles and woody material grown on marginal land with a minimum of resources, including irrigation (Braid, 25). This approach raises the potential for promoting little-used and non-conventional feeds, such as oil-palm products (Wan Zahari, Alimon and Wong, 13; de Albuquerque *et al.*, 14), micro-algae (Ravishanker *et al.*, 24), *Jatropha* species (Makkar, Kumar and Becker, 21), lipid co-products (Wiesman, Segman and Yarmolinsky, 18), *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes (Dutta, Panda and Kamra, 22), sugar cane bagasse (Anandan and Sampath, 16) and *Camelina sativa* (Cherian, 17). Some may require detoxifying to produce safe livestock feed (Anandan and Sampath, 16; Dutta, Panda and Kamra, 22; Abbeddou and Makkar, 19; Makkar, Kumar and Becker, 21).

ETHANOL**Cereal feedstocks**

The European Union has set targets both for the inclusion of non-fossil fuels for road transport and for reduction of GHG emissions, embodied in the Renewable Energy Directive (RED) and the Fuel Quality Directive (FQD) (Lywood and Pinkney, 2). The USA introduced the

Renewable Fuel Standard (RFS) in 2005, which led to the Energy Independence and Security Act of 2007 that sets targets for blending of biofuels with fossil fuels through to 2022 (Cooper and Weber, 1). In Europe, GHG emissions have been reduced in all areas of activity except public energy production (small increase) and road transport (large increase) (Lywood and Pinkney, 2). By 2020, 10 percent of fuels used for surface transport should come from non-fossil sources, and GHG emissions should be reduced by 60 percent with a 6 percent reduction in carbon emissions compared with 100 percent fossil fuel usage (Shurson, Tilstra and Kerr, 3). The present generation of petrol engines can tolerate 10 percent ethanol in the fuel mix. However, diesel engines currently have a maximum tolerance of 7 percent, which, because of the age of the global transport pool, points to a need for rapid improvement in tolerance levels if the 2020 target is to be met.

The feedstocks from which ethanol is produced largely reflect the agriculture area. In the United States of America (USA), maize [corn] (Table 2) is the dominant source (Shurson, Tilstra and Kerr, 3). The USA has also built an export trade in dried distillers grain with added solubles (DDGS), initially to Canada for beef production, but now expanded to a wider market, with an emphasis on pig and poultry production (Shurson, Tilstra and Kerr, 3). The development of wet processing encouraged the siting of plants near beef feedlots to minimize costs of drying and transporting distillers grain. This also encouraged many beef producers to become croppers. However, in the Southern Great Plains of the USA, sorghum is an important feedstock, thus giving rise to considerable quantities of co-products (Galyean *et al.*, 4). In Europe (Hippenstiel *et al.*, 11; Noblet, Cozannet and Skiba, 9) and parts of Canada (Christensen *et al.*, 26) the major cereal contributing to the industry is wheat. Christensen *et al.* (26) have traced the development of the ethanol industry in Western Canada from the beginning, when DDGS was imported from the USA, to the present time. Although imports are still important, locally grown Canadian wheat is now contributing significantly to the distillers grain market.

Other cereals – triticale, barley and rye – can be used, either alone or in combination, but are not significant ethanol feedstocks compared with maize and wheat. The European targets for biofuel production will be met mainly through increased crop yields and continuing cropping of arable land that should have been released from use. The increased availability and use of co-products in livestock feed would partially replace a mixture of EU cereals and imported soyabean meal (Lywood and Pinkney, 2).

Sugar cane and other non-cereal feedstocks

Sugar cane (Table 2) is also a major feedstock for ethanol production. Patino *et al.* (15) estimated that, at the present

time, on a global scale, 90 percent of ethanol output is accounted for by maize and sugar cane. In tropical regions of Central and Southern America and Asia, sugar cane is one of the most important crops, and its value as a feedstock is recognized (Anandan and Sampath, 16). Between 1990 and 2009, production of sugar cane in Asia increased by 53 percent, while the land area devoted to its growing only increased by 34 percent, suggesting an improvement in cultivation and harvesting techniques. Two of the major prerequisites for a successful sugar cane industry are a warm environment and water. Cooper and Webber (1) stress the importance of sugar cane as a feedstock in tropical countries with a high rainfall, quoting the example of Brazil, where 98 percent of ethanol production comes from this source. The same authors estimated that in 2010, 93 percent of ethanol production took place in the USA, Brazil and Europe. Other feedstocks listed by Rao *et al.* (12) and Cooper and Webber (1) included tropical sugar beet, sweet potato, cassava and sweet sorghum. In contrast sweet sorghum is favoured by Rao *et al.* (12) because of its tolerance to a wide range of harsh conditions and the number of options for its use, including human food, forage and biofuel production.

Sweet or forage sorghum requires 25 percent of the water needed by sugar cane, and substantially fewer growing days (Rao *et al.*, 12). In the decentralized process, developed for small-scale farmers to operate at a village level, they describe how crushing of the sorghum plant to obtain the juice and then boiling to concentrate this are key actions, with the two principle co-products, or residues, being the bagasse and grain. Grain free from mould is used for human consumption. The juice can then go forward for ethanol extraction or be retained in the village for fermentation to give a mash containing 6–10 percent ethanol. Currently, the system operates for the rainy season crop only because the needs of farmers for food and livestock feed are more easily met from crops grown in drier weather.

New and unconventional feedstocks

The feedstocks discussed above are regarded as first-generation crops. One of their limitations is that they could be seen as being in conflict with what are regarded as the prime objectives of cropping land, namely the provision of food and livestock feed. To combat this, and also to utilize materials traditionally regarded as unusable, there is increasing interest in what have become known as second-generation feedstocks (Shurson, Tilstra and Kerr, 3). These contain large amounts of cellulose (Table 2) and include crop residues (straws and stubble), shrubs and trees. An example is short rotation eucalypts grown for coppicing, which currently account for less than 5 percent of cleared land in Australia (Braid, 25). Trees can provide shade and shelter to the extent that lamb survival, especially those from twin

births, is improved by their presence. The use of trees in alley farming is another possibility. Use of stubble requires moving the residue from the field and needs examining in the whole farm context because of disturbance to the nutrient cycle on arable land that might traditionally have been grazed. The total fuel ethanol capacity in Australia is estimated at 330 million litres per year (Braid, 25). Wang and Dunn (27) found that growing feedstocks without irrigation greatly reduced the water footprint of biofuels. They also reported the contribution of cellulosic by-products as a source of electricity. Unconventional raw materials should also be considered, the desirable characteristics being good levels of sugar or starch, good agronomic production, tolerance of low soil fertility, pest and disease resistance, and the ability to withstand environmental stress (Patino *et al.*, 15). Among the crops suggested are sweet sorghum, sweet potato and cassava. Development of technology to produce biofuels and manage the co-products for livestock feed by farmers with little education and financial resources are the aims of the Rural Social Biorefineries (RUSBI) programme (Patino *et al.*, 15).

Several lipid co-products are produced during the biofuel production from a range of feedstock sources, and are likely to increase with greater sophistication of fractionation techniques during processing. They can provide both supplements and feeds for ruminants and have a role in meeting guidelines for human health, which call for a reduction in the saturated fatty acid content of the diet, with the essential and non-essential fatty acids coming from unsaturated sources (Wiesman, Segman and Yarmolinsky, 18). The inclusion of Megalac-protected fat or pre-formed calcium soaps in the diet, which avoid rumen degradation, do not adversely affect fibre digestion and also decrease the

amount of stearic acid deposited in body tissues (Wiesman, Segman and Yarmolinsky, 18). Reductions in saturated fat in milk and increased omega-3 fatty acids in meat have been observed. However, for animal and public health security, the authors recommend adequate risk assessment of new products.

Ethanol production

Ethanol can be obtained from any cereal grain that stores starch in its endosperm, the choice between the major cereals being governed by environmental factors (Kalscheur *et al.*, 7). Distillers grain were originally obtained as by-products of distilling and brewing industries, the authors quoting the value attached to the slops recovered from George Washington's distillery in the late 1700s and fed to pigs and cattle. With the development of the biofuels industry during the 1970s and 1980s a large number of wet milling plants were built in the USA (Shurson, Tilstra and Kerr, 3), and at the same time dry grind facilities were also developed. The dry grind plants were small and for various reasons initially, many went out of business, although currently they are now dominant. Expansion of the industry has been helped in some States by legislation specifying inclusion levels of ethanol in motor fuel and by direct subsidies (Shurson, Tilstra and Kerr, 3). Shurson *et al.* (10) in their chapter describe diagrammatically dry grind (Figure 1 in Chapter 10) and wet milling (Figure 2 in Chapter 10) fuel ethanol production, and list the co-products from each process (see also Erickson, Klopfenstein and Watson, 5). They also confirm that these plants can handle any grain source or combinations of grain. A result of these activities has been the introduction of several co-products as livestock feed. This trend is on-going, increasing both in complexity and in the number of livestock

TABLE 2
Feedstocks used for ethanol production, their co-products and major areas of utilization

Feedstock	Co-product	Co-product utilization
Maize (3, 4, 7, 10, 23, 26); Sorghum (4); Wheat (2, 9, 11, 26); Triticale (5); Rye, barley (26); Co-products from biodiesel production (10)	DG; WDG or DDG; with added S (DDGS); HP additive (3, 4, 5, 6, 7, 10, 11, 23, 26); Maize oil, maize-condensed distillers soluble, maize gluten feed (5); Maize steep water, whole stillage (26); Ethanol co-products (6).	DG, DDGS, WDG, DDGS-HP for beef cattle (3, 4, 5, 11, 26) DG for dairy cattle (5, 7, 11) DG for pigs (3, 9, 10, 11) DG for poultry (3, 9, 11) DDGS as grazing supplements for ruminants (25, 26) Maize oil, maize solubles, maize gluten feed (5) DDGS for aquaculture (23) Manure (4)
Sugar cane (15, 16); Sugar beet, sweet sorghum (12); Cassava (15)	Vinasse (multi-nutritional blocks/pellets/meal) (16). Fertilizer, bagasse, paper and board (16); Sugar cane tops, bagasse and molasses (15); Sugar beet tops, fermentable palatable waste; Grain/bagasse/foam/froth/steam/vinasse/syrup from 'sugary' stems (12); Cassava residue plus sludge from cane processing (15).	Sugar cane co-products, including use of effluents [simple technology essential] for cattle (15); Food and commercial uses/cattle and other ruminants/poultry and composting (12); Some bagasse direct to forage traders (12); Sugar cane bagasse with supplements and cassava residue for cattle and other ruminants (15, 16), Electricity generation (27); Biogas (15).
Micro-algae (24)	Algae residues left after extraction of oil and/or materials used for ethanol production (24)	Fuel, food, feed and chemicals (24)

Notes: Numbers in the body of the table denote chapter numbers in this book. For a list, see Appendix 1. DG = distillers grain; WDG = wet distillers grain; DDG = dried distillers grain; S = DDG with added solubles (i.e. DDGS); HP = with high protein additive.

species that are benefiting. The increased efficiency of front-end fractionation for ethanol production and the potential for increasing the range of co-products available are discussed (Shurson, Tilstra and Kerr, 3; Cooper and Weber, 25). Rear-end oil extraction is also possible with dry milling, the oil being available as maize oil for livestock or to contribute with other vegetable oils in biodiesel production (Shurson, Tilstra and Kerr, 3; Cooper and Weber, 1). Within the USA, Shurson, Tilstra and Kerr (3) do not foresee an immediate increase in the number of wet milling plants, but possibly a small increase in the number of dry grind plants.

Comparisons of wet and dry processing of DGS (distillers grain with solubles) have been inconclusive, but there are practical considerations, such as handling and storage costs, with the wet product having a relatively short shelf life of up to seven days (dependent on ambient temperature, unless anaerobic storage is available, such as bunkers, pits or silage bags); it is advisable to avoid vertical tower storage because of problems of compaction and flow, creating problems with hygiene and auger-based mixing and delivery systems (Kalscheur *et al.*, 7). However, in 2007, dry mills sold a third of their distillers grain with solubles wet, rather than dry (Wang and Dunn, 27). For usage close to the plant, wet co-products avoid the costs of drying. In some situations, heat for drying can be supplied by burning process residues. Sorghum bagasse (Rao *et al.*, 12), biogas from sugar cane vinasse (Patino *et al.*, 15), and from sugar cane bagasse (Anandan and Sampath, 16) are suggested as sources of fuel.

The current extraction process for ethanol necessitates the use of sulphuric acid, thus increasing the level of sulphur in DG above that in the original grain and creating a potential cause of excess ruminal hydrogen sulphide (Galyean *et al.*, 4; Schoonmaker and Beitz, 6). Sugar and starch fermentation to produce ethanol is described by Lywood and Pinkney (2), as is the hydrolysis of lingo-cellulose feeds, which is then followed by fermentation to give ethanol. Both processes show high levels of efficiency. Appropriate processing plants for cellulosic materials are being developed (Shurson, Tilstra and Kerr, 3).

Co-products resulting from ethanol production

Notwithstanding the debate regarding the use of land for fuel rather than feed, the production of ethanol as a biofuel is the largest growth sector in the USA, where there are now 200 plants producing 35 million tonne of co-products annually (Shurson, Tilstra and Kerr, 3). Mjoun and Rosentrater (23) estimated ethanol production at 51 billion litres in 2010, over three times as much as in 2005, with 32.9 million tonne of distillers grain being produced, of which 2.7 percent came from the beverage industry and the remainder from maize-based ethanol production. Currently, in the USA, the beef industry uses 66 percent

of the available DDGS, the dairy industry 14 percent, pigs 8 percent and poultry 12 percent, with little evidence of meaningful amounts being used in aquaculture (Mjoun and Rosentrater, 23). However, the authors note substantial increases in the amount of fish coming from aquaculture during the last decade, coupled with the high price of the traditional protein sources, fishmeal and soybean meal, and the comparatively low price of DDGS.

In Western Canada, the current annual demand for DDGS is estimated at 1.4 million tonne, but the local industry, based on wheat, can only produce around half a million tonne, the shortfall being met from the USA (Christensen *et al.*, 26). In Europe, the dominant feedstock for ethanol production is also wheat, although some other cereals, especially barley, may be added to the mix (Noblet, Cozannet and Skiba, 9). Rye is also used as a feedstock, but is restricted to colder areas (Kalscheur *et al.*, 7). The products of fermentation are expected to be 93 percent ethanol, 3 percent yeast and 4 percent glycerol (Noblet, Cozannet and Skiba, 9). Distillers grain from various feedstocks can be mixed with minimal changes in animal performance responses, although Kalscheur *et al.* (7) rate barley as the least productive cereal feedstock, because of the relatively high fibre and low starch content of the grain.

Shurson, Tilstra and Kerr (3) address food safety and note possible causes of contamination resulting from the process, including excess sulphur, mycotoxins (in adverse climatic conditions, especially excessive heat or moisture), harmful bacteria, and transfer of antibiotics to animal and human tissue. The formation of H₂S and the dangers it represents to both ruminants and non-ruminants are described by Schoonmaker and Beitz (6), who consider that it rivals cyanide in its toxicity. Endogenous H₂S is produced by the catabolism of S-containing amino acids, cysteine being important in this process, or by sulphate-reducing bacteria present in the digestive tract. But it is important to note that added sulphur used in the fermentation process is the primary culprit for ruminally produced hydrogen sulphide, not the dietary S-containing amino acids. At low levels, H₂S functions as a gaseous signalling molecule in animal tissues; at higher levels it inhibits oxidative processes in nervous tissue, which in ruminants can lead to a disorder of the nervous system known as polioencephalomalacia (PEM) (Schoonmaker and Beitz, 6).

Co-products from sweet sorghum processed in the decentralized system being promoted in India are the grain, bagasse, foam and froth, steam and vinasse (Rao *et al.*, 12). The grain produced in the wet season is often mouldy and unsuitable for human consumption and therefore used for alcohol production and livestock feed (there are three growing seasons per year); the bagasse can be used as a feed, either fresh or after ensiling; as fuel for a variety of

uses, including in the evaporation stage of the process, but also increasingly can be seen as a ligno-cellulose source of ethanol, justifying further processing; the foam and froth can be used as livestock feed or fertilizer; if captured, the steam can be used as heat within the process; and the vinasse for irrigation (but it should not be allowed to enter a water course), as fertilizer or in an anaerobic digester as a source of methane (Rao *et al.*, 12).

Patino *et al.* (15) described the Rural Social Biorefineries (RUSBI) approach developed in Brazil for the production of 'local-use biofuels'. The vinasse (effluent) from the process, which is based on sugar cane, has been incorporated into multi-nutritional blocks, pellets and meal, primarily as a supplement for cattle. Depending on the feedstock and process used to produce the ethanol, up to 50–80 percent inclusion of vinasse is possible, the other ingredients being those normally associated with multi-nutrient block manufacture. Other uses include organic fertilizer, either wet, where there could be contamination of the soil or water courses depending on the distillation process used, or dried and mixed with other materials (Patino *et al.*, 15).

In 2008, Asian production of sugar cane produced 167.4 million tonne of bagasse, which has a variety of uses, including provision of low quality livestock feed, heating, electricity generation, biogas, paper and board manufacture, or as fertilizer. However, this material is also a cellulosic material with potential as an ethanol feedstock (Anandan and Sampath, 16). The authors also suggest various treatments to improve the nutritive value of the bagasse. Hydrolysis of ligno-cellulose feeds followed by fermentation can be used to produce bio-ethanol; gasification of ligno-cellulosic waste leaves a residue that can then be subjected to biodiesel synthesis (Lywood and Pinkney, 2). Wiesman, Segman and Yarmolinsky (18) describe the micro-nutrients found in lipid co-products, and their contribution to the well-being of the animal.

Nutritive value of ethanol co-products for livestock

Ruminants

Distillers grain (DG) is regarded as a cost-effective energy feed that also contain substantial amounts of crude protein (CP) with useful amounts of amino acids (although supplementary lysine may need to be added for high yielding dairy cows). DG is also rich in digestible phosphorus (P) compared with other feeds (Shurson, Tilstra and Kerr, 3). Because the process of producing ethanol reduces the starch but not the fibre content, the residual DG is higher in fibre than the whole grain from which it originated. However roughage should still be included in the diet because of the fineness of the fibre particles coming from the grain. There is also evidence that the rumen degradability of crude protein (RDP) is reduced, and un-degraded protein increased by

the addition of DG, so the authors recommended a small urea supplement at 15 percent wet DG, but unnecessary at 30 percent DG where urea recycling should make up the dietary shortfall in RDP (Galyean *et al.*, 4). The authors noted that the fat in sorghum DG had beneficial effects, which could be replicated by the addition of yellow grease. Galyean *et al.* (4) also reported that DG in the diet increased the amount of manure and the amount of P excreted, which may have a bearing on the way in which the manure is complemented with traditional fertilizers. The authors found that wet DG at more than 10–15 percent of the diet might increase urinary N excretion and ammonia and nitrous oxide emissions.

Erickson, Klopfenstein and Watson (5) suggest that maize co-products are seen primarily as a source of dietary protein in feedlot diets, although at high levels of inclusion, when they replace substantial amounts of whole grain, the fat and fibre will contribute meaningful amounts of energy. They describe maize gluten feed (a product of wet milling) and DG with added solubles (DGS) as having a low starch content, thus removing the negative effects of diets containing large amounts of whole grain on fibre digestibility, and also reducing the acidosis challenge of grain-rich feedlot diets. It should be noted that DG can contain up to 10 percent glycerine, but as described by authors it is suggested that the effects of this on fibre digestion will be minimal (see also Drouillard, 8).

Conversely, with high forage diets, DGS can add the necessary CP and P, thus improving the rumen ecology for microbial protein production and digestion of fibre. Erickson, Klopfenstein and Watson (5) and Cooper and Weber (1) reported similar responses in intake and growth rate when wet, modified or dried DGS was added at up to 40 percent of the diet of feedlot cattle, and contributed to un-degraded or bypass protein (UDP) that could then be recycled to the rumen as urea, again contributing to microbial protein synthesis. Cooper and Weber (1) rated the feeding value of DDGS at approximately 1.2 that of maize. At up to 40 percent of the diet, modified and DDGS can have a feeding value up to 30 percent greater than maize, although the difference narrows at inclusion rates above 40 percent (Erickson, Klopfenstein and Watson, 5). However, if the level of sulphur exceeds 0.47 percent, which is common at the recommended level of dietary inclusion, performance can be reduced, and in some cases PEM can occur. Sulphuric acid is used in the treatment process to control pH, and although steps are taken to reduce residues, the amounts remaining in the DG vary. Erickson, Klopfenstein and Watson (5) suggest that ruminally degradable sulphur is a better measure of likely H₂S production than total sulphur in the diet. Schoonmaker and Beitz (6) give levels of acceptable sulphur similar to those given by Erickson, Klopfenstein and Watson (5), while pointing to

variation in the ability of cattle to tolerate excess sulphur in the diet, with mild intoxication reducing daily liveweight gain (DWG) and feed efficiency, but when H_2S bypasses hepatic detoxification a more serious situation can develop. The problem can be mitigated by chemical analysis and careful formulation of feeds, but sulphur concentration can change between batches as well as among sources (Schoonmaker and Beitz, 6). Suggestions for managing diets with a high sulphur content include limiting where possible the amount of dietary sulphur (choice of mineral mix); adapting cattle to the high sulphur diet; and use of appropriate feed additives to combat the excess sulphur (suggestions include supplementary thiamine, appropriate antibiotics, minerals) (Schoonmaker and Beitz, 6).

Storage of DDGS can be problematic because of bridging, especially in vertical stores and if movement by auger is involved. The situation is worsened if the fat content of the product is above 10 percent or if water is added (Kalscheur *et al.*, 7). Mjoun and Rosentrater (23) reported that while DDGS should not replace fishmeal in aquafeeds it can be used in lieu of other plant proteins, such as soybean meal. However, the authors noted the degree of variation in DG, both among and within processing plants, but this may be less with DG derived from maize than DG from the beverage industry. They also drew attention to the density of DDGS, which could be related to the amount of solubles added to the dried DG, and again noted the importance of having a product that flows, particularly in aquaculture, to meet delivery requirements. Other concerns were the costs of transport and storage. The colour of DG is regarded as important, in that a dark colour is indicative of a Maillard reaction caused by overheating during processing, signalling a reduction in the digestible lysine content (Mjoun and Rosentrater, 23).

In Germany, wheat-based DDGS have successfully replaced traditional protein sources in dairy cows at up to 200 g of the protein per day, and can also be used as the main dietary protein source for fattening cattle (Hippenstiel *et al.*, 11). However, DDGS may be from a mixture of feedstocks, which will have a bearing on nutritive value. For instance, the CP of wheat is more likely to escape rumen degradation than CP of barley, the grain with the most neutral-detergent fibre (Hippenstiel *et al.*, 11). To stimulate a large increase in the feeding of DDGS in Canadian feedlots, Christensen *et al.* (26) asked that reducing variability in the composition of the product be addressed, particularly variability in fat and protein. They also reported trials where diets containing 40 percent of DDGS were successfully incorporated in feedlot diets, and that although the product could be provided in wet form, the expense of drying could in some circumstances be justified by ease of transport and a longer shelf life. Wet products such as WDG contain 23–24 percent solids, and thin stillage (liquid residue after removal of the grain) contains 8.5 percent sol-

ids. Condensed distillers solubles (CDS) result from evaporation of the thin stillage and can be added to either wet or dried DG to give wet distillers grain with added solubles (WDGS), or dried with the grain fraction to produce DDGS (see Figure 3 in Chapter 26). In one feedlot, situated next to an ethanol plant, thin stillage is pumped through the drinking system, thus eliminating the need for drinking water (Christensen *et al.*, 26).

Research into the use of DG for dairy cattle started in the middle of the twentieth century. The list of co-products available has increased considerably and is likely to continue increasing as the technology for extraction and fractionation becomes more sophisticated (Kalscheur *et al.*, 7). These authors make suggestions for feeding WDGS to dairy cattle through growth into lactation. For lactating cows, WDGS from maize is judged to be a good source of un-degradable (bypass) protein when fed at up to 30 percent of the diet, although peak milk production response will probably be around 21 percent. Supplementation with lysine may be necessary if the amino acid profile of the milk indicates that it is low. For dairy heifers, where restricted growth is often desirable to encourage development of mammary tissue, feeding WDGS will allow use of poorer quality forages, examples being soybean stalks or maize stover. For dry cows there is little direct information, but a similar feeding regime to that of growing heifers is probably adequate, although a 15 percent supplement of WDGS during the last four weeks of pregnancy has improved energy balance and resistance to ketosis in early lactation. With calves, 25–30 percent of the maize can be replaced with DGS if the rumen is fully functional, but lysine and methionine levels should be checked for adequacy (Kalscheur *et al.*, 7).

The value of DDGS produced from both wheat and other sources will depend on the original feedstock, although the method of processing is the dominant factor, with colour indicating the degree of heating involved (Noblet, Cozannet and Skiba, 9). After removal of the starch for ethanol, other components of the grain residue (such as fat, fibre and protein) are approximately three times as concentrated as in the original feedstock, although levels of the essential amino acids lysine and arginine will be reduced (Noblet, Cozannet and Skiba, 9). The authors suggest that processing should receive attention to assure a high quality, uniform product capable of diversification to allow production of more specific by-products, examples being with or without hulls, protein concentrates and germ separation. For poultry and pig diets, the authors suggest a link between colour of the product and digestibility of energy and amino acids.

Of the sorghum grain in rural India, the best (free of mould) is kept for human consumption, especially of the white varieties, but the remainder will be used for livestock (Rao *et al.*, 12). Because of its relatively high content of

insoluble fibre sorghum is usually ascribed a feeding value of 95 percent that of yellow dent maize. The dairy industry in India (Rao *et al.*, 12), especially in the north of the country, is a major user of sorghum, both grain, the whole plant, and bagasse, which is important because every 10 tonne of sorghum crushed results in 5–6 tonne of bagasse. The bagasse can be fed fresh or ensiled, or sold into the forage supply chain. Fresh bagasse leaf residue can be successfully ensiled without additives, and then used as a general ruminant feed (dairy cows, buffalo and small ruminants). The fresh leaves can also be incorporated into feed blocks (Rao *et al.*, 12). Intake of bagasse could be enhanced by chopping. Other uses include paper making, fertilizer (limited because of possible deleterious effects on soil), and co-generation of energy (process heat and electricity).

Anandan and Sampath (16) stress that sugar cane bagasse is fibrous, of low nutrient density, and must be supplemented with other feed ingredients to support maintenance. The extent of its use is related to the availability of conventional cereal straws (paddy rice, wheat and sorghum). Tax breaks for using the sugar cane bagasse as fuel could also negatively influence its acceptance as a livestock feed. The amount of bagasse to be incorporated in ruminant diets will depend on the level of production expected, with a range of 30–40 percent in the diet for medium levels of production, and up to 60 percent for low-level production (Anandan and Sampath, 16). Supplements for use with bagasse will be those suitable for mixing with any low grade forage, including urea, molasses and locally available concentrates. Treatment of bagasse to improve its nutritive quality and digestibility has included physical, chemical and biological approaches, with the first two being the most successful so far. However steam treatment with alkali can cause changes in the bagasse that are harmful to livestock (Anandan and Sampath, 16). To improve the digestibility of fibrous forages (possibly the major source of ruminant feed globally), Kalscheur *et al.* (7) discuss the technique of ammonia fibre expansion (AFEX), which, together with enzymatic hydrolysis treatment of forages, may result in a high energy diet that is relatively low in degradable CP.

Non-ruminants

Cooper and Weber (1) noted a shift from the traditional use of DDGS as a substitute for the higher priced maize and soybean in cattle diets, towards pigs, poultry and fish, although the optimum levels of inclusion are still being determined.

Regular DDGS or high protein DDGS (HP-DDGS) after dehulling of the maize can be fed to pigs at all stages of the production chain. The energy of DDGS is similar to maize, unless the oil has been removed, but the energy content of HP-DDGS is slightly higher due to the reduced fibre content. The digestibility of P in DDGS is high. Growing

pigs, from two to three weeks after weaning, can be fed diets containing 30 percent maize DDGS (gestating sows 50 percent) as long as all amino acid requirements are met. With finishers it may be necessary to withdraw DDGS three to four weeks before slaughter because the high level of polyunsaturated fatty acids in the maize oil (measured by iodine value – which is the ratio of unsaturated to saturated fatty acids in a lipid) could reduce pork fat quality. Diets for gestating sows can contain up to 50 percent DDGS, and lactating sows have acceptable performance when fed diets containing 30 percent DDGS, while dramatically reducing or replacing the soybean meal in the diet (Shurson *et al.*, 10). While more research is needed to understand the mechanisms, the authors report that DDGS in the diet may improve intestinal health in pigs. Inclusion of DDGS will also increase the amount of manure produced, reflecting reduced dry matter digestibility, although the loss of N and P can both be controlled (Shurson *et al.*, 10).

Hippenstiel *et al.* (11) found that wheat DDGS up to 20 percent of the diet of pigs did not affect growth, fattening and carcass composition. With laying hens, inclusion levels between 15 and 30 percent wheat DDGS had no effect on laying intensity, egg quality and hen health, but with broilers there was a suggestion that levels above 10 percent may reduce performance unless non-polysaccharide-degrading enzymes are added to the diet (Hippenstiel *et al.*, 11).

Wheat DDGS is seen as a source of energy, protein and P for poultry and pigs (Noblet, Cozannet and Skiba, 9). Crude protein in DDGS can be as high as 30 percent, but lysine levels are low and variable, with ileal digestibility lower than with whole wheat especially if the DDGS has any heat damage. The energy value of wheat DDGS is lower than whole wheat, the difference being dependent on the fibre content of the DDGS. However, wheat DDGS can be included at up to 30 percent in poultry and pig diets as long as the diet meets overall nutrient requirements (Noblet, Cozannet and Skiba, 9). In ruminants, H₂S can be a major problem; in non-ruminants, H₂S formed in the gastrointestinal tract is largely excreted or absorbed and detoxified in the liver, although there may be a link between inorganic sulphur and chronic intestinal disease (Schoonmaker and Beitz, 6).

With sweet sorghum it is the stalk that is used for ethanol production and the grain is a by-product. Most of the sorghum grain produced in India goes into the poultry industry (77 percent), followed by the dairy industry (16 percent), alcohol production (6 percent), and 1 percent for the production of starch (Rao *et al.*, 12). The inclusion levels of sorghum grain in poultry diets are normally 10 percent for layers and 15 percent for broilers, although the actual levels will depend on the price of maize, increasing in years when the price of maize is high (Rao *et al.*, 12).

Fish

Fish require specific amino acids (AA) rather than crude protein. Although DDGS has a similar AA profile to maize, it is deficient in lysine (Mjoun and Rosentrater, 23). Differences between species of fish should also be noted. The authors suggest two ways in which the diet can be balanced, either by including DDGS in a cocktail of protein feeds, or by the addition of synthetic AA. DDGS is rich in vitamins and P, but is low in Ca, Cl and trace minerals. Mjoun and Rosentrater (23) note that cereal feedstocks other than maize are being used in practice, but currently only DDGS from maize, and high protein DDGS (HP-DDGS), also from maize, have been tested for use in aquaculture. The use of barley is limited because of its high content of beta-glucans (Mjoun and Rosentrater, 23). Growth, feed utilization and flesh composition in a number of aquatic organisms, including Nile, hybrid and red tilapia; channel catfish; rainbow trout; yellow perch; common carp; freshwater prawn; Pacific white shrimp; re claw crayfish; and sunshine bass, are summarized in Table 5 of Chapter 23 (Mjoun and Rosentrater, 23), together with the ingredients replaced by DDGS. Tilapia and channel catfish require supplementary lysine if DDGS exceeds 30 percent of the diet (Mjoun and Rosentrater, 23). Feed efficiency in rainbow trout is reduced if DDGS is included in the diet. The other species listed show some positive results, but more information is needed (Mjoun and Rosentrater, 23). In several trials, the flesh contained more protein and fat when DDGS was fed, but taste was not affected. If the protein and fat content of the flesh are unchanged, it could indicate an imbalance in the amino acid profile of the diet. There are few large-scale trials reported where DDGS is fed to fish, but there are indications that the digestibility of DDGS is lower than that of soybean meal or fishmeal, thus indicating that more of the feed is excreted into the pond and thereby becoming a possible source of pond pollution (Mjoun and Rosentrater, 23).

BIODIESEL

In 2010, a total of 140 plants produced 1.2 billion litres of biodiesel, but relatively little glycerol was used for livestock feeding, possibly due to its relatively high value elsewhere in pharmaceuticals and other industry applications. One litre of diesel production is accompanied by 0.08 kg of glycerine (Shurson, Tilstra and Kerr, 3), although Cooper and Weber (1) indicated a lower figure of 0.04 L of glycerine per litre of biodiesel produced. Stoichiometrically, 1 L of biodiesel production should result in the production of 1 kg of glycerine. Biodiesel production peaked in the USA in 2008 and has since fallen, to the extent that glycerol for livestock feed could become scarce because of its demand by other sectors (Shurson, Tilstra and Kerr, 3). However, the USA economy could handle 9.5 billion litres of biodiesel by 2015 (Cooper and Weber, 1). Biodiesel production is by one

of three methods, all based on the use of methanol as the alcohol source (low cost and can be recycled) with sodium methoxide and potassium hydroxide used as catalysts (Cooper and Weber, 1).

Algae contain lipids, along with starch and cellulose present in cell walls. However, their feeding value, and also that of seaweed, is not yet known (Shurson, Tilstra and Kerr, 3).

Europe is the world leader in biodiesel production from vegetable oils, although currently rapeseed oil supported by imported soybean meal is the backbone of the industry (Abbeddou and Makkar, 19). The European need for biodiesel to meet inclusion targets in transport fuels by 2020 will depend on the division between petrol- and diesel-engined transport, which in turn will be price related and largely dependent on government support and taxation levels. If more biodiesel is required, this will be provided by rapeseed oil, providing residual rape meal, as well as through imports of biodiesel or vegetable oils, but the amount of co-products available for livestock feed will not increase tremendously. If the fuel demand and policy shift is toward needing more ethanol, then improvements in crop yields and cropping of underutilized arable land, together with production of livestock co-products of between 23 and 35 million tonne per year, would maintain the total arable output for food and feed at its current level (Lywood and Pinkney, 2).

The importance of the oil palm industry to the Malaysian economy cannot be understated, with palm oil and palm kernel oil in 2008 representing 30 percent of total global production, from 4.5 million hectare of land (Wan Zahari, Alimon and Wong, 13). Major products include palm oil, oleo-chemicals and biodiesel. In Brazil, two palms of importance are the oil palm, *Elaeis guineensis*, and babassu (*Orbignya phalerata*), both originally used in food, charcoal and soap production, but now increasingly as a source of biodiesel. The residue is available as a low-cost energy source for livestock (de Albuquerque *et al.*, 14).

There are other potentially productive sources of biodiesel, but for their residues to contribute fully as livestock feed, detoxification is required. These include *Jatropha* (Makkar, Kumar and Becker, 21; Anandan, Gowda and Sampath, 20) and castor (Anandan, Gowda and Sampath, 20). The possibilities for detoxification of other potential feed sources is discussed by Abbeddou and Makkar (19), Makkar, Kumar and Becker (21) and Dutta, Panda and Kamra (22).

Feedstocks used for biodiesel production

In the USA, soybean is the major feedstock for biodiesel, but in Europe rape is the chief home-grown source of oil (Hippenstiel *et al.*, 11), supplemented with imported soybean, animal fats and yellow grease. However, a number of 'non-conventional' crops and resources have been or

are being investigated for potential use where they are abundant (Table 3).

Camelina sativa, also known as false flax, is an oilseed crop of the brassica family. For over 2000 years it has been cultivated in Europe for its oil and as a livestock fodder. It survives well on marginal land, needs very few inputs and no irrigation, thereby keeping conflict for scarce resources of land, water and fertilizer at a minimum. Because of its increasing use as a biofuel feedstock, more information is needed on the potential role of camelina as a feed ingredient, although there is some evidence of its suitability for ruminants. In Chapter 17, Cherian examines its role specifically as a feed for poultry.

Biofuel policy in India is based on the use of non-food feedstocks to avoid the possibility of conflict between the requirements of humans, livestock and biofuels targets, and also to create a tool in rural development to bring marginal

land into production (Anandan, Gowda and Sampath, 20). However, the authors consider that the industry is unlikely to achieve its 2017 target contribution to transport fuel because of slow progress in establishing crops such as *Jatropha* (see also Makkar, Kumar and Becker, 21), low productivity and poor market infrastructure, compounded with competition for the same land by expansion of the sugar cane industry.

In Australia, Braid (25) describes the current biofuels industry as small (total current capacity 280 million litre per year), and biodiesel has been produced from tallow and used cooking oil. However, *Brassica juncea* and *Pongamia pinnata* are low-rainfall oilseed crops, both with residues (juncea and pongamia meals, respectively) with feed potential after detoxification (Braid, 25). *Pongamia pinnata* is a native species of India and South-east Asia, where the oil is used for cooking and lighting, and along the coast of

TABLE 3
Feedstocks used for biodiesel production, their co-products and major areas of utilization

Feedstock	Co-product	Co-product use by livestock
Soybean (3) Rapeseed (11) Vegetable oils (2) Maize oil (27)	Crude glycerine (3, 7, 8, 23); several uses, human foods/ pharmaceuticals/commercial, etc. (8) Oil seed cake (mechanically extracted) and meal (solvent extracted); methanol should be removed (11)	Pigs (3, 10) Beef cattle (8) Fish (23) Dairy, beef, pigs and poultry (11) Glycerol as drench and supplement for dairy cattle (7)
<i>Camelina sativa</i> (17)	Camelina meal: derives from member of the brassica family that grows on marginal land, no irrigation needed. Meal is rich in amino acids and antioxidants (17)	Poultry (broilers and layers) (17)
<i>Jatropha</i> (20, 21)	Heated <i>J. platyphylla</i> kernel meal (21) Detoxified <i>J. curcas</i> kernel meal and detoxified protein isolate (21) Heated kernel meal from non-toxic genotype of <i>J. curcas</i> (21)	Fish, turkeys and pigs (21)
Oil palm (13, 14) Oil palm (<i>Elaeis guineensis</i>) and babussa (<i>Oribgnya phalerata</i>) (14)	Rapidly expanding industry with several by-products from refining of crude palm oil or palm kernel oil: oil palm fronds, trunks, pressed fibre, empty fruit bunches, kernel cake and oil mill effluent are products available in the field and ex-processing (also solubles), with aim of integrating livestock industry with oil palm production (13) Oil palm and babussa oil used for food, charcoal, soap and now biodiesel (14)	Ruminant feeding and complete diets based on oil palm for poultry, pigs and freshwater fish (13) Oil palm and babussa feed for collared peccary (<i>Pecari tajacu</i>) (14)
Seed oils (18)	Co-products derived during bioethanol and biodiesel production (18)	In livestock feed as feed additives (but also used in human food and cosmetics) (18)
Micro-algae (25, 24)	Algal residues left after extraction of oil (24)	Fuel, food, feed and chemicals (24)
<i>Brassica juncea</i> (25) <i>Pongamia pinnata</i> (25, 19) <i>Pongamia glabra</i> (22) <i>Azadirachta indica</i> (22, 19)	Juncea meal (residue after oil extraction, 25) <i>P. pinnata</i> meal (residue after oil extraction, 19, 25) <i>P. glabra</i> meal (Karanj seed cake) – de-oiling needed for complete detoxification (22) <i>A. indica</i> (neem seed cake) – water washing reduces toxicity (19, 22)	Juncea meal (pigs, 25) <i>P. pinnata</i> meal at low levels as livestock feed (25) (possible toxicity problems, 19) Karanj and neem seed cakes after treatment fed to ruminants and poultry (22)
Non-edible oils (19) <i>Ricinus communis</i> (castor) (20) <i>Jatropha</i> (21)	Oil cakes and meals; detoxification needed; meals that can be fed after treatment are <i>R. communis</i> ; <i>Hevea brasiliensis</i> (livestock trials needed); <i>Crambe abyssinica</i> ; <i>A. indica</i> ; <i>P. pinnata</i> (19). Need for industrial and commercial uptake of detoxifying techniques for castor (6) With <i>Jatropha</i> , removal of phorbol esters necessary (21)	Ruminants used where oil cakes and meals were tested (19) <i>Jatropha</i> requires testing (21)

Notes: Numbers in the body of the table denote chapter numbers in this book. For a list, see Appendix 1.

northern Australia. The integration of trees into pasture land has many potential benefits for sheep and cattle (Braid, 25).

In India, four strategies were proposed to overcome the shortage of protein for livestock: (1) restricting exports of oilseed meals; (2) increasing areas of cultivation for growing high quality green forage crops; (3) increasing efficiency of use of existing protein feeds; and (4) identifying non-conventional oilseeds and, if necessary, taking measures to detoxify the resulting seed cake (Dutta, Panda and Kamra, 22). This last approach matches the Indian government's policy of increasing production of biodiesel without aggravating the conflict of interest between biofuel and food production, and resulted in identification of karanj and neem. In the past, the karanj plant (*Pongamia glabra*) has had many uses, including as a traditional medicine, with the oil supplying heat and light (Dutta, Panda and Kamra, 22: Table 1). However, extraction of the oil results in a seed cake that at present is often used as fertilizer, but which needs detoxifying before feeding to livestock (Dutta, Panda and Kamra, 22). Abbeddou and Makkar (19) discuss nine oleaginous crops suitable for oil extraction but that leave behind toxic co-products, which after detoxification could be used as protein feeds. The authors stress that detoxification techniques need to be suitable for up-scaling if sufficient material is to be handled to have an impact in the market. Makkar, Kumar and Becker (21) outline the potential for *Jatropha* spp., a hardy shrubby tree that grows in wild or semi-cultivated areas, often on degenerated land in Africa, Asia, and Central and Southern America. Its seeds contain 55–60 percent oil that yields good quality biodiesel and the residue is rich (60–66 percent) in CP (Makkar, Kumar and Becker, 21).

With the increased use of algae for oil production, research into technical aspects of using these sources is needed. Currently there is no commercial activity with algae, but as an industry suited to development in coastal regions of the world, it could be developed in Australia, with the co-products being used for energy generation or possibly in livestock nutrition (Braid, 25).

Biodiesel co-products

Crude glycerine is an important co-product from the biodiesel industry (Table 3). Its purity is measured by the amount of water it contains. Pure glycerol has less than 5 percent water and is also colourless. Crude glycerol contains increasing amounts of water and other impurities that affect the colour, with increasing shades of brown as the water and impurities increase (Shurson *et al.*, 10; Drouillard, 8; Cooper and Weber, 1). In the USA in 2010, 48 percent of glycerol was sold for high value uses, while 33 percent went to the livestock feed industry (Cooper and Weber, 1).

Glycerine at different purities may help to stabilize the hygienic quality of pelleted feeds without affecting the

physical quality of the pellets. Mature cattle can consume 1 kg of glycerine per day, as a source of rapidly fermentable carbohydrate, while it is not clear if the sweet taste of this product acts as an intake stimulator (Hippenstiel *et al.*, 11). Drouillard (8) estimates that the yield of glycerine is approximately 10 percent of that of the oil or fat from which it is derived, with pure glycerine being used in human food and industrial processes including; beverages (glycerine contains 60 percent of the sweetness of sugar); pharmaceuticals; synthetic polymers; cosmetics and personal care products; and, after modification, as an emulsifying agent. Glycerine also has humectant properties beneficial in both food and feed production systems, in the latter for texturing properties and dust control, although reduced production costs of pellets and improved hygiene have also been noted (Drouillard, 8).

Camelina meal contains 36–40 percent crude protein, 11–12 percent fat and 4600 kcal/kg gross energy. Its protein is rich in essential AA, including lysine and methionine. The fat is rich in alpha-linolenic acid, the parent fatty acid of omega-3, and the antioxidant tocopherol, both necessary for healthy, productive poultry and quality poultry products for humans (Cherian, 17).

Castor cake is a high-protein product, but its use as livestock feed is restricted because of toxins, especially ricin, which means that a large proportion of the residue cake produced is used as organic fertilizer. However, treatments involving heat, water and alkali, especially the use of NaOH, have reduced the problem (Anandan, Gowda and Sampath, 20; see also Table 6 of Chapter 20 for a summary). If marketed at the current (2011) price, plus the cost of treatment, it would still be competitive with other protein feeds. The authors suggest that the use of castor cake, through its promotion and marketing, should be handled by a united approach involving all interested parties. All the major castor producing countries, namely India, China and Brazil, also have large numbers of livestock and therefore a large demand for protein feeds, to which detoxified castor cake could make a significant contribution (Anandan, Gowda and Sampath, 20).

Pongamia cake (karanj) is available in two forms, from either a mechanical-extraction process or a solvent-extraction process, but both contain anti-nutritional factors (Braid, 25). The use of karanj cake, both expeller and solvent extracted, is limited by the presence of three types of toxins: furanoflavones, tannins and trypsin inhibitors (Dutta, Panda and Kamra, 22). The AA profile of Karanj compares favourably with traditional proteins, and it contains more Ca, P and Na than soybean meal, but less Cu and Fe (Dutta, Panda and Kamra, 22).

Neem oil has traditionally been used for soaps, creams, toothpaste, etc., with the cake, which contains 35–49 percent CP, used as fertilizer or as a pesticide (Dutta, Panda

and Kamra, 22). The bitter taste and variable composition of neem seed cake and neem seed kernel cake, due to de-pulping, de-corticating and oil extracting, affect its value as a feed. In addition, crude fibre and CP are both affected by the methods employed and degree of processing (Dutta, Panda and Kamra, 22).

Abbeddou and Makkar (19) summarized the potential for detoxification of seed cakes from non-conventional sources that could contribute protein for livestock. *Azadirachta indica*, the source of neem cake, after washing can be used at up to 45 percent of the concentrate in calf diets, while other treatments for this product include methanol, urea and alkali extraction. *Ricinus communis* meal cooked at 100 °C for 50 minutes could be added as 15 percent of chick diets, and, with the addition of 4 percent lime, included at 10 to 15 percent of the diet for sheep and beef cattle. HCN levels in *Hevea brasiliensis* meal could be reduced by soaking in water to allow fermentation, but livestock trials have not as yet been conducted. *Crambe abyssinica* meal de-hulled and subjected to a heat-carbonate treatment is acceptable to beef cattle, and can replace up to two-thirds of the soybean meal in the diet. *Pongamia pinnata* meal after washing with water or alkali treatment can be included at up to 13.5 percent of the concentrates in lamb diets. *Brassica juncea* has been selected as a break crop for cereal lands, particularly in hot areas and an extracted oilseed cake is available (Braid, 25).

The benefits of lipid co-products are summarized by Wiesman, Segman and Yarmolinsky (18), although many are also available from the production of ethanol. The advantages include acting as a source of vitamin E, required for many essential functions in both humans and livestock including growth and reproduction; as a source of carotenes, normally available to the grazing animal but lost when forage is conserved as hay or silage; and providing phyto-sterols, important in reducing the absorption of cholesterol, thereby helping to reduce cardiovascular disease (squalene has similar properties in this respect). They also have anti-inflammatory, anti-bacterial, anti-ulcerative and anti-tumour properties, and are beneficial to the immune system of piglets. Polyethenols are able to improve the efficiency of protein use in ruminants, reduce urea content of manure, inhibit bloat, and help combat sub-clinical helminth infections. Lecithins act as dust suppressors (dustiness has been identified as a constraint to intake by ruminants), emulsifiers and as a source of essential fatty acids (Wiesman, Segman and Yarmolinsky, 18). The authors stress the need for thorough testing of these products obtained from biodiesel production to avoid toxic compounds reaching humans and livestock. Shurson *et al.* (10) stress the problems likely to be encountered from an excess of methanol in the diet and in particular the need to control intake of glycerine in pigs because of the slow rate of excretion of methanol.

Nutritive value of biodiesel co-products

Ruminants

The two major co-products from the biodiesel process are protein-rich cakes or meals, and glycerol. The cakes and meals have long been major sources of CP in commercial livestock and poultry production, the market being dominated by soybean meal (Makkar, Kumar and Becker, 21). Glycerol, a glucose precursor, has traditionally been used as a drench for dairy cows to combat ketosis, often shortly after calving, because it is rapidly fermentable within the rumen and favours a decrease in the acetate-to-propionate ratio (Kalscheur *et al.*, 7). Increasing propionate benefits the supply of gluconeogenic substrate reaching the liver, and increasing butyrate encourages ruminal epithelial tissue growth, possibly leading to improved absorption of nutrients (Kalscheur *et al.*, 7). However, it can also be used as a supplement for transition cows, or as a replacement for maize at 10–12 percent of the diet, but its effect in causing a reduction in fibre digestibility is similar to that of starch (Kalscheur *et al.*, 7). The authors recommend analysis of individual batches of feed rather than depending on book values when formulating diets, and warn that some agricultural crops may not be ideal co-components in diets based on DG. For example, a combination of DDGS plus alfalfa hay results in a feed containing too much CP. Adding glycerine to the diet will favour a propionate-butyrate, rather than acetic, rumen fermentation, although this may be affected by the level of glycerine and the composition of the rumen flora (Drouillard, 8). Young cattle fed glycerine early in life and then fed a diet containing maize gluten feed, which had a glycerol content of 4.9 percent in the finishing period, have performed better than cattle fed the same finishing diet but without the addition of glycerine at the earlier stage, suggesting that rumen adaptation to glycerine may have a relatively long carry-over period (Drouillard, 8).

In Europe, rapeseed co-products are widely used in cattle, pig and poultry diets (Hippenstiel *et al.*, 11). Recommendations from Germany are available for daily amounts of both rapeseed meal (solvent extracted) and rapeseed cake (mechanically extracted), which range from 4 kg of rapeseed meal for a dairy cow (2 kg of rapeseed cake) to 0–100 g of the meal and 50–100 g of the cake for laying hens (Hippenstiel *et al.*, 11, especially Table 16). A safety quality assessment of rapeseed cake for cattle is required because variations in processing can affect the chemical composition, particularly that of crude fat and CP, making ration formulation using this product difficult. Rapeseed meal can completely replace soybean meal in dairy cow rations, although there may be differences in intake of energy, rumen degradability and amino acid profiles between the two sources (Hippenstiel *et al.*, 11). Hippenstiel *et al.* (11) also comments on the use of glycer-

ine, stressing that methanol should be removed as far as is technically possible and that the methanol content of each batch should be declared.

Pongamia cake (Braid, 25; Abbeddou and Makkar, 19) is similar to soybean meal in many respects, but contains anti-nutritional factors (karanj and pongamol) that also make it unpalatable, although Abbeddou and Makkar (19) consider it a safe feed within limits after detoxification. However, because of the anti-nutritional characteristics and relative unpalatability, Braid (25) suggests *Pongamia pinnata* as a useful tree for incorporating into extensive pasture because of a relatively low risk of grazing damage. Feeding of untreated pongami or karanj cake to livestock reduced dry matter intake and caused histological changes to vital organs of ruminants and poultry, and this has led to various attempts to detoxify it, although they have been general rather than targeting a specific toxin (Dutta, Panda and Kamra, 22; Braid, 25). The most successful of these was de-oiling to ensure removal of the toxins during the extraction process, which is achieved through treatment with an alkali solution (1.5% NaOH plus 3% lime) or ammoniation with urea (Dutta, Panda and Kamra, 22). Carcass weight of lambs was reduced more with expeller cake than with solvent-extracted karanj, but chemical and physical attributes of the lambs were not affected. Both forms of karanj resulted in lighter carcasses than de-oiled groundnut meal (Dutta, Panda and Kamra, 22). The same authors reported that masking the taste of neem or urea ammoniation improved intake of neem seed kernel cake (NSKC) in ruminants. Neem seed cake (NSC) was found to reduce growth, impair the male reproductive system and, in some cases, result in haematuria. Treatments showing positive results with neem seed kernel cake include adding NaOH (1 percent) and boiling (this can reduce CP) and washing with water, which can result in a loss of dry matter. Another approach is to ensile the NSKC with either 2 percent NaOH for 24 hours or 2.5 percent urea for 5–6 day, followed by sun drying and grinding (Dutta, Panda and Kamra, 22). Although responses in feeding trials have been mixed, there are no reports of changes in rumen pH or total volatile fatty acids. Dutta, Panda and Kamra (22) suggest that both karanj and neem seed cake, if properly prepared, could replace 50 percent of the nitrogen in the diets of lambs. Although farmers in India show reluctance to feed castor meal (this could be related in part to a high economic return from sugar cane), there is evidence that ruminants can use it (Anandan, Gowda and Sampath, 20; see also Table 7 in Chapter 20), in some cases without detoxification of the ricin.

The two major by-products from palm oil processing are palm kernel cake (PKC), also known as palm kernel expeller (PKE), and crude palm oil (CPO) (Wan Zahari, Alimon and Wong, 13). There are two dominant processing methods

used: solvent extraction and expeller. These result in palm products with a range of nutritive values arising from differences in agronomic factors and processing procedures. Expeller palm kernel meal (PKM) has a substantially higher oil content than the solvent-extracted material and the AA profile shows deficiencies in lysine, methionine and tryptophan, which are currently being addressed (Wan Zahari, Alimon and Wong, 13). PKC is free of aflatoxins, heavy metals and chemicals, and can be stored for up to three months. However, the Malaysian palm oil industry also produces valuable by-products resulting directly from the field operations. These include oil palm fronds (OPF) from pruning, felling and harvesting that are available throughout the year, the yield being around 82.5 kg/palm/year (Wan Zahari, Alimon and Wong, 13). The fronds can be chopped and fed fresh, which is the common practice, ensiled, or processed for cubing or pelleting. Freshly chopped OPF is a common source of forage and can be fed at 40 percent of the diet, often with some added PKC, to buffalo, cattle and sheep. If ensiled, the diet will benefit from a urea supplement to offset the low level of CP in the silage. The second field residue is oil palm trunks (OPT), the life of a tree being 25–30 years (the criteria for felling and clearing are height of palm >13 m and/or a diminishing yield). The trunks can be chipped and ensiled, and, with added urea, have a similar nutritive value to that of rice straw, with the parenchyma being an excellent source of roughage for beef cattle (Wan Zahari, Alimon and Wong, 13). With beef cattle, a maximum inclusion of 85 percent PKC is recommended, and for dairy cows 30–50 percent PKC is recommended, often fed as a pellet with grass and other concentrates. However, with sheep, 30 percent PKC should be regarded as the maximum because of the high Cu content of the cake, which can cause long-term problems in this species (Wan Zahari, Alimon and Wong, 13).

Other products from the oil palm industry, which either have some use at present or merit research for future use, include palm oil mill effluent (POME), which after decantation can be used for ruminants; empty fruit branches, a field product, suitable for coarse forage, mulching and fibreboard production; palm press fibre (PPF), used for fuel, paper, fibreboard, etc., as well as for coarse forage (treatment with alkali or steam is not assured of success); and crude palm oil (CPO) is rich in vitamins A and D and can be used to reduce dustiness in the diet. Derivatives from CPO include palm fatty acid distillates (PFAD) and spent bleached earths (SBEs) (Wan Zahari, Alimon and Wong, 13).

Non-ruminants

Crude glycerine contains similar energy to that of maize for pigs. If affordable, sow diets can contain up to 9 percent and weaners at least 6 percent glycerine, which can be increased up to 15 percent for finishers. Inclusion of

glycerol in a mechanized system can improve feed flow, but amounts of Na and methanol (toxic) in the diet should be checked (Shurson *et al.*, 10).

In poultry diets, Cherian (17) found that camelina meal could be incorporated at 10 percent in layer and broiler diets without affecting the performance of the birds or quality of the products, and reduce the omega-6 to omega-3 ratio in meat and eggs. Castor cake, after treatment to detoxify the ricin, has been fed successfully to poultry, but because of its high fibre and lignin contents is more likely to be better used by ruminants (Anandan, Gowda and Sampath, 20). Pigs fed *Brassica juncea* cake at up to 18 percent of the diet exhibited no ill effects, but at 24 percent of the diet *B. juncea* cake caused a reduction in intake, and thus growth rate declined (Braid, 25). Hippenstiel *et al.* (11) call for a greater understanding of the role of glucosinolates, more common in rapeseed cake than meal, in the diets of both pigs and poultry. Rapeseed meal is lower in lysine than soybean meal, and the crude protein is less digestible than in soybean meal, but contains more sulphur AA. Rapeseed products are not commonly used in poultry diets, and, when used, supplementary iodine may be necessary.

Limited amounts of PKC can be fed to poultry because of its high crude fibre content and the presence of polysaccharides and shells. A maximum of 20 percent PKC in the diet for broiler chicks and 20–25 percent for layers, while 30 percent is the maximum recommended for muscovy ducks (Wan Zahari, Alimon and Wong, 13). Higher levels of PKC in poultry diets would require balancing with fat, which would not be cost effective. Enzyme treatment and solid-state fermentation of the PKC are being investigated. After processing, POME can be fed to poultry, although at present this is not economical (Wan Zahari, Alimon and Wong, 13). Pigs, both growers and finishers, are often fed 20–25 percent of the diet as PKC, although the inclusion rate varies throughout the Malay peninsula. In Nigeria, inclusion levels can be as high as 40 percent.

Solvent-extracted karanj meal, after treatment with NaOH or lime, and expeller karanj cake treated with NaOH, have been fed to poultry, but were unpalatable as a sole feed (Dutta, Panda and Kamra, 22). The expeller cake was also unacceptable because of pathological changes in the vital organs of the birds (Dutta, Panda and Kamra, 22). Solvent-extracted karanj (complete removal of the oil renders this product safe for livestock) can be included at 6.4 percent of the diet of quail up to four weeks of age, after which supplementary methionine would be required. However, de-oiled karanj meal reduced the growth rate in quail chicks when it was above 4.45 percent of the diet, and in layer male chicks above 5 percent reduced growth. More research is needed (Dutta, Panda and Kamra, 22). De-oiled neem seed cake (NSC), raw NSC and un-decorticated expeller reduced growth in chicks. However, soaking

expeller NSC and adding charcoal (0.4 percent w/w) and solvent extracted NSC improved growth, while a combination of acid, alkali and washing removed the bitter taste, making the cake acceptable to chicks. Saponification of neem oil (present in the cake) with 10 percent KOH completely detoxified the cake (Dutta, Panda and Kamra, 22.). Replacing groundnut meal with NSC at above 25 percent markedly reduced egg production, but replacing groundnut at 10 percent neem kernel meal treated with 2 percent NaOH had no effect on egg production (Dutta, Panda and Kamra, 22). Changes in carcass characteristics were small but some abnormalities were noted, including pale and shrunken muscles and fatty changes in the vital organs, and the anti-fertility effect of neem was confirmed (Dutta, Panda and Kamra, 22).

Research has shown that 40 and 22 percent of dietary energy can come from babussa (replacing maize) and oil palm (replacing wheat bran), respectively, thus reducing the cost of feed and not impairing production (de Albuquerque *et al.*, 14).

Detoxified *J. curcas* kernel meal (DJCKM) has also been fed successfully to turkeys from 3 weeks of age, up to 20 percent of the diet, and growing pigs, where it has replaced 50 percent of the soybean meal protein in the diet (Makkar, Kumar and Becker, 21). The authors suggest DJCKM as a substitute protein when fishmeal and other conventional protein-rich feeds are in short supply and expensive.

Fish

With fish, the amount of PKC in the diet will depend on the species, with current recommended inclusion levels ranging from 30 percent for catfish to 20 percent for tilapia. However, ongoing work involving treatment with enzymes indicates that the levels of PKC could be increased, thus allowing a reduction in the amounts of imported maize in the diet (Wan Zahari, Alimon and Wong, 13).

Makkar, Kumar and Becker (21), seeking non-conventional alternative feedstocks, studied two species of *Jatropha*. The first of these, *Jatropha curcas*, contains toxic phorbol esters, but after oil extraction from the kernel and detoxification, the kernel meal has a CP content of 60–66 percent. The second species, *J. platyphylla*, has a CP content in the kernel meal of 65–70 percent after oil extraction, and although not toxic, its kernels contain the trypsin inhibitors lectin and phytate. Detoxified *J. curcas* kernel meal, heated (to inactivate trypsin inhibitors and lectins), *J. platyphylla* kernel meal and detoxified *J. curcas* protein isolate can replace 50, 62.5 and 75 percent of fish meal protein, respectively, in fish diets without compromising growth performance, nutrient utilization and health indicators (Makkar, Kumar and Becker, 21). A non-toxic genotype of *J. curcas* (free of phorbol esters, but contain-

ing trypsin inhibitors and lectins) is also available in Mexico. The heated kernel meal of this genotype is also an excellent feed resource (Makkar, Kumar and Becker, 21). Since jatropha meals are rich in phytate, addition of phytase in the diets of monogastric animals is necessary for effective utilization of the meals.

Crude glycerine derived from the production of biodiesel from pure or waste vegetable oil or rendered animal fat can contain between 38.4 and 96.5 percent glycerol, although the normal range is between 75 and 85 percent (Mjoun and Rosentrater, 23). The large-scale biodiesel producers supply high grade glycerol to the food, pharmaceutical and cosmetic industries, while that from the smaller producers is likely to contain more impurities, thus limiting its usage. Animal fat derivatives contain less glycerol and more impurities than from vegetable oil feedstocks. Trials with channel catfish and rainbow trout have shown that glycerol can be added to the diet at 10–12 percent and acts as a precursor for gluconeogenesis, but not lipogenesis. However, rainbow trout do not use glycerol efficiently as an energy source (Mjoun and Rosentrater, 23).

MICRO-ALGAE

All of the feedstocks considered above have been produced from agricultural land, either suitable for cropping or currently regarded as marginal. Phytoplanktons are the largest biomass producers in global aquatic systems, both marine and freshwater, at levels that sunlight can facilitate photosynthesis. Algae, the primary producer, are responsible for half of the annual global output of organic carbon (Ravishanker *et al.*, 24). The viability of biofuel production from micro-algae depends on full use of the algal biomass, which is rich in proteins and vitamins and therefore useful for food and feed. They contain chemicals, pigments, fatty acids, sterols and polysaccharides. They have anti-viral, anti-tumour and anti-bacterial properties and act as an antidote against HIV. Their 'farmed' production could be centred on coastal seawaters, thus removing competition for land and water resources needed for agriculture. Ravishanker *et al.* (24) propose five areas to be considered in developing their use: (1) algal biodiversity; (2) large-scale culture of micro-algae; (3) downstream processes for conversion to biofuels; (4) use of micro-algae for food and feed; and (5) technical and economic analysis of the bio-refinery concept to assess and promote adaptation. Algae thrive under a wide range of extreme conditions and have simple nutrient needs and a very fast growth rate, with the ability to accumulate fat up to 50 percent of their biomass. The authors describe two methods of cultivating micro-algae, either in open ponds, which are relatively cheap and most of those used do not compete for land, or in closed system cultivation that can be more closely regulated (Ravishanker *et al.*, 24). Algae yield biofuels (diesel) by trans-esterification of algal

lipids or hydrocracking (i.e. cracking and hydrogenation of biomass containing hydrocarbons). Ethanol can be released from either algal biomass or algal cake (Ravashanker *et al.*, 24). In Table 6 of Chapter 24, the authors give the food applications for micro-algae, together with the cultivation system and the countries currently involved, and in Table 7 compare the vitamin content of some algae with traditional foods. Many micro-algae contain vitamin B₁₂ and some brown algae contain tocopherol. Micro-algae containing astaxanthin are also used as feed in aquaculture production, where they can be fed with, or replace, fishmeal, acting as colouring agents in such species as salmon, rainbow trout and koi carp. Improved growth rate and survival, and yolk colour have also been recorded in poultry (Ravishanker *et al.*, 24). Micro-algae have also been fed to ruminants and pigs. They are a good source of carbohydrates, and some contain cellulose, usable by ruminants. They tend to be deficient in the sulphur-containing AA, cysteine and methionine. Other uses listed by the authors include the presence of bio-active molecules (e.g. phycobiliproteins, polysaccharides) and production of biogas, which can provide bio-electricity as an alternative energy source to biofuel. This is an area of great promise waiting for economically viable technology to release its potential.

ECONOMICS

Cooper and Weber (1) foresee the future use of agricultural crops for biofuel resulting in a small increase in livestock feed costs, which will be offset to some extent by the use of co-products as feed and by increases in crop yields over time. Poultry production is a fast growing industry because of a rising world demand for animal protein. Feed costs represent 65 percent of poultry production costs, which could be reduced by largely un-researched co-products such as camelina meal, non-toxic jatropha, and detoxified jatropha meal (Cherian, 17; Makkar, Kumar and Becker, 21). Christensen *et al.* (26) discuss the difficulty of getting accurate data for the costs of wheat DDGS, including the costs of nutrient management. The authors explain the sensitivity of the industry in North America to the exchange rate between the USA and Canadian dollars, in that a strong Canadian dollar will favour importation of DDGS from the USA rather than developing the local industry. The same authors also register concern regarding the growth of the ethanol industry in Western Canada, where wheat is a major feedstock available in Saskatchewan, whereas the beef feedlot industry is concentrated in Southern Alberta. Full economic appraisal must include co-products because of their influence on pathway selection and economics of biofuel production (Wang and Dunn, 27). They suggest that wet distillers grain may be economically viable within a radius of 80 km of the ethanol plant because savings in drying costs will offset higher transport costs and a

shorter shelf life (without ensiling). Patino *et al.* (15) call for upgrading of the vinasse produced from bioethanol production from cassava, sugar cane, sweet potato, and sweet sorghum from small-scale on-farm and rural group activities. The techniques should be simple, efficient and sustainable, but result in a product that can be added direct to feed or included in a multi-nutritional block. Larger-scale operations, from which more sophisticated products can be developed and promoted especially for cattle feeding, should also promote social inclusion and extension of knowledge (Patino *et al.*, 15).

Galyean *et al.* (4) considered economics to have been a major driver in growth of the industry. The need for leadership to drive a new industry is taken up by Christensen *et al.* (26), who suggest a combination of public and private forces to ensure adequate regulation of the market and maintenance of the profit motive (see Tables 4 and 5 of chapter 26). A counter argument is proposed by Drouillard (8), in that the recent rapid expansion in biodiesel production, which is predicted to continue until 2020, has caused a market glut of glycerol and thus is expected to cause the price of this product to fall, thereby increasing its acceptability as a livestock feed.

Decentralized groups producing syrup from sweet sorghum are a feature of production in India (Rao *et al.*, 12), where groups of small-scale farmers work together to produce syrup for ethanol production, leaving the co-products available for local use. This is in contrast to centralized production, based on large-scale producers. Feeding of sugarcane bagasse has not been successful economically, and using it for fuel currently shows a better return (Anandan and Sampath, 16). Wan Zahari, Alimon and Wong (13) suggested that market forces will drive the use of oil-palm by-products as livestock feed in Malaysia because of the acute shortage of traditional forage and the need for a large increase in livestock production to satisfy demand. Castor cake, of which there are large quantities in India, China and Brazil, even after the cost of detoxification is taken into account, could probably be marketed well below the price of traditional protein sources (Anandan, Gowda and Sampath, 20). Shurson, Tilstra and Kerr (3) make a case for co-products such as DG to be available on Futures Markets, and a recent development is that DDGS are now tradable on the Chicago Mercantile Exchange (CME) (G. Cooper, pers. comm.). A stumbling block to this being quality variation, which resulted in 2007 in a call for standard analytical procedures and clear definitions of the products. These authors present data that show USA exports to have increased from 1 million tonne to 9 million tonne between 2004 and 2010, to an increasingly wide global market and for an increasing number of livestock species. Cherian (17) estimates that between 70 and 80 percent of the harvested weight of *Camelina sativa*

is co-product, camelina meal, and 65 percent of the costs in poultry production are accounted for by the cost of feed. Establishing a demand for camelina meal may enhance the overall value of the crop and reduce the cost of feeding poultry. India, the country with the greatest population of livestock, is short of protein- and energy-rich feeds, a worsening situation because of shrinking grazing lands and liberalized export policies. This situation is forcing attention to non-conventional feeds, two of which, *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) are discussed by Dutta, Panda and Kamra (22), with a third, *Jatropha* spp., described by Makkar, Kumar and Becker (21).

Socio-economics

The economics of production are not solely confined to finance. Abbeddou and Makkar (19), in their assessment of potential use of co-products from non-edible-oil-based biodiesel production as feedstuffs call for socio-economic analysis alongside the development and use of the detoxified materials. They foresee sustainability from feedstocks that are not in competition with human food and animal feed, and that grow in poor and marginal soils. They also note that many of the emerging co-products contain toxic or anti-nutritional factors, thus generating a need for detoxification or nutritional improvement. The case for micro-algae development is based partly on the lack of competition for land and water resources with traditional agriculture (Dutta, Panda and Kamra, 22).

Wang and Dunn (27) discuss the water footprint of biofuels, which is a combination of that needed to grow the feedstock and that needed in the production process. The demand for irrigation is, and will be, an important component, although the authors note that improved practices have reduced irrigation by 27 percent in the last 20 years, with some reduction of water use also in the production of ethanol. They present a series of allocation methodologies to create a life-cycle analysis. The parameters include displacement, massed-based, energy-based, market-value-based and process purpose, which can be combined into a hybrid methodology (Wang and Dunn, 27).

When calculating reductions in GHG emissions, the savings in fossil fuel and use of a cleaner fuel are only one side of the equation, as energy expenditure and GHG emissions implicit in growing, transporting and processing the biofuel must be also be accounted for (Lywood and Pinkney, 2). These authors go on to explain the formula by which savings of GHG are calculated so that a 'trading balance' can be established. Over the next decade, it is likely that the biofuels industry will expand less rapidly than in the previous decade in its traditional areas because of controls put on expansion by several governments, such as China and the USA (Cooper and Weber, 1).

In Brazil, the Rural Social Biorefineries (RUSBI) approach has been developed for small-scale farmers, especially in remote and marginal areas, to promote agricultural development, food safety and energy self-sufficiency, as cooperatives rather than as associations in order to benefit most from the prevailing tax system (Patino *et al.*, 15). Similar developments in Colombia were adopted where petrol prices were high (Patino *et al.*, 15).

Braid (25) suggests that the biofuels industry is being driven by needs such as fuel security and government demand for a pricing mechanism for carbon. Wiesman, Segman and Yarmolinsky (18) comment on incentives to the biofuels industry, but also raise the question of penalties for non-inclusion of biofuels in transport fuel within government timeframes.

The approach to small-scale farmers has also been used in India with sweet sorghum being a major feedstock in a 'decentralized' system designed to encourage rural development (Rao *et al.*, 12). This allows small groups of farmers to develop local installations to produce syrup and sweet sorghum co-products and to send the syrup to a centralized unit for ethanol extraction (Rao *et al.*, 12), thus avoiding the high cost of transporting the whole crop to the centralized unit, and allowing local retention of the co-products. The viability of this approach depends on the sale of fodder bagasse, and producers are rapidly becoming aware of enhancing the value of this through chopping and supplementation (Rao *et al.*, 12).

Erickson, Klopfenstein and Watson (5) point to the increased N and P content of properly handled manure and the GHG benefits to the rating of ethanol compared with gasoline if DGS is produced, the amount of P often being sufficient to adopt a four-year rotation for this element. The savings in GHG largely accrue through the greater average daily gain (ADG) of feedlot cattle fed DGS, reducing the number of days in the feedlot, and, where transport distances allow, the feeding of wet DGS saves emissions associated with drying the DG (Erickson, Klopfenstein and Watson, 5).

Ravishanker *et al.* (24) argue that all photosynthetic processes should be subjected to a full audit at all stages of energy production, an approach currently missing. In Brazil, increased availability of potentially cheap energy sources for livestock, as a result of the expansion of biodiesel production, has created opportunities for rural farmers to intensify domestication of a wild game species, the collared peccary (de Albuquerque *et al.*, 14).

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

The biofuels industry has evolved rapidly over the last two or three decades with developments in processing techniques and an expansion of the range of plants and other natural energy sources being considered as feedstocks. On-farm

application of the co-products, on which the viability of the industry depends, is often ahead of unbiased research to support its use, and there is a growing demand for standardization of products. This has generated a need for research to fill in the gaps of knowledge from existing progress, to seek answers to problems that are known to exist, and to be ready to answer questions raised by future developments. This is against the backdrop of an industry that started as an outlet for grain-based residues from the production of alcoholic beverages, which were fed to pigs and cattle, to one that has grown to importance in protecting the environment and safeguarding dwindling supplies of fossil fuels.

Tables 4 to 6 summarize the research seen as necessary at the present time, which includes assessing current and potential feedstocks, and the nutritional needs of most species of livestock, poultry, and aquaculture. Much of the potential research identified as needed is concerned with co-product feeding value, the need for standardization of products from within an individual plant and between plants, and the search for new feedstocks, particularly those indigenous to an area but underutilized, together with safety standards (including detoxification of seed meals where necessary). Coupled with this is the need to consider the species to which the co-product is to be fed.

The knowledge gaps identified in Chapters 1 to 7 inevitably show a degree of overlap, such that in some cases the positioning of a topic within the four tables may appear arbitrary. Table 4 concentrates on DG, including some of the potential constraints in its use. Table 5 brings together suggestions for investigating co-products from feedstocks other than cereals, including the programme on micro-algae. Table 6 lists areas for nutritional research relating to a specific livestock species, although it is accepted that the work involving jatropha co-products and camelina meal would have been equally at home in Table 5. Table 6 presents the areas that belong in neither Table 4 nor 5, but all of which have relevance if the co-products industry is to remain economically viable and to benefit all sectors of the livestock industry.

A major impetus to progress is the need to meet international targets to use biofuels for road transport and to reduce GHG emissions within an agreed timeframe. The success of the industry will depend in part on governments creating the enabling conditions for meeting the targets, and Lywood and Pinkney (2) suggest that this will be easier in Europe for bio-ethanol than for biodiesel. In Australia, sustainability will depend on re-examination of the criteria and indicators of standards for biofuels (Braid, 25). Establishment of a DDGS industry in Western Canada will have to be done against the backdrop of cheap imports from the USA and is unlikely to succeed unless public and private bodies work together (Christensen *et al.*, 26).

TABLE 4
A summary of researchable topics to complement current knowledge relating to distillers grain

Nutritional value of DDGS	Reduction of variability in batches of DDGS produced in the same plant and between plants Linking of chemical and physical characteristics of distillers grain co-products to better define energy values and amino acid profiles Use of infrared technology to evaluate DDGS Assessment of micronutrients and vitamins in DDGS (shortages and excesses of both) Nutritional comparisons between DDGS and WDGS Effects of maize oil extraction on the feeding value of DDGS	1, 3, 4, 5, 7, 9, 10, 26
Storage of DDGS	Role of antioxidants to prevent the growth of moulds and mycotoxins, especially in hot and humid conditions and where long-term storage of DDGS is likely	3
Environmental issues of WDG	An assessment of the reduction of negative environmental effects of wet DG used in feedlots, including water and electricity usage, especially compared with production of DDG Carbon footprints of livestock feeds, including cost of transport LCA studies on the use of co-products of biofuel industry as livestock feed	2, 4, 7, 9, 11, 12, 25
Dietary inclusion rates of DDGS	Appraisal of nutritional strategies to increase inclusion rates of DDGS in diets for livestock and poultry, while maintaining product quality	3, 10, 11
Higher added value co-products	Development and refinement of technology protocols for animal feeds, leading, for example, to a system of product warranty The production of yeast from sugar cane-based vinasse	15, 26
Associative effects of feeds	Interacting factors between elements of the diet including DMI, forage type and inclusion level, age and class of animal to be fed Forage replacement values of DGS, information particularly needed within the dairy sector	5, 7
DDGS in pig and poultry nutrition	Effects of feed processing techniques on energy and fibre digestibility Reduction in dietary fibre to enhance the CP content of the feed Effects of addition of enzymes on DDGS utilization Effects of DDGS on the immune system Impact of wheat DDGS on gut health Evaluation of new products resulting from improved fractionation in the ethanol manufacturing process	9, 10, 21, 26
DDGS in aquaculture	Standardization of product quality of DDGS as feed for fish Reduction of fibre levels in DDGS to improve digestibility Flowability of product needed in transport, storage and diet preparation, processes often involving use of augers Development of processing techniques specific for aquaculture, with adequate consideration of health and safety issues Product testing of new co-products coming on stream	23
Anti-nutrients in DG and the use of additives	Tannin concentrations (in sorghum WDG especially) and their impact on productivity, and possible harmful effects of mycotoxins in the diet The addition of probiotics and feed additives needs assessing	4
Effects of Maillard reaction	Understanding of Amadori compounds, especially how they affect both the destruction and unavailability of lysine	9
Hydrogen sulphide	The synthesis, nutritional and environmental factors needed to understand cellular and physiological effects of H ₂ S The role of diet composition and environmental strategies leading to better diagnosis and treatment for PEM	6
Wider use of DDGS	Evaluation of DDGS for use in aquaculture and in the diets of domestic pets, horses and rabbits	3
Distillers co-products	Assessment of nutraceutical properties of distillers co-products in respect of their role in human health and nutrition	3

Notes: Numbers in column 3 denote chapter numbers in this book. For a list, see Appendix 1. DDGS = dried distillers grain with added solubles; WDGS = wet distillers grain with added solubles; DG = distillers grain; DDG = dried distillers grain; LCA = life cycle analysis; DMI = dry matter intake; DGS = distillers grain with added solubles; CP = crude protein; WDG = wet distillers grain; PEM = polioencephalomalacia

TABLE 5
A summary of researchable topics to complement current knowledge relating to co-products from feedstocks other than cereals

Sugar cane bagasse	Economic analysis and feasibility studies to incorporate bagasse into appropriate livestock feeding systems	16
Castor cake as livestock feed	Detoxification (removal of ricin) before feeding of castor cake Promotion of castor cake as valuable protein source through linking of laboratory and field trials and collaboration with the feed supply industry	20
Oil palm by-products	Use of specialty fats, produced from oil palm, as feed for dairy cattle, poultry, swine and aquaculture Commercial applications, local use and export opportunities for oil palm by-products Use of co-products from oil palm and other locally occurring crops to develop a livestock industry based on currently non-domesticated livestock species (e.g. collared peccary)	13, 14
Rapeseed cake	Feeding rapeseed cake to pigs and poultry to best advantage; levels of inclusion, influence of the processing conditions on variation in nutritive value and the reduction of glucosinolates	11,
Glycerine (livestock)	Removal of methanol from glycerine which is injurious to livestock health Understanding of the mode of action and optimum inclusion levels of glycerine as a dietary energy source, and the role of glycerine feeding in the control of pathogens (e.g. <i>E. coli</i>). Effects of residual glycerine in distillers grain on fibre digestion	8, 11
Glycerine (aquaculture)	Recommendations for levels for feeding crude glycerine to fish Variability of product needs reducing Assessment of potential problems from the presence of residual methanol Assessment of long-term effects on fish health and the quality of the meat produced Processing, handling and storage of glycerine to be used in fish diets	21, 23
Lipid co-products and toxicity of unconventional seed meal	Examination of biodiesel lipid co-products for the presence of compounds toxic to animals and humans Development of methods for selective removal of primary toxins from <i>Pongamia glabra</i> and <i>Azadirachta indica</i> , both potential sources of feed protein, leading to an industrial process for detoxification Adequate testing of the efficiency of the detoxification process selected on the feeds, and also of the animal product resulting from their use before promotion on-farm Development of a detoxification processes for non-edible oil seed meals, including improvement of procedures that currently exist, and up-scaling where appropriate (these studies need relating to socio-economic analysis) Development of protein isolates and peptides to assist in eliminating toxins and other antinutritional factors	18, 19, 22
Development of micro-algae	Selection of the best organisms, together with sustainable culture methodologies, including use of marginal land, coastal areas, sea surfaces, etc., to minimize conflict with land-based resources Assessment of co-products from micro-algae, both for their feeding value and commercial application (potential use in diets for livestock, poultry and aquaculture)	24
Camelina meal for poultry	Nutritional value assessment of camelina meal for poultry of all age groups whether for meat or egg production Assessment of the need for additional enzymes The impact of camelina meal on meat quality Investigation of techniques for enhancing the nutritional value of camelina meal	17

Notes: Numbers in column 3 denote chapter numbers in this book. For a list, see Appendix 1.

TABLE 6
A summary of researchable topics to complement current knowledge and having relevance to the use of co-products as feed for livestock, poultry and fish

Effluent handling	Development of methods to reduce effluents from processing plants and that are suitable for both large and small-scale operations Conversion of vinasse into biogas (to be used as a source of energy and fertilizer) Identification and validation of flocculants and agglomerants	15
Decentralized systems suitable for groups of small-scale farmers in India	Identification of crops to extend the period of use of processing plants (currently one crop per year is processed) Identification of multi-purpose crops to meet household and livestock requirements Juice extraction and syrup conversion needs to be more efficient Improvement of quality of syrup produced Extension and training at all levels	12
Assessment of improved production methods, improved co-products and co-products resulting from new and unconventional feedstocks	Testing of new and unconventional feedstocks, developed from improved production Testing of new co-products leading to changing end uses Life cycle analysis of the use of these products required coupled with traditional nutritional appraisal Understanding of interactions between cropping, grazing and bio-energy production Nutritional assessment of co-products should be linked to studies on animal health and feed safety in livestock and poultry Effects of feeding new or enhanced co-products on milk quality	3, 5, 7, 25, 27
Marketing	Evaluation of: nutrient management costs; indicators for import and export criteria; differences between feedstocks; full economic appraisal encompassing field costs; and the net value of biofuel and co-product Understanding of associative relationships between traditional feeds and co-products is not understood and needs clarifying, supported by up to date information on production	1, 7, 26

Notes: Numbers in the body of the table denote chapter numbers in this book. For a list, see Appendix 1.

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Appendix 1

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G. Cooper and J.A. Weber
- 2 An outlook on EU biofuel production and its implications for the animal feed industry
W. Lywood and J. Pinkney
- 3 Impact of United States biofuels co-products on the feed industry
G.C. Shurson, H. Tilstra and B.J. Kerr
- 4 Utilization of wet distillers grains in high-energy beef cattle diets based on processed grain
M.L. Galyean, N.A. Cole, M.S. Brown, J.C. MacDonald, C.H. Ponce and J.S. Schutz
- 5 Utilization of feed co-products from wet or dry milling for beef cattle
G.E. Erickson, T.J. Klopfenstein and A.K. Watson
- 6 Hydrogen sulphide: synthesis, physiological roles and pathology associated with feeding cattle maize co-products of the ethanol industry
J.P. Schoonmaker and D.C. Beitz
- 7 Feeding co-products of ethanol and biodiesel to dairy cattle
K.F. Kalscheur, A.D. Garcia, D.J. Schingoethe, F. Diaz Royón and A.R. Hippen
- 8 Utilization of crude glycerin in beef cattle
J.S. Drouillard
- 9 Nutritional value and utilization of wheat dried distillers grain with solubles in pigs and poultry
J. Noblet, P. Cozannet and F. Skiba
- 10 Use of distillers grain co-products and crude glycerol in swine diets
G.C. Shurson, R.T. Zijlstra, B.J. Kerr and H.H. Stein
- 11 Co-products from biofuel production for farm animals – an EU perspective
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- 12 Opportunities and challenges in utilizing co-products of the sweet sorghum-based biofuel industry as livestock feed in decentralized systems
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- 13 Utilization of oil palm co-products as feeds for livestock in Malaysia
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- 15 Sustainable and competitive use as livestock feed of some co-products, by-products and effluents generated in the bio-ethanol industry
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- 21 Use of detoxified jatropha kernel meal and protein isolate in diets of farm animals
H.P.S. Makkar, V. Kumar and K. Becker
- 22 Use of *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes for feeding livestock
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- 24 Cultivation of micro-algae for lipids and hydrocarbons, and utilization of spent biomass for livestock feed and for bio-active constituents
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- 25 Land use in Australia for biofuels and bio-energy: opportunities and challenges for livestock industries
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- 27 Biofuels: their co-products and water impacts in the context of life-cycle analysis
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T. Smith and H.P.S. Makkar

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During the past 13 years his research programme has focused primarily on evaluating the nutritional value of dried distillers grains with solubles in swine diets. He has developed an extensive network of collaborators, including researchers at other universities, in various industries and in international research communities. His research work has resulted in numerous scientific publications and presentations to national and international audiences. He works closely with the U.S. Grains Council to provide educational programmes and assess export market opportunities for DDGS. He also serves as a consultant for a wide variety of ethanol and feed companies and organizations, as well as government agencies.

Fabien Skiba is in charge of the Nutritional Value Unit at Arvalis-Institut du végétal, France. With his team, he is working to improve knowledge on the value of pulses and of cereals and their co-products for pigs and poultry. In recent years he has supervised several experiments on wheat DDGS, including those of the PhD project of Pierre Cozannet and also with European ethanol producers.

T. Smith After college, Tim first went to Africa as a volunteer in the sixties, helping establish a village in Tanzania. He then joined the National Dairy Research Institute at Reading, where work on poor quality forages was the subject of his PhD. In 1986 he joined a World Bank team in Zimbabwe, where he addressed aspects of ruminant nutrition affecting resource-poor smallholder crop-livestock farmers. He was also head of Matopos Research Station in Zimbabwe. Since 1996 he has been involved with a number of livestock-related projects in several African and Asian countries. He has also acted as a consultant for a number of international organizations such as the International Atomic Energy Agency (IAEA), Vienna, Austria and the Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.

S. Smyth is a Research Scientist at the University of Saskatchewan, Canada. He received his PhD from the University of Saskatchewan in 2005, and his research has focused on how societies regulate innovation. The focus of this research is on the innovation of agricultural biotechnology. Dr Smyth is part of a group of academics that received \$CAN 5.4 million in funding in 2009 from Genome Canada to examine the genomic, economic, environmental, ethical, legal and social (GE³LS) issues pertaining to bio-products and biofuels. In addition to this, Dr Smyth is leading the GE³LS component on two other Genome Canada-funded projects that have a combined value of \$CAN 22.5 million. Much of his recent research has focused on marketplace liabilities created by innovation, which has been compiled in a 2010 book—*Innovation and Liability in Biotechnology: Transnational and Comparative Perspectives*—published by Edward Elgar.

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Harold Tilstra is a 1975 graduate of the College of Veterinary Medicine, University of Minnesota, USA. Dr Tilstra's current position with Land O' Lakes Purina Feed LLC includes supervising several swine feed sales consultants, organizing swine feed sales training programmes, and coordinating national and international technical support for distillers grain utilization and marketing. He represents Land O' Lakes Purina Feed LLC as a delegate to the US Grains Council and as a director on the

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Michael Wang is the manager of the Systems Assessment Group of the Center for Transportation Research (CTR) at Argonne National Laboratory, USA. Dr Wang's research areas include the evaluation of energy and environmental impacts of advanced vehicle technologies and new transportation fuels, including biofuels. Dr Wang's accomplishments include the development of Argonne's GREET (Greenhouse gases, Regulated Emissions, and Energy use in Transportation) software model for life-cycle analysis of advanced vehicle technologies and new fuels. At present, GREET has more than 15 000 registered users worldwide. Dr Wang's research and the GREET model have been used by governmental agencies in North America, Asia and Europe to develop transportation fuel policies, such as low-carbon fuel standards and vehicle greenhouse gas emission regulations.

Andrea K. Watson is a research technician and PhD student in Animal Science at the University of Nebraska-Lincoln, USA. She assists faculty members in coordinating research projects involving the utilization of ethanol co-products by the cattle industry.

J. Alan Weber is a founding partner of MARC-IV, a consulting company that specializes in the development of industrial products from agricultural resources. Active with biodiesel commercialization activities since 1991, Mr Weber assisted with the establishment of the National Biodiesel Board's (NBB) Washington, D.C., office in 2006–07 and continues to provide economic and technical support to NBB efforts. He currently leads industry feedstock development efforts to increase raw material sources such as algae, jatropha, minor oilseeds (winter canola and camelina), halophytes and traditional oilseed commodities. In addition to his activity with MARC-IV, Mr Weber is actively engaged with the management and operation of an 875-acre family farm in central Missouri. Weber is a recipient of the 2007 NBB Outstanding Service Award and the NBB Industry Outstanding Commitment Award in 2000. He completed his undergraduate and graduate training in agricultural economics at the University of Missouri, USA.

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Climate change and predicted shortages of fossil fuels present major challenges. Currently, biofuel production is from agricultural crops grown primarily on arable land. Conflict with the traditional use of arable land, itself a limited resource, to produce food and animal feed must be avoided and economic sustainability assured. At present cereals, especially maize and wheat, and sugar cane are used for ethanol production, with soybean, oil palm and rapeseed for biodiesel production.

The expanding transport industry requires increasing amounts of biofuels, and an increasing market for co-products has generated a need for new feedstocks. Cellulosic material, often available from sub-prime land with minimal inputs, and other non-conventional sources are being investigated. Before being used as feeds, some seeds and cakes will require detoxification. The contribution of micro-algae, production of which can be achieved in coastal waters, is likely to grow in importance. These developments are mirrored the broadening of the animal species receiving the co-products, from ruminants, especially cattle, and pigs to poultry and fish (aquaculture). Further developments include enhancement of the use of existing co-products and the introduction of new ones.

This publication collates, discusses and summarizes state-of-the-art knowledge on the use as livestock feed and future availability of co-products from the biofuels industry. The levels at which the co-products could be safely used in livestock diets are also presented. Throughout the book, gaps in knowledge and research topics needed to address them have been identified. These include standardization of product quality to assist ration formulation; testing of new products; development of detoxification procedures; research on micro-algae; and life cycle analysis linked to traditional nutritional appraisal.

This publication covers a wide array of co-products and is a timely contribution, as people's aspirations are rising, evident from the increasing demand for livestock products and an ever greater reliance on transport, coupled with the challenge of maintaining agricultural production when faced with global warming. We hope that the information here synthesized will be useful to policy-makers, researchers, the feed industry, science managers and NGOs, supporting them in making information-based decisions on issues such as food-feed-fuel competition. Hopefully it will help confront the emerging challenges of global warming, in addition to making efficient use as livestock feed of a wide range of currently available and future co-products from the biofuel industry.

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