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-
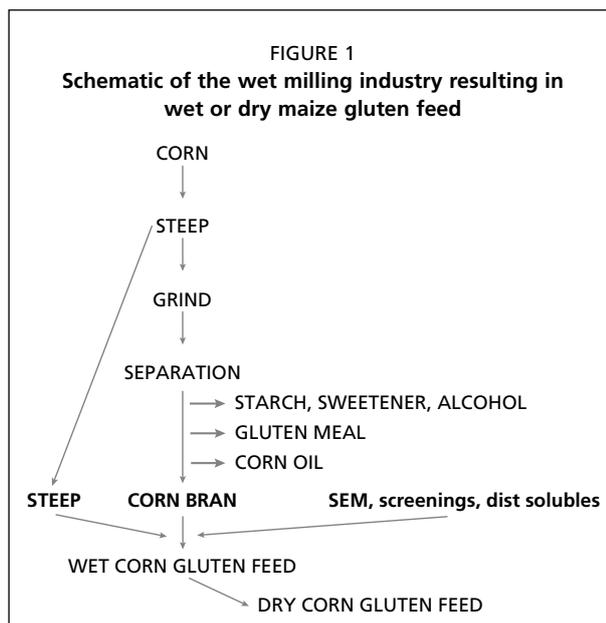
tle maize residue, almost no fat, and is high in protein from the remnants of yeast cells from the fermentation process. The distillers solubles and a portion of the steep liquor are added to the bran fraction of the maize resulting in WCGF. The WCGF can have a portion of the germ meal added if the plant 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).
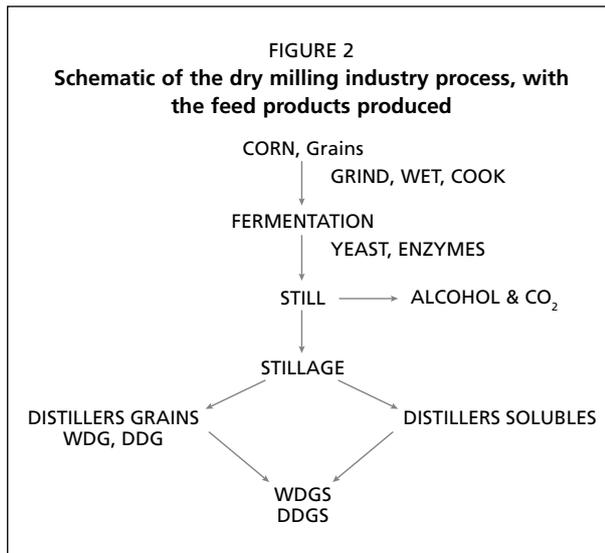
Wet CGF contains 16 to 23 percent CP, of which about 70 percent is degraded in the rumen (degradable intake protein, DIP) and used by rumen microbes. During wet milling, maize gluten meal is removed and marketed in higher value markets. Maize gluten meal should not be confused with WCGF because they 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 CO_2 (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.

FIGURE 1
Schematic of the wet milling industry resulting in wet or dry maize gluten feed

CORN
↓
STEEP
↓
GRIND
↓
SEPARATION
→ STARCH, SWEETENER, ALCOHOL
→ GLUTEN MEAL
→ CORN OIL

STEEP   CORN BRAN   SEM, screenings, dist solubles
↓
WET CORN GLUTEN FEED
↘ DRY CORN GLUTEN FEED

FIGURE 2
Schematic of the dry milling industry process, with the feed products produced

CORN, Grains
| GRIND, WET, COOK
FERMENTATION
| YEAST, ENZYMES
STILL ⟶ ALCOHOL & CO_2
|
STILLAGE

DISTILLERS GRAINS   DISTILLERS SOLUBLES
WDG, DDG

WDGS
DDGS

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

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

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 = phosphorous, 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		
Alfalfa level	0	3.75	7.50	0	3.75	7.50
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 × 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)

	Treatment (see notes)						
	Control	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 × 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)
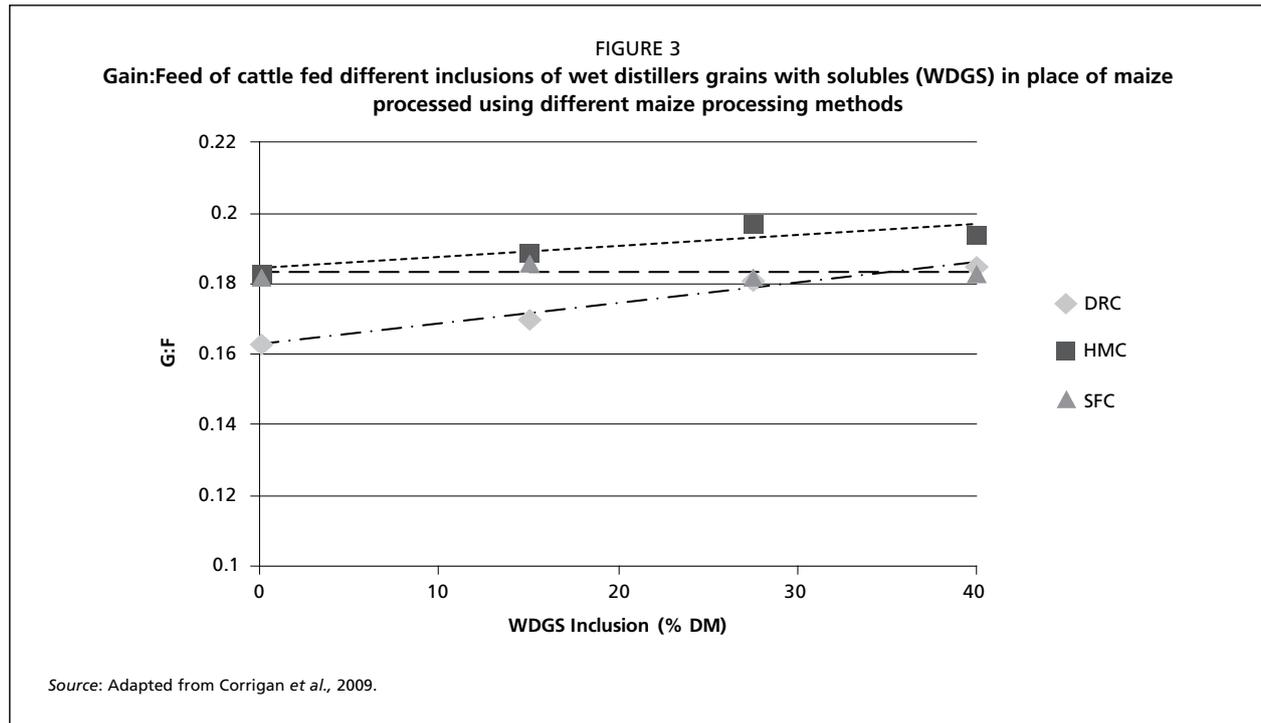
	Processing method (sse notes)			
	25% WCGF			
	GHMC	**DRC**	**RHMC**	**SFC**
ADG (kg)	1.93	1.92	1.91	1.97
G:F	0.198 b	0.182 c	0.195 b	0.204 a
NEg (maize) (Mcal/kg)	1.71	1.54	1.68	1.77
Faecal starch (%)	8.4 b	19.2 c	10.6 ab	4.1 a
	32% WCGF with calves			
	Whole	**DRC**	**RHMC**	**SFC**
ADG (kg)	1.9	1.93	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.

FIGURE 3
Gain:Feed of cattle fed different inclusions of wet distillers grains with solubles (WDGS) in place of maize processed using different maize processing methods

Source: Adapted from Corrigan *et al.,* 2009.

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 (H_2S) 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 2×2×3+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 H_2S 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 outcompete 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.
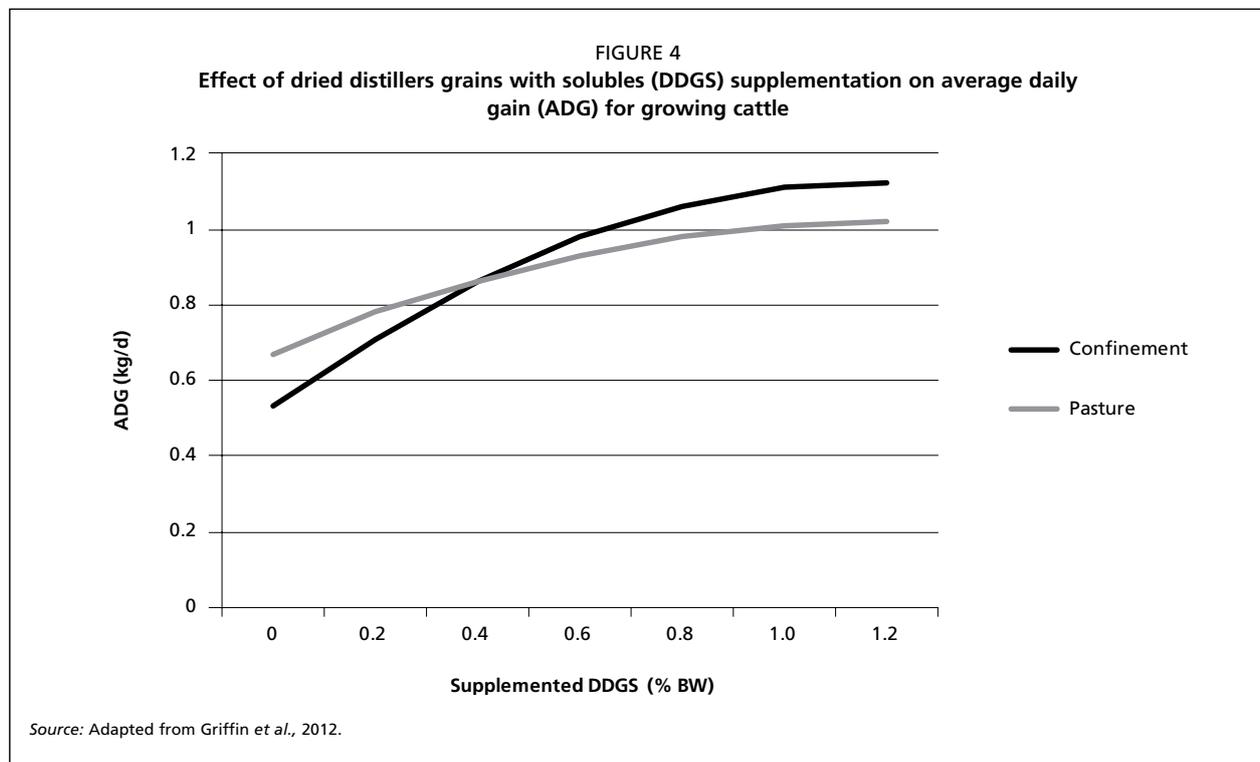
An analysis of 14 separate grazing trials in which cattle were supplemented with DDGS was conducted by Griffin *et al.* (2012) to determine effects of supplementation on ADG and final BW in pasture grazing situations. Additionally, pen studies were evaluated to determine the effects of DDGS supplementation 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

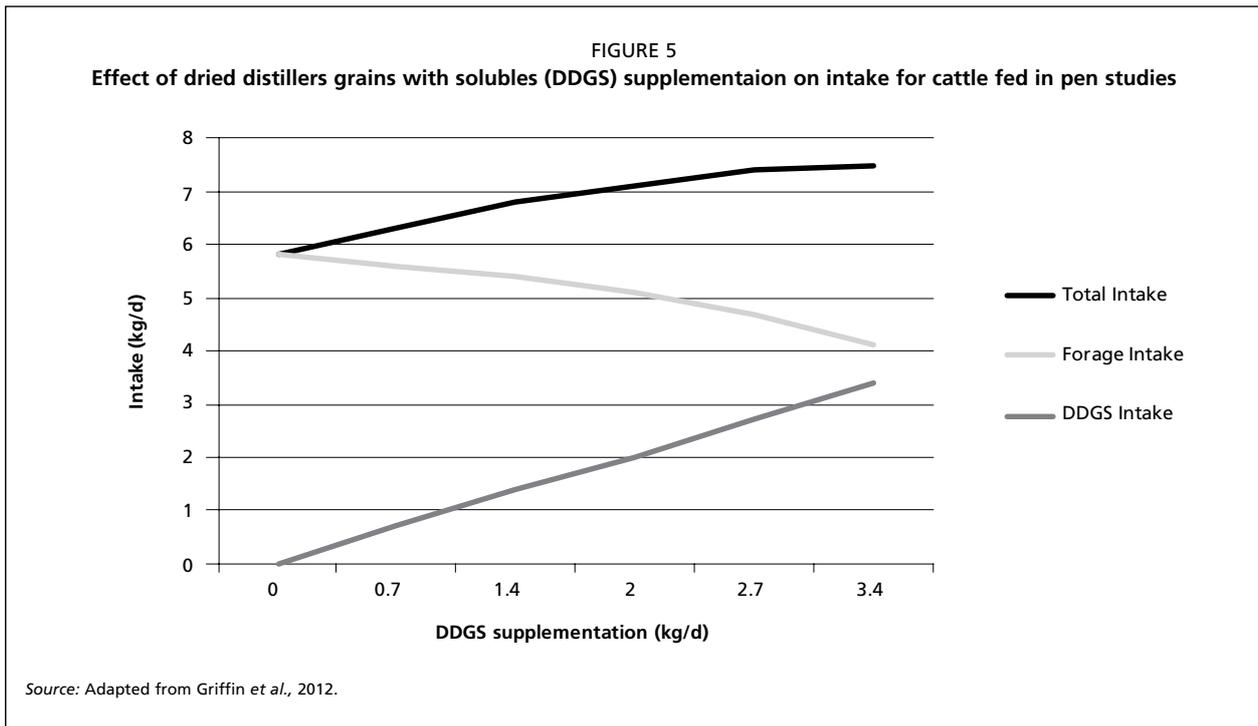| | Diet | | | | | F-Test | |
	0	33	67	100	133	SEM	*P*-value
Individually fed							
Initial BW (kg)	278	278	280	280	279	5	0.99
Final BW (kg)	315	317	309	319	319	7	0.85
ADG (kg)	0.48	0.47	0.42	0.46	0.47	0.03	0.77
DMI (kg/day)	5.1	5.2	5.2	5.2	5.2	0.09	0.95
G:F	0.200	0.185	0.167	0.185	0.189	0.004	0.54
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.

FIGURE 4
Effect of dried distillers grains with solubles (DDGS) supplementation on average daily gain (ADG) for growing cattle

Source: Adapted from Griffin *et al.*, 2012.

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-

FIGURE 5
Effect of dried distillers grains with solubles (DDGS) supplementaion on intake for cattle fed in pen studies

Source: Adapted from Griffin *et al.,* 2012.

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 Galyean, 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 Galyean, 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 higher 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			
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 CO_2eqv/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. Galyean 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 H_2S 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 (H_2S) rivals cyanide in toxicity. Endogenous H_2S 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, H_2S functions as a gaseous signalling molecule in animal tissues. At high concentrations, H_2S 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 H_2S by ruminal bacteria, eructated, and then inhaled by the animal, thus bypassing liver detoxification. In contrast, H_2S 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 H_2S production in the rumen, development of diagnostic tools, and development of practical approaches to alleviation of the symptoms of H_2S 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 H_2S all may be present in the ruminant animal. Elemental S, sulphates, and sulphuric acid are relatively non-toxic. However, H_2S can be highly toxic at high concentrations, particularly when the H_2S catabolizing systems of the liver and kidney are bypassed. At low concentrations, H_2S functions as a signaling molecule in animal tissues (Kabil and Banerjee, 2010). At high concentrations, H_2S 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

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MAIN MESSAGES

- Co-products of the ethanol industry are high in sulphur.
- H_2S is produced by sulphate-reducing bacteria in the rumen of cattle.
- H_2S is a signal molecule in animal tissues.
- H_2S has significant effects in several tissues.
- H_2S chemically reacts with metalloproteins and oxidized cysteine residues of proteins to exert its biological effects.

- H_2S, when produced in excess, causes polioencephalo-malacia in cattle.
- Cattle seem to vary in their susceptibility to H_2S toxicity.
- H_2S mitigation strategies are currently being investigated and can decrease H_2S 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 H_2S 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 H_2S, 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 tetrathiomolybdate. 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 seco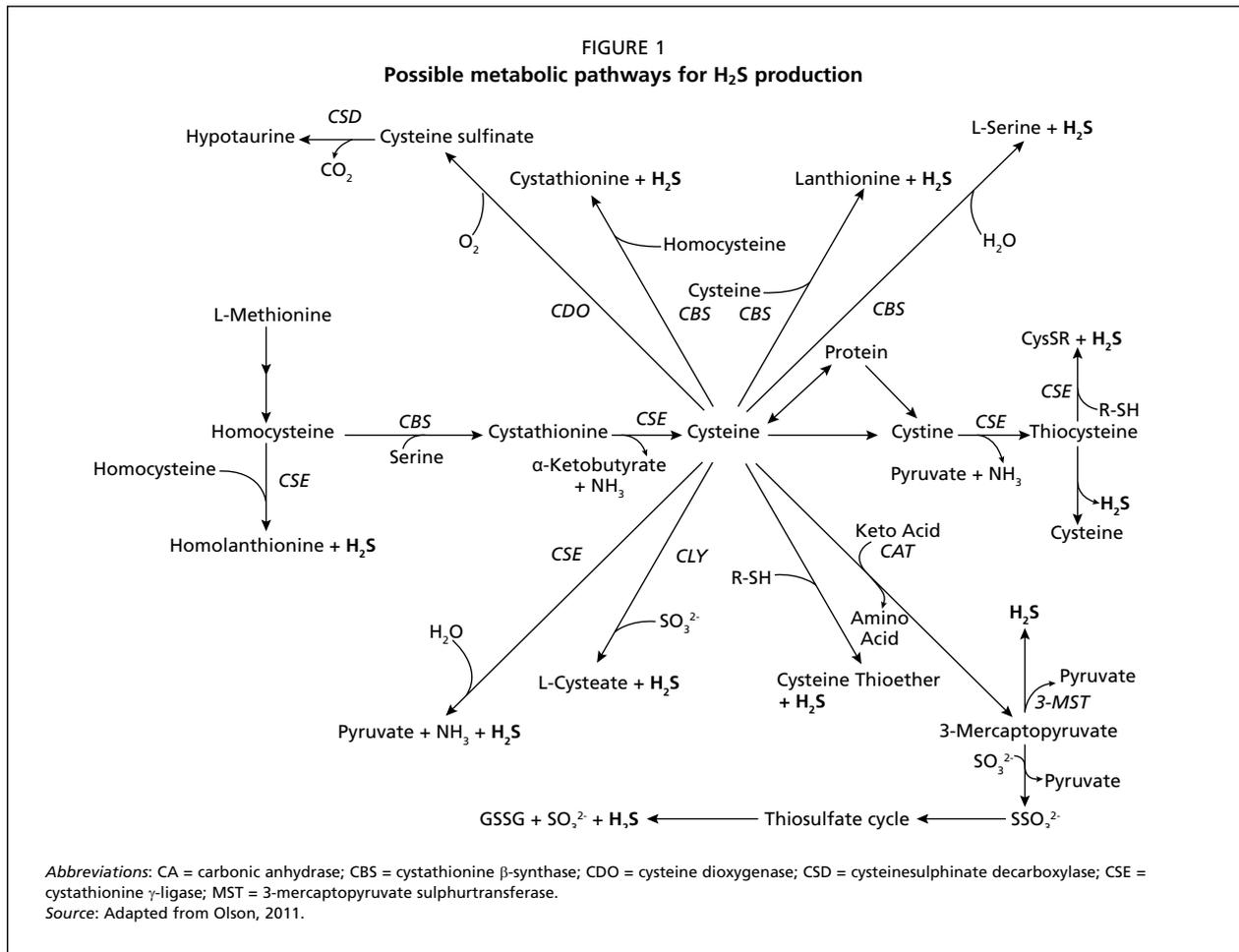ndary copper deficiency can result in impaired reproduction and performance (NRC, 1996). Gould (1998) also reported that the bio-availability of other minerals, particularly iron and zinc, may be limited because of the formation of insoluble salts with sulphide. Availability of selenium also may be limited 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 H_2S and its ionic forms, which are highly toxic substances that interfere with cell respiration (Beauchamp, Bus and Popp, 1984; Bray, 1969; Kandylis, 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 H_2S 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 H_2S 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 H_2S (Figure 1; Olson, 2011). Cysteine may be catabolized by several biochemical pathways involving transsulphuration or oxidation reactions to generate H_2S. As shown in Figure 1, the cysteine may be derived from methionine as a donor of the S. The biogenesis of H_2S 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

FIGURE 1
Possible metabolic pathways for H₂S production

Abbreviations: CA = carbonic anhydrase; CBS = cystathionine β-synthase; CDO = cysteine dioxygenase; CSD = cysteinesulphinate decarboxylase; CSE = cystathionine γ-ligase; MST = 3-mercaptopyruvate sulphurtransferase.
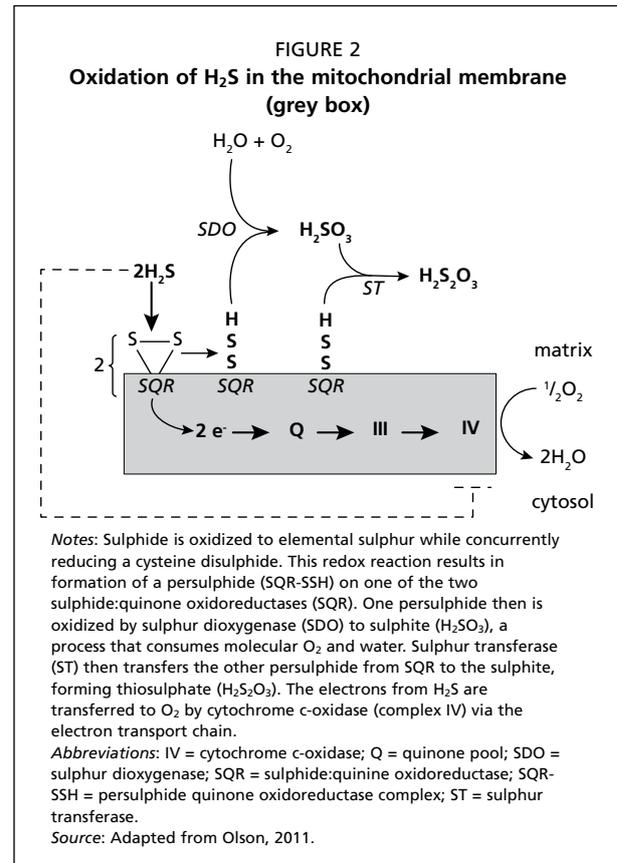*Source*: Adapted from Olson, 2011.

(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 cysteinesulphinate 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 in 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*, *Butyvibrio* 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 H_2S. Organic forms of sulphur, such as those found in amino acids, are not readily available in the rumen for production of H_2S, whereas inorganic forms of sulphur (e.g. sulphuric acid and sulphur salts) are more readily available for production of H_2S. Calculating rumen degradable sulphur intake was able to explain 64.9 percent of the H_2S 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 H_2S 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^-, HSO_3^-, S^{2-} and S^0 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 H_2S, which has a pKa value for first and second dissociation steps of 7.04 and 11.96, respectively. One third of H_2S 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 H_2S present in the rumen also increases. With a change of pH from 6.8 to 5.2, the percentage of H_2S 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 H_2S concentrations in the gas phase and induce clinical symptoms of H_2S 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; Uwituze *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 H_2S production in the rumen, although rumen pH is actually slightly increased when sulphur content of the diet is increased (Uwituze *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 (Uwituze *et al.*, 2011b). Further H^+ ions, in the form of H_2S, are eructated, which further relieves rumen acidity. As such, strategies that buffer H^+,

FIGURE 2
Oxidation of H_2S in the mitochondrial membrane (grey box)

Notes: Sulphide is oxidized to elemental sulphur while concurrently reducing a cysteine disulphide. This redox reaction results in formation of a persulphide (SQR-SSH) on one of the two sulphide:quinone oxidoreductases (SQR). One persulphide then is oxidized by sulphur dioxygenase (SDO) to sulphite (H_2SO_3), a process that consumes molecular O_2 and water. Sulphur transferase (ST) then transfers the other persulphide from SQR to the sulphite, forming thiosulphate ($H_2S_2O_3$). The electrons from H_2S are transferred to O_2 by cytochrome c-oxidase (complex IV) via the electron transport chain.
Abbreviations: IV = cytochrome c-oxidase; Q = quinone pool; SDO = sulphur dioxygenase; SQR = sulphide:quinine oxidoreductase; SQR-SSH = persulphide quinone oxidoreductase complex; ST = sulphur transferase.
Source: Adapted from Olson, 2011.

such as addition of forage or monensin, have been shown to competitively inhibit H_2S production and improve feed intake (Felix and Loerch, 2011).

Sulphide is readily absorbed through the rumen wall into the blood stream (Bray, 1969). Protonated H_2S, however, is not absorbed across the rumen wall (NRC, 2005). Catabolism of H_2S seems to be ubiquitous is animal tissues with the exception of brain (Lagoutte *et al.*, 2010). Oxidation of H_2S 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 H_2S 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 H_2S 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 H_2S 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.
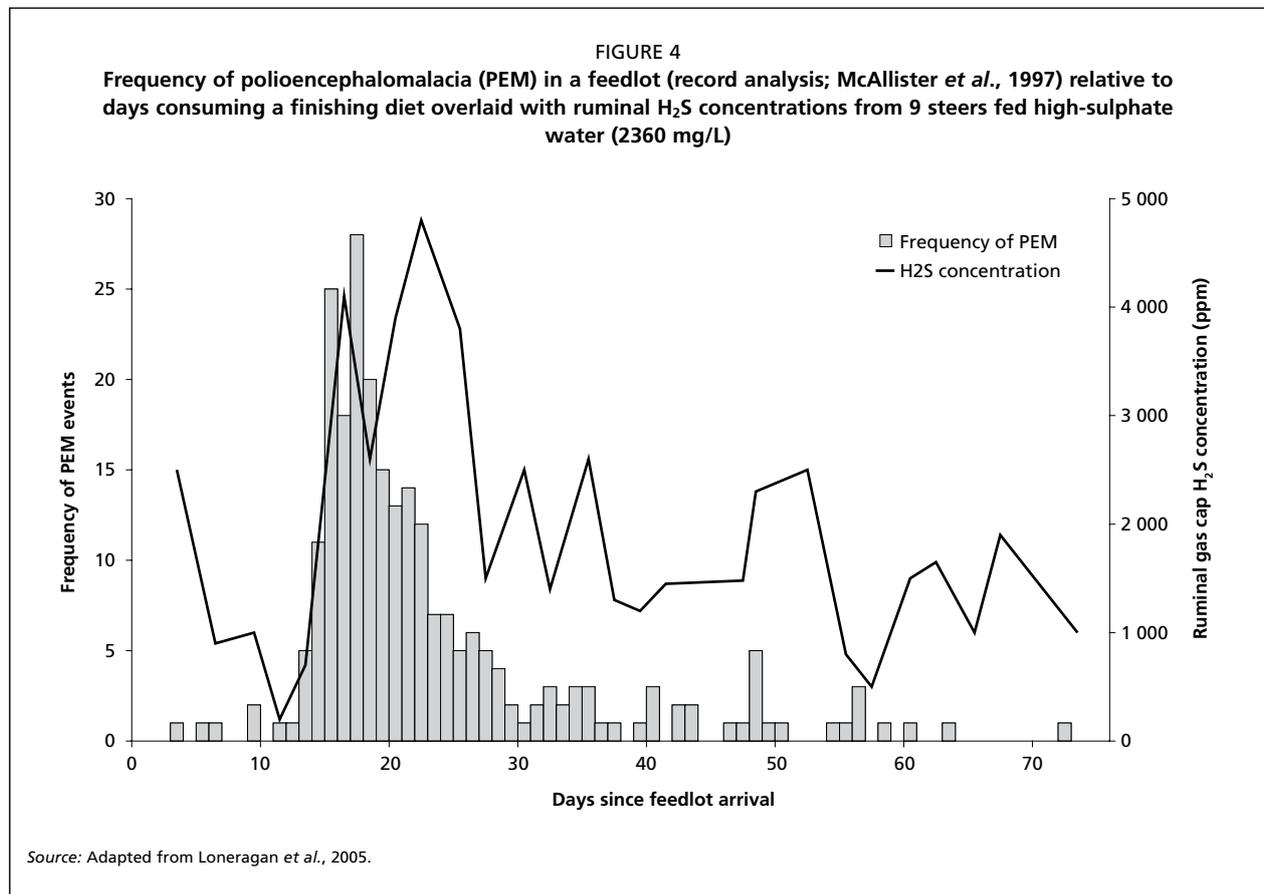

FIGURE 3
Proposed mechanism for high-sulphate-induced polioencephalomalacia (PEM)

Source: Adapted from Kung *et al.*, 1998.
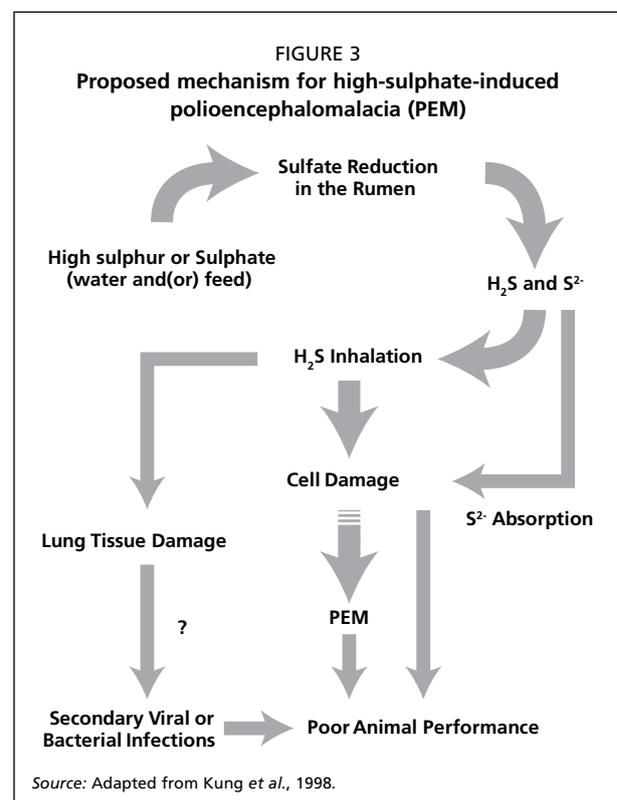
Hydrogen sulphide relaxes smooth muscles from the stomach through the colon (Olson, 2011). Moreover, H_2S is thought to have anti-inflammatory effects in the colon because it enhances ulcer healing independent of nitric oxide synthase and ATP-sensitive K channel involvement (Olson, 2011). Further, in model systems, H_2S protects against and promotes healing in colitis (Olson, 2011). In contrast, excessive sulphate entering the lower gastro-intestinal 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 H_2S 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 H_2S. 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 H_2S 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 H_2S 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 H_2S (Figure 4; McAllister *et al.*, 1997; Loneragan *et al.*, 2005). After this peak, H_2S 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

FIGURE 4
Frequency of polioencephalomalacia (PEM) in a feedlot (record analysis; McAllister *et al.*, 1997) relative to days consuming a finishing diet overlaid with ruminal H_2S concentrations from 9 steers fed high-sulphate water (2360 mg/L)

Source: Adapted from Loneragan *et al.*, 2005.

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 H_2S 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 H_2S 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; Uwituze *et al.*, 2011b), which may compete with H_2S for H^+, effectively lowering ruminal H_2S 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 micro-organisms, however, is unclear.

Thiamine and PEM

The lack of adequate dietary thiamine will inhibit thiamin-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 H_2S, 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, bambermycin, lasalocid, chlortetracycline and oxytetracycline, as well as an experimental compound, anthraquinone, on sulphide production *in vitro*. Anthraquinone, bambermycin, chlortetracycline, oxytetracycline and lasalocid all decreased *in vitro* H_2S production, with the greatest decreases occurring with anthraquinone, chlortetracycline and oxytetracycline (Kung, Bracht and Tavares, 2000). The effect of these compounds on *in vivo* H_2S production are

unclear. *In vitro* studies evaluating the effect of monensin on H_2S production have been inconclusive. Some researchers observed no change in *in vitro* H_2S 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* H_2S 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 H_2S and S^{2-} concentrations when diets containing 0.5 percent S were fed (Felix *et al.*, 2011).

Inclusion of molybdate successfully inhibits H_2S 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 H_2S production. Cross, Rust and Powers (2010), however, demonstrated that the addition of 60 ppm copper and 6 ppm molybdenum did not decrease *in vivo* H_2S 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 H_2S concentration in feedlot cattle (Kelzer *et al.*, 2010). Ferric ions also show promise as a strategy to decrease ruminal H_2S 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 H_2S 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 H_2S concentration in the rumen gas cap but did not improve feedlot performance by steers consuming high-sulphate water ($\geq$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 H_2S have only recently been elucidated, much information regarding the biochemistry and biology of H_2S remains to be determined. The typical concentrations of H_2S 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 H_2S 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 H_2S 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 H_2S production in the rumen. Much research, however, is needed to characterize the role of diet composition, and other environmental strategies to mitigate H_2S 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 H_2S are sufficient to exert the demonstrated effects. Metalloproteins and oxidized cysteine residues of proteins are postulated to serve as the target molecules for H_2S action in a cell. In fact, H_2S 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 H_2S toxicity may result in decreased average daily gain and feed efficiency; severe cases of H_2S 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 H_2S 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. Depenbusch *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 e*t 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 e*t 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; Bhatty, 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 similat 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

	Product			
	RFDDGS	**HPDDG**	**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 gem 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 feedstuff 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, ß-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 stud,y 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 Birkelo (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 trans-esterification 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 glycerin 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 pot-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 ± 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 ± 44.7 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 ± a standard deviation of 305 μm 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-expended 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 peroxyl 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	Day					SEM1
	0	3	7	14	129	
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 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 ×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 rumens 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 centrifugication, 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.

ACKNOWLEDGEMENTS

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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.
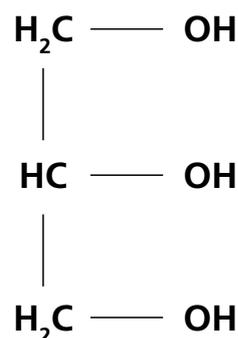
INTRODUCTION

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.
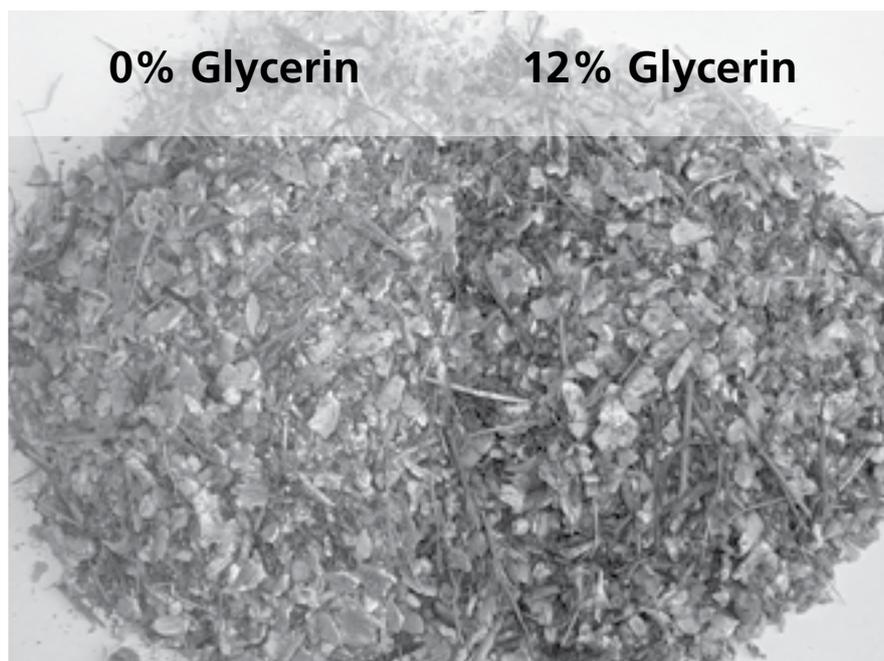

Photo 1
Effect of glycerin addition to a maize-based cattle finishing diet. Small particles are aggregated, minimizing segregation and dust

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.
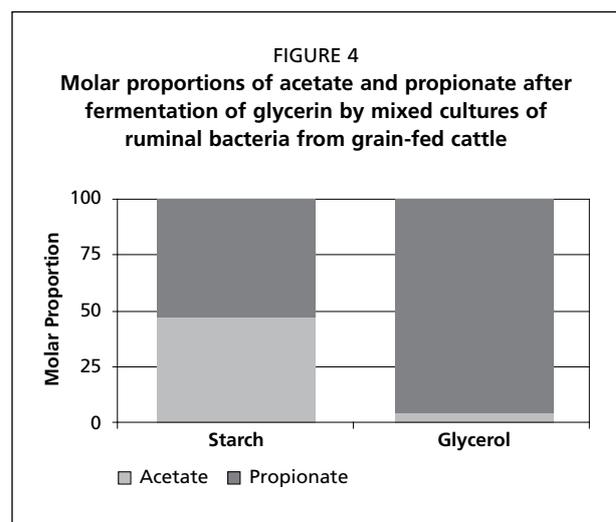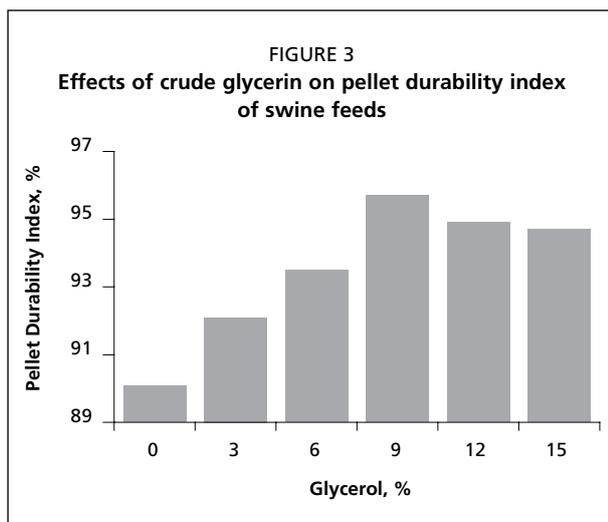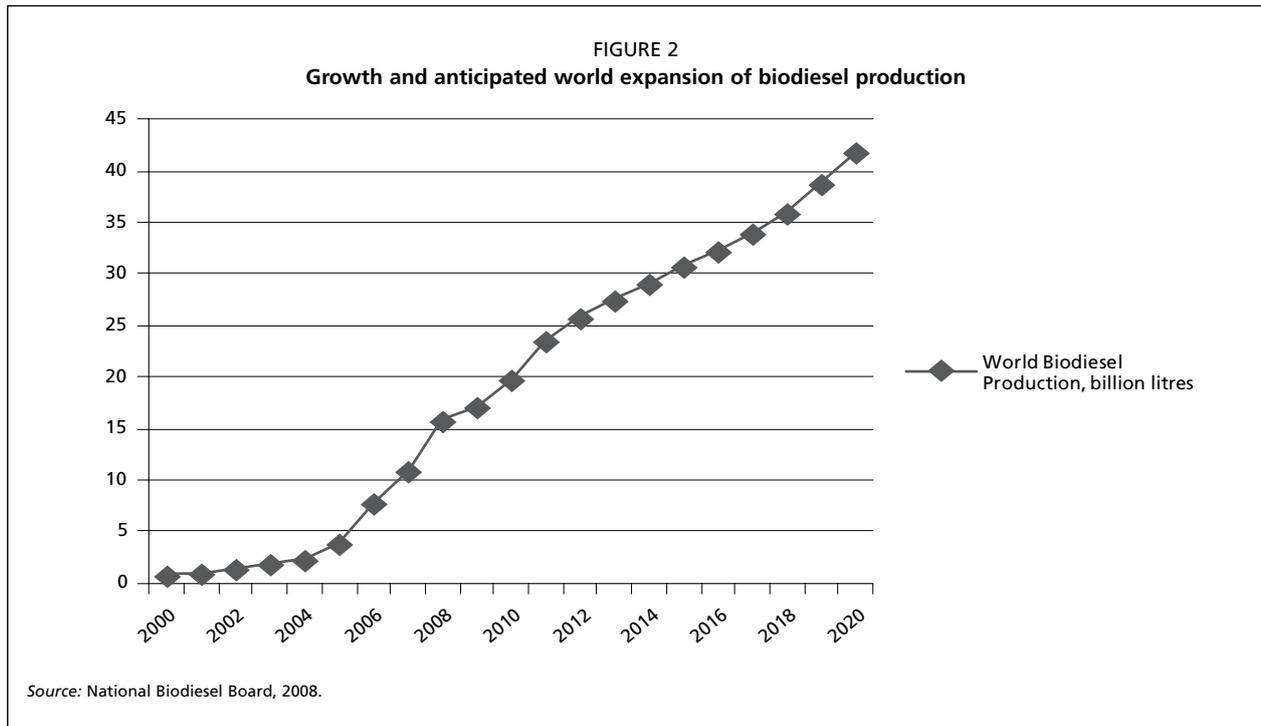
Figure 2 illustrates the recent dramatic growth in world biodiesel production and anticipated expansion through 2020 (OECD/FAO, 2011). Similar increases have been observed in the United States, resulting in large surpluses of crude glycerin that have caused market prices to plummet. The relatively low 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.

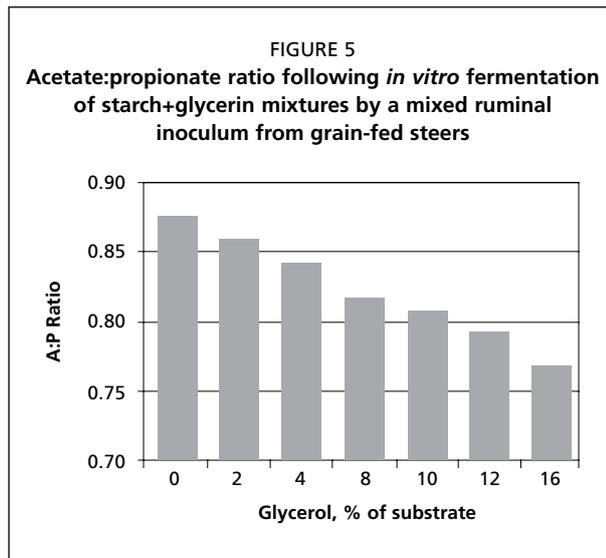## FERMENTATION BY RUMINAL MICROBES

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

FIGURE 2
Growth and anticipated world expansion of biodiesel production

Source: National Biodiesel Board, 2008.

FIGURE 3
Effects of crude glycerin on pellet durability index of swine feeds

FIGURE 4
Molar proportions of acetate and propionate after fermentation of glycerin by mixed cultures of ruminal bacteria from grain-fed cattle

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 ^{14}C-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

FIGURE 5
Acetate:propionate ratio following *in vitro* fermentation of starch+glycerin mixtures by a mixed ruminal inoculum from grain-fed steers

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 glycerin. 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. Aperce *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 gastro-intestinal 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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