Chapter 21
Use of detoxified jatropha kernel meal and protein isolate in diets of farm animals

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ABSTRACT

Jatropha curcas L. (physic nut) is a drought-resistant shrub or tree, which is widely distributed in wild or semi-cultivated areas in Central and South America, Africa, India, China and South East Asia. It is a hardy plant and thrives on degraded land. Jatropha kernels (de-shelled seeds) contain 55–60 percent oil that can be transformed into good quality biodiesel through transesterification and used as a substitute for diesel. The kernel meal obtained after oil extraction is an excellent source of nutrients and contains 60–66 percent crude protein; while jatropha protein isolate obtained from jatropha seed cake (residue obtained after mechanical pressing of the whole seeds) has about 81–85 percent crude protein. The contents of essential amino acids (EAAs) (except lysine) are higher in jatropha kernel meal than in soybean meal (SBM), and higher in jatropha protein isolate than soy proteins isolate. However, presence of toxic factors (phorbol esters) and anti-nutritional constituents (trypsin inhibitors, lectins and phytate) restricts the use of Jatropha meal and protein isolate in animal feed. Phorbol esters are the toxic compounds in *J. curcas*. Kernel meal and protein isolate from *J. curcas* have been detoxified. Another *Jatropha* species, *J. platyphylla* is free of phorbol esters and hence non-toxic; however, its seed kernels and kernel meal contain trypsin inhibitors, lectin and phytate. The kernel meal from *J. platyphylla* obtained after oil extraction contains 65–70 percent crude-protein. Detoxified *J. curcas* kernel meal (DJKM), heated *J. platyphylla* kernel meal (H-JPKM) and detoxified *J. curcas* protein isolate (DJPI) can replace 50, 62.5 and 75 percent of fishmeal protein, respectively, in fish diets without compromising their growth performance and nutrient utilization. In addition, DJKM could also replace 50 percent of fishmeal protein without adversely affecting growth and nutrient utilization in shrimp. Increased DJKM inclusion in diets (>50 percent replacement of fishmeal protein) caused a significant lowering of the digestibility of protein, lipid and energy. No such effects were observed when DJPI was used in fish diets. Feeding DJKM to common carp and H-JPKM to Nile tilapia did not change the energy budget (routine metabolic rate, heat released and metabolizable energy) compared with the fishmeal-fed group. No mortalities, unaffected haematological values and no adverse histopathological alterations in stomach, intestine and liver of fish suggested that they were in normal health.

DJKM has also been fed to turkeys with no significant difference in feed intake and weight gain compared with the SBM-containing diet, with feed efficiency (gain:feed ratio) was higher in the DJKM-fed groups. The precaecal amino acid digestibilities of DJKM varied from 0.48 (cystine) to 0.91 (methionine) in turkeys. In pigs, average weight gain and feed:gain ratio were similar for DJKM-fed groups and the SBM-based control group. In addition, the serum and haematological parameters did not differ amongst the groups and values were within the normal range. Histopathological studies revealed that the liver and kidney of pigs fed DJKM and control diets exhibited normal histomorphology. Overall, the DJKM can replace SBM protein in fish, shrimp, turkey and pig diets by as much as 50 percent. DJKM, H-JPKM and DJPI are thus good quality protein sources for animal feeds.

INTRODUCTION

There is an urgent need to increase animal production in order to meet the increasing demand for animal protein driven by increasing human population and the growing economies of developing countries. The rapid world-wide expansion of aquaculture and livestock production strongly indicates that a crisis is imminent in the livestock and aquaculture feed industries in the near future due to unavailability of good quality feed resources (Spinelli, 1980; Belewu *et al.*, 2009). More than 1000 million tonne of animal feed is produced globally every year, including 600 million tonne of compound feed. In terms of species, use of the compound

MAIN MESSAGES

- **Detoxified *Jatropha curcas* kernel meal, heat-treated *J. platyphilla* kernel meal and detoxified *J. curcas* protein isolate can replace 50, 62.5 or 75 percent fishmeal protein, respectively, without compromising growth performance and nutrient utilization in fish, and without adversely affecting fish health, as illustrated by blood parameter evaluation and histopathological investigations on fish organs.**
- **Detoxified *J. curcas* kernel meal can also replace 50 percent fishmeal protein without any adverse effects on growth and nutrient utilization in shrimp.**
- **High inclusion (>50 percent fishmeal protein replacement) of detoxified *J. curcas* kernel meal decreases the efficiency of conversion of feed to body mass. No such effects were observed on using detoxified *J. curcas* protein isolate.**
- **Based on good growth performance, nutrient utilization and high amino acid digestibility, detoxified *J. curcas* kernel meal is valuable protein source for turkeys.**
- **Detoxified *J. curcas* kernel meal can replace 50 percent soymeal protein in diets of growing pigs.**
- **Detoxified *J. curcas* kernel meal and heat-treated *J. platyphilla* kernel meal contain approximately 65 percent crude protein, which is similar to the level in fishmeal, and therefore these could substitute for fishmeal on an equal-weight basis.**
- **The acceptability of DJKM, H-JPKM and DJPI-based diets by fish, as measured by immediate consumption and no waste in the tanks, is good.**
- **Detoxified *J. curcas* kernel meal, heat-treated *J. platyphilla* kernel meal and detoxified *J. curcas* protein isolate are deficient in lysine. Therefore lysine monohydrochloride should be supplemented at a level of 1.5 percent of these jatropha-based products (w/w) in the diet to compensate for the deficiency.**
- **Detoxified *J. curcas* kernel meal and heat-treated *J. platyphilla* kernel meal contain approximately 9–10 percent phytate, which is almost 3-fold that in soybean meal. To mitigates its effect, addition of 1500 FTU phytase per kg of diet is suggested.**

feed is most for poultry, followed by pigs and then cattle. Although feed production for aquaculture is relatively low (14 million tonne) currently, there is an increasing demand for feed for farmed fish and crustaceans.

Substantial progress has been made towards the use of different plant ingredients, including soybean meal (SBM), lupin, maize, wheat, sorghum, peas, rapeseed meal and sunflower meal in animal feed. Typical compositions of commonly used animal feed ingredients are presented in Table 1. Among plant ingredients, SBM is currently the most commonly used plant protein source in animal feeds because of its reliable supply and high content of protein with a high concentration of essential amino acids (EAAs). On a worldwide basis, soybean supplies over one-quarter of the fats and oils and two-thirds of the protein concentrates for animal feeds, and is three-quarters of the total world trade in high-protein meals (Peisker, 2001; Best, 2011). However, soybean, together with maize, has been a staple food of mankind since ancient times. In human diets, soybean has been used as a protein source for over 5 000 years (Peisker, 2001). A vast array of products can be derived from soybean and these are found nowadays in more than 20 000 items on the food shelves of supermarkets worldwide. Also, nutrition of high performing animals is unthinkable without soy products (Peisker, 2001). Soybean competes with human food and hence there is a need to identify other protein-rich plant resources that could be used in animal diets. The world is becoming increasingly aware of the looming food scarcity, and hence the possibility of raising animals on unconventional but easily sourced and available feedstuffs in the tropics and subtropics deserves more attention (Belewu *et al.*, 2009). Worldwide, the growing scarcity of conventional animal feed has therefore motivated nutritionists to find alternative sources of protein for livestock.

JATROPHA

Botanical and agro-climatic description

The genus *Jatropha* belongs to the tribe Joannesieae of Crotonoideae in the Euphorbiaceae family (well known for its toxicity) and contains approximately 175 known species. It is considered to have originated in Central America, most probably Mexico. Jatropha species for which the toxicity has been widely studied are *Jatropha curcas*, *J. elliptica, J. glauca, J. gossypifolea, J. aceroides, J. tanoresisi, J. macarantha, J. integerrima, J. glandulifera, J. podagrica* and *J. multifida* (Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a). Among these, *J. curcas* (toxic genotype) is the most studied as

TABLE 1
Typical composition of commonly used animal feed ingredients

Ingredient	Proximate composition (g/kg DM except Gross energy)					Essential Amino Acida (g/kg DM)								
	DM	CP	Total lipid	Ash	GE	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Fishmeal[(1)]	917	770	68	142	21.3	43	25	28	55	46	21	29	32	34
Fishmeal[(2)]	920	720	84	104	21.6	38	19	25	46	43	18	23	26	32
SE soybean meal	909	518	47	69	19.6	42	14	23	44	28	9	27	24	24
SE canola meal	962	431	22	86	19.6	32	26	3	25	41	30	27	16	78
EX canola meal	898	381	136	66	23.1	39	28	3	28	46	37	29	18	66
Yellow lupin[(3)]	903	547	87	44	20.9	61	15	20	45	23	4	21	20	19
NL lupin	885	415	53	33	20.4	47	10	15	29	14	3	16	16	14
Groundnut meal	928	481	13	58	20.3	67	16	19	32	20	5	29	14	20
Sunflower meal	930	422	29	76	20.7	36	11	17	26	12	7	20	13	23
Maize gluten meal	900	602	18	21	21.1	19	13	25	102	10	14	38	21	28
Cotton seed meal	900	414	18	64	20.6	45	12	13	25	17	7	22	14	18
Pea meal	928	252	13	38	16.4	19	11	14	41	27	6	19	17	14
Rapeseed meal	900	385	39	67	20.8	21	11	14	24	20	8	16	15	17
Wheat gluten	937	856	13	9	21.1	43	21	43	69	16	17	49	24	43
Wheat meal	941	145	16	14	18.7	6	3	4	9	3	7	2	4	5
DJKM	945	665	11	137	18.3	70	22	27	47	23	11	30	22	32
PPC	910	738	15	20	20.9	38	17	71	76	58	17	49	43	49
JPI	945	808	97	10	21.3	86	24	34	56	19	39	12	26	59
LPC	942	690	93	31	22.2	78	15	27	51	25	5	28	23	23
SPC	939	590	54	79	20.3	45	15	26	48	28	9	30	25	27
SPI	957	922	10	38	22.0	69	24	36	68	52	43	12	31	37

Notes: (1) Chilean anchovetta meal; (2) Herring; (3) *Lupinus luteus* (cv. Wodjil) kernel meal. DM = dry matter; CP = Crude Protein; GE = Gross energy expressed as MJ/kg; NL = Narrow-leaf lupin (*Lupinus angustifolius*) (mixed cultivars) kernel meal; LPC = *Lupinus angustifolius* (mixed cultivars) protein concentrate. SE = solvent extracted; EX = expeller; DJKM = detoxified jatropha kernel meal; SPC = soybean protein concentrate; SPI = soy protein isolate; JPI = jatropha protein isolate; PPC = potato protein concentrate.

Sources: Miller and Young, 1977; Nwokolo, 1987; NRC, 1983, 1998; Glencross, Booth and Allan, 2007; Makkar, Francis and Becker, 2008; Makkar and Becker, 2009a.

TABLE 2
Common and vernacular names of *Jatropha curcas*

Language or country	Common name
Angola	Mupuluka
Arabic	Dand barrî, habel meluk
Brazil	Mundubi-assu
Chinese	Yu-lu-tzu
Costa Rica	Coquillo, template
Côte d'Ivoire	Bagani
Dutch	Purgeernoot
English	Physic nut, purging nut, pulza
French	Pourghère, pignon d'Inde
German	Purgiernuß, Brechnuß
Guatemala	Pinón
Hindi (India)	Ratanjyot, bagbherenda, jangli arandi, safed arand, bagaranda
Indonesia	Jarak budeg
Italian	Fagiola d'India
Mexico	Piñoncillo
Nepal	Kadam
Nigeria	Butuje
Peru	Piñol
Philippines	Túbang-bákod, tuba-tuba
Portuguese	Turgueira
Puerto Rico	Tártago
Sanskrit	Kanananaeranda, parvataranda
Senegal	Tabanani
Tanzania	Makaen
Thailand	Sabudam
Togo	Kpoti

Sources: Schultze-Motel, 1986; Münch, 1986; Divakara *et al.*, 2010; Mabberley, 1987.

a result of its oil (as a source of biofuel) and associated co-product utilization (Makkar and Becker, 2009a, b). A non-toxic genotype of *J. curcas* has also been recorded, which is found only in Mexico (Makkar and Becker, 2009a). *Jatropha curcas* (toxic genotype) is found in parts of tropical America (central and southern regions) and many tropical and subtropical regions of Africa and Asia. It is believed that *Jatropha* species were introduced into other regions from the Caribbean, where it was used during the Mayan period (Schmook and Seralta-Peraza, 1997; Gaur, 2009), by sailors on Portuguese ships travelling via the Cape Verde islands and Guinea Bissau (Heller, 1996). The name *Jatropha curcas* (Euphorbiaceae) was first given by Linnaeus (Linnaeus, 1753). The genus name *Jatropha* derives from the Greek words jatr´os (doctor) and troph´e (food), which implies its medicinal uses. Table 2 presents some vernacular names of *J. curcas*. *J. curcas* is monoecious, flowers are unisexual but occasionally hermaphrodite flowers occur, each inflorescence yielding a bunch of approximately 10 or more ovoid fruits (Dehgan and Webster, 1979; Kumar and Sharma, 2008). The young *J. curcas* plant with both flowers and developing seed pods is shown in Photo 1A. Photo 1B shows the *J. curcas* inflorescence containing both male staminate flowers and female pistillate flowers. The seeds of *J. curcas* form within seed pods. Each seed pod typically contains three seeds (Photo 1C) (King *et al.*, 2009). The seeds mature 3–4 months after flowering. Mature seeds of *J. curcas* are presented in Photo 1D. The plant can be easily propagated from seeds or cuttings. It grows under a wide range of rainfall regimes, from 250 to over 1200 mm per annum (Katwal and Soni, 2003; Kumar and Sharma, 2008). The trees are deciduous, shedding their leaves in the dry season. One major trait associated with the plant is its hardiness and sustainability in warm and arid climates. It prefers well-drained alkaline soil (pH 6–9) (Kumar and Sharma, 2008). It is a small perennial tree or large shrub, which normally reaches a height of 3–5 m, but can attain 8–10 m under favourable conditions (Gaur, 2009). Seed yields of 5–8 tonne/ha have been reported (Gübitz, Mittelbach and Trabi, 1999).

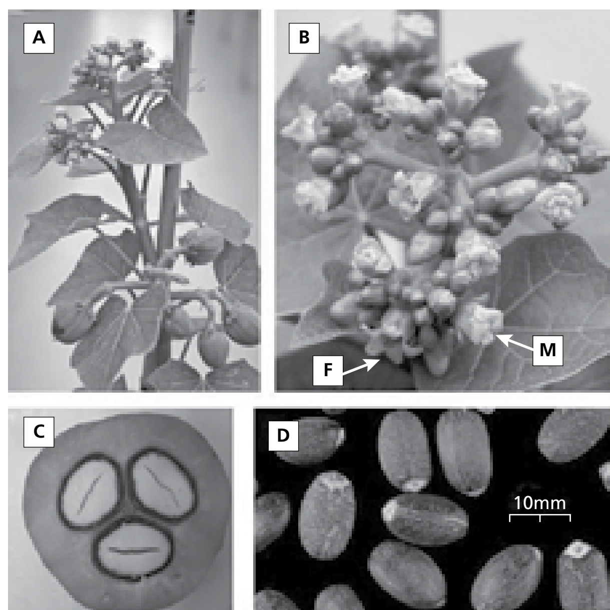


Photo 1
Images of Jatropha curcas
Notes: (A) Young *J. curcas* plant with both flowers and developing seed pods. (B) *J. curcas* inflorescence containing both male staminate flowers (M) and female pistillate flowers (F). (C) Cross-section of a *J. curcas* seed pod containing three developing seeds. (D) Mature seeds of *J. curcas*.
Source: King *et al.*, 2009.

A new, non-toxic, species of Jatropha, *Jatropha platyphylla,* has been identified (Makkar *et al*., 2011). *J. platyphylla* (locally called 'sangregrado' in Mexico) is a drought-resistant shrub or tree, 2–5 m high, almost glaborous. The species is restricted to warm areas (average temperature 20–29 °C) on the Pacific coast from Sinaloa to Michoacán, including Nayarit and Jalisco states in Mexico, and is usually found in and around deciduous forests. It has thick succulent branches, large peltate glaborous leaves (25–35 cm) on long petioles, and white urceolate flowers that are held on a long and branched florescence (Dehgan, 1982). The kernel (white potion after removal of shells) contains about 50–60 percent oil, which can be used as edible oil or can be converted into biodiesel of high quality (Makkar *et al.*, 2011). The kernel meal obtained

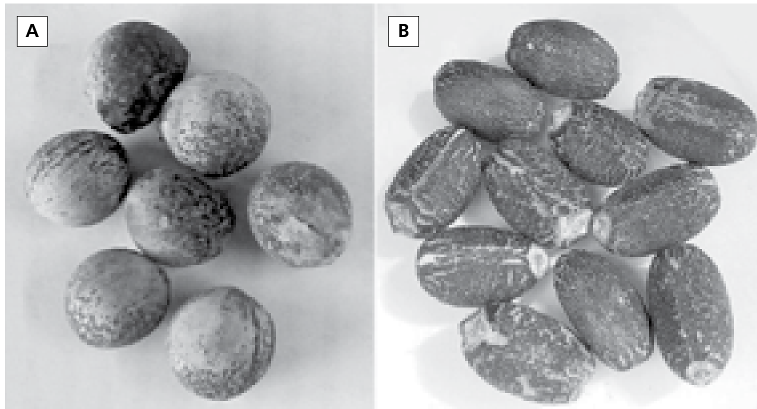


Photo 2
Seeds of (A) Jatropha platyphylla *and (B)* Jatropha curcas
Source: Makkar *et al.*, 2011.

after oil extraction is an excellent source of nutrients and contains 60–65 percent crude protein (Makkar *et al.*, 2011). The levels of EAAs (except lysine) are higher in defatted *J. platyphylla* kernel meal than in SBM (Makkar *et al.*, 2011). In addition, *J. platyphylla* kernel meal is free of phorbol esters, the main toxin present in most Jatropha species (Makkar *et al.*, 2011). However, anti-nutrients, e.g. a trypsin inhibitor, lectin and phytate, are present in the meal at high levels (Makkar *et al.*, 2011). Heat labile anti-nutrients, protease inhibitors and lectins are easy to inactivate by moist heating, and phytase could be incorporated into the diet for degradation of phytate.

Applications of jatropha

Jatropha seeds have been extensively investigated as a source of oil. *J. curcas* seeds contain 25–35 percent crude oil (Makkar and Becker, 2009a; King *et al.*, 2009). The oil contains 21 percent saturated fatty acids and 79 percent unsaturated fatty acids (Gübitz, Mittelbach and Trabi, 1999; Makkar and Becker, 2009a). Jatropha oil fatty acid composition includes 14–16 percent palmitate (16:0), 5–8 percent stearate (18:0), 34–46 percent oleic acid (18:1), 29–44 percent linoleic acid (18:2) and a trace of longer-chain saturated fatty acids (Foidl *et al.*, 1996; Gübitz, Mittelbach and Trabi, 1999; King *et al.*, 2009). *Jatropha curcas* oil has good feedstock qualities for biodiesel production, the biodiesel meeting the European Union (EN14214) and North American standards (ASTM D6751) (Makkar and Becker, 2009a; King *et al.*, 2009). A number of countries, including India, Pakistan, China, Mexico, Brazil, Nigeria, Indonesia, Madagascar, Mali, Thailand, Ghana, Bangladesh, Kenya, Zimbabwe and Cape Verde, have initiated programmes for planting *J. curcas* as an energy plant. The cultivation of Jatropha species as a source of oil for biodiesel production will in turn produce a number of by-products and co-products. The utilization of these products may increase the overall value of the jatropha biodiesel production chain. However, the presence of toxic components limits the utilization of many unprocessed jatropha-based products. Jatropha and its components have several uses, which are summarized in Table 3.

Comparative physical and chemical characteristics of seeds and kernel meals from toxic and non-toxic *Jatropha curcas* genotypes and *Jatropha platyphylla*

The seeds of *J. curcas* (toxic and non-toxic genotypes) are elliptical whereas seeds of *J. platyphylla* are almost circular (Photo 2) (Makkar *et al.*, 2011). The seed, shell and kernel masses are similar for both the toxic and non-toxic genotypes (Table 4). Composition of jatropha seed is presented in Figure 1. The seeds are rich in crude protein and lipids. The chemical composition of seeds of these two *Jatropha* species – *J. curcas* and *J. platyphylla* – is similar (Table 4). Sugar and starch contents and the mineral composition (except

FIGURE 1
Composition of jatropha seeds (% in DM)

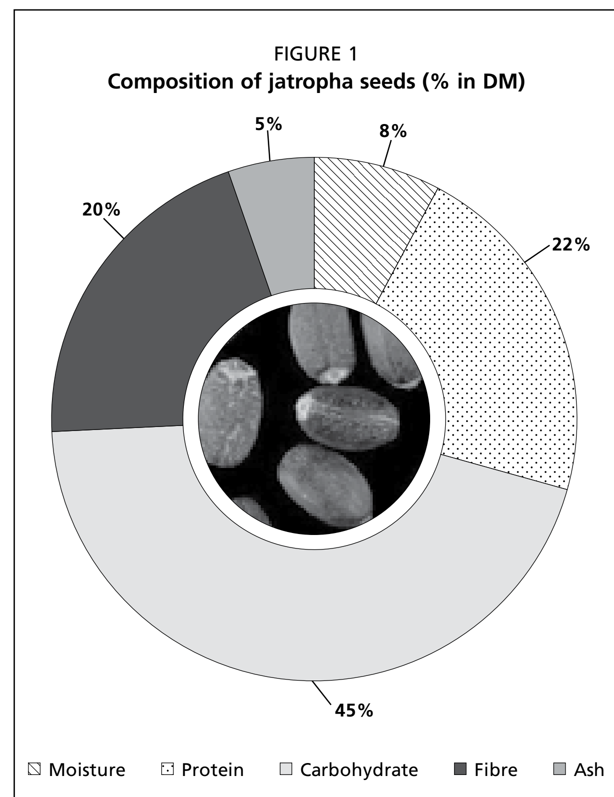


TABLE 3
Uses of jatropha-based products

Plant part and use	References
Use in ethnomedicine	
Latex Contains an alkaloid Jatrophine, which has anti-carcinogenic properties, and latex also strongly inhibits the watermelon mosaic virus	Parajuli, 2009; Devappa, Makkar and Becker, 2011a.
Leaves and sap Used to control parasites. Sap is used for staining linen. Sometimes used for marking and labelling	Rug *et al*., 1997; Kisangau *et al*., 2007; Devappa *et al*., 2010b.
Leaf extracts Used to clean sores, treat skin rashes and oral candidiasis. It is also used for fever, mouth infections, jaundice, guinea-worm sores, and joint rheumatism	Kisangau *et al.,* 2007; Devappa, Makkar and Becker, 2010b.
The juice of the whole plant Used for stupefying fish	Gübitz, Mittelbach and Trabi, 1999.
Emulsion of the twig sap with benzyl benzoate Effective against scabies, wet eczema and dermatitis	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009.
Roots Acts as an antidote to treat snake-bite. Used as mouthwash for bleeding gums and toothache. Applied on skins to treat eczema, ringworm and scabies. Used to treat dysentery and venereal diseases like gonorrhea, leprosy	Irvin, 1961; Oliver-Bever, 1986; Devappa, Makkar and Becker, 2010b.
Root oil (yellow in colour) Used as strong anthelmintic. Has wound healing and anti-inflammatory effects	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009; Nath and Dutta, 1997; Staubmann *et al.,* 1997; Kumar and Sharma, 2008.
Seeds Acts as an anthelmintic	Gübitz, Mittelbach and Trabi, 1999.
Seed oil Used to treat rheumatism, eczema and skin diseases and, also reported to be abortificient and efficacious in dropsy, sciatic and paralysis	Heller, 1996; Gübitz, Mittelbach and Trabi, 1999; Kumar and Sharma, 2008; Parajuli, 2009.
Jatropha seeds enzyme (ß-1,3-glucanase) Antifungal against *Rhizoctonia solani* Kuha and *Gibberelle zeae* Schw.	Wei *et al*., 2005; Makkar and Becker, 2009a.
Use as source of phytochemicals and its agro-pharmaceutical importance	
Phorbol esters Tumour-promoting, irritant, cytotoxic, anti-inflammatory, antitumour, molluscicidal, insecticidal and fungicidal activities	Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a.
Pesticidal effects against *Sitophilus zeamays* and *Callosobruchus chinesis*	
Kills snails of the *Physa* species, which are also known to be intermediary hosts of schistosomes that causes the deadly disease schistosomiasis in humans	
Compound (12-deoxyphorbol-13-phenylacetate) synthesized from phorbol ester Acts as antidote against HIV by inhibiting the HIV entry into target cells	Wender, Kee and Warrington, 2008; Makkar and Becker, 2009a.
A proteolytic enzyme, curcain from jatropha latex Wound-healing properties	Nath and Dutta, 1997.
Biologically active cyclic peptides Mahafacyclin, pohlianin, chevalierin and curcacyclin have anti-malarial properties Jatrophidin has antifungal activity Labaditin and biobollien have immuno-modulatory effects	Makkar and Becker, 2009a; Devappa, Makkar and Becker, 2010a, b, 2011a
Phytates from seeds Cancer prevention, hypercholesterolemic effects, reduction in iron-induced oxidative injury and reversal of colorectal tumorigenesis initiation, and prevention of lipid peroxidation	Singh, Bhat and Singh, 2003; Kumar *et al.,* 2010a.
Other uses	
Bark Yields a dark blue dye which is reported to be used in Philippines for colouring cloth, finishing nets and lines	Gübitz, Mittelbach and Trabi, 1999; Parajuli, 2009.
Jatropha proteins (approximately 50 kDa) Production of wood/paper adhesive – polyketone-based wood adhesive formulations	Hamarneh *et al.,* 2010.
Jatropha wood and husks/shells Jatropha seed shells have 45–47% lignin and has a high energy value (ca 19.5 MJ/kg)	Makkar and Becker, 2009.
Jatropha-derived biodiesel Mixed with jet fuel and used as an aviation fuel	Gaur, 2009.
Jatropha oil Used for making soap and candles in addition to direct use as energy and as biodiesel	Gübitz, Mittelbach and Trabi, 1999.
Oil with iron oxide Preparation of varnish	Gübitz, Mittelbach and Trabi, 1999.
Jatropha seed cake Fertilizer Briquettes for use as fuel Production of biogas Raw material for plastics and synthetics fibres As a substrate for solid state fermentation to produce: (a) proteases and lipases using *Pseudomonas aeruginosa* and (b) xylanase using *Scytalidium thermophilum* Source of fermentable sugars and solubilized proteins	Gübitz, Mittelbach and Trabi, 1999; Vyas and Singh, 2007; Singh, Bhat and Singh, 2003; Sharma and Singh, 2008; Carels, 2009; Mahanta, Gupta and Khare, 2008; Makkar and Becker, 2009a; Ali, Kurchania and Babel, 2010; Joshi and Khare, 2011; Liang *et al.,* 2010.

TABLE 4
Physical and chemical parameters of *Jatropha curcas* (toxic and non-toxic genotypes) and *J. platyphylla* seeds and kernel meals

	Jatropha curcas		*Jatropha platyphylla*
	Toxic	**Non-toxic**	
Seed weight (g)	0.80 ± 0.08	0.73 ± 0.09	1.80 ± 0.15
Shell weight (g)	0.31 ± 0.05	0.26 ± 0.03	0.92 ± 0.01
Kernel weight (g)	0.49 ± 0.0.7	0.47 ± 0.07	0.85 ± 0.13
Proximate composition (g/kg DM) of kernel			
Crude protein	266 ± 11.2	268 ± 12.4	271 ± 20
Oil	574 ± 5.0	575 ± 6.9	603 ± 354
Ash	40 ± 6.7	45 ± 5.6	39 ± 0.9
Nutrients in defatted kernel meal (g/kg on DM basis)			
Crude protein	637 ± 11	624 ± 26	664 ± 20
Crude lipid	11.4 ± 0.52	12.1 ± 0.41	11.4 ± 0.29
Crude ash	94 ± 10.1	91 ± 10.4	90 ± 5.8
Neutral-detergent fibre	182	180	–
Total sugar	7.7–10.3	10.2	–
Starch	9.4–11.2	10.6	–
Minerals in defatted kernel meal (mg/kg on DM basis)			
Boron	14.0–15.0	23.1–25.6	41.5–43.1
Calcium	8 995–9 769	6 660–7 077	6 771–7 396
Copper	48–52	40–44	48–53
Iron	304–344	251–278	209–231
Potassium	19 882–21 064	21 381–22 878	22 965–24 259
Magnasium	17 947– 19 452	14 432–15 715	15 094–13 801
Maganase	69–74	53–57	76–84
Sodium	26 652– 27 190	219–226	24–28
Phosphorus	21 171–22 676	17 533–18 815	21 456–23 288
Zinc	105–114	80–89	116–135

Sources: Makkar and Becker, 2009a; Makkar *et al.*, 2011.

sodium) in kernel meals of *J. platyphylla* and toxic and non-toxic genotypes of *J. curcas* are almost similar (Table 4). The amino acid composition of *J. platyphylla* and *J. curcas* (toxic and non-toxic genotypes) kernel meal is almost identical (Table 5). The levels of EAAs (except lysine) are higher than those quoted in the FAO reference protein for a growing child of 2–5 years of age (Makkar and Becker, 2009a). The amino acid composition of jatropha kernel meal and SBM is similar (except lysine and the sulphur-containing amino acids cystine and methionine); lysine is less and sulphur-containing amino acids are more in the jatropha kernel meal compared with SBM. EAA contents in jatropha kernel meals are higher than or similar to those in castor bean meal (Makkar, Aderibigbe and Becker, 1998; Makkar and Becker, 2009a). Jatropha kernel meal contains low level of non-protein nitrogen (9.0 percent of total nitrogen), suggesting a high level (91 percent) of true protein (Makkar, Aderibigbe and Becker, 1998; Makkar and Becker, 2009a). When a non-toxic genotype from *J. curcas* kernel meal (JCM) was fed to fish and rats, high growth rate and good protein utilization were observed, suggesting that the quality of protein in jatropha kernel meal is good (Makkar and Becker, 1999, 2009a).

Jatropha kernel meal (heated to 121 °C at 66 percent moisture for 30 minutes) from toxic and non-toxic genotypes has similar digestibility and metabolizable energy; however, these meals have lower digestibility and metabolizable energy than SBM (Table 6) (Menke *et al.*, 1979; Makkar and Becker, 2009a). The pepsin plus trypsin digestibilities of jatropha kernel meal protein were similar to those of the heated SBM, whereas the *in vitro* rumen digestibility of proteins in the kernel meal of the non-toxic jatropha genotype was lower (ca 50 percent) compared with that of SBM, suggesting that the former meal has substantial amounts of rumen undegradable protein, which could be used post-ruminally. These results demonstrate that kernel meal from the non-toxic jatropha genotype can be used as a good quality protein source in animal nutrition (Makkar and Becker, 2009a). Furthermore, it is inferred from these results that a similar level of application could also be expected of jatropha kernel meal from the toxic genotype, provided it is detoxified.

Constraints: toxic component and antinutrients in *Jatropha curcas*

Makkar and Becker (1997) unequivocally established that the main toxic factor in *J. curcas* seeds, oil and cake, is the diterpene derivatives of a tigliane skeleton classified as phorbol esters. A number of anti-nutrients are present in

TABLE 5
Amino acid composition (g/16 g nitrogen) of kernel meals of *Jatropha curcas* (toxic and non-toxic genotypes), *J. platyphylla* and SBM, versus FAO reference dietary protein requirement values

Amino acid	*Jatropha curcas*		*J. platyphylla*	SBM	FAO reference protein (2–5-year-old child)
	Toxic	Non-toxic			
Essential					
Methionine	1.56–1.91	1.38–1.76	1.58	1.32	2.50(1)
Cystine	1.77–2.24	1.58–1.81	1.55	1.38	
Valine	4.35–5.19	3.79–5.30	6.91	4.50	3.50
Isoleucine	3.93–4.53	3.08–4.85	4.10	4.16	2.80
Leucine	6.55–6.94	5.92–7.50	6.68	7.58	6.60
Phenylalanine	4.08–4.34	3.93–4.89	4.71	5.16	6.30(2)
Tyrosine	2.45–2.99	2.62–3.78	2.69	3.35	
Histidine	2.81–3.30	2.65–3.08	2.66	3.06	1.90
Lysine	3.63–4.28	3.40–3.49	3.16	6.18	5.80
Threonine	3.33–3.96	3.15–3.59	3.64	3.78	3.40
Tryptophan	1.31	ND	1.06	1.36	1.10
Non-essential					
Serine	4.67–4.80	4.59–4.91	5.05	5.18	–
Arginine	11.8–12.2	11.4–12.90	12.46	7.64	–
Glutamic acid	14.68–16.7	15.91–16.50	16.21	19.92	–
Aspartic acid	9.49–11.8	9.92–11.7	9.33	14.14	–
Proline	4.13–4.96	3.80–4.21	5.16	5.99	–
Glycine	4.40–4.92	4.18–4.61	4.56	4.52	–
Alanine	4.36–5.21	4.26–4.94	4.04	4.54	–

Notes: (1) Methionine plus cystine; (2) Phenylalanine plus tyrosine. ND = not detected. *Sources*: Makkar and Becker, 2009a; Makkar *et al*., 2011.

TABLE 6
Pepsin plus trypsin digestibilities, available lysine, digestible organic matter, metabolizable energy and rumen-degradable nitrogen of heat-treated jatropha kernel meals

	Jatropha curcas		*Jatropha platyphylla*	SBM
	Toxic	Non-toxic		
Pepsin plus trypsin digestibility (% of total nitrogen)	89	90	97.1	91
Available lysine (mg/100 mg sample)	3.10	3.16	3.29	–
Available lysine (g/16 g N)	4.87	5.06	4.95	–
Digestible organic matter (%)	78	77.3	–	87.9
Metabolizable energy (MJ/kg)	10.9	10.7	–	13.3
24-hour *in vitro* rumen-degradable nitrogen (% of total nitrogen)	43.3	28.9	–	80.9

Notes: SBM = Soybean meal. *Sources*: Makkar and Becker, 2009a; Makkar *et al*., 2011.

defatted kernel meal obtained from *J. curcas* genotypes (toxic and non-toxic) and these are listed in Table 7.

Phorbol esters are naturally-occurring compounds that are widely distributed in plant species in the Euphorbiaceae and Thymelaeceae. They are tetracyclic diterpenoids of phorbol type and esters of tigliane diterpenes (Evans, 1986; Devappa, Makkar and Becker, 2010b, 2011a). Six phorbol esters (jatropha factors C1–C6) have been characterized from *J. curcas* seed oil (Haas, Sterk and Mittelbach, 2002; Devappa, Makkar and Becker, 2010b, 2011a) and designated as C1 (A), C2 (B), C3 (C), epimers C4 (D), C5 (E) and C6 (F), with the molecular formula $C_{44}H_{54}O_8Na$ (MW 733.37) (Figure 2). The phorbol esters are lipophilic, present mainly in oil or kernel, and are not affected by heat treatment. The concentration of phorbol esters varies from 1 to 3 mg/g kernel meal and from 2 to 7 mg/g oil (Makkar and Becker, 1997, 2009a; Devappa, Makkar and Becker, 2010b, 2011a). Table 8 shows phorbol ester content of different parts of a toxic *J. curcas* plant. Figure 3 represents the phorbol ester content in different part of the toxic *J. curcas* kernel (Devappa, Makkar and Becker, 2011b).

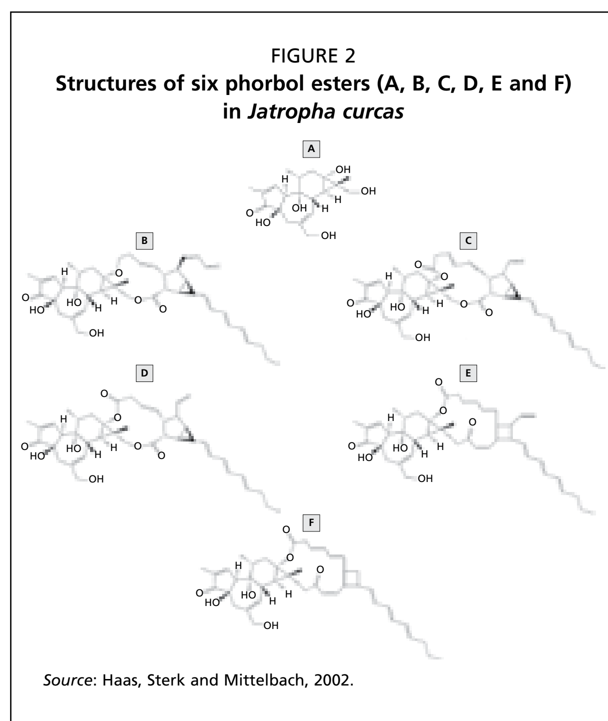
Rumen microbes cannot degrade phorbol esters (Makkar and Becker, 2010b) and they cause as severe toxic symptoms in ruminants as they do in monogastric animals. These mimic the action of diacylglycerol, an activator of protein kinase C which regulates different signal transduction pathways (Devappa, Makkar and Becker, 2010a, b, 2011a). Phorbol esters affect a number of processes including phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression. These are considered to be a co-carcinogen and have strong purgative and membrane-irritant effects (Goel *et al.*, 2007; Devappa, Makkar and Becker, 2010a, b, 2011a).

TABLE 7
Levels of toxic and anti-nutritional factors in unheated kernel meals of *Jatropha curcas* (toxic and non-toxic genotypes) and *J. platyphylla*

Component	*Jatropha curcas*		*Jatropha platyphylla*
	Toxic	Non-toxic	
Phorbol esters (mg/g kernel)[(1)]	2.79	ND	ND
Total phenols (% tannic acid equivalent)	0.36	0.22	0.33
Tannins (% tannic acid equivalent)	0.04	0.02	0.17
Condensed tannins (% leucocyanidin equivalent)	ND	ND	ND
Phytate (% DM)	9.4	8.9	8.7
Saponins (% diosgenin equivalent)	2.6	3.4	1.9
Trypsin inhibitor (mg trypsin inhibited per g sample)	21.3	26.5	20.8
Lectin activity (inverse of mg meal per mL of the assay that produced haemagglutination)	51–102	51–102	51–102
Glucosinolates	ND	ND	ND
Cyanogens	ND	ND	ND
Amylase inhibitor	ND	ND	ND
Non-starch polysaccharides (% in DM)			
Rhamnose	0.2	0.2	0.3
Fucose	0.1	0.1	0.1
Arabinose	2.5	2.7	3.1
Xylose	1.2	1.4	2.0
Mannose	0.3	0.3	0.5
Galactose	1.2	1.2	1.4
Glucose	4.7	4.7	5.7
Glucuronic acid	0.9	0	0
Galacturonic acid	2.6	3.0	3.0
Total non-starch polysaccharides	13.7	13.6	16.0

Notes: (1) As phorbol-12-myristate 13-acetate equivalent. ND = not detected. *Sources*: Makkar and Becker, 2009a; Makkar *et al.*, 2011.

FIGURE 2
Structures of six phorbol esters (A, B, C, D, E and F) in *Jatropha curcas*



Source: Haas, Sterk and Mittelbach, 2002.

TABLE 8
Phorbol esters in different parts of toxic *J. curcas* plants

Plant part	Phorbol esters (mg/g DM)[(1)]
Kernel	2.00–6.00
Leaves	1.83–2.75
Stems	0.78–0.99
Flower	1.39–1.83
Buds	1.18–2.10
Roots	0.55
Latex	Not detected
Bark (outer brown skin)	0.39
Bark (inner green skin)	3.08
Wood	0.09

Notes: (1) As phorbol-12-myristate 13-acetate equivalent.
Source: Makkar and Becker, 2009a.

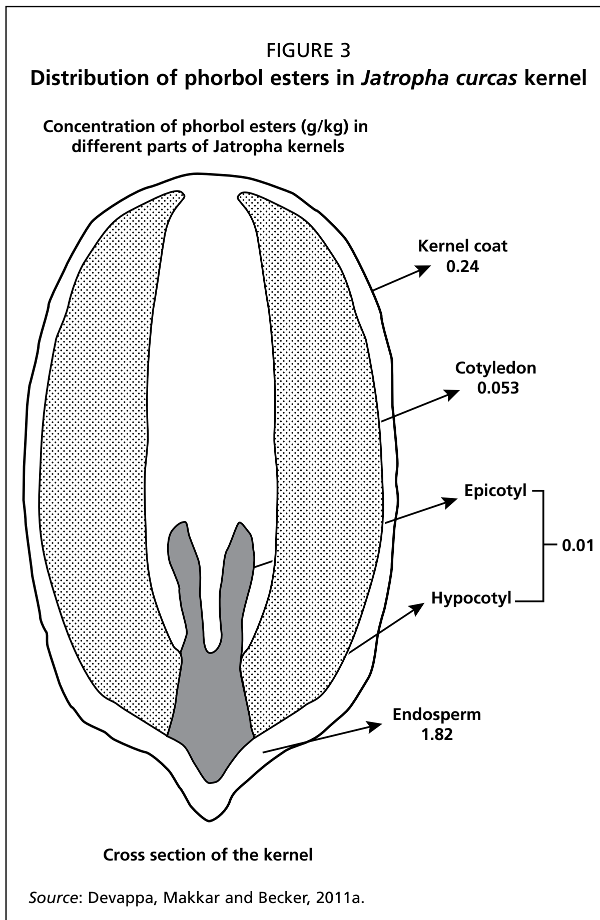
The main antinutrients present in the seeds of kernel meal are curcin, trypsin inhibitors and phytate.

For effective utilization of kernel meal the removal of antinutrients and toxic principles is necessary. Antinutrients such as trypsin inhibitors and lectin (curcin) can be deactivated by heat treatment, and the adverse effects of phytate can be mitigated by supplementation with phytase enzyme. However, the main toxic compounds, the phorbol esters, are heat stable to a large extent. Other strategies must therefore be applied for their removal.

Different approaches evaluated for detoxification of *Jatropha curcas* products

In the past two decades, several approaches (active chemicals or organic solvents) have been tried for detoxifying defatted cake and kernel meal. Makkar and Becker (1997) reported that ethanol (80 percent) or methanol (92 percent) [1:5 w/v] reduced both the saponins and phorbol

FIGURE 3
Distribution of phorbol esters in *Jatropha curcas* kernel



Source: Devappa, Makkar and Becker, 2011a.

esters by 95 percent after four extractions. Heat treatment in presence of alkali was also effective in reducing phorbol esters. Martinez-Herrera *et al.* (2006) studied the effect of various treatments, such as hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with $NaHCO_3$, and ionizing radiation, to inactivate the antinutritional factors in jatropha kernel meal. Trypsin inhibitors were easily inactivated with moist heating at 121 °C for 20 minutes (Makkar and Becker, 1997). Extraction with ethanol, followed by treatment with 0.07 percent $NaHCO_3$ considerably reduced lectin activity. The same treatment also decreased the phorbol ester content by 97.9 percent. Chivandi *et al.* (2004) reported that petroleum ether extraction reduced phorbol ester content in kernels of *J. curcas* seeds by 67.7 percent, and double solvent extraction followed by moist heat treatment reduced phorbol esters by 70.8 percent. Double solvent extraction accompanied with wet extrusion, re-extraction with hexane and moist-heat treatment diminished phorbol ester content by 87.7 percent. Rakshit and Bhagya (2007) reported that up to 90 percent of the phorbol esters could be removed by treating the meal with 20 g/L of calcium hydroxide. Gaur (2009) developed a process that obtains high yields of jatropha oil and detoxifies the defatted (oil-free) jatropha meal. The principle of solid-liquid extraction was utilized to detoxify the meal. Various organic solvents were used for the extraction. Extraction of ground jatropha seed kernels in a Soxhlet apparatus involving a sequential combination of hexane, followed by methanol proved highly efficient in detoxifying the meal. Phorbol ester content was reduced by 99.6 percent from 6.05 mg/g in untreated meal to about 0.06 mg/g in solvent-treated meal.

Chivandi *et al.* (2006) detoxified defatted *J. curcas* kernel meal (JCM) using 95 percent ethanol at 35 °C to remove most of the highly lipo-soluble phorbol esters in the kernels. The ethanol-extracted meal was heated with pressurized steam at 90 °C for 30 minutes to distil off the ethanol, after which the meal was sun-dried. The re-extracted meal was autoclaved at 121 °C for 30 minutes to inactivate the heat-labile antinutrients. This "detoxified" JCM was then fed to pigs for 8 weeks. Haematological and biochemical parameters were measured and it was found that dietary 'detoxified' JCM caused severe adverse effects in pigs. This demonstrates that the detoxification procedure had failed to remove and/or neutralize the toxic factors in the JCM. Some of the toxicity observed could be ascribed to the residual phorbol esters in the JCM. In the study of Belewu, Belewu and Ogunsol (2010), autoclaved (121 °C, 15 psi for 30 minutes) *J. curcas* seed cake was treated with fungi (*Aspergillus niger* and *Trichoderma longibrachiatum*) and fed to West African dwarf goats for 70 days. Phorbol ester content was reported for neither the treated nor untreated *J. curcas* kernel cakes. The growth and nutrient utilization was lower in *J. curcas* cake-fed groups compared with the control, implying that the cake was not detoxified and could not be used as a component in animal feed.

The solid-state fermentation (SSF) of seed cake using the white-rot fungi *Bjerkandera adusta* and *Phlebia rufa* decreased phorbol ester content by 91 and 97 percent, respectively, under optimized laboratory conditions (28 °C for 30 days) (de Barros *et al.*, 2011). Similarly, SSF using *Pseudomonas aeruginosa* PseA strain decreased phorbol esters to an undetectable level within nine days under optimized conditions (30 °C, pH 7.0 and relative humidity 65 percent) (Joshi, Mathur and Khare, 2011). Animal studies have not been conducted using material treated thus.

In Hohenheim, Germany, a new method has been developed to detoxify jatropha kernel meal and protein isolate (Makkar and Becker, 2010a). This detoxification of kernel meal and protein isolate is based on extraction of phorbol esters using organic solvents (alkaline methanol) and inactivation of trypsin inhibitors and lectin by heat treatment. Furthermore, these authors reported a one-step detoxification method in which the proteins from mechanically pressed jatropha seed cake were solubilized at pH 11, and then the solubilized proteins were precipitated and detoxified using ethanol at pH 8. These procedures are available in patent (WIPO Patent, WO/2010/092143). The detoxified

JCM and protein isolate obtained using this process have been intensively investigated as soybean and fishmeal protein replacers in diets of a number of farm animal species, and these studies are discussed below.

DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL AS A PROTEIN SOURCE IN AQUA FEED

Aquaculture continues to grow at a faster pace than the farming of terrestrial animals. For fish and shrimp feeds, the most pressing need is to find alternative protein sources. Several studies performed on partial replacement of protein sources, especially fishmeal, by detoxified *J. curcas* kernel meal (DJKM), heated *J. platyphylla* kernel meal (H-JPKM) and detoxified jatropha protein isolate (DJPI) in fish and shrimp diets are presented.

Use of detoxified jatropha kernel meal in common carp (*Cyprinus carpio* L.) diet

Feed intake, feed utilization and growth performance

Two experiments were performed by Kumar, Makkar and Becker (2011a) wherein 50 and 75 percent (Table 9), and 50 and 62.5 percent (Table 11) of fishmeal protein was replaced by DJKM, with synthetic lysine added in the DJKM-containing diets. Based on visual observations during feeding time, acceptability and palatability of the DJKM-based feeds was similar to the control diet (Kumar, Makkar and Becker, 2011a). High inclusion (>50 percent replacement of fishmeal protein) of the detoxified meal resulted in reduced protein utilization, measured as protein efficiency ratio and protein productive value (Kumar, Makkar and Becker, 2011a, 2010c). These results showed that ≤50 percent replacement of fishmeal protein by DJKM in common carp diet met the dietary demands for protein and energy.

The blends of DJKM with fishmeal, at different levels, were found to have excellent protein, lipid and energy digestibilities in common carp (Kumar, Makkar and Becker, 2011a). Protein and energy digestibilties were statistically similar ($P > 0.05$) for the control and the group in which 50 percent fishmeal protein was replaced by DJKM, and these values were higher ($P < 0.05$) than those for the group fed a diet in which 75 percent fishmeal protein was replaced by DJKM. The digestibility of the DJKM protein fraction was greater than 90 percent (Kumar, Makkar and Becker, 2011a). The protein digestibility coefficients compare favourably with those of any other high-quality

TABLE 9
Growth performance, nutrient utilization, digestibility measurement, digestive enzymes activity and haematological parameters of common carp fed detoxified jatropha kernel meal (DJKM)-based diets

Parameter	Control	Jatropha inclusion	
		50% (J_{50})	75% (J_{75})
Initial body mass (g)	3.2 ± 0.1	3.2 ± 0.10	3.2 ± 0.10
Final body mass (g)	32.0^a ± 1.96	33.3^a ± 0.64	28.3^b ± 1.21
Feed conversion ratio	1.00^b ± 0.05	1.01^b ± 0.02	1.21^a ± 0.24
Protein efficiency ratio	2.6^a ± 0.12	2.6^a ± 0.04	2.2^b ± 0.37
Protein productive value (%)	38.8^b ± 2.41	41.3^a ± 0.88	34.4^b ± 5.56
Digestibility measurements, relative intestinal length, digestive and metabolic enzymes activity			
Protein digestibility (%)	92.3^a ± 0.45	92.2^a ± 0.39	90.6^b ± 0.07
Lipid digestibility (%)	97.2^a ± 0.68	95.0^b ± 0.91	92.1^c ± 0.90
Energy digestibility (%)	87.7^a ± 1.33	87.6^a ± 1.11	83.1^b ± 0.95
Relative intestinal length (mm/g)	2.55^b ± 0.13	2.93^{ab} ± 0.10	3.30^a ± 0.18
Amylase (U/g protein)	14.2^a ± 1.71	11.6^b ± 1.80	11.0^b ± 1.59
Protease (U/g protein)	31.1^a ± 5.04	24.8^b ± 1.92	20.1^c ± 2.37
Lipase (U/g protein)	4.9^a ± 0.74	4.4^{ab} ± 0.38	4.2^b ± 0.62
Alkaline phosphatase (U/L)	85^a ± 55.1	115^a ± 52.1	75^a ± 32.5
Alanine transaminase (U/L)	92.0^a ± 6.63	85.7^b ± 4.57	80.2^b ± 10.2
Blood parameters			
Red blood cells (10^6 cells/mm^3)	1.58^b ± 0.10	1.74^{ab} ± 0.20	1.85^a ± 0.13
Albumin (mg/dl)	1.98^a ± 0.15	1.73^{ab} ± 0.13	1.63^b ± 0.35
Globulin (mg/dl)	0.88^c ± 0.17	1.03^b ± 0.19	1.20^a ± 0.12
Lysozyme activity (IU/ml)	336.4^a ± 32.0	447.7^a ± 172.9	401.8^a ± 186.7
Glucose (mg/dl)	73^b ± 4.03	88^b ± 4.03	98^a ± 5.63

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Jatropha inclusions levels are 50% (J_{50}) and 75% (J_{75}) fishmeal protein replaced by DJKM. Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A_{280} per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 microequivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 at 37 °C. Alkaline phosphatase and Alanine transaminase expressed as 1 U = 16.66 nKat/L; nKat = amount of glandular kallikrein that cleaves 0.005 mmol of substrate per minute. Lysozyme activity IU is the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25 °C, using a suspension of *Micrococcus lysodeikticus* as the substrate.
Sources: Kumar *et al.*, 2010b; Kumar, Makkar and Becker, 2011a.

protein feedstuff, such as fishmeal (NRC, 1993). High availability of amino acids from DJKM for this fish species is expected. In general, the digestibility coefficients obtained for various jatropha constituents have been high, indicating that a large percentage of those constituents are digested and absorbed by the fish for further metabolism. Lipid digestibility of DJKM-based diets ranged from 74 to 90 percent (Kumar, Makkar and Becker, 2011a). High inclusion levels of DJKM (>50 percent fishmeal protein replacement) decreased lipid digestibility probably because of its high content of non-starch polysaccharides (NSPs) (Kumar, Makkar and Becker, 2011a, b).

Intestinal amylase, protease and lipase activities for the control group were significantly higher ($P < 0.05$) than for DJKM-fed groups (Kumar, Makkar and Becker, 2011a). Heat labile antinutrients, such as trypsin inhibitors and lectins, are absent in the DJKM, whereas a heat stable antinutrient (phytate) is present. Phytate is known to inhibit digestive enzymes such as pepsin, trypsin and α-amylase (Robaina *et al.* 1995; Alarcon, Moyano and Diaz, 1999). It also forms complexes with minerals (Teskeredzic *et al.*, 1995; Sugiura *et al.*, 1999) and proteins (Lopez *et al.,* 1999), thereby modifying digestion processes and impairing intestinal absorption. Kumar, Makkar and Becker (2011a) observed a decrease in digestive enzyme (amylase, protease and lipase) activity in the intestine on inclusion of DJKM in the common carp diets, which might be caused by the presence of phytate in the DJKM-based diets. However, this study used phytase 500 FTU phytase per kg for DJKM-based feeds, which might not be sufficient because of the high phytate content (9–10 percent) in DJKM. An increase in the level of DJKM in the diets led to a decrease in protein availability, probably caused by the presence of unhydrolysed phytate.

Effects of DJKM-containing feeds on growth performance, nutrient utilization, digestibility measurement, digestive enzymes activity and haematological parameters of common carp are presented in Table 9. Based on these results it is concluded that 50 percent of the fishmeal protein can be replaced by DJKM in common carp diets without compromising growth and nutrient utilization. However, >50 percent replacement of fishmeal protein by DJKM leads to significantly lower growth and higher feed conversion ratio (feed/body mass gain) in common carp, which could be attributed to factors such as:

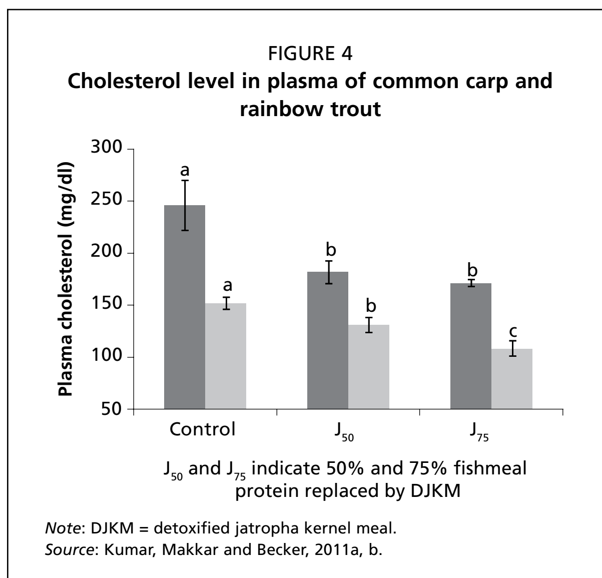
- Lower digestibilities of protein and energy in the diets, leading to lower protein and energy availability from DJKM (plant protein structures in general are much more compact than fishmeal protein, so digestive enzymes act slowly on DJKM proteins).
- The DJKM contains large concentrations of antinutrients such as phytate and non-starch polysaccharides (NSPs), and these could adversely affect feed utilization.
- The digestibility of synthetic lysine, which was added as a supplement to the diets, may be less than that of the natural amino acid present in the feed ingredients.

Retention of nutrients in the whole body

The efficiency with which nutrients and energy are retained from feeds provides a useful assessment of the efficiency of nutrient utilization from diets (Cho and Kaushik, 1990; Booth and Allan 2003; Glencross *et al.*, 2004). Feeding trials performed by Kumar, Makkar and Becker (2011a, 2010c) showed that inclusion of DJKM in a common carp diet exhibited significantly higher lipid deposition in the whole body than in the control group. The increase in whole body fat content on using dietary DJKM-based diets could be due to the higher content of total carbohydrate in these diets. Carbohydrates can be converted to lipids in the body by lipogenesis (Kumar, Makkar and Becker, 2011a). There is evidence that replacement of fishmeal protein by plant protein sources such as maize gluten meal and soy protein concentrates increases hepatic lipogenic enzyme activities in fish (Dias 1999; Kaushik *et al.*, 2004), leading to higher whole body lipid. In fish (salmonids), increases found in whole body fat content with the use of dietary plant proteins, were explained by imbalances in amino acid concentrations (Kaushik *et al.*, 2004; Bjerkeng *et al.*, 1997). Furthermore, it is suggested that unbalanced amino acid composition influences energy metabolism. Vilhelmsson *et al.* (2004) found an up-regulation of several proteins involved in energy metabolism in fish liver when fed plant proteins (maize gluten meal, wheat gluten, extruded whole heat, extruded peas and rapeseed meal) and concluded that the plant proteins increase energy demands of fish. Another possible reason could be a greater supply of some of the dispensable amino acids, such as glutamic acid, in excess by the DJKM proteins that could have led to higher lipid retention. Involvement of possible metabolic or endocrine mechanisms in eliciting such differences in whole body lipid deposition is suggested (Kumar, Makkar and Becker, 2011a, b). Proficient protein synthesis requires adequate availability of all EAAs. Unbalanced amino acid concentrations in a common carp diet resulted in increased protein degradation, and thereby increased protein turnover (Langar *et al.*, 1993; Kumar, Makkar and Becker, 2011a, b; Martin *et al.*, 2003). Generally, the plant protein-based diets decreased nitrogen retention in fish and shrimp because these diets have less digestible energy and an amino acid profile that is sub-optimal for muscle growth. Interestingly, Kumar, Makkar and Becker (2011a) showed that when compared with fishmeal, feeding DJKM to common carp led to higher whole body crude protein content, showing that DJKM contains optimum digestible energy and has a balanced amino acid profile ideal for fish growth.

Dietary inclusion of DJKM reduced the cholesterol level

FIGURE 4
Cholesterol level in plasma of common carp and rainbow trout



Note: DJKM = detoxified jatropha kernel meal.
Source: Kumar, Makkar and Becker, 2011a, b.

in plasma and muscle when compared with the fishmeal-fed group (Kumar *et al.*, 2010b). As DJKM level increased in the common carp diets the cholesterol level in muscle and plasma decreased (Figure 4). This hypocholesterolaemia in response to increasing dietary DJKM supply could be due either to an increased excretion of bile salts, to an inhibition of cholesterol intestinal absorption, or just to the withdrawal of fishmeal, rather than to the direct effects of plant protein (Kaushik *et al.*, 2004; Kumar *et al.*, 2010b). Further, fibre and antinutrtional factors (NSPs and phytate) reduce absorption of total fat, including cholesterol, when these factors are increased in the diet (Krogdahl, Bakke-McKellep and Baeverfjord, 2003; Hansen, 2009). Faecal excretion of steroids (bile acids) is the major pathway for elimination of cholesterol from the body (Hansen, 2009).

Energy budget and metabolic efficiency

Growth and production can be described in terms of partition of dietary energy between catabolism as fuels and anabolism as storage in tissues. Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure. Kumar, Makkar and Becker (2010c) reported that common carp fed DJKM and fishmeal-based diets exhibited similar values for routine metabolic rate (Table 10). These observations suggest that energy requirement for digestion and absorption of nutrients from DJKM and fishmeal are similar, and that DJKM is a promising good quality protein source for incorporation in feed for common carp. An energy budget was constructed by Cui and Liu (1990) for fish fed ad libitum and found that heat loss was always the largest component, 50–69 percent of the consumed energy, whereas the energy used for growth was much smaller, 21–35 percent. Kumar, Makkar and Becker (2010c) also observed that for DJKM-fed fish, energy retained for growth was 37 percent and energy expenditure was 41.4 percent of the gross energy fed. Metabolizable energy of the DJKM-based diet and metabolizable energy for growth in common carp were 78 percent and 47 percent, respectively. Metabolizable energy and energy expenditure per gram of protein retained in the fish body for growth in fish was also similar for DJKM- and fishmeal-fed groups (Table 10). It is evident that the protein quality of DJKM is equivalent to fishmeal protein, and both these protein sources result in similar growth performance, energy expenditure and energy retention (Kumar, Makkar and Becker, 2010c).

Impact of feeding detoxified jatropha kernel meal on common carp health

Haematological, biochemical and histological measurements are an integral part of evaluating the health status of commercially important fish. The activities of alkaline phosphatase (ALP) and alanine transaminase (ALT) in blood are used as indicators of liver cell condition. Usually, the level of ALP and ALT rises in blood during acute liver damage (Goel, Kalpana and Agarwal, 1984). Feeding DJKM and fishmeal did not change (P >0.05) levels of ALP and ALT activity in the blood (Kumar *et al.*, 2010b; Kumar, 2011) (Table 9). In addition, ALP and ALT levels in all groups were in the normal range as reported by Zhang *et al.* (2009) for healthy fish. Other health-related blood parameters, such as blood urea nitrogen, total bilirubin and creatinine contents, which

TABLE 10
Energy budget of common carp (*Cyprinus carpio* L) fed fishmeal (Control) and DJKM-based diet

Parameter	Control	DJKM
Initial body mass (g)	11.2 ± 1.14	10.6 ± 0.63
Final body mass (g)	$49.0^{a} \pm 7.9$	$48.3^{a} \pm 3.0$
Energy expenditure (EE; % of GE fed)	$44.3^{a} \pm 8.4$	$41.5^{a} \pm 0.9$
Energy retention (ER; % of GE fed)	$33.5^{a} \pm 0.7$	$36.9^{a} \pm 1.5$
Apparently unmetabolized energy (AUE; % of GE fed)	$22.2^{a} \pm 8.2$	$21.6^{a} \pm 1.10$
Efficiency of energy retention (ER/EE)	$0.77^{a} \pm 0.13$	$0.89^{a} \pm 0.05$
Average metabolic rate (mg O_2 $kg^{0.8}$/hour)	$363^{a} \pm 83.3$	$442^{a} \pm 120.9$
Energy expenditure (EE)/g protein fed	$19.6^{a} \pm 3.9$	$18.9^{a} \pm 1.1$

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscripts differ significantly (P <0.05). DJKM diet had 75% fishmeal protein replaced by DJKM. *Source*: Kumar, Makkar and Becker, 2010c.

are indicators of liver, kidney and gill function (Stoskopf, 1993; Tietz, 1986) were also in the normal range (Kumar *et al.*, 2010b). This suggested that the liver, kidney and gills of the common carp were in a normal functional condition in the DJKM-fed groups (Kumar *et al.*, 2010b). Also when common carp were fed DJKM as a protein source (Kumar *et al.*, 2010b; Kumar, 2011), haematology [haematocrit, haemoglobin and red blood cell count] values were within normal ranges (Ghittino, 1983; Rosenlund *et al.*, 2004).

One of the few unusual effects observed was a significant reduction in blood cell size (measured as mean cell volume) as the content of DJKM proteins increased in common carp diets (Kumar *et al.*, 2010b). As this observation appeared to coincide with increased spleen size, it was suggested that some of the plant ingredients may have caused early release of immature erythrocytes (Kumar *et al.*, 2010b). It may be noted that the spleen was larger in DJKM protein-fed groups than in the control group (fishmeal-based diet) (Kumar *et al.*, 2010b). Blood protein is considered a basic index for health and nutritional status in fish (Martinez, 1976). Among the blood proteins, albumin and globulin are the major proteins, which play a key role in the immune response. Lysozyme is regarded as the first line of defense, with high activity in mucus, serum, gills and the alimentary tract (Lie *et al.*, 1989). Feeding DJKM to common carp led to significantly higher (P <0.05) albumin, globulin and total protein concentrations in blood, and numerically higher lysozyme activity in serum, than in the control diet, indicating an immuno-stimulatory effect of DJKM on the common carp (Kumar *et al.*, 2010b; Kumar, 2011). Albumin, globulin and total protein concentrations in blood were within the normal range for DJKM-fed groups (Wedemeyer and Chatteron, 1970; Sandnes, Lie and Waagbo, 1988). Also DJKM diets exhibited no abnormal changes in intestine and liver (Kumar *et al.*, 2010b). The intestinal mucosa was well developed, no morphological alteration was found, and the intestinal mucosa appeared to be normal for common carp. Liver also showed no pathological alteration or signs of steatosis or hepatic lipidosis in the DJKM-fed group (Kumar *et al.*, 2010b).

Based on the above findings, it was concluded that DJKM can replace 50 percent fishmeal protein without comprising growth, nutrient utilization or health of the fish.

Use of detoxified jatropha kernel meal in rainbow trout (*Oncorhynchus mykiss*) diet

Impacts on growth and feed utilization

The utilization of detoxified jatropha kernel meal (DJKM) as a protein source in a carnivorous fish species, rainbow trout (*Oncorhynchus mykiss*), was investigated (Kumar, Makkar and Becker, 2011b). In this study, 50 percent (J_{50}) and 62.5 percent ($J_{62.5}$) fishmeal protein was replaced by DJKM. Palatability and acceptability of DJKM-based diets were similar to that of the fishmeal-based diet. Growth performance, and nutrients and energy digestibilities were statistically similar (P >0.05) for control and J_{50} group, but were higher (P <0.05) than for $J_{62.5}$ group (Table 11). Feed conversion ratio, protein efficiency ratio, protein productive value and energy retention were similar for control and DJKM-fed groups (Table 11). The lower growth response of the $J_{62.5}$ group could be due to lower protein and energy availability from the DJKM, poor availability of crystalline lysine added to DJKM-containing diets to equalize lysine content, or the presence of antinutrients such as phytate and NSPs, which are present in high amounts in the kernel meal. According to NRC (1983) the sulphur-containing amino acid (methionine and cystine) requirement of rainbow trout is 13 g/kg diet. In the $J_{62.5}$ diet the total sulphur amino acid was 11.3 g/kg, which is slightly lower than the optimum requirement. This could have led to lower growth performance in this group. Another constraint related to the digestion of DJKM-based diets is its relatively high carbohydrate content; carbohydrates are generally not well digested by rainbow trout (Kumar, Makkar and Becker, 2011b).

Retention of protein and lipid in the whole body of rainbow trout was higher in DJKM-fed groups compared with control groups, suggesting that DJKM contains optimum digestible energy and a balanced amino acid profile optimal for rainbow trout growth.

As DJKM increased in the rainbow trout diets the activity of digestive enzymes (amylase, protease and lipase) in the intestine significantly decreased (P <0.05), which might be because of phytate present in the DJKM-based diets (Kumar, Makkar and Becker, 2011b) (Table 11). Phytate is well known for inhibition of digestive enzymes. It also forms complexes with minerals and proteins, thereby modifying digestion processes and impairing intestinal absorption in rainbow trout. It is known that carnivorous fish require more time to digest plant protein-based diets compared with animal protein-based diets (Buddington, Krogdahl and Bakke-McKellep, 1997). A direct relationship between the amount of dietary plant protein and relative intestine length (RIL; mm/g) has been reported in fish (Kramer and Bryant, 1995). In rainbow trout, DJKM-based diets exhibited higher (P <0.05) RIL than the control group (Table 11). The RIL value increased as the DJKM inclusion increased. From a physiological view point, a greater RIL would facilitate an increase in digestibility and retention time by enhancing contact time of the digestive enzymes and the feed components, resulting in increases in their digestion and absorption. Carnivorous fish species like rainbow trout showed compensation mechanisms, such as an increase in RIL and as a result an increase in digestive activity, to achieve a digestive balance and growth rates similar to those observed for control groups (Kumar, Makkar and Becker, 2011b).

TABLE 11
Growth performance, nutrient utilization, digestibility measurements, digestive enzyme activities and haematological parameters of rainbow trout (*Oncorhynchus mykiss*) and common carp over an experimental period of 16 weeks

Parameters	Rainbow trout			Common carp		
	Control	Jatropha inclusion level		Control	Jatropha inclusion level	
		50%	62.5%		50%	62.5%
Initial body mass (g)	4.12 ± 0.26	4.17± 0.49	4.31 ± 0.54	21.5 ± 0.74	21.6 ± 1.04	21.8 ± 1.03
Final body mass (g)	61.0[a] ± 9.90	61.8[a] ± 6.88	54.6[b] ± 5.18	149[a] ± 23.0	128[a] ± 8.0	104[a] ± 31.6
FCR	1.3[a] ± 0.10	1.2[a] ± 0.10	1.3[a] ±0.20	1.7[a] ± 0.26	1.8[a] ± 0.05	2.2[a] ± 0.48
PER	1.6[a] ± 0.10	1.7[a] ± 0.11	1.6[a] ± 0.40	1.6[a] ± 0.16	1.4[a] ± 0.02	1.2[a] ± 0.26
PPV (%)	22.8[a] ±1.10	26.3[a] ±2.37	25.2[a] ± 4.59	26.5[a] ± 4.26	26.1[a] ± 0.29	21.9[a] ± 3.88
Digestibility measurements, relative intestine length (RIL), digestive and metabolic enzymes activity						
PD (%)	89.8[a] ± 0.55	89.7[a] ± 0.83	84[b] ± 1.41	85.9[a] ± 0.98	83.2[b] ± 0.80	78.6[c] ± 0.37
LD (%)	95.2[a] ± 0.43	95.2[a] ±0.80	89.8[b] ± 0.86	89.6[a] ± 0.51	86.2[b] ± 0.76	80.1[c] ± 1.65
ED (%)	86.8[a] ± 0.83	86.1[a] ± 0.71	81.8[b] ± 1.13	82.0[a] ± 1.62	77.7[b] ± 1.83	73.4[c] ± 0.49
Amylase (U/g protein)	4.6[a] ± 0.40	3.2[b] ± 0.20	2.5[c] ± 0.15	17.4[a] ±1.32	13.6[b] ± 0.83	11.0[c] ± 0.76
Protease (U/g protein)	50.3[a] ± 3.59	41.0[b] ± 2.16	32.5[c] ± 1.29	36.5[a] ± 1.31	28.1[b] ± 0.83	20.7[c] ± 1.24
Lipase (U/g protein)	13.9[a] ± 1.02	10.8[b] ± 0.38	8.6[c] ± 0.48	6.8[a] ± 0.29	5.3[b] ± 0.32	4.3[c] ± 0.22
RIL (mm/g)	0.47[c] ± 0.02	0.56[b] ± 0.02	0.63[a] ± 0.01	2.24[c] ± 0.07	2.78 [b] ± 0.10	3.17[a] ± 0.10
Alkaline phosphatase (U/L)	101[a] ± 8.3	96[a] ± 25.0	84[a] ± 30.7	60.8[a] ± 5.6	64.8[a] ± 29.6	84.8[a] ± 24.4
Alanine transaminase (U/L)	48.4[a] ± 13.1	75.2[a] ± 41.8	71.0[a] ± 35.5	80.3[a] ± 17.2	61.0[a] ± 6.1	64.8[a] ± 13.1
Blood parameters						
RBC (10^6 cells/mm^3)	0.96[a] ± 0.05	0.97[a] ± 0.07	1.05[a] ± 0.08	1.32[c] ± 0.02	1.41[b] ± 0.06	1.52[a] ± 0.06
Albumin (mg/dl)	2.18[b] ± 0.28	2.66[a] ± 0.15	2.36[b] ± 0.53	2.25[a] ± 0.48	2.05[a] ± 0.71	2.13[a] ± 0.05
Total protein (mg/dl)	3.8[b] ± 0.20	4.1[a] ± 0.30	3.8[b] ± 0.50	2.78[a] ± 0.39	2.83[a] ± 0.50	2.88[a] ± 0.13
Creatinine (mg/dl)	1.70[a] ± 0.86	0.98[ab] ± 0.22	0.34[b] ± 0.21	1.55[a] ± 1.12	0.28[b] ± 0.15	0.20[b] ± 0.00

Notes: Values are mean ± standard deviation. For each species, mean values in the same row with different superscript differ significantly ($P <0.05$). Jatropha inclusion levels are 50 and 62.5% of fishmeal protein replaced by DJKM. FCR = feed conversion ratio; PER = protein efficiency ratio; PPV = protein productive value; PD = protein digestibility; LD = lipid digestibility; ED = energy digestibility; RBC = red blood cells. Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A_{280} per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 micro-equivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 and 37 °C. ALP and ALT expressed as 1 U = 16.66 nKat/l; nKat = amount of glandular kallikrein that cleaves 0.005 mmol of substrate per minute.
Sources: Kumar, Makkar and Becker, 2011b; Kumar, 2011.

Inclusion of DJKM in rainbow trout diets decreased cholesterol level in muscle and plasma (Figure 6) (Kumar, Makkar and Becker, 2011b). This could be due to an increased excretion of bile salts, an inhibition of cholesterol intestinal absorption, or just the taking away of fishmeal rather than to the direct effects of DJKM (Kumar, Makkar and Becker, 2011b). Higher ($P <0.05$) concentrations of phosphorus and calcium ions in blood of rainbow trout were observed for DJKM-fed groups. The DJKM-based diets were supplemented with phytase (500 FTU/kg feed), which would increase release of phosphorus and calcium from feed and make them available for rainbow trout.

Impacts on health of fish

Metabolic enzyme (ALP and ALT) activities, metabolites (urea nitrogen, total bilirubin and creatinine) and ion concentrations in blood were in the normal range in the groups in which 50 percent and 62.5 percent of fishmeal protein was replaced by DJKM. Blood parameters such as red blood cell (RBC) and white blood cell counts, haematocrit and haemoglobin level were also not affected ($P >0.05$) by dietary treatments (Kumar, Makkar and Becker, 2011b) and their ranges were also in the normal range reported by Blaxhall and Daisley (1973) for healthy trout.

After feeding DJKM as a protein source to the rainbow trout, no signs of histopathological lesions were observed in the organs (Kumar, 2011). The gastric glands were well developed and the epithelium lining the luminal surface that consists of highly columnar cells and produces protective mucous was not altered. The case for the branched tubular glands was similar, as they were also well developed. There was no change in the shape and cellular morphology of pepsin- and hydrochloric acid-producing cell-types (oxyntopeptidic cells), indicating no leucocyte immigration and therefore no signs of inflammation (Kumar, 2011). In addition, there was no alteration in intestinal loops, pyloric appendices, the terminal hind gut, and the villi of the appendices or terminal intestine. There was also no sign of hepatic steatosis or lipidosis in rainbow trout when fed with DJKM as a protein source.

Conclusively, DJKM can replace 50 percent fishmeal protein without compromising the growth, feed utilization and health of rainbow trout.

USE OF DETOXIFIED JATROPHA KERNEL MEAL AS A PROTEIN SOURCE IN WHITE LEG SHRIMP FEED

Growth performance and nutrient utilization parameters on incorporation of detoxified jatropha kernel meal (DJKM)

TABLE 12
Growth performance and nutrient utilization of white leg shrimp (*Litopenaeus vannamei)* fed control and DJKM-based diets for eight weeks

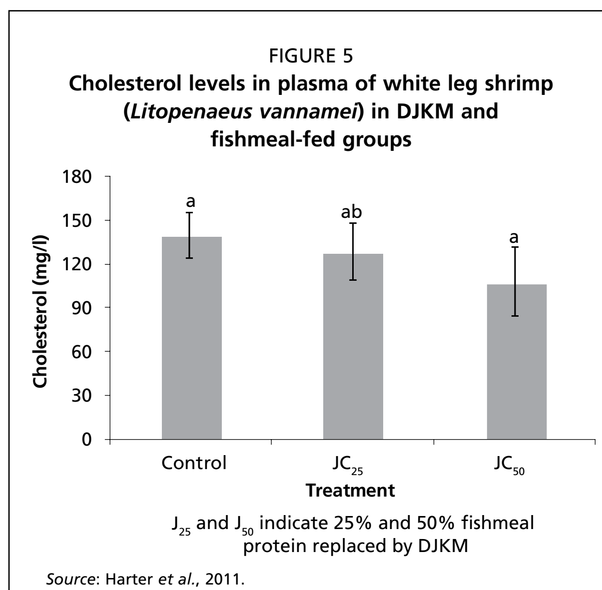
Treatment	Initial body mass (g)	Final body mass (g)	MGR (g $kg^{0.8}$ day^{-1})	Feed conversion ratio	Protein efficiency ratio
Control	4.46 ± 0.60	10.54^b ± 3.17	5.51^b ± 0.70	3.18^a ± 0.37	1.01^b ± 0.11
JC_{25}	4.47 ± 0.64	12.59^a ± 3.98	6.67^a ± 0.38	2.46^b ± 0.28	1.24^a ± 0.21
JC_{50}	4.45 ± 0.69	13.60^a ± 3.18	7.22^a ± 0.75	2.28^b ± 0.39	1.39^a ± 0.23

Notes: JC_{25} and JC_{50} are 25% and 50% of fishmeal protein replaced by DJKM. MGR = metabolic growth rate. Values are mean (n = 4) ± standard deviation. Mean values in the same column with different superscript differ significantly (P <0.05). *Source*: Harter *et al.*, 2011.

in the diet of white leg shrimp are presented in Table 12. Greater growth response and nutrient utilization were observed in DJKM-fed groups (25 or 50 percent fishmeal protein replaced by DJKM) compared with fishmeal-fed group in white leg shrimp (*Litopenaeus vannamei*) (Harter *et al.*, 2011). There is a possibility of synergistic effects between the feed ingredients used (fishmeal and DJKM), being complementary to each other in their amino acid composition. The DJKM protein in combination with fishmeal protein gave excellent nutrient and energy digestibility, leading to higher growth performance and nutrient utilization (Harter *et al.*, 2011). These results, along with the amino acid composition of the diets tested, indicated that the requirements of shrimp (Akiyama and Tan, 1991; Van Wyk, 1999) for amino acids were met.

In the whole body of the shrimp, there was no significant effect (P >0.05) on lipid deposition after feeding DJKM, whereas protein and energy deposition were significantly higher (P <0.05) in the control group compared with DJKM-fed groups (Harter *et al.*, 2011). Cholesterol is reported to be an essential nutrient for growth and survival of all crustacean species (Kanazawa *et al.*, 1971). Usually, shrimp diets are supplemented with cholesterol, because they like other crustaceans cannot synthesize cholesterol *de novo* (Teshima and Kanazawa, 1971). Cholesterol levels in plasma of white leg shrimp decreased when fed with DJKM-based diets (Figure 5). Reduction in plasma cholesterol level in shrimp as dietary fishmeal levels decreased and DJKM levels increased was a consequence of the reduced amount of cholesterol available in the diet (Kaushik *et al.*, 1995; Harter *et al.*, 2011).

FIGURE 5
Cholesterol levels in plasma of white leg shrimp (*Litopenaeus vannamei*) in DJKM and fishmeal-fed groups



J_{25} and J_{50} indicate 25% and 50% fishmeal protein replaced by DJKM

Source: Harter *et al.*, 2011.

Overall, growth performance and nutrient utilization in white leg shrimp for DJKM-fed groups were better (P <0.05) than for the control group, which suggests that white leg shrimp, can efficiently use DJKM as a good quality protein source. Additional studies with DJKM-based diets at a larger scale and under commercial pond conditions are suggested.

USE OF *JATROPHA CURCAS* KERNEL MEAL OF A NON-TOXIC JATROPHA GENOTYPE IN AQUA FEED

Heat-treated (121 °C at 66 percent moisture for 15 minutes) and unheated *J. curcas* kernel meals of a non-toxic variety were used as protein sources for common carp diet. The heat treatment was done to inactivate trypsin inhibitor and lectins. Similar growth performances were observed for both the groups, suggesting no physiological relevance of heat-labile factors such as trypsin inhibitor and lectins in jatropha meal and of the heat-stable factors such as antigenic proteins, if any, for common carp. Incorporation of jatropha kernel meal that was subjected to heat treatment for >15 min decreased growth performance of common carp. Lower growth performance and nutrient utilization were also observed with 30- and 45-minute-heated jatropha kernel meal compared with the unheated group (Makkar and Becker, 1999). These findings imply the loss of amino acids and their lower availability due to Maillard reaction products or heat-induced changes in the structure of jatropha proteins, or a combination, which are less digestible by the fish intestinal proteases. The energy retained in the fish was also lower in the 30- and 45-minute-heated jatropha meal-fed groups compared with the unheated meal-fed group. However, heat treatment has been shown to increase protein digestibility of jatropha protein by rumen proteases (Aderibigbe *et al.*, 1997) and also to inactivate the trypsin inhibitor and lectin (Makkar and Becker, 1997). Nutrient retention in the whole body was similar for control, unheated and heated (15, 30 or 45 minutes at 121 °C) jatropha meal-fed groups. It would be interesting to investigate the effects of incorporating unheated jatropha kernel

meal from the non-toxic jatropha genotype in the diets of other fish species.

The results of Makkar and Becker (1999) demonstrate that the availability of protein from the unheated jatropha meal is higher than from heat-treated jatropha meal. Furthermore, the nutritional value of jatropha meal of the non-toxic genotype is high, and potential exists for its incorporation into the diets of monogastrics and aquaculture species.

USE OF *JATROPHA PLATYPHYLLA* KERNEL MEAL AS A PROTEIN SOURCE IN AQUA FEED

Impacts of *Jatropha platyphylla* kernel meal (heat treated) on growth performance of Nile tilapia

The kernel meal of *J. platyphylla* contains 65–70 percent crude-protein with a well-balanced EAA profile, in addition to heat labile antinutritional factors, trypsin inhibitor and lectin. The heat treated (121 °C at 66 percent moisture for 15 minutes) kernel meal (H-JPKM) was fed to Nile tilapia (*Oreochromis niloticus* L.) for 12 weeks with two levels of replacement (50 percent and 62.5 percent) of fishmeal protein). H-JPKM-based diets were supplemented with phytase (500 FTU per kg feed) to mitigate the adverse effects of phytate. The utilization of proteins from H-JPKM and fishmeal was similar ($P > 0.05$). Also, growth performance of H-JPKM-fed groups was similar; indicating that availability of protein (amino acids) from the H-JPKM for protein synthesis was similar to that from fishmeal. These findings showed that H-JPKM is a good quality dietary protein source for Nile tilapia feed. The level of NSPs in H-JPKM was about 16 percent; however, no detrimental effects were observed. The anti-nutritional effects of NSPs are not yet fully understood in fish. However, these compounds are assumed to cause increased intestinal viscosity in fish similar to that in poultry. Usually, NSPs in diets for Atlantic salmon tended to reduce digestibility of protein and lipid due to increased intestinal viscosity and reduced diffusion and activity of the digestive enzymes (Refstie *et al.*, 2000). However, Makkar *et al.* (2011), Kumar *et al.*, (2011c) and Akinleye *et al.* (2011) did not observe any such adverse effects in Nile tilapia after feeding H-JPKM as the protein source. Retention of protein and lipid in the whole body of Nile tilapia were similar ($P > 0.05$) for H-JPKM-fed and fishmeal-fed groups (Kumar *et al.*, 2011c; Akinleye *et al.*, 2011). These results suggest that H-JPKM containing diets were ideal for fish growth.

Impacts of *Jatropha platyphylla* kernel meal (heat treated) on energy budget and health of Nile tilapia

In a feeding trial performed by Kumar *et al.* (2011c) wherein Nile tilapia were fed H-JPKM-based diet (62.5 percent fishmeal protein replaced by H-JPKM) and a control diet (fishmeal-based diet), no significant difference ($P > 0.05$) for oxygen consumption, average metabolic rate, energy retention, energy expenditure and metabolizable energy were observed among the groups. The energy retention for growth was 35 percent, energy expenditure 40 percent and metabolizable energy 74 percent for the H-JPKM-based diet (Kumar, Makkar and Becker, 2011c). This finding suggests that dietary protein sources H-JPKM can be efficiently utilized for growth of Nile tilapia, and the efficiency is as high as that for fishmeal.

Inclusion of H-JPKM in the diet elicited no adverse effects on biochemical changes such as metabolic enzymes (ALP and ALT) and electrolytes and metabolites (urea nitrogen, bilirubin, calcium, potassium and sodium in the blood) (Akinleye *et al.*, 2011). The prominent changes include increased RBC count, haematocrit content and blood glucose concentrations, and decreased cholesterol concentration in plasma when compared with the control group (Akinleye *et al.*, 2011). However, haematological parameters were within normal ranges for fish (Ghittino, 1983; Rosenlund *et al.*, 2004).

The results showed that H-JPKM can replace fishmeal with no negative impacts on growth, feed utilization and physiological parameters (Makkar *et al.*, 2011; Kumar, Makkar and Becker, 2011c; Akinleye *et al.*, 2011). Conclusively, the H-JPKM can replace fishmeal protein up to 62.5 percent in the diet of Nile tilapia without any unfavorable effects on growth performance, nutrient utilization, energy budget and biochemical activities in the fish, and it can be utilized in Nile tilapia diet as a good quality protein source. Further research should be conducted to examine the possibility of increasing the inclusion of H-JPKM beyond 62.5 percent fishmeal protein replacement in the diet of Nile tilapia. Also studies on the utilization of H-JPKM in other fish species are required.

USE OF DETOXIFIED *JATROPHA CURCAS* PROTEIN ISOLATE IN COMMON CARP FEED

Impacts on feed intake and growth performance

Kumar, Makkar and Becker (2011d) observed that detoxified jatropha protein isolate (DJPI)-based diets had excellent palatability for common carp and there was no wastage of feed during the experiment. Common carp fed a diet containing a lower level of DJPI (50 percent replacement of fishmeal protein) grew significantly better ($P < 0.05$) than those on the fishmeal-based control diet (Kumar *et al.*, 2011d; Nepal *et al.*, 2010). However, a higher level (75 percent replacement of fishmeal protein) of DJPI exhibited growth performance similar ($P > 0.05$) to that with the control diet (Table 13) (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010). Since overall growth performance, and protein

TABLE 13
Growth performance, nutrient utilization, digestibility measurements, digestive enzyme activities and haematological parameters of common carp (*Cyprinus carpio* L) fed DJPI-based diets

Parameters	Control	Jatropha	
		50% (J_{50})	75% (J_{75})
Initial body mass (g)	20.3 ± 0.12	20.3 ± 0.11	20.2 ± 0.08
Final body mass (g)	124^a ± 9.0	118^a ± 13.5	118^a ± 13.5
Feed conversion ratio	1.36^a ± 0.06	1.31^a ± 0.03	1.39^a ± 0.10
Protein efficiency ratio	1.91^a ± 0.11	2.01^a ± 0.05	1.86^a ± 0.14
Protein productive value (%)	30.4^c ± 2.84	34.2^b ± 1.37	33.7^b ± 2.83
Nutrient digestibility and digestive and metabolic enzymes activity			
Protein digestibility (%)	90^b ± 1.03	93^a ± 1.57	89^b ± 2.01
Lipid digestibility (%)	94^a ± 1.58	95^a ± 1.67	94^a ± 2.53
Energy digestibility (%)	88^b ± 1.28	91^a ± 1.64	89^b ± 2.08
Amylase (U/g protein)	20.1^a ± 3.36	18.6^a ± 5.81	18.4^a ± 4.40
Protease (U/g protein)	40.0^a ± 2.82	37.1^a ± 3.91	32.7^a ± 3.04
Lipase (U/g protein)	7.8^a ± 1.39	8.4^a ± 0.46	8.5^a ± 0.85
Alkaline phosphatase (U/L)	65.7^a ± 13.3	62.7^a ± 3.5	68.3^a ± 7.4
Alanine transaminase (U/L)	74.3^a ± 17.2	79.0^a ±11.8	68.0^a ± 10.1
Blood parameters			
Red blood cells (10^6 cells/mm^3)	1.1^c ± 0.02	1.3^b ± 0.03	1.4^a ± 0.01
Albumin (mg/dl)	2.1^b ± 0.2	2.5^a ± 0.1	2.6^a ± 0.3
Globulin (mg/dl)	0.6^b ± 0.2	0.8^a ± 0.2	0.9^a ± 0.1
Lysozyme activity (IU/ml)	384^b ± 24	419^a ± 18	431^a ± 34
Cholesterol (mg/dl)	139^a ± 14	116^b ± 22	93^c ± 15
Glucose (mg/dl)	67.0^a ± 8.0	61.0^a ± 11.8	87.0^a ± 25.2

Notes: Values are mean ± standard deviation. Mean values in the same row with different superscript differ significantly (P <0.05). Amylase U expressed as millimoles of maltose released from starch per minute. Protease U expressed as amount of enzyme needed to release acid soluble fragments equivalent to 0.001 A_{280} per minute at 37 °C and pH 7.8. Lipase U expressed as hydrolysis of 1.0 micro-equivalent of fatty acid from a triglyceride in 24 hours at pH 7.7 at 37 °C. ALP and ALT expressed as 1 U = 16.66 nKat/l; nKat = amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute. Lysozyme activity IU expressed as the amount of enzyme required to produce a change in the absorbance at 450 nm of 0.001 units per minute at pH 6.24 and 25 °C, using a suspension of *Micrococcus lysodeikticus* as the substrate.
Sources: Kumar *et al.*, 2011d; Nepal *et al.*, 2010.

and energy utilization of this group were similar to those of the fishmeal-fed group, it is plausible to surmise that a high replacement level (up to 75 percent replacement of fishmeal protein) of fishmeal by a single plant protein-based source such as DJPI is possible in common carp diet. Higher levels of replacement of fishmeal (up to 100 percent) by DJPI should be investigated in future studies.

Impacts on digestive physiology

DJPI in combination with fishmeal protein showed excellent nutrient and energy digestibilities in common carp (Table 13) (Kumar *et al.*, 2011d). Compared with fishmeal protein, DJPI had similar (P >0.05) apparent protein and lipid digestibility, which could be attributed to the absence of a trypsin inhibitor and lectin, the presence of lower levels of NSPs (NSPs in DJPI were 10 percent), and the addition of phytase to mitigate the effects of phytate, if any (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010). Energy digestibility of the DJPI protein-based diets was considerably lower than protein digestibility (Kumar, Makkar and Becker, 2011d; Nepal *et al.*, 2010).

Dietary inclusion of DJPI did not (P >0.05) alter the intestinal digestive enzyme (amylase, protease and lipase) activities (Table 13). Phytate content in DJPI was 2.9 percent, and the DJPI-based feeds were supplemented with 500 FTU phytase/kg. This level of phytase appears to be sufficient to hydrolyse the phytate in the DJPI-based diets. No change in activities of digestive enzymes could be attributed to the absence of trypsin inhibitors and lectins and addition of phytase (500 FTU phytase/kg) in the DJPI-based diets (Kumar, Makkar and Becker, 2011d).

Impacts on nutrients retentions

Inclusion of DJPI in feed exhibited higher (P <0.05) lipid retention in the whole body of fish compared with control (fishmeal) fish. As DJPI protein increased in the common carp diet, lipid retention in the whole body also increased, which led to a higher value of lipid productive value and energy productive value (Kumar, Makkar and Becker, 2011d). DJPI could have increased the lipogenic enzyme activities in fish. Protein deposition in the whole body of common carp was more pronounced (P <0.05) in DJPI-fed groups compared with the fishmeal-fed group, which concurs with the higher value of protein productive value in the former group (Table 13). Protein synthesis in the body requires an optimum level of dietary EAAs. Unbalanced amino acid concentrations in a diet or different availability of individual amino acids results in increased protein

degradation, leading to increased protein turnover. Usually plant protein sources such as soy protein decrease protein retention because soy protein-based diets are not able to provide well balanced EAAs and energy for growth (Cheng, Hardy and Usry, 2003). Interestingly, Kumar Makkar and Becker (2011d) found that protein retention in the body of common carp was significantly higher (P <0.05) in DJPI-fed groups than the control group. This finding reveals that that DJPI-containing diets have optimum digestible energy and a balanced amino acid profile for optimum growth and optimum nutrient deposition in fish.

Impacts on biochemical parameters and haemato-immunology

Feeding DJPI as a protein source significantly decreased (P <0.05) cholesterol level in plasma and muscle compared with the control group (Table 13) (Kumar *et al.*, 2009; Nepal *et al.*, 2010). The hypocholesterolaemic effect of plant proteins compared with animal proteins is well documented (Forsythe, 1995) and could be due to increased excretion of the bile salts, resulting in inhibition of cholesterol absorption through the intestine (Kumar, Makkar and Becker, 2009; Kumar *et al.*, 2010b; Nepal *et al.*, 2010). The amino acid composition of dietary protein was partially responsible for its effect on cholesterol concentration (Tasi and Huang, 1999). These authors observed a significant positive correlation between the lysine/arginine ratio of the diet and serum cholesterol concentration. Nepal *et al.* (2010) and Kumar Makkar and Becker (2009) also observed that the lysine/arginine ratio in DJPI-based diets was positively correlated with cholesterol concentration in plasma of common carp.

RBC counts increased with increased level of DJPI in common carp diet (Table 13) (Kumar, Makkar and Becker, 2009; Nepal *et al.*, 2010); however these values were in the normal range (1.10–2.20 $\times 10^6/mm^3$) for healthy carp (Ghittino, 1983). Higher RBC in the DJPI-fed group might be due to a higher proportion of immature erythrocytes released from the spleen (Härdig and Hoglund, 1983). Kumar *et al.* (2009) and Nepal *et al.* (2010) also observed higher haematocrit value in the DJPI protein-fed groups than the control group due to higher RBC count in these groups. Haematocrit level in all groups was in the normal range of 44–59 percent for common carp (Radu *et al.*, 2009).

Significantly higher (P <0.05) albumin and globulin concentrations in blood and lysozyme activity in serum were observed for DJPI-fed groups (Table 13), which suggest an immuno-stimulatory effect of DJPI in common carp (Kumar, Makkar and Becker, 2009; Nepal *et al.*, 2010). Albumin and globulin concentrations in blood for DJPI-fed groups were within the normal range (Wedemeyer and Chatterton, 1970; Sandnes, Lie and Waagbo, 1988). Feeding DJPI as a protein to common carp exhibited levels of metabolic enzyme (ALP and ALT) activities similar (P >0.05) to those in the control group, suggesting normal organ function and absence of toxic factors in DJPI (Nepal *et al.*, 2010). Blood glucose concentration was unaffected (P <0.05) by dietary inclusion of DJPI in common carp (Nepal *et al.*, 2010).

An overview of the results of jatropha-based feed ingredients when used to replace fishmeal in fish and shrimp diets are presented in Table 14.

CONCLUSIONS REGARDING USE OF DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* AS AQUA FEED

Effects on growth and nutrient utilization

- Detoxified jatropha kernel meal, H-JPKM and DJPI can replace 50, 62.5 or 75 percent fishmeal protein, respectively, without compromising growth performance and nutrient utilization in fish. In addition, DJKM can also replace 50 percent fishmeal protein without any adverse effects on growth and nutrient utilization in shrimp. If the replacement levels are exceeded, the nutrient profile of the feeds must be carefully examined to ensure that desired production levels can be achieved and fish and shrimp health maintained.
- High inclusion (>50 percent fishmeal protein replacement) of DJKM decreases the efficiency of conversion of feed to body mass. No such effects were seen on using DJPI in common carp diets.
- Increased DJKM inclusion (>50 percent fishmeal protein replacement) in diets caused a significant lowering of protein, lipid and energy digestibilities. No such effects were observed when DJPI was used in common carp diets.

Effects on energy budget

- Feeding DJKM and H-JPKM to common carp and Nile tilapia respectively did not change the major components of the energy budget (routine metabolic rate, heat released and metabolizable energy) compared with fishmeal and SBM-fed groups. These results showed that, as dietary protein sources, DJKM and H-JPKM can be efficiently utilized for growth by common carp and Nile tilapia respectively, and as good as soymeal and fishmeal.

Effects on clinical health parameters and gut health

- No mortalities and unaffected haematological values suggested that the fish were in normal health. ALP and ALT activities, urea nitrogen, bilirubin and creatinine concentrations in blood were in the normal ranges, showing no liver or kidney dysfunction.
- The plasma nutrient levels measured gave no indications of stress, but increasing the level of plant protein in the diet decreased plasma cholesterol. A decrease in muscle

TABLE 14
An overview of the results of replacing fishmeal with jatropha-based feed ingredients in fish and shrimp diets

Jatropha-based ingredient	Species	Inclusion level in diet (%)	CP in diet (%)	Experi-mental period (weeks)	Fishmeal protein replaced in diet (%)	Biological effects	References
Detoxified jatropha kernel meal (DJKM)	Common carp (*Cyprinus carpio*)	24 and 36	38	8	50 and 75	At up to 50% replacement level growth performance and nutrient utilization were similar to those in control; >50% replacement level decreased performance. Inclusion of DJKM in the diets did not change blood metabolite, ion and enzyme levels. Also no adverse histopathological changes were observed.	Kumar *et al.,* 2010b; Kumar, Makkar and Becker, 2011a.
DJKM	Common carp	38	38	6	75	Growth performance, nutrient utilization, oxygen consumption and metabolic rate were similar to those in control.	Kumar, Makkar and Becker, 2010c.
DJKM	Common carp	25 and 31	38	16	50 and 62.5	No significant difference in growth rate among the groups.	Kumar, 2011.
DJKM	Rainbow trout (*Oncorhynchus mykiss*)	34 and 43	45	12	50 and 62.5	Growth rate and feed efficiency for 50% replacement group were similar to those for control; 62.5% replacement significantly depressed these parameters.	Kumar, Makkar and Becker, 2011b.
DJKM	White leg shrimp (*Litopenaeus vannamei*)	12.5 and 25	35	8	25 and 50	Shrimp on DJKM-based diet grew better than control; nutrient deposition in the body was similar; hypocholesterolaemic effect observed in fish fed DJKM-based diet.	Harter *et al.,* 2011.
Detoxified jatropha protein isolate (DJPI)	Common carp	20 and 30	38	12	50 and 75	Growth performance, nutrient utilization and digestive enzyme activity were similar to those in control, and improved protein utilization in DJPI-fed group. Blood parameters were in the normal range. Also no adverse histopathological changes were observed.	Kumar, Makkar and Becker, 2011d.
Heated *Jatropha platyphylla* kernel meal (H-JPKM)	Nile tilapia (*Oreochromis niloticus*)	20 and 25	36	12	50 and 62.5	No differences in growth rate, feed utilization, oxygen consumption and average metabolic rate among the H-JPKM diets and control.	Makkar *et al.*, 2011; Akinleye *et al.,* 2011; Kumar *et al.,* 2011c.

cholesterol level is also expected, which could be considered good for human health.

- Histopathological evaluation showed no damage to stomach, intestine or liver of common carp or rainbow trout.

USE OF DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL IN POULTRY FEED

Soybean and canola meals (i.e. rapeseed meal) are the major protein meals used worldwide in poultry feed (USDA, 2010). However, SBM competes with human food and there is a need to search for alternative plant-protein sources for poultry feed. Recent research with fish species has shown that detoxified *J. curcas* kernel meal (DJKM) can be an excellent source of dietary protein in animal feeds, especially in situations where fishmeal and conventional protein-rich feed ingredients are in short supply and expensive (Makkar and Becker, 2009a; Kumar, Makkar and Becker, 2011a, b). The nutrient and energy concentrations of DJKM compare well with that of SBM, with a higher content of EAAs (except lysine).

Boguhn *et al.* (2010) evaluated the nutritional quality of DJKM in turkeys (3-week-old) by including at levels of 0 (control), 10 (J_{10}) or 20 percent (J_{20}) into a basal diet based on maize, SBM and wheat gluten, at the expense of maize starch. Body mass gains were 42, 54 and 57g/day for control, J_{10} and J_{20} groups respectively. Feed efficiency (gain:feed ratio) was significantly higher (P <0.05) in DJKM-fed groups (0.81 and 0.82 vs 0.70). Precaecal amino acid digestibilities of amino acid from DJKM varied from 0.48 (cystine) to 0.91 (methionine) (Table 15). Mean digestibility of the non-essential amino acids was 80 percent, while that of EAAs was 83 percent.

Considering growth performance, nutrient utilization and amino acid digestibility of DJKM, it can be concluded that DJKM is valuable protein source for turkeys.

USE OF DETOXIFIED *JATROPHA CURCAS* KERNEL MEAL IN PIG FEED

The most commonly used source of supplemental protein in diets for non-ruminants is SBM because of its excellent

TABLE 15
Amino acid content of detoxified jatropha kernel meal (g/kg DM) and calculated coefficients of their precaecal digestibility (mean ± standard error)

	Detoxified jatropha kernel meal	Precaecal digestibility cofficient
Crude protein	630	0.83 ± 0.042
Alanine	31.0	0.85 ± 0.037
Arginine	74.0	0.90 ± 0.034
Aspartic acid	62.0	0.70 ±0.033
Cystine	4.9	0.48 ± 0.057
Glutamic acid	103.3	0.85 ± 0.056
Glycine	29.2	0.79 ± 0.035
Isoleucine	25.2	0.88 ± 0.049
Leucine	45.2	0.86 ± 0.049
Lysine	21.7	0.87 ± 0.068
Methionine	10.7	0.91 ± 0.083
Phenylalanine	29.4	0.89 ± 0.041
Proline	29.0	0.83 ± 0.072
Serine	33.1	0.79 ± 0.041
Threonine	24.1	0.83 ± 0.051
Tryptophan	6.9	0.83 ± 0.042
Valine	29.0	0.88 ± 0.041

Source: Boguhn *et al.*, 2010.

amino acid profile and dependable supply. In a typical pig diet, soybean supplies about 50 percent of the protein and amino acids and about 25 percent of the metabolizable energy. Wang *et al.* (2011) investigated the effects of replacing SBM by detoxified *J. curcas* kernel meal (DJKM) in the diet of the growing pig. The DJKM protein replaced 0, 25 or 50 percent of SBM protein in the diets, and the DJKM-containing diets were supplemented with lysine (~2 percent of DJKM inclusion). There were no significant differences (P >0.05) in growth performance and feed utilization on substituting 25 or 50 percent of SBM protein with DJKM (Table 16). These results show that the nutrient value of a DJKM-supplemented diet containing additional lysine is comparable with that of SBM for growing pigs. Dietary inclusion of DJKM did not (P >0.05) affect carcass weight, dressing percentage, back fat thickness or visceral organ weight and its ratio to body weight when compared with the control group.

Also, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase and ALP activities and the concentrations of albumin, urea, glucose and triglycerides in serum did not change (P >0.05) in growing pigs. There were no histopathological changes in liver and kidney of growing pigs fed DJKM diets (Wang *et al.*, 2011).

The above data show that incorporation of DJKM had no ill effects on health, and it can replace 50 percent soymeal protein in diets of growing pigs.

CHALLENGES AND OPPORTUNITIES IN USING AS LIVESTOCK FEED BY-PRODUCTS OBTAINED DURING THE PRODUCTION OF BIODIESEL FROM JATROPHA OIL

During the process of biodiesel production, acid gum and fatty acid distillate are produced during the de-gumming and de-odorization processes, respectively, before the transesterification process; and glycerol is produced during the transesterification process. Amongst these by-products, glycerol is recovered in substantial amounts (10 percent of

TABLE 16
Growth performance, nutrient utilization and health parameters of pigs fed DJKM-based diets

Parameter	Control	Jatropha		SEM
		25% (J_{25})	50% (J_{50})	
Growth and feed utilization				
Initial body mass (kg)	21.45	21.43	21.44	0.244
Final body mass (kg)	38.76	38.40	39.24	0.695
Feed to gain ratio	2.167	2.127	2.150	0.098
Biochemical				
Total protein (g/L)	60.5^{ab}	58.0^{b}	63.6^{a}	1.46
Albumin (g/L)	34.6	33.3	36.3	0.94
Urea nitrogen (mmol/L)	2.89	2.80	3.04	0.146
Glucose (mmol/L)	5.40	4.99	4.95	0.350
Triglyceride (mmol/L)	0.39	0.35	0.36	0.026
Enzyme activities				
Superoxide dismutase (U/mL)	148.6^{ab}	138.7^{b}	157.3^{a}	4.25
Lactate dehydrogenase (U/mL)	14.0	15.1	14.7	1.02
Lysozyme (U/mL)	70.0	62.8	70.3	4.22
Glutamic-pyruvic transaminase (U/L)	11.7	12.3	12.5	0.81
Glutamic-oxalacetic transaminase (U/L)	10.8	11.4	10.9	1.02
Alkaline phosphatase (U/100 mL)	20.9	18.1	20.6	1.15
Acid phosphatase (U/100 mL)	11.6	10.8	11.7	0.81

Notes: Means with different superscripts within a rows differ significantly (P <0.01). J_{25} and J_{50} = 25 and 50% of SBM protein replaced by detoxified *Jatropha curcas* kernel meal, respectively. SEM = standard error of the mean. *Source*: Wang *et al.*, 2011.

the biodiesel). In the study of Makkar and Becker (2009a) no phorbol esters were detected in the glycerol fraction; however phorbol esters were detected in glycerol samples obtained from two different laboratories associated with biodiesel producing companies that produced biodiesel from jatropha oil (company #1: 0.58–0.97 mg/g glycerol; company #2: 0.061 mg/g glycerol). Similarly, fatty acid distillate produced by the procedure adopted by Makkar and Becker (2009a) was free of phorbol esters; however, an earlier study (Haas and Mittelbach, 2000) reported the presence of phorbol esters in the fatty acid distillate fraction. Compared with the Makkar and Becker (2009a) study, the study of Haas and Mittelbach (2000) used mild conditions during the stripping or de-odorizing step that gives fatty acid distillate. These observations suggest that the process conditions used in the Makkar and Becker (2009a) study led to destruction of phorbol esters, and that process parameters for biodiesel production could be established that gives glycerol and fatty acid distillate fractions free of phorbol esters. It should be be noted that at present no information is available on the nature of the phorbol ester degraded products and their possible toxicity. There is need for further research to evaluate the innocuous nature of fatty acid distillate and glycerol so produced. The acid gum fraction obtained during the de-gumming stage was rich in phorbol esters (2.02 mg/g) (Makkar and Becker, 2009a) and hence not usable in animal feeds. At the same time, these fractions obtained during biodiesel production from the oil from the non-toxic *J. curcas* would be safe for inclusion in livestock diets.

To enable safe use of these by-products, a process was needed for isolation of phorbol esters from the toxic jatropha oil before the oil goes for biodiesel production, and efforts in this directions have been successful (Devappa, Makkar and Becker, 2010c; Devappa *et al.*, 2010d). The phorbol esters isolated could be used for various agricultural and pharmaceutical applications since they have strong molluscicidal and pesticidal activities (Makkar and Becker, 2009a).

GUIDELINES FOR USING DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* AS A PROTEIN SOURCE IN ANIMAL FEED

Based on our studies, the detoxified jatropha kernel meal and detoxified jatropha protein isolate should have the traits presented in Table 17.

Detoxified jatropha kernel meal, H-JPKM and DJPI can replace 50, 62.5 and 75 percent fishmeal protein, respectively, in fish diets, without sacrificing growth and nutrient utilization, and without affecting physiological and haematological parameters. For shrimp, 50 percent fishmeal protein could be replaced by DJKM. The guidelines described below would increase the efficiency of DJKM, H-JPKM and DJPI utilization in fish and shrimp.

- Take into account that DJKM and H-JPKM contain approximately 65 percent crude protein, which is similar to the level in fishmeal, and can therefore substitute for fishmeal on an equal weight basis.
- The acceptability of DJKM, H-JPKM and DJPI-based diets by fish, as measured by immediate consumption and no waste in the tanks, is good.
- DJKM, H-JPKM and DJPI are deficient in lysine. Therefore lysine monohydrochloride should be supplemented at a

TABLE 17
Recommended quality parameters for detoxified jatropha kernel meal and detoxified jatropha protein isolate

	Detoxified jatropha kernel meal	Detoxified jatropha protein isolate
Protein (%), minimum	60–66	81–88
Fat (%), minimum	0.9–1.2	0.8 –1.0
Fibre (%), maximum	8–9	1
Ash (%)	< 11%	< 3%
Gross energy (KJ/g)	18.5	21.4
Moisture (%)	6–8	4–6
Non-starch polysachharides (%), maximum	16	10
Lysine (%)	> 2.3	> 2.5
Available lysine	Near 100%	Near 100%
Pepsin plus trypsin digestibility (% of total nitrogen)	> 92.0	> 97.0
Protein dispersibility index	15–40%	-
Trypsin inhibitor (mg trypsin inhibited per g sample)	Not detectable	Not detectable
Lectin activity(1)	Not detectable	Not detectable
Texture	Homogenous, free flowing, no lumps, not dusty	Homogenous, free flowing, no lumps, not dusty
Taste	Bland	Bland
Contaminants	Free of PEs Free of urea Free of ammonia Free of mycotoxins and mould	Free of PEs Free of urea Free of ammonia Free of mycotoxins and mould

Notes: (1) Based on haem-agglutination. PEs = phorbol esters (sensitivity: <3 µg/g material).

level of 1.5 percent of the DJKM, H-JPKM and DJPI (w/w) inclusion in the diet to compensate for the deficiency.

- DJKM and H-JPKM contain approximately 9–10 percent phytate, which is almost 3-fold that in SBM. To mitigate its effect, add 1500 FTU phytase per kg of diet (Kumar *et al.*, 2011f).
- Detoxified jatropha kernel meal-, H-JPKM- and DJPI-based diets could be fed to fish at 5 times maintenance requirements. Single maintenance requirement equals 3.2 g feed/kg metabolic body mass ($kg^{0.8}$) per day. Shrimp (juveniles, >10 g) should be fed 3-4 percent of the total body weight per day.

POTENTIAL CHALLENGES IN USING DETOXIFIED KERNEL MEAL AND DETOXIFIED PROTEIN ISOLATE FROM *JATROPHA CURCAS* IN FEEDS

- Inadequately heated jatropha kernel meal that contains significant amounts of trypsin inhibitor and lectin could reduce the performance of monogastric animals. Similarly, inadequately detoxified material containing phorbol esters could cause adverse effects. Phorbol esters must be below the detectable limit (<3µg/g meal).
- Overheating jatropha kernel meal could increase the portion of 'bound protein', which is indigestible. A laboratory measurement of ADIN (acid-detergent insoluble nitrogen) could be used as an indicator of the extent of bound protein.
- Incorporating a high level of DJKM into a diet requires rebalancing the ingredients in order to maintain a proper ratio of energy to other nutrients, especially protein.
- Fish and shrimp fed high levels of plant protein such as DJKM or DJPI could deposit more fat in their fish muscles, which could be more unsaturated and hence more susceptible to oxidation.

ENVIRONMENTAL CONSIDERATIONS

Detoxified jatropha kernel meal and DJPI contain almost three times the phytate content of SBM and soy protein isolate, respectively. Detoxified jatropha kernel meal-based feeds have higher phosphorus concentrations than traditional feeds. Feeding large quantities of these feeds increases the amount of phosphorus excreted by the aquatic organism. In any given aquaculture enterprise, the excess phosphorus would increase the acreage needed for spreading waste in order to comply with waste management regulations, which could potentially limit future expansion. To minimize these potential problems, it is suggested that supplemental phosphorus should not be included in the diet when DJKM-based feeds containing phytase are fed. These diets would provide adequate phosphorus and meet the requirements indicated in National Research Council (NRC) 1993, recommendations without further supplementation. Several studies have demonstrated that feeding excess phosphorus does improve neither the reproduction efficiency nor health of fish or shrimps. When phosphorus is fed in excess of NRC recommendations, additional calcium may be required to maintain normal calcium-phosphorus ratios in the diet.

FUTURE STUDIES

Further studies are warranted on the utilization of DJKM and DJPI in other fish (e.g. Nile tilapia (*Oreochromis niloticus*); major Indian carps (*Catla catla*, *Cirrhinus mrigala* and *Labeo rohita*); Atlantic Salmon (*Salmo salar*); and shrimp species (e.g. freshwater prawn (*Macrobrachium rosenbergii*) and giant tiger prawn (*Penaeus monodon*). In addition, long-term feeding trials at farm level should be conducted on fish, shrimp, turkey, poultry, pigs and other domestic animals. Organoleptic properties, while not a common assessment, have been used to evaluate the potential impact of novel ingredients on product quality. Such assessments could also be conducted. Other related studies that would enhance the utilization of jatropha-based products as animal feed are: comparative evaluation of toxic and non-toxic genotypes of jatropha with respect to yields, disease susceptibility and nutrient and water requirements; and breeding studies to produce jatropha cultivars that are adapted to different agroclimatic conditions and with reduced toxins.

FINAL COMMENTS

The animal feed industry is growing at a rapid pace, and production is switching to intensively managed high-input systems. The main input in any livestock production system is feed, and demand for feed for aquatic organisms is expected to nearly triple by the end of the decade. This growth dictates greater use of protein sources other than fishmeal or soymeal. Jatropha products could be leading candidates to supply a large amount of the protein required for the expanding feed market. The work reported in this chapter enlarges the portfolio of feed ingredients available to the feed industry. It also highlights synergies between the bio-energy and feed industries, and shows how food and energy security can be better integrated.

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Chapter 22
Use of *Pongamia glabra* (karanj) and *Azadirachta indica* (neem) seed cakes for feeding livestock

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ABSTRACT

India, with the world's largest livestock population, intertwined intimately with its crop production, has immense potential for future growth and development of the livestock sector. However, chronic shortage of protein- and energy-rich feeds, shrinking grazing land and liberalized export policies are posing serious threats to developing the livestock industry into an economic enterprise. As the conventional protein-rich feeds are costly, the poor and marginal farmers of the country are unable to incorporate them in livestock diets to obtain optimum production. Animal nutritionists constantly seek cheaper and easily available non-competitive unconventional agroforestry-based industrial co-products for feeding livestock. Agroforestral industrial co-products include *Pongamia glabra* (*karanj*) and *Azadirachta indica* (*neem*) seed cakes, commonly used only as manure, which is highly uneconomical and almost unethical in a country having the largest livestock population in the world and facing chronic shortage of good quality feeds for them. In India, 1.3 milion tonne of karanj cake and 0.9 million tonne of neem seed cake are available annually. The toxic principles present in these cakes make them unpalatable, but these toxins can be removed by various techniques. Industry and progressive farmers are being recommended to include these cakes in the diet of animals after partial or complete detoxification. De-oiling of karanj cake results in complete removal of fat-soluble toxic compounds, and water washing of karanj cake and neem seed cake can detoxify them partially. Therefore, these treated cakes might replace conventional oil cakes (soybean meal or groundnut cake) at up to 50 percent of the nitrogen in diets, without any adverse effect on nutrient metabolism, growth and health of the animals.

INTRODUCTION

Protein-rich feeds are one of the costliest feed ingredients in animal diets. The roughage-based diets that are the primary ruminant feed in India are deficit in protein. Poor dietary supply of proteins results in low rates of reproduction and production, as well as increased susceptibility of livestock, including poultry, to metabolic disorders and infectious diseases. The strategy for improving livestock production has therefore been to maximize the efficiency of utilization of the available feed resources in the rumen by providing optimum conditions for microbial growth and by supplementation with protein-rich feeds like oil seed cakes, thus optimizing the ratio of energy to protein. Animal nutritionists constantly seek cheaper, easily available, non-competitive (with human food), unconventional agro-forestal and industrial co-products for feeding to ruminants and poultry. The feed deficit in India is currently 11 percent for dry fodder, 28 percent for concentrate and 35 percent for green fodder (NIANP, 2005).

Oilseed cakes are commonly used as protein supplements in India. Dikshit and Birthal (2010) estimated the feed consumption rates for different livestock species and the generated demand for different types of feeds by the year 2020, when India is forecast to require 56 $\times 10^6$ tonne of concentrate feeds, comprising 27.4 $\times 10^6$ tonne of cereals, 4.0 $\times 10^6$ tonne of pulses, 20.6 $\times 10^6$ tonne of oilseeds, oilcakes and meals and 3.6 $\times 10^6$ tonne of manufactured feed. The present requirement for concentrate feeds in the country is 47.3 $\times 10^6$ tonne (Dikshit and Birthal, 2010), but the availability is only 34 $\times 10^6$ tonne. There has been serious concern over the question of protein scarcity emerging as the major constraint to dairy development in the coming years. Several strategies have been examined by scientists, policy-makers and administrators, amongst which are:

- restricting oilseed meal exports as a means of increasing domestic availability of oilseed proteins;
- developing ways to increase rapidly the cultivated area under high-protein green fodders;

MAIN MESSAGES

- **By 2020, India can be expected to have an annual requirement for 56×10^{6} tonne of concentrate feeds, comprising 27.4×10^{6} tonne of cereals, 4.0×10^{6} tonne of pulses, 20.6×10^{6} tonne of oilseeds, oilcakes and meals, and 3.6×10^{6} tonne of manufactured feed.**
- **Conventionally, different parts of *Pongamia glabara* are used for different purposes: the oil is used as a lubricant, water-paint binder, pesticide, in soap making, for tanning, fuel for cooking and lamps, in rheumatism, herpes, enhancing the pigmentation of skin affected by leucoderma or scabies; and the leaf juice is used in treating colds, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy, and as an anthelmintic, digestive and laxative aid.**
- **Neem oil and other products of the neem tree are used traditionally for making cosmetics (soaps, mild detergents, creams, tooth cleanser) and in traditional Indian medicine (skin infections, inflammations, fever, leprosy, malaria, tuberculosis, worm infestation, eczema, etc.), in addition to use as an anti-bacterial and anti-fungal agent in bio-manure and in plant protection.**
- **Karanj and neem seed cakes are rich in protein. The crude protein content of rotary-pressed karanj cake ranges from 6 to 24 percent, while it varies from 22.0 to 28.7 percent in expeller-pressed karanj cake and 30.0 to 34.0 percent in solvent-extracted karanj cake. On a dry matter basis, neem seed cake contains12.4 to 19.6 percent crude protein, de-oiled NSC contains 17.9–18.4 percent crude protein, and neem seed kernel cake contains 33.5–40.8 percent crude protein.**
- **Karanj cake toxins include furanoflavones (karanj, pongamol, pongapin, pongaglabron, kanjone, isopongaflavone lanceolatin B), tannins and trypsin inhibitors. Karanj and pongamol are the most important toxic factors, and its bitterness is attributed to these two compounds.**
- **Neem seed cake contains toxic triterpenoids (azadirachtin, salanin, nimbin, nimbidiol) and its bitterness is attributed to these compounds.**
- **The anti-nutritional factors of karanj cake are soluble in oil. Complete removal of oil from cake appears to be more effective than other treatment methods.**
- **Water-washed or de-oiled karanj cake and water-washed neem seed cake may be incorporated at up to 50 percent of the nitrogen moiety of conventional protein supplements like soybean meal or groundnut cake without any adverse effect on nutrient metabolism, growth or health of animals**.

- developing technical interventions to improve utilization of existing protein sources in the rumen through protection of degradable proteins; and
- identifying unconventional oil cake sources, and their detoxification for use as animal feed.

In the present chapter, efforts have been made to consolidate information available on *Pongamia glabra* (*karanj*) and *Azadirachta indica* (*neem*) seed cakes with respect to their chemical composition, toxic compounds present, detoxification and the effects of their inclusion in the diets of ruminants and poultry on the physiology and health of these animals and the quality of their products.

KARANJ (*PONGAMIA GLABRA*) CAKE

The ambitious National Biodiesel Mission aims to meet 20 percent of India's diesel requirements through bio-diesel by 2016–2017. Since the demand for edible vegetable oil exceeds supply, the government has decided to use non-edible oil seeds as biodiesel feedstock. Biofuels offer a number of environmental, social and economic advantages, including lower emissions of harmful pollutants; decreased greenhouse gas emissions; increased employment opportunities; increased energy security, especially in rural areas; decreased dependence on oil imports; and good fuel properties for vehicles. The national mission on biofuels has already been launched in two phases. Under the first phase, jatropha and karanj plantations would be established on 400 000 ha of government-owned land. Among the various agroforest based industrial co-products, the current use of karanj cake primarily as manure is highly uneconomical and almost unethical in a country having the largest livestock population in the world and facing chronic shortage of quality feeds for them. Karanj cake will be available as a co-product from the biodiesel plants in appreciable quantities in various parts of the country, and could be used as a source of protein for economic livestock production.

Availability and conventional uses of *Pongamia glabara*

Pongamia glabra (syn. *Pongamia pinnata*), commonly known as karanj (pongam oil tree), belongs to the family Leguminosae. It is a medium-sized, deciduous, glabrous, fast growing tree with a spreading crown of up to 25 m, and capable of growing under a wide range of agroclimatic conditions (Parmar, Sahrawat and Mukherjee, 1976). In India, it is found abundantly in Andhra Pradesh, Bihar, Jharkhand, Karnataka, Maharashtra, Tamil Nadu and West Bengal. The tree is adapted to humid and subtropical envi-

TABLE 1
Conventional uses of *Pongamia glabra*

Plant part	Conventional uses
Oil	Commonly used as a lubricant, water-paint binder, pesticide, in soap-making, for tanning, as fuel for cooking and lamps, as medication in rheumatism, for itches, herpes, effective in enhancing the pigmentation of skin affected by leucoderma or scabies.
Leaf juice	Medicinally for colds, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy, and as an anthelmintic, digestive and laxative aid.
Roots	Cleaning gums, teeth, and ulcers.
Bark	Bleeding piles.
Flowers	Biliousness and diabetes.
Fruit and seed	Keratitis, piles, urinary discharges and diseases of the brain, eye, head and skin.
Medicinal activity of various products	
70% ethanolic extract of leaves	Anti-inflammatory activity without any side effects; anti-pyretic action
Decoction of leaves	Anti-diarrhoeal action.
Ethanolic extract of flowers	Anti-hyperglycaemic and anti-lipidperoxidatives effects.

Sources: Adapted from Chopade *et al.,* 2008*;* Srinivasan, Muruganandan and Lal, 2001; Brijesh, Daswani and Tetali, 2006; Punitha and Manoharan, 2006.

ronments and thrives in areas having an annual rainfall of 500–2500 mm. In its natural habitat in India, the maximum temperature ranges from 27 to 38 °C, with a minimum of 1 to 16 °C. Mature trees can withstand waterlogging and slight frost. These trees can grow even at a height of about 1200 masl, although in the Himalayan foothills it is not found above 600 masl (Government of India, 1983).

It is known by different names in different Indian languages: 'karanj' in Hindi, 'pongam' and 'punnai' in Tamil, 'honge' in Kannada, 'Indian beech' in English, 'kanuga' in Telugu and 'karanja' in Bengali. It bears compound pinnate leaves consisting of 5–7 leaflets arranged in 2 or 3 pairs. Leaflets are 5–10 cm long, 4–6 cm wide and pointed at the tip. Flowers are borne on racemes, and are pink, light purple or white in colour. Pods are elliptical, measuring 3–6 cm in length and 2–3 cm in width, with a thick wall and usually containing a single seed. Seeds are 1–2 cm long or oblong and light brown in colour.

Karanj thrives on all sorts of soils, ranging from stony, through sandy to clayey, including vertisols, but prefers well-drained light porous soil. It is a common sight to find the tree near perennial water sources, on the banks of rivers, streams, tanks, canals and lakes. It is also a well-known avenue tree, grown in parks, gardens and roadsides. It is highly tolerant of salinity and hence it is common along waterways or seashores, with its roots in fresh or salt water. It does not do well on dry sands, and the highest growth rates are observed on well-drained soils with assured moisture.

The tree starts bearing seeds at 4–7 years. The fruit come to harvest at different periods of the year in different parts of the country, but the harvest season extends in general from November-December to May-June. The pods are collected and the shells are removed either by hand or separated by a decorticator before oil extraction. The seed yield ranges from 10 kg to more than 90 kg per tree (Anon., 1969). Mature seed contains 5 percent shell and 95 percent oleaginous kernel. Pressing the seed produces 25 percent oil and 70 percent residue, known as cake, assuming 5 percent of losses. This is a high oil yield compared with other oil seeds. The main drawback is that the cake is non-edible as such, due to its toxicity.

Karanj seed production in India is 110 000 to 130 000 tonne annually (Ministry of Agriculture, 1992; NOVODB, 1995), of which about 85 500 tonne go uncollected. Seeds are mainly used for oil extraction and production is nearly 30 000 tonne per annum (De *et al.,* 1998). The oil is dark in colour, with an unpleasant odour. Technology has been developed to upgrade oil quality for soap manufacture and other industrial purposes.

Different plant parts of the karanj tree have different uses, and their extracts have medicinal values, as listed in Table 1. The karanj oil has varied uses in industry (leather dressing, soap making, lubrication, bio-diesel, illumination, etc.), as an insecticide or in medicine. The oil is known for its curative effect on skin problems, such as leucoderma, psoriasis, scabies and skin itches (Bringi and Mukerjee, 1987). At present, the use of karanj oil for the production of biodiesel is being explored (De and Bhattacharyya, 1999; Srivastava and Prasad, 2000; Vivek and Gupta, 2004; Meher *et al.*, 2006). Increased production of biodiesel from karanj may enhance the availability of karanj cake, which is the residue left after oil extraction. The cake, which is bitter and pungent, is used as manure, fungicide or insecticide. Although the cake is a protein-rich co-product potentially of great value for animal feeding, it is seldom used in animal feeding due to its poor palatability and the presence of various toxic constituents.

Chemical composition of karanj cake

Three main types of karanj cakes are available, namely rotary pressed, expeller-pressed (EKC) and solvent-extracted (SKC), the composition of which depends on the degree of decortication and method of oil extraction. The crude protein content of rotary-pressed karanj cake (Natanam, Kadirvel and Balagopal, 1988; Chandrasekaran, Kadiravel

TABLE 2
Chemical composition of karanj cake (as % of DM)

Parameter	Kernel[(1)]	EKC[(2)]	SKC[(3)]	Rotary pressed[(4)]
Crude protein	20.5–24.1	24.1–28.7	30.0–34.0	24.6
Ether extract	33.2–43.3	6.1–14.2	0.1–2.2	14.0
Crude fibre	3.8–4.6	3.9–10.7	5.0–5.6	6.0
NFE	24.5–39.7	49.9–56.4	54.3–59.9	–
Total ash	2.8–3.5	3.2–7.1	4.4–6.9	5.2
NDF	–	18.2	18.0–28.2	37.7
ADF	–	10.6	1.7–20.0	11.3
Karanjin	–	0.29–0.32	0.01–0.19	–
Ca	0.5	0.6–0.8	0.6–0.9	0.74
P	0.38	0.48–0.70	0.55–0.61	0.89

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; NFE = nitrogen-free extract; ADF = acid-detergent fibre; NDF = neutral-detergent fibre. *Sources*: (1) Natanam, Kadirvel and Ravi, 1989; Vinay and Kanya, 2008. (2) Gupta *et al*., 1981; Natanam, Kadirvel and Ravi, 1989; Ravi *et al*., 2000; Prabhu, 2002; Panda, 2004; Soren *et al.,* 2007. (3) Mandal, Banerjee and 1974; Konwar, Banerjee and Mandal, 1984; Konwar and Banerjee, 1987; Gupta *et al*., 1981; Ravi *et al*., 2000; Prabhu, 2002; Panda, 2004; Soren, 2006. (4) Chandrasekaran, Kadiravel and Viswanathan, 1989.

TABLE 3
Amino acid composition (g/16 g N) of karanj cakes compared with soybean meal

Amino acid	SKC [(1)]	EKC [(2)]	SBM [(3)]
Histidine	2.07	5.11	1.13
Isoleucine	3.59	5.95	2.56
Lysine	5.60	3.34	2.96
Leucine	7.72	7.87	3.47
Methionine	0.99	1.43	0.62
Arginine	4.33	4.62	3.27
Phenylalanine	5.22	3.64	2.24
Tryptophan	0.61	–	0.60
Threonine	2.64	4.13	1.73
Valine	5.26	6.44	2.44
Aspartic acid	10.92	6.19	–
Serine	4.53	3.98	–
Glutamic acid	13.45	17.56	–
Proline	5.01	2.90	–
Glycine	3.85	3.39	3.27
Alanine	5.10	2.31	–
Cystine	6.06	1.23	0.69
Tyrosine	3.67	1.27	1.43

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; SBM = soybean meal. *Sources*: (1) Mandal and Banerjee, 1975. (2) Natanam, Kadirvel and Ravi, 1989. (3) Adapted from Kellems and Church, 1998.

TABLE 4
Mineral composition of Karanj cake compared with soybean meal

Mineral	Seed kernel	EKC	SKC	SBM
Macro minerals (as % in DM)				
Ca	0.51	0.76	0.87	0.38
P	0.38	0.48	0.55	0.71
Na	0.29	–	0.49	0.48
K	0.27	0.23	0.20	0.15
Mg	0.47	0.27	–	0.28
Trace minerals (ppm)				
Cu	1.37	1.96	1.97	20.0
Fe	3.28	14.32	17.81	150.0
Co	0.27	0.09	0.15	0.16
Mn	35.78	76.21	70.82	22.0
Pb	0.11	0.73	1.00	–

Notes: EKC = expeller-pressed karanj cake; SKC = solvent-extracted karanj cake; SBM = soybean meal. *Sources*: Natanam, Kadirvel and Ravi, 1989; Georgievskii, Annenkov and Samokhin, 1982.

and Viswanathan, 1989) ranges from 6 to 24 percent, while it is 22.0–28.7 in EKC and 30.0–34.0 percent in SKC. The crude fibre (CF) level varies from 3.9 to 5.6 percent. The variation in ether extract (EE) content is mainly due to method of oil extraction. Usually, cakes obtained by expeller extraction have a higher EE value (9–14.5 percent), while lower levels (0.1–2.2 percent) are found in the cake produced by solvent extraction. A summary of various reports by different workers regarding the chemical composition of various types of karanj cake are presented in Table 2.

Amino acid composition is very important in determining the protein quality of any cake. Values for 18 amino acids, including essential amino acids, have been reported by various workers (Mandal and Banerjee, 1975; Parmar, Sahrawat and Mukherjee, 1976). Mandal and Banerjee (1975) observed that the amino acid composition of karanj cake was almost similar to that of sesame, groundnut cake (except for lysine, which was higher in karanj cake) and soybean meal (SBM) (except for methionine, which was lower in karanj cake). Further, karanj cake was found to be rich in cystine. Amino acid values reported by various workers are presented in Table 3. The mineral composition of karanj cake (Natanam, Kadirvel and Ravi, 1989) is presented in Table 4. In comparison with SBM, karanj cake has higher calcium, phosphorus and sodium; however, the concentrations of copper and iron are very low.

Toxic compounds in karanj cake

On the basis of chemical nature, the toxic compounds in karanj cake can be grouped into three categories, viz. furanoflavones, tannins and trypsin inhibitors.

Furanoflavones

The furanoflavones present in karanj seed include karanjin, pongamol, pongapin, pongaglabron, kanjone and isopo-

ngaflavone lanceolatin B (Limaye, 1925; Rangaswami and Seshadri, 1940; Roy, Sharma and Khanna, 1977). Among them, karanjin and pongamol are the most important toxic factors due to their potentency. The bitter taste of karanj cake is attributed to the presence of these two compounds.

Karanjin

Karanjin ($C_{18}H_{12}O_4$; 3-Methoxyfurano-(2′,3′:7,8)-flavone) is the first flavonoid compound that was isolated, identified and characterized, and hence it is the earliest known furanoflavone and the most important physiologically active factor of *Pongamia* sp. Its concentration in karanj oil is approximately 1.25 percent. Limaye (1925, 1926) isolated karanjin from pongamia oil. Seshadri and Venkateshwarlu (1943) identified, characterized and established the structure of karanjin. Its melting point is 158–159 °C. In the seeds of *P. glabra*, pongamol was subsequently identified along with karanjin (Rangaswami and Seshadri, 1940). The karanjin content of the karanj oil has been reported to be 147 mg/100 ml and 10–15 mg/100 g in SKC (Punj, 1988). Similarly, karanjin content of EKC was found to be in the range of 0.19 to 0.324 percent and 0.01–0.132 percent in SKC (Prabhu *et al.*, 2002; Panda *et al.,* 2006; Soren, 2006). Vinay, Appu Rao and Sindhu Kanya (2006) reported karanjin content in the whole karanj seed to be 1.95 percent.

Of the 24 furanoflavonols isolated, karanjin has been studied extensively and found to be hypoglycaemic. Oral administration at a dose of 2 mg/kg per day for 7 days caused a reduction in blood glucose level both in normal and alloxan-induced diabetic rats (Mandal and Maity, 1987). It also showed antitubercular (suppressing growth of *Mycobacterium tuberculosis*; Ramaswamy and Sirsi, 1960), antifungal (Pan *et al.,* 1985), antibacterial, phytotoxic (Simin *et al.*, 2002), and central nervous system stimulant activities (Mahali *et al.*, 1989). Apart from these activities, karanjin is also a nitrification inhibitor (Majumdar, 2002), juvenomimetic (Mathur *et al.,* 1990) and synergist to insecticides (Sighamony, Naidu and Osmani, 1983). It was found to haemolyse red blood cells, with release of LDH (lactate dehydrogenase) (Gandhi and Cherian, 2000).

Pongamol

Pongamol, a crystalline compound from the oil of *P. glabra,* was identified by Rangaswami and Sheshadri (1942). Compared to karanjin, it is a minor component, far more soluble in oils. It has the molecular formula $C_{18}H_{14}0_4$ and contains a methoxyl group. Demethylation with aluminium chloride yields nor-pongamol, whereas treatment with HCl gives rise to a product which is probably isomeric but does not possess a phenolic group. Oxidation with $KMnO_4$ or decomposition with alkali yields benzoic acid. These properties and colour reaction suggest that pongamol is a flavone derivative (Rangaswamy and Sheshadri, 1942). Narayanaswamy, Rangaswami and Seshadri (1954) established the structure of pongamol as benzoyl-O-methyl karanjoyl methane (S-benzolacetyl-4-methoxy benzofuran). It is the first example of a naturally occurring diketone related to flavones. Pongamol melts at 128 °C and has relatively less bitterness compared with karanjin. Among the flavanoid diketones, pongamol has been explored extensively and found to have sedative and depressant effects (Mahali *et al*., 1989). It is commercially used in cosmetic and sun-screen preparations (Noriaki, Masamichi and Masanori, 2001).

Tannins

Apart from karanjin, the cake is also reported to contain tannins to the extent of 3.2–3.4 percent (Natanam, Kadirvel and Ravi, 1989). Tannins are a naturally occurring group of phenolic compounds with a molecular weight of 500–3000 daltons (Haslam, 1966). These have alkaloids, gelatins and protein precipitation properties. However, the total tannins and condensed tannins content of karanj cake was reported to be lower and protein-precipitation capacity was not detected by Makkar, Singh and Negi (1990), suggesting that these co-products could be safe for incorporation in livestock feed. The tannin content was found to be slightly higher in SKC than in the expeller cake (Panda *et al.*, 2006). The SKC contained 0.94 percent tannins, along with other antinutritional factors, such as phytate (0.65 percent) and trypsin inhibitors (31 units/mg) (Vinay and Kanya, 2008).

Protease inhibitors

Protease inhibitors are well known anti-nutrients that are responsible for lower digestibility of plant proteins. Protease inhibitors, namely trypsin and chymotrypsin inhibitors, are found in karanj oil seed residue (Rattansi and Dikshit, 1997). These are a group of anti-nutritional factors, protein in nature, with a molecular weight between 6000 and 25000 daltons, and are generally present in leguminous seeds (Birk, 1976; Liener, 1979). The expeller- and solvent-extracted cakes contain trypsin inhibitor up to 8.7 and 8.2 percent of protein, respectively (Natanam, Kadirvel and Ravi, 1989). The adverse effect of trypsin inhibitors is mainly on the pancreas, which responds to the inhibitors by enhanced synthesis and secretion of proteolytic enzymes. The pancreatic enzyme secretion is regulated by a negative feedback mechanism mediated by intestinal trypsin and chymotrypsin, and complex formation of trypsin with the inhibitors leading to a reduction of free trypsin in the small intestine (Alumot and Nistan, 1961). This reduction activates the pancreas-stimulating hormone, cholecystokinin, the release of which from the intestinal mucosa is inhibited by free trypsin (Wilson *et al.,* 1978). As a consequence of cholecystokinin action, the pancreas becomes hyperactive.

Use of karanj cake as ruminant feed

The karanj cake can be used as livestock feed as it contains a fairly good amount of crude protein However, its use as protein supplement is limited due to the presence of toxic compounds. The detoxification processing of karanj cake significantly reduces the toxic effects and therefore its use as a livestock feed has been tested. The results of some of these experiments are summarized in this section.

Detrimental effects of feeding karanj cake in ruminants

Feeding of EKC has been reported to depress feed intake, cause histopathological changes in vital organs and produce toxicity symptoms. A concentrate mixture containing 4 percent EKC was found to be unpalatable to buffalo calves, and the animals developed symptoms like loss of appetite and weight, frequent and strong-coloured micturation, swelling in the intermaxillary spaces and facial muscles, discoloration of skin and loss of hair, watery to sticky lacrimation, and gangrene of tail, followed by its sloughing (Gupta *et al.,* 1981). Konwar and Banerjee (1987) found no harmful effect on red (RBC) and white blood cell (WBC) counts, packed cell volume (PCV) and haemoglobin, Fe, Ca and P content in growing calves, except for blood plasma protein concentration, which was significantly lowered at 75 percent of the level of de-oiled karanj cake (DKC) incorporation in the diet.

Detoxification of karanj cake

Karanjin and pongamol are soluble in oil and their levels vary in cake depending on the residual oil content in it. Both are insoluble in water but easily soluble in organic solvents like ethyl alcohol, methyl alcohol or benzene. Some karanjin is removed by water washing due to washing away of EE attached with other ingredients of the SKC, which is evident from the lowered EE of the water-washed SKC (Soren *et al.,* 2007). Dilute acid treatment causes change in the nature of oil due to hydrolysis of oil and production of fatty acids, which render karanjin and pongamol insoluble in it, and these compounds precipitate. Alkaline decomposition of karanjin yields four products: C-acetyl coumarone ($C_{11}H_{10}O_4$), karanjic acid ($C_9H_6O_4$), kanjol ($C_8H_6O_2$) and benzoic acid (Limaye, 1926); whereas alkaline decomposition of pongamol yields a single product, namely benzoic acid (Rangaswami and Sheshadri, 1942). All these intermediate decomposition products are said to be non-bio-active and non-toxic.

Various attempts have been made so far to detoxify the cake, initially without specifically targeting any of the particular toxins, and later targeting specific toxins, namely karanjin, tannin, trypsin inhibitors and phytates. Non-specific detoxification attempts include de-oiling (Konwar and Banerjee, 1987), oven drying (100 °C for 24 hours), autoclaving (242 kPa pressure, 30 minutes), cold water extraction (1:3, w/v, 24 hours) (Natanam, Kadirvel and Ravi, 1989), hot water extraction (60 °C) and toasting (15 minutes) (Mandal and Banerjee, 1974). Prabhu *et al.* (2002) detoxified karanj cake by various physico-chemical methods, including solvent extraction, water washing, pressure cooking, alkali and acid treatments. They found that de-oiling of karanj cake was the best method of detoxification as it substantially reduced the karanjin content (from 0.19 down to 0.01). Panda *et al.* (2006) observed that pressure cooking (30 minutes), treatment with alkali (1.5 percent NaOH) and lime (3.0 percent) were effective in reducing the karanjin content in SKC. Similarly, various methods, namely water washing, water soaking, dry heat treatment, pressure cooking, urea ammoniation, alkali (calcium hydroxide, potassium hydroxide, sodium hydroxide and sodium bicarbonate) treatments, biological treatments (*Saccharomyces cerevisiae, Aspergillus oryzae*) and toxin binder (HSCAS) were tried to reduce karanjin content of cake by Soren *et al.* (2009). They reported that pressure cooking was found to be the most effective method, followed by sodium hydroxide treatment, for removing karanjin. However, none of the treatments removed karanjin completely from the cake.

Effect on rumen fermentation

In vitro studies involving incubation of karanj cake with rumen liquor for 48 hours resulted in disappearance of 92.3 percent of DM and 93.5 percent of organic matter (OM) (Chandrasekaran, Kadiravel and Viswanathan, 1989). However, in buffaloes, *in sacco* DM degradation was reported to be 49.5 percent and protein degradation 22.2 percent (Paul *et al*., 1995). Decreased degradability of DM, OM and neutral-detergent fibre (NDF) was reported when SKC and EKC were incorporated in the concentrate mixture at a 20 percent level (Saha *et al.,* 2004a, b). The *in vitro* DM degradability due to inclusion of raw and treated SKC were comparable to control, although NDF degradability was significantly lower in the raw-SKC-containing concentrate mixture (Soren, 2006).

Effect on rumen fermentation

Ravi *et al.* (2001) reported a significantly higher pH in growing lambs fed EKC, whereas the pH in the SKC-fed group was comparable with the control. Though other nitrogen fractions were comparable, ammonia nitrogen was significantly lowered in karanj cake-fed groups (Table 5). Contrary to the above finding, the pH and concentration of NH_3-N, total N and tricholoroacetic acid ppt.-N (TCA) was found similar in sheep given isonitrogenous diets containing de-oiled groundnut cake (GNC), SKC and EKC (replacing 50 percent N of de-oiled groundnut cake (DGNC) in full) for 210 days of experimental feeding, while total volatile fatty acid (TVFA) concentration was lower in the karanj cake-fed group (CASAN, 1999–2000).

TABLE 5
Effect on ruminal metabolites in lambs of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake

Parameter	Groups		
	DGNC	EKC	SKC
pH	6.20 b	6.54 a	6.28 b
TVFA (mEq/dl)	7.86	7.24	8.04
Total-N (mg/dl)	135.0	115.0	127.5
TCA-ppt-N (mg/dl)	85.00	69.17	80.28
Ammonia-N (mg/dl)	18.05 a	14.35 b	14.50 b

Notes: TVFA = total volatile fatty acids; DGNC = de-oiled groundnut cake; TCA = tricholoroacetic acid. Means with different letters in a row differ significantly (P <0.05). *Source*: Ravi *et al.,* 2001.

Prabhu (2002) and Soren (2006) reported comparable pH and concentrations of NH_3-N, total volatile fatty acid (TVFA) and other nitrogen fractions in rumen liquor of lambs fed either soybean- or karanj cake-based isonitrogenous supplements replacing 50 percent N of DGNC with EKC and SKC. TVFA concentration was comparable except in binder-treated cake, in which it was lower than control. TCA-ppt.-N was significantly higher in the control group than in the test groups. Urinary excretion of purine derivatives and microbial protein synthesis did not differ significantly among diets. However, urinary excretion of creatinine was lower (P <0.05) in lambs fed control and water-washed diet as compared with LM (lime treated) and BN (binder treated) treated SKC diets (Table 6) (Soren and Sastry, 2009). The higher excretion of creatinine in the LM and BN groups might be due to catabolism of body protein to meet energy needs and the residual karanjin in the processed cake might have affected protein anabolism.

TABLE 6
Urinary excretion of purine derivatives (PD) and creatinine, absorption of PD and microbial nitrogen supply in lambs fed processed karanj cake

Variables	Diets			
	Control	WW	LM	BN
Urinary excretion of PD (mmol/day)				
Allantoin	3.88	3.44	3.70	2.87
Uric acid	1.29	1.67	1.25	1.08
Xanthine	0.38	0.20	0.38	0.43
Hypoxanthine	0.16	0.19	0.20	0.12
Total PD	5.73	5.51	5.54	4.51
Creatinine	1.53 b	1.48 b	2.94 a	2.46 a
PD absorption (mmol/day)	6.50	6.26	6.31	4.96
Microbial N synthesis (g N/day)	4.72	4.55	4.58	3.60
Efficiency of microbial N synthesis				
g N/kg DOMI	15.65	20.33	23.88	21.34
g N/kg DOMR	24.08	31.28	36.73	32.83

Notes: Control = soybean meal; WW = water-washed SKC; LM = 25 g/kg lime-treated SKC; BN = 4 g/kg binder-treated SKC; SKC = solvent-extracted karanj cake; DOMI = digestible organic matter intake; DOMR = digestible organic matter. Means with different letters in a row differ significantly (P <0.01). *Source*: Soren and Sastry, 2009.

Palatability and voluntary feed intake

Karanj cake as sole feed is highly unpalatable. A palatability trial conducted with male buffalo calves given EKC-based supplement revealed that even at a 4 percent level it was quite unpalatable and led to development of toxic symptoms (Gupta *et al*., 1981), although they found that de-oiling could completely remove the bitter taste and pungent smell of the karanj cake, and SKC could be included at up to 32 percent, replacing 80 percent of mustard cake without any adverse effect on DM intake (DMI). In general, growing animals are expected to be more sensitive than adults in accepting any unconventional feed. Complete replacement of de-oiled GNC with SKC significantly reduced feed intake, however. Konwar and Banerjee (1987) reported that absolute replacement of de-oiled GNC nitrogen with SKC did not affect DMI in growing cross-bred bulls. In an another study involving growing calves, Konwar, Banerjee and Mandal (1984) found no adverse effect on feed intake when SKC was incorporated at up to 25 percent in the concentrate mixture. Srivastava *et al.* (1990) also found no difference in DMI of kids when de-oiled GNC nitrogen was replaced with de-oiled karanj cake at the 40 percent level.

Ravi (1999) reported significantly lower DMI in lambs given concentrate mixture containing EKC at 24 percent level, although comparable DMI was observed when they fed a concentrate mixture incorporating 20 percent SKC (Ravi *et al.,* 2000). The reduced intake on EKC-containing diets might be due to the presence of bitter and pungent compounds (Parmar, Sahrawat and Mukherjee, 1976) in the oily portion of the karanj cake, rendering it unpalatable. Prabhu (2002) also observed comparable DMI in growing lambs fed diets incorporating 16.5 percent raw and alkali-treated SKC in a 180-day feeding trial.

Nutrient digestibility

Only a few reports are available on the effect on the digestibility of nutrients of feeding EKC, which may be due to the fact that animals did not readily consume EKC because of its pungent and repulsive smell. Chandrasekaran, Kadiravel and Viswanathan (1989) determined the nutritive value of EKC in adult ewes and reported that it could be used in the concentrate mixture by replacing a maximum of 75 percent N of GNC as a temporary short-term feed source without any adverse effect. They further reported *in vitro* digestibility coefficients of 0.853, 0.797, 0.859, 0.308 and 0.756 for DM, OM, CP, CF and EE, respectively.

Konwar and Banerjee (1987) reported digestibility coefficient of 0.593, 0.622, 0.607, 0.580, 0.698 and 0.615 for DM, OM, CP, CF, EE and NFE, respectively, in growing calves fed rations containing 25 percent de-oiled karanj cake (DKC). They further reported no adverse effect on nutrients digestibility up to 25 percent DKC inclusion. Feeding of rations containing 0, 6, 9 or 12 percent DKC showed no

TABLE 7
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on nutrient utilization in lambs

Parameter	Diet group		
	DGNC	EKC	SKC
Body weight (kg) during trial period	13.4	12.8	13.6
Metabolic body weight (kg $W^{0.75}$)	7.0	6.7	7.0
Dry matter intake (g per day)	525.6	490.6	540.0
Daily DMI (g/kg $W^{0.75}$)	74.9	71.9	75.9
Digestibility (%)			
DM	59.1a	53.5 b	59.1 a
OM	61.9 a	56.2 b	61.6 a
CP	55.9 a	46.7 b	53.0 ab
NDF	51.5 a	44.3 b	49.4 ab
ADF	47.9 a	38.7 b	48.3 a
Daily intake of nutrients (g/kg $W^{0.75}$)			
TDN	42.6	37.3	43.0
DCP	5.37	4.26	5.0

Notes: DMI = Dry matter intake; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; TDN = total digestible nitrogen; DCP = digestible crude protein; DGNC = concentrate mixture containing de-oiled groundnut cake as major protein source; EKC = concentrate mixture replacing 50 percent of DGNC N by EKC; SKC = concentrate mixture replacing 50 percent of DGNC N by SKC; $W^{0.75}$ = metabolic weight (size). Means with different letters in a row differ significantly (P <0.05). *Source*: Ravi *et al.,* 2000.

significant difference with respect to digestibility coefficients of DM, CF and NFE amongst kids in different dietary groups (Srivastava *et al.*, 1990), although they found that the digestibility of CP and EE was significantly (P <0.05) depressed by feeding 12 percent DKC.

Ravi *et al.* (2000) reported significant reduction in digestibility of various nutrients in lambs fed concentrate mixture containing 24 percent EKC, while SKC inclusion up to 20 percent did not adversely affect the digestibility of proximate principles and fibre fractions. Prabhu (2002) observed no adverse effect on the digestibility of various nutrients by feeding raw and processed SKC at the 16.5 percent level in the diet of growing lambs. Digestibility of fibre fractions (NDF, ADF, hemicellulose and cellulose) were also found to be comparable among the control and SKC-fed groups (Table 7). Similarly, no significant difference was observed between the digestibility of DM, OM and CP in the control feed (27 percent SBM in concentrate) and the processed SKC feed (22.5 percent in concentrate) (Soren and Sastry, 2009).

Nutrient balance

The balance of various nutrients is an important parameter when assessing the suitability of any feed in the diet of farm animals. Srivastava *et al.* (1990) reported a significant reduction in the retention of N and Ca with inclusion of more than 9 percent SKC in the concentrate mixture for growing kids, although no adverse effect was noticed with respect to the balance of P. Nutrient balance studies conducted with growing calves showed similar Ca and P retention when SKC was incorporated at up to 17 percent in the concentrate mixture, though at the 25 percent level SKC resulted in significantly lowered retentions (Konwar and Banerjee, 1987). Chandrasekaran, Kadiravel and Viswanathan (1989) reported positive N (1.6 g), Ca (0.7 g) and P (0.3 g) balances in ewes fed EKC. Lactating cows given a supplement replacing 25 percent protein of mustard oil meal with DKC protein showed no adverse effect on the balances of N, Ca and P (Dutta *et al.,* 1984).

TABLE 8
Effect on intake and balances of N, Ca and P in lambs of feeding processed karanj cake

Variables	Diets			
	Control	WW	LM	BN
N balance				
Daily N intake	12.24 a	9.86 b	8.59 bc	7.19 c
N retained	4.78 a	3.71 b	2.71 bc	1.87 c
Ca				
Daily Ca intake	3.60 a	3.16 ab	2.47 b	2.54 b
Ca retained	0.34 b	0.65 a	0.29 b	0.12 b
P				
Daily P intake	4.06 a	2.40 b	2.09 c	1.94 c
P retained	2.67 a	1.22 b	1.09 b	0.96 b

Notes: Control was a soybean meal diet; WW = water-washed SKC; LM = 25 g/kg lime treated SKC; BN = 4 g/kg binder-treated SKC; SKC = solvent-extracted karanj cake. Means with different letters in a row differ significantly (P <0.05). *Source*: Soren and Sastry, 2009.

In a short-term 98-day study with lambs, Ravi *et al.* (2000) observed no adverse effect on retention of N, Ca and P with inclusion of 20 percent SKC in the concentrate mixture, though inclusion of EKC at 24 percent adversely affected the retention of N and Ca. Similarly, Prabhu (2002) and Soren and Sastry (2009) observed no adverse effect on the balance of N, Ca and P in lambs fed concentrate mixtures replacing 50 percent of SBM of the control concentrate mixture with SKC and alkali-treated SKC for a period of 90–180 days (Table 8).

Growth and feed conversion efficiency

Studies (Srivastava *et al.,* 1990) with small ruminants, viz. lambs and kids, have shown diverse responses in growth rates due to karanj cake feeding as compared with conventional protein supplements. An average daily gain (ADG) of 35–38 g was obtained in kids when SKC was fed at up to 9 percent, while further incorporation up to 12 percent resulted in a significant reduction in gain (ADG of 22 g). Srivastava *et al.* (1990) further reported comparable feed conversion efficiency (FCE) of 10.1 when feeding levels of up to 6 percent in the diet; however, beyond a 6 percent level, inclusion of SKC lowered the FCE (15.9).

Gupta *et al.* (1981) reported a reduced growth rate (236 g/day vs 409 g/day) in growing calves fed diets replacing mustard cake protein with more than 40 percent SKC. Corresponding values of ADG and FCE were observed by

TABLE 9
Effect on body weight changes in lambs of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake

Parameter	Groups		
	DGNC	EKC	SKC
Initial body weight (kg)	11.2	9.9	10.7
Final body weight	17.1	14.7	16.5
Average daily gain (g)	60.5 a	48.8 b	59.6 a
Total DMI in 98 days (kg)	54.8	50.5	54.1
Feed conversion efficiency	9.2	10.6	9.3

Notes: DMI = dry matter intake; DGNC: concentrate mixture containing de-oiled groundnut cake as major protein source; EKC = concentrate mixture replacing 50% of DGNC-N by EKC; SKC = concentrate mixture replacing 50% of DGNC-N by SKC. Means with different letters in a row differ significantly (P <0.05). *Source*: Ravi *et al.,* 2000.

TABLE 10
Effect of feeding processed karanj cake on body weight changes, intake and digestibility of nutrients in lambs

Variables	Diets			
	Control	WW	LM	BN
Initial body weight (kg)	12.6	12.9	12.8	12.9
Final body weight (kg)	23.7 a	22.4 a	17.5 b	17.0 b
Body weight change	10.7 a	9.5 a	4.7 b	4.1 b
Dry matter intake (g/day)	536 a	402 b	357 b	332 b
Apparent digestibility				
Dry matter	0.54	0.59	0.53	0.54
Organic matter	0.58	0.61	0.56	0.57
Crude protein	0.58	0.58	0.55	0.53

Notes: Control was a soybean meal concentrate; WW = water-washed SKC; LM = 25 g/kg lime-treated SKC; BN = 4 g/kg binder-treated SKC; SKC= solvent-extracted karanj cake. Means with different letters in a row differ significantly (P <0.01). *Source*: Soren *et al.,* 2009.

Ravi *et al.* (2000) in growing lambs when SKC was fed at up to 20 percent in concentrate mixture replacing 50 percent de-oiled GNC; however, EKC inclusion at 24 percent in the same experiment resulted in lowered ADG without any effect on FCE (Table 9).

Prabhu (2002) observed similar ADG and FCE in lambs fed SBM and raw or processed SKC containing diets up to te 16.5 percent level. Similar growth was reported by Soren *et al.* (2009) in lambs fed either a SBM control or water-washed SKC-based diets, although growth was significantly reduced in lime- and toxin-binder (HSCAS)-treated SKC-based diets (Table 10).

Blood biochemistry

Blood parameters are important indicators to assess the wholesomeness and suitability of any unconventional feed for the presence of negative factors. Short-term or longer duration feeding may have immediate effects on the blood picture. Assessment of serum enzymes gives a clear status of different organs. Thus greater transaminases is normally observed due to liberation of enzymes into the circulating blood stream because of cell destruction (La Due, Wroblewski and Karmen, 1954).

TABLE 11
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on blood bio-chemicals in lambs

Parameter	Diet groups		
	DGNC	EKC	SKC
Glucose (ml/dl)	37.50	39.75	44.05
Urea-N (ml/dl)	12.61 a	16.06 b	14.20 ab
AST (IU/L)	66.34	70.02	65.87
ALT (IU/L)	17.72	20.29	18.00
SDH (IU/L)	16.38	17.34	15.69

Notes: DGNC = concentrate mixture containing de-oiled groundnut cake as the major protein source; EKC = concentrate mixture replacing 50% of DGNC N by EKC; SKC = concentrate mixture replacing 50% of DGNC N by SKC; AST = aspartate aminotransferase; ALT = alanine aminotransferase; SDH = sorbitol dehydrogenase. Means with different letters in a row differ significantly (P <0.05). *Source*: Ravi *et al.,* 2000.

Incorporation of 16.6 and 25 percent de-oiled karanj cake to substitute 50 and 75 percent de-oiled GNC nitrogen had no adverse effect on RBC and WBC counts, packed cell volume (PCV), haemoglobin, iron, calcium and phosphorus concentration of blood in growing calves, however, blood plasma protein content was significantly reduced at 75 percent level of de-oiled karanj cake incorporation (Konwar and Banerjee, 1987). Similarly, Ravi *et al.* (2001) observed no adverse effect on serum glucose and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) in lambs fed SKC, though level of serum urea was significantly increased when EKC was incorporated up to 24 percent in the diets of lambs (Table 11).

Prabhu (2002) also found no adverse effect on blood glucose, total protein, globulin, albumin, urea nitrogen and creatinine in lambs fed either SKC or alkali-processed SKC. They further reported statistically similar antibody titre among lambs sensitized with sheep pox virus on various diets, though antibody titres in general were lower in lambs fed raw SKC. Similarly, Soren (2006) observed comparable haemoglobin, serum glucose, calcium, albumin, globulin, A:G ratio, blood urea nitrogen and activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase in lambs fed either SBM based or the processed SKC diets, while LDH activity was increased significantly in groups fed SKC compared with the control. Singh *et al.* (2006) reported lower (P <0.05) levels of SGOT, LDH and acetylcholine esterase in sheep fed EKC and SKC diets versus sheep fed a DGNC (control) diet.

Carcass characteristics

The results of a study in which the animals were slaughtered after 280 days of experimental feeding with karanj cake (CASAN, 1999–2000) revealed that the fasted live weight and weight of hot carcass was significantly (P <0.05) reduced in sheep fed EKC based diet compared with diets containing de-oiled GNC and SKC. The comparable weights

TABLE 12
Effect of feeding expeller-pressed (EKC) and solvent-extracted (SKC) karanj cake on carcass characteristics in various groups

Parameter	Treatment group		
	DGNC	EKC	SKC
Live body weight (after 12 h fasting) (kg) **	19.5 b	13.5 a	16.2 ab
Dressed hot carcass weight (kg) *	7.74 b	5.67 a	6.52 ab
Weight of skin (kg)	2.35	1.65	1.82
Weight of head (kg)	1.64	1.22	1.37
Weight of gastro-intestinal tract (kg) **	6.12	3.77 a	5.41 ab
Weight of hooves (g)	495	412	462
Weight of vital organs (g)			
Liver **	270 a	375 b	309 ab
kidneys *	68 a	85 a	80 ab
Heart	80	87	75
Testes **	170 b	87 a	91a
Spleen	28	25	27
Lungs and trachea	275	287	294
Weight of various cuts (g)			
Loin *	910 b	600 a	650 a
Breast and shank *	1620 b	1325 a	1512 ab
Neck *	566 b	337.5 a	387 a
Nack *	1175 b	925 a	1019 ab
Thigh (hind legs) *	2820 b	1825 a	2415 ab

Notes: DGNC = de-oiled groundnut cake. a,b = Means with same suffixes in a row differ significantly. * = $P<0.05$, ** = $P<0.01$.
Source: Singh *et al.,* 2006.

of various meat cuts between de-oiled GNC and SKC diets were significantly ($P<0.05$) heavier than in sheep on an EKC diet. The organoleptic evaluation of pressure-cooked meat (with 1.5 percent salt) revealed no untoward taste due to karanj cake feeding.

Prabhu (2002) observed that dietary variation did not affect the physical and chemical characteristics of the carcass in lambs fed raw or alkali-processed SKC replacing 25 and 50 percent nitrogen of a SBM-based diet. Contrary to earlier studies, Soren (2006) reported significantly higher carcass weight in lambs fed SBM or water-washed SKC diets compared with lime- and binder-treated SKC-based diets, although the yields of bone and meat, the meat:bone ratio and the yields of wholesale cuts in the control and test diets did not differ significantly.

Singh *et al.* (2006) reported that the fasted live weight and weight of hot carcass ($P<0.05$) was significantly lower in sheep fed a diet containing EKC compared with weights in sheep receiving DGNC and SKC diets. However, weight of skin, head and hooves did not differ significantly among the three groups (Table 12). The weights of various primal cuts such as loin, breast and shank, neck, rack and thigh were significantly higher ($P<0.05$) in sheep fed DGNC and EKC diets.

Gross pathology and histopathological changes

After feeding karanj cake-based diets for 280 days (CASAN, 1999–2000), no gross pathological lesions were found in the vital organs of sheep. However, karanj cake feeding interfered with normal spermatogenesis, due to testicular degeneration. Spleens also showed moderate haemosiderosis. Similarly, Prabhu (2002) observed no gross or histopathological lesions in the tissues of vital organs in lambs fed for 180 days on diets containing SKC replacing the nitrogen moiety of SBM at either 25 or 50 percent in the concentrate mixture. In the same study, lambs fed 8.25 percent SKC and 16.50 percent processed SKC showed loss of striations, and thin and increased intermyofibrillar space due to oedema.

Long-term feeding of karanj cake (EKC, 24 percent) resulted in abnormalities of long bones and lameness. The radiographic examination of radius, carpal and metacarpal bones of lambs revealed thinner and less dense cortices, widened medullary cavity of the radius and presence of swollen soft tissues around carpal joints, similar to osteoporosis (Sastry *et al.*, 2000). No gross histopathological lesions could be noticed in the vital organs of the lambs fed an SBM-based control diet. Histopathological lesions were found in testes, epididymis, parathyroid, liver and small intestine of some lambs fed processed SKC-based diets (Soren, 2006), who also observed reduced length of long bones (metacarpals and metatarsals), reduced radiographic density, thinner cortices and increased diameter of the medullary cavity with binder (HSCAS)-treated SKC diets, and thicker cortices and narrowing of the medullary cavity in lime-treated SKC-fed lambs, all suggestive of poor mineralization of the bones, while mineralization of the long bones was normal in the control group and the group fed water-washed SKC.

On the basis of the above results, it can be inferred that long-term feeding of expeller-pressed, and to some

extent also solvent-extracted, karanj cake had deleterious effects on nutrient utilization, blood biochemical profile, rumen fermentation pattern and carcass characteristics, with clinico-pathological changes in bones and vital organ tissues. Water-washing and de-oiling of kananj cake may be the most feasible detoxification methods. Water-washed karanj cake may be incorporated at up to 22.5 percent in concentrate mixtures by replacing 50 percent of the nitrogen moiety of a conventional protein supplement like SBM, without any adverse effect on nutrient metabolism, growth and health of lambs.

Karanj cake as poultry feed

Detoxification

As discussed above, various techniques have been used for detoxification of karanj cake for the feeding of ruminants. Some of these techniques could also be applicable in the case of poultry, but the levels of incorporation in the diet will have to be standardized for each of the animal categories. In addition to the detoxification studies of Natanam, Kadirvel and Ravi (1989), Prabhu *et al.* (2002) and Panda *et al.* (2006) tried 36 possible detoxification methods for karanj cake. Based on reduction in karanjin, tannin and trypsin inhibitor activities, two methods of detoxification of SKC (1.5 percent NaOH or 3 percent $Ca(OH)_2$, w/w) and one for EKC (2 percent NaOH, w/w) have been recommended.

Palatability

Karanj cake as such is unpalatable to poultry as a sole feed. Substitution of 25 percent black til cake (black-seeded *Sesamum indicum,* commonly used as a protein source) with raw EKC in chick starter ration drastically reduced the feed intake with 50 percent mortality at 4 weeks old. However, incorporation of SKC to replace 25 percent black til cake on an isonitrogenous basis did not affect the quantity of feed consumed, with no mortality, and the feed conversion ratio (FCR) was found to be equivalent to the control group (Mandal and Banerjee, 1974). Inclusion of 5 percent raw karanj kernels in diets of male White leghorn chicks chicks up to 4 weeks of age reduced feed consumption by about 50 percent (Natanam, Kadirvel and Ravi, 1989), while the inclusion of either EKC or SKC at the 10 percent level did not result in any significant variation from control diet with respect to feed intake by White Leghorn pullets (Natanam, Kadirvel and Vishwanathan, 1989). In another study involving quail chicks, substitution of 20 percent red til cake (red-seeded *Sesamum indicum*) with de-oiled karanj cake up to 4.45 percent of the ration had no adverse effect on feed intake (Dhara *et al.*, 1997), but increasing the level of DKC beyond this resulted in poor growth and reduced feed consumption. The results obtained from different laboratories working on karanj cake co-products indicate that EKC or SKC had no adverse effect, while the raw products affected intake and nutrient utilization adversely, confirming that the anti-nutritional factors in karanj are either fats or fat-soluble.

Growth and feed efficiency

Twenty-five per cent substitution of til cake with SKC on an equi-protein basis did not result in any significant difference with respect to average daily gain (212 g vs 206 g) in broiler chicks up to 4 weeks old (Mandal and Banerjee, 1974). Mandal and Banerjee (1982a) also reported that de-oiled karanj cake could replace black til cake up to 30 percent in the diet of pullets from 9–18 weeks of age without affecting growth rate and feed conversion efficiency. The inclusion of either EKC or SKC at 10 percent level in the diets of White Leghorn pullets did not show any significant variation with respect to body weight gain and feed intake compared with those fed a control diet. However, birds fed on EKC had maturity delayed by 14–17 days (Natanam, Kadirvel and Vishwanathan, 1989). In contrast, Natnam and Kadrivel (1990) reported significantly reduced body weight gain with incorporation of EKC in the diet of White Leghorn pullets at a 10 percent level (18–22 weeks) compared with the control. The broiler chicks on diets incorporating 1 percent karanj oil and 10 percent expeller-pressed karanj cake had a growth depression of 51 percent, while those fed 2 percent oil and 20 percent cake showed 82 percent depression, compared with those receiving the basal diet. At the same time, chicks fed on a diet containing 40 percent cake (EE = 14.4 percent) suffered 100 percent mortality (Natanam, Kadirvel and Ravi, 1989). The increase in mortality as the cake level increased from 10 to 40 percent could be due to the corresponding increase in karanj oil content going from 1.4 to 5.6 percent in different groups. Such an adverse effect could be attributed to the presence of toxic factors such as karanjin and pongamol in the oil or oil fraction of the cake. Inclusion of raw karanj kernels at 5 percent level in the diets of broiler chicks to 4 weeks of age depressed growth rate by 50 percent, and processing of kernels (autoclaving/water washing) did not improve their performance (Natanam, Kadirvel and Ravi, 1989; Natanam, Kadirvel and Chandrasekaran, 1989a). Similarly, inclusion of de-oiled karanj meal at a 5 percent level in the diet of male chicks up to six weeks of age did not support good growth (Chaudhury *et al.*, 1991). In an experiment with unsexed Japanese quail from 14 to 42 days old, Dhara *et al.* (1997) reported that de-oiled karanj cake could safely be included in the diet to a maximum level of 4.45 percent of feed replacing 20 percent red til cake protein without affecting growth, but a further increase (7 to 22.5 percent) adversely affected their daily gain. Dietary incorporation of alkali-treated SKC at 6.43 percent did not have any adverse effect on body weight gain or feed conversion efficiency during 0–4 weeks old. However, there

was growth retardation subsequently and the body weight gain during 0–6 weeks old age was significantly ($P < 0.05$) lowered in birds fed a diet incorporating alkali-processed SKC at 6.43 percent. Supplementation of methionine to the diet with alkali-processed SKC at 6.43 percent was found to be beneficial in alleviating the growth depression (Panda, Sastry and Mandal, 2005).

Egg production

Inclusion of 10 percent SKC in the diet of layers did not affect egg weight and egg production, whereas a 15 percent inclusion had an adverse effect, although egg quality was not affected by the incorporation of SKC at either a 10 or 15 percent level (Mandal and Banerjee, 1981). Verma, Gupta and Srivastva (1984) reported poor egg production and feed consumption in addition to leg weakness in birds given 10 percent SKC in the diet. In a 22-week trial with White Leghorn pullets from 18-week old age, Natanam, Kadirvel and Vishwanathan (1989c) found that inclusion of either expeller-pressed or solvent-extracted karanj cake in the diet at a 10 percent level did not result in any significant variation from the pullets fed the control diet with respect to weight of first egg, but birds fed on expeller-pressed cake had poor hen-day production and depressed feed efficiency. Egg production was significantly lowered in groups receiving karanj cake diets (at 31.2–43.2 percent) compared with those fed the control diet. The reduction in egg production and poor feed conversion efficiency observed in birds fed karanj cake diets was due to poor protein quality or presence of toxic flavonoid compounds, or both (Parmar, Sahrawat, and Mukherjee, 1976; Natanam, Kadirvel and Chandrasekaran, 1989)

Blood biochemical profile

Incorporation of SKC up to 6 percent level in cockrel rations had no adverse effect on the erythrocyte sedimentation rate, PCV, Fe or haemoglobin content of blood (Mandal and Banerjee, 1982a). The feeding of karanj cake at 10 percent level in the diet of broiler chicks significantly ($P < 0.05$) lowered the haemoglobin (7.75 vs 4.50 g/100 ml) and PCV (26.7 vs 18.2 percent) compared with the control. However, Natnam and Kadrivel (1990) found no adverse effect on haemoglobin level and PCV by dietary incorporation of 10 percent karanj cake fed to 18-week old White Leghorn pullets. It was attributed to the age of the birds, as adult birds are more tolerant than younger ones.

Liver is the primary site of detoxification and any material suspected of toxicity is frequently tested with reference to its potential to cause liver damage. Due to higher tissue turn over and synthesis of dispensable amino acids during stages of early life, the active level of serum transaminases (AST and ALT) is expected to be relatively high (Lehninger, 1984), but elevated levels are suggestive of damage to vital organs (Oser, 1971). Mandal and Banerjee (1982b) found no adverse effect on SGOT activity in layers fed rations containing 6 percent SKC. These workers reported that incorporation of the extracted karanj cake replacing 30 percent N of black til cake is not detrimental to bird liver function. Samanta and Sasmal (1986) studied the effect of different solvent extracts of karanj seeds (neutral and acid fractions of petroleum ether extract, benzene extract, chloroform extract, phenolic and non-phenolic extracts of alcohol and water soluble part of alcoholic extract) in chicks. They observed that only the neutral fraction of petroleum ether extract significantly increased AST activity, while the other extracts had no adverse effect on its activity.

Carcass characteristics

There was no difference in organ weights (liver, heart, kidney and spleen) of cockerels due to dietary replacement of black til cake with de-oiled karanj cake at a 30 percent level (Mandal and Banerjee, 1982a). Similarly, Dhara *et al.* (1997) found no variation in weight of different organs (giblet, liver, heart and gizzard) and commercial cuts (neck, wing, thigh, shank, breast and trunk) due to incorporation of de-oiled karanj cake up to 22.4 percent in the diet of Japanese quail. However, dietary inclusion of 1 percent oil and 10 percent karanj cake in the diet of broiler chicks significantly increased the weight of liver and pancreas (Natanam, Kadirvel and Ravi, 1989), and the adverse effect of diet on organ weights relative to the body weight was attributed to the growth depression. None of the slaughtered and dressing characteristics differed significantly due to incorporation of SKC at 6.43 percent in the diet of broiler chickens (Panda *et al.*, 2006). No untoward and abnormal qualities with regard to appearance, odour, taste, texture, tenderness, juiciness and overall acceptability were found in meat of broilers fed processed karanj seed cake (Panda *et al*., 2007).

Harmful effects on health

The neutral fraction of the petroleum ether extract of the karanj seed exhibited high toxicity, with 100 percent mortality when fed to Rhode Island Red chicks (Samanta and Sasmal, 1986). Grossly, the heart was dilated with accumulation of fluid in the pericardium, and the liver and kidney showed red infarction. On histological examination, the myocardium, especially the peripheral zone, showed moderate degenerative fatty changes. Liver sections showed dilation and congestion of central veins. Kidneys exhibited hypercellularity, atrophy, mesangial cell proliferation, capsular epithelial cell crescent formation and tubular degenerative changes with coagulative necrosis. These workers attributed the harmful effects of feeding karanj to the toxic compounds present in the neutral part of petroleum ether extract. Verma (1988) reported

histopathological changes in the liver at a higher level of inclusion (16.8 percent w/w) of SKC. Mild degenerative changes were noticed in the form of cloudy swelling in the liver of chicks fed a diet containing a mixture of agro-industrial co-products having 63 percent SKC replacing 29 percent of a standard diet on an isonitrogenous basis (Haque *et al*., 1996). The liver and pancreas of chicks receiving 2 percent karanj oil and 20 percent EKC in the diet showed necrosis, fatty changes and disrupted structures (Natanam, Kadirvel and Ravi, 1989). Pathological studies showed no remarkable gross changes in vital organs at lower levels of inclusion (i.e. at 20 percent replacement of red til cake) of SKC (4.45 kg in 100 kg of feed) in the diet of Japanese quail, but higher levels of inclusion induced minor pathological changes in liver, heart, kidney and lungs (Dhara *et al.*, 1997). Dietary incorporation of either processed or unprocessed karanj cake beyond a 25 percent replacement (6.43 percent in diet) level, except for NaOH-treated SKC (12.86 percent in diet), resulted in histopathological abnormalities and the severity increased with increase in the level of replacement (Panda *et al.*, 2008). The severity of lesions was comparatively higher in the group fed a diet incorporating 25.72 percent NaOH (2 percent)-treated EKC. Livers showed hepatic degeneration, with distortion; kidneys showed tubular degeneration with necrotic lesions; spleen cells showed degeneration with necrotic foci and depletion of lymphocytes; and testes had degenerative changes of testicular follicles and vaculation. Feeding of SKC after treatment with either NaOH or $Ca(OH)_2$ was found to be beneficial instead of feeding SKC as such, since untrated SKC induced more severe histopathological lesions in the vital organs of broiler chickens. Treating SKC with 1.5 percent NaOH effectively minimized the toxic effects of karanjin.

The results from different laboratories confirm that EKC as such is unsuitable for poultry feeding. However, after detoxification with alkali (2 percent NaOH, w/w) it can be incorporated, but only at a low level (3.24 percent in diet), replacing 6.25 percent of the N moiety of SBM for broiler diets without adversely affecting performance. However, SKC can be incorporated after alkali (1.5 percent NaOH, w/w) processing at an enhanced level of 6.43 percent in the diet, replacing 12.5 percent of SBM N, in broiler diets up to 4-weeks old, beyond which the observed growth depression on this diet could be alleviated by 0.2 percent methionine supplementation. Such a diet, by partially substituting for the costly and scarce conventional oil cake, can support optimum nutritional performance in broiler chickens. However, further research should be focused on developing improved methods for detoxification to reduce the bitterness and toxic factors in karanj cake, permitting its inclusion at a higher level, making poultry production more economic.

NEEM SEED CAKE

Neem oil and other products of the neem tree are used traditionally for making cosmetics (soaps, mild detergents, creams, teeth cleansers) and traditional Indian medicines (for skin infections, inflammations, fever, leprosy, malaria, tuberculosis, worm infestation, eczema, etc.), in addition to being a source of anti-bacterial and anti-fungal agents in bio-manure and plant protection. In 1995, the European Patent Office granted a patent on neem as an anti-fungal agent to the United States Department of Agriculture and multinational company W.R. Grace, to which the Government of India objected, as neem has been used as an anti-microbial agent for more than 2000 years. This was decided in favour of India in 2000, but when the multinational mounted an appeal, it took five more years before dismissal of the appeal, in March 2005.

Distribution of the neem tree

Neem or margosa (*Azadirachta indica*; syn. *Melia azadirachta* Linn.) is a fast growing evergreen perennial tree with a height up to 20 m, and belongs to the family Maliaceae. It is found widely in semi-arid to sub-humid areas of the tropics, but it can thrive well even in warm, dry arid regions having rainfall less than 500 mm annually. Though neem is native to India, it has spread to Pakistan, Bangladesh, Sri Lanka, Malayasia, Indonesia, Thailand and the Near East. In Africa, it was introduced by Indian settlers and is abundant in the whole tropical belt from East to West Africa. Neem is also reported to occur in the West Indies Islands and some countries of Central and South America (Anon., 1948). Neem can grow in a wide range of climatic conditions. Such a wide adaptation and tolerance to varied soil and climatic conditions confirms its high degree of heterozygosity and potential scope for increasing production through selection, if the nutritional worth of its co-products are proved and found safe for feeding. India has about 25 million neem trees, with an average annual production potential of 900 000 tonne of neem seed cake (NSC) as a residue after oil extraction (Singh, 1993).

Bitter and toxic neem compounds

Neem seed kernel cake, a protein rich (35–40 percent CP) agro-industrial co-product hitherto utilized as fertilizer-cum-pesticide, was found unsuitable for animal feeding due to presence of bitter and toxic triterpenoids (azadirachtin, salanin, nimbin, nimbidiol, etc.). The bitterness of neem is attributed to limonoids, which are the triterpenoids. The pioneer work of Siddiqui (1942) revealed that the bitter principles (1.2 percent of dry matter) comprised both water- and fat-soluble fractions. The main feature of these compounds is that they are mostly tri- or tetraterpenoids. The structure and chemistry of these compounds has recently been reviewed by Devakumar and Dev (1993),

TABLE 13
Chemical composition of various type neem cakes (percentage of DM basis)

Type of cake	Crude protein	Ether extract	Crude fibre	NFE	Total ash	Ca	P	Source
Neem seed cake (NSC)	12.4–19.6	1.8–3.3	17.9	52.5–64.3	13.9–14.3	1.5	0.4	Bedi, Vijan and Ranjihan, 1975a; Nath, Vijjan and Ranjhan, 1978.
Deoiled NSC	17.9–18.4	0.4–3.6	25.9–30.1	35.0–46.2	5.5–16.2	0.7–1.0	0.2–0.6	Christopher, Ahmed and Sastry, 1976; Garg, 1989.
Neem seed kernel cake (NSKC)	33.5–40.8	7.9–10.4	11.4–23.0	19.6–26.6	12.3–15.0	–	–	Rajgopal and Nath, 1981; Nath, Rajagopal and Garg, 1983; Reddy, 1992.

Notes: NFE = nitrogen-free extract.

according to whom these can be classified into several groups: protomeliacins; limonoids with modified side chain (e.g. γ-hydroxybutenolides, azadirone and its derivatives); vilasinin-type compounds; and those belonging to 3c-seco-meliacins, namely nimbin, solanin and azadirachtin. The chemical structure of these compounds indicates the presence of polar and non-polar groups, a property that has been exploited in extraction of these compounds.

Chemical composition

The chemical composition of neem seed cake (NSC) and neem seed kernel cake (NSKC) varies greatly and depends on many factors. Crude protein and crude fibre contents of cake are inversely co-related and largely depend upon the type of seeds and method of oil extraction. When decorticated kernels are processed for oil, the cake obtained has high crude protein and low crude fibre, while the undecorticated cake is low in crude protein and high in crude fibre. Cakes obtained from partially de-pulped and decorticated seeds are intermediate depending upon the degree of de-pulping and/or decortication of the seeds (Table 13). The mineral composition of NSC, as well as of leaves, fruit and seed (Singhal and Mudgal, 1984) are summarized in Table 14, together with the amino acid composition reported by Singhal and Mudgal (1983) and Tewari (1992).

TABLE 14
Mineral composition and amino acid profile of neem seed cake

Amino Acid Profile		Mineral Composition	
Amino acid	g/16 g N	Mineral	Content
Aspartic	7.31–8.19	Ca %	0.96
Threonine	1.88–3.13	P %	0.30
Serine	2.88–3.63	Mg %	0.44
Glutamic	15.00–15.13	Na %	0.40
Proline	5.25	K %	0.98
Glysine	2.44–6.75	Cu, ppm	19
Alanine	2.88	Zn, ppm	19
Cystine	2.13–10.81	Fe, ppm	2705
Valine	3.00–4.75	Co, ppm	1.5
Methionine	0.88–4.38	Mn, ppm	70
Isoleucine	2.06–3.75	Cr, ppm	1
Tyrosine	1.63	Pb, ppm	10.5
Phenylalanine	3.88–5.00	Cd, ppm	–
Histidine	1.00–1.31		
Lysine	1.75		
Arginine	3.56–4.56		

Sources: Adapted from Singhal and Mudgal, 1983, 1984, and Tewari, 1992.

Feeding of neem seed cake to ruminants

Initially, Christopher (1970) showed the possibility of using of NSC as a protein source in cattle feed. Later, several feeding studies were conducted in the country to determine its palatability, nutritive value and possible use as animal feed. Studies with calves (Rao and Nath, 1979), buffalo bulls (Bedi, Vijan and Ranjihan, 1975a), cross-bred bulls (Ananthasubramanian, Menacherry and Devasia, 1979) and sheep (Gupta and Bhaid, 1980) showed that NSC as such was unpalatable, although the water extracts of neem seed cake showed no adverse effect on the hydrolytic enzymes of the rumen (Agarwal *et al.*, 1991) when tested *in vitro*. Most of the later studies concentrated on improving the palatability of NSC by feeding it together it with highly palatable ingredients such as starch, molasses, maize or jaggery [crude sugar from palm sap] (Christopher, 1970). Urea-ammoniated NSKC was found to be quite palatable to buffalo calves (Reddy, 1992) and kids (Anandan, 1994).

Effect of neem seed cake on performance of ruminants

Neem seed cake, when fed as such, besides being unpalatable, is harmful to animals as it adversely affects growth, the male reproductive system, and has at times led to haematuria (Nath, Vijjan and Ranjhan, 1978; Rao and Nath, 1979). Various attempts have since been made to detoxify the cake, making it suitable for feeding ruminants with optimum growth and better nutrient utilization.

Bedi, Vijan and Ranjihan (1975a) observed poor palatability, depressed growth rate and reduced nutrient digestibility (DM, CP, CF and NFE) in cross-bred calves fed concentrate mixtures containing 25 and 57 percent NSC. When NSC was substituted at rates of 25 and 50 percent digestible crude protein (DCP) for GNC in concentrate mixtures, loss of body weight with poor palatability was noted in buffalo calves, and there was significantly depressed nutrient digestibility. especially at the higher level of incorporation (Bedi, Vijan and Ranjihan, 1975b), indicating that

untreated NSC was not suitable even for maintenance of animals. Adverse effects on protein utilization were also recorded in buffalo calves (Arora, Singhal and Ludri, 1975) when fed concentrate mixtures containing 50 or 27 percent NSC. The neem derivative nimbin did not adversely affect microbial protein synthesis in buffalo calves fed on rations containing 20 percent NSC, although nutrient intake and growth were significantly reduced (Ludri and Arora, 1977). Impaired protein metabolism, as indicated by presence of albumin and bile salts in the urine, was recorded in cattle receiving 10 and 20 percent NSC in the concentrate mixture (Anon., 1977-78).

Pyne, Moitra and Gangopadhyar (1979) noted no change in milk composition and general health of lactating buffaloes fed on 10, 15 or 20 percent NSC-supplemented concentrate mixture for a period of 60 days. However, RBC, WBC and haemoglobin levels were higher and serum protein was lower in experimental animals than controls. In view of the unaltered serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase activities and blood calcium and phosphorus levels, Gangopadhyay *et al*. (1981) suggested it was safe to incorporate NSC at up to 20 percent of the concentrate mixture for lactating buffaloes. Later, NSKC, a neem seed by-product rich in protein but low in crude fibre content in comparison with NSC, was tried by Rajagopal and Nath (1981). They observed significant (P <0.01) depression in growth rate without affecting nutrient digestibility and DMI in cross-bred bull calves fed a ration containing 45 percent NSKC, showing that untreated NSKC is toxic to the animals. Even after solvent extraction, when de-oiled NSC was incorporated at 45 percent level in the concentrate mixture for cross-bred cow calves, it resulted in pronounced growth depression, higher DMI to compensate for 17 percent lower intake, along with reduced blood haemoglobin content, higher serum glutamate pyruvate transaminase (SGPT) and similar serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase and cholesterol when compared with calves on GNC-based control ration (Garg, 1989), and hence was considered unsuitable for animal feeding.

Soaking NSC in 1 percent NaOH overnight and then washing with water reduces the content of bitter principles and alkaloids (Anon., 1977-78). Nath, Vijjan and Ranjhan (1978) also reported that boiling of NSC with NaOH (8 g/kg cake) in 2.5 litre of water for 30 minutes, followed by water washing, resulted in a product palatable to cattle. However, feeding a concentrate mixture containing 50 percent NaOH-treated and water-washed NSC significantly depressed the DM digestibility and growth rate due to lower availability of energy, though the DMI was comparable to the control at the end of 50 days of feeding. But alkali treatment followed by water washing also has limitations, as along with the toxic compounds it removes part of CP, most of the soluble sugars and sulphur. In contrast, Vijjan, Rao and Nath (1978) noticed no ill effects from such alkali-treated NSC on creatinine excretion in urine, but rather serum icteric index, blood inorganic phosphorus and serum alkaline phosphatase activity were unaffected in cross-bred calves. Calves fed 45 percent alkali-treated NSC (ATNSKC) in their ration showed similar DM intake, nutrient digestibility and calcium, phosphorus and sulphur balances, but significantly (P <0.05) reduced growth rate (Rao and Nath, 1979). There was no difference in respect of serum icteric index, inorganic phosphorus and alkaline phosphatase activity between the groups fed control and experimental diets, but significantly reduced haemoglobin content in ATNSKC-fed calves, which suggests that treating the cake with lower levels of alkali followed by water washing was only partially effective in removing toxic compounds, even though the calves did not exhibit any palatability problem over the ATNSKC incorporation. Later, water washing of NSKC as an alternative to alkali treatment was tried by Nath, Rajagopal and Garg (1983). Such water-washed NSKC (WWNSKC), after sun-drying and grinding, when incorporated in concentrate mixture and fed to male cattle calves for a period of 273 days at a level of 45 percent showed statistically insignificant reduced growth rate in the group fed the experimental diet, but DMI, digestibility of nutrients, balance of calcium, phosphorus and nitrogen, and TDN intake were comparable to control. The blood haemoglobin content, serum acid phosphatase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase did not differ significantly, implying the removal of toxic bitter compounds to a greater extent by water washing. In another study, WWNSKC was incorporated in the concentrate mixture of buffalo calves at a 40 percent level in a feeding trial that continued for 179 days and involved a balance trial. It resulted in significantly (P <0.05) lower digestibility of DM and total carbohydrates, but higher (P <0.05) nitrogen balance in the experimental group. However, faster (P <0.05) growth rate was observed in animals receiving WWNSKC compared with those on the control diet (Table 15).

A full lactation experiment (300 days) involving a balance trial on cross-bred milch cows (32) divided into two groups was carried out. The control group was given a concentrate mixture consisting of 40 percent GNC, 30 percent maize, 27 percent wheat bran, 2 percent mineral mixture and 1 percent common salt. In the experimental group, the GNC was replaced with WWNSKC. The results showed that there was no difference (P <0.05) in the milk yield, butter fat content and organoleptic evaluation of milk (Table 16), DMI, digestibility of nutrients, haemoglobin content, SGOT, SGPT, acid phosphatase and alkaline phosphatase in blood, and reproductive ability of the cows in the two groups. The nitrogen balance was higher (P <0.05) in the WWNSKC

TABLE 15
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on the performance of buffalo calves

Parameter	Treatment	
	Control	Experimental
Initial body weight (kg)	83.2	83.2
Growth rate (g/day)	507 a	606 b
Dry matter intake (kg/day)	2.63	2.87
Digestibility (%)		
Dry matter	61.3 b	55.5 a
Crude protein	71.4	68.7
Ether extract	56.4 a	69.9 b
Total carbohydrates	61.8 b	56.1 a
Nitrogen balance (g/day)	27.5	37.4

Source: Agrawal, Garg and Nath, 1987.

TABLE 16
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on milk yield and composition

Parameter	Treatments	
	DGNC	WWNSKC
Average daily milk yield (kg)	7.68	7.20
Average daily milk yield corrected for initial yield (kg)	7.21	7.55
Average daily fat-corrected milk yield (kg)	8.46	7.43
Fat (g/kg)	45.0	41.3
Crude protein (g/kg)	34.4	34.3
Total solids (g/kg)	122.4	115.6
Solids-not-fat (g/kg)	76.4	72.4
Ash (g/kg)	7.7	7.6
Calcium (g/kg)	1.52	1.55
Phosphorus (g/kg)	0.53	0.55

Notes: DGNC = de-oiled groundnut cake. *Source*: Nath *et al.*, 1989.

TABLE 17
Effect of feeding water-washed need seed kernel cake (WWNSKC) on dry matter intake, digestibility of nutrients and nitrogen balance

Parameter	Treatments	
	DGNC	WWNSKC
DM intake (kg/day)	9.07	8.74
DM intake as % of body weight	2.56	2.61
Digestibility		
Dry matter	0.55	0.55
Crude protein	0.67	0.67
Organic matter	0.58	0.58
Ether extract	0.66	0.67
Crude fibre	0.43	0.48
Nitrogen-free extract	0.62	0.59
Total carbohydrate	0.56	0.55
Nitrogen intake (g/day)	244.0	245.0
Nitrogen excretion (g/day)		
In urine	101.67 a	75.0 b
In faeces	78.75	80.06
In milk	26.98	31.21
Nitrogen balance (g/day)	36.6 a	57.9 b

Notes: DGNC = de-oiled groundnut cake. a,b = means in a row with different suffixes are significantly different ($P < 0.05$).
Source: Nath *et. al.,* 1989.

TABLE 18
Effect of feeding water-washed neem seed kernel cake (WWNSKC) on blood measurements

Parameter	Treatments	
	DGNC	WWNSKC
Haemoglobin (g/L)	94.0	93.3
SGOT (IU/L)	36.0	34.70
SGPT (IU/L)	10.50	11.50
Acid phosphatase (IU/L)	5.99	7.01
Alkaline phosphatase (IU/L)	36.63	39.34
Urea (mg/L)	422.5 a	345.0 b

Notes: SGOT = serum glutamate oxaloacetate transaminase; SGPT = serum glutamate pyruvate transaminase; DGNC = de-oiled groundnut cake. a,b = Means in a row with different suffixes are significantly different ($P < 0.05$). *Source*: Nath *et. al.*, 1989.

group due to less excretion of urinary nitrogen and a concomitant decrease in blood urea nitrogen (Tables 17 and 18).

The water washing technique developed above for preparing WWNKSC can be adopted in all countries producing neem seed oilcake, so that the material, hitherto unused, can be used for animal feeding. Feeding NSKC (untreated) and WWNSKC to cross-bred calves had no adverse effect on rumen pH, TVFA concentration, holotrich protozoa count, activity of amylase, xylanase and CM-cellulose (Table 19), but there was significant depression in total-N, NH_3-N, TCA-soluble N, total protozoal count and urease and protease activity. TCA-ppt.-N and medium-sized and total spirotrich protozoa were significantly reduced with feeding of NSKC, whereas these were comparable between WWNSKC and control (DGNC) groups (Mondal and Garg, 2002).

In another experiment, metabolic studies showed that feeding of NSKC and WWNSKC had no adverse effect on intake of DM, OM and TDN, and on digestibility of DM,

TABLE 19
Effect of feeding neem seed kernel cake (NSKC) and water-washed NSKC (WWNSKC) on rumen fermentation (units/dl SRL) and enzyme specific activity (U/mg) in rumen contents

Parameter	Ration group		
	DGNC	NSKC	WWNSKC
pH	6.33	6.57	6.53
TVFA (mmole)	8.46	7.51	7.12
Total-N (mg)	145.21 a	105.78 b	116.36 b
NH_3-N (mg)	24.50 a	12.13 b	16.33 b
TCA soluble-N (mg)	55.13 a	46.85 b	45.11 b
Amylase	6.65	6.26	5.50
CMCellulase	1.35	1.55	1.26
Xylanase	5.07	5.22	4.47
Urease	24.71 a	14.14 b	14.19 b
Protease	13.77 a	8.38 b	9.55 b

Notes: TVFA = total volatile fatty acids; TCA = tricholoroacetic acid. a,b = means with different suffixes in a row differ significantly ($P < 0.05$). One unit of enzymic activity = amount of enzyme needed to produce 1 µmole of glucose/xylose/NH_3 (for CMCellulase/xylanase/urease) or for degradation of 1 mg of protein (for protease) per minute under assay conditions. *Source:* Mondal and Garg, 2002.

TABLE 20
Effect of feeding alkali treated neem seed kernel cake (ATNSKC) or urea-ammoniated neem seed kernel cake (UNSKC) on growth rate, feed intake, nutrient utilization and plane of nutrition in buffalo calves

Parameter	Control	ATNSKC	UNSKC
Initial body weight (kg)	235.5	203.0	221.3
Growth rate (g/day)	357.4	375.9	371.3
DM intake (g/kg $W^{0.75}$)	84.2	84.3	84.4
Concentrate:roughage ratio	51.49	46.54	54.46
Nutrient digestibility (%)			
Dry matter	55.1	52.5	52.9
Organic matter	57.6	54.6	56.2
Crude protein	63.9	61.9	63.7
Ether extract	60.7	64.9	66.5
Crude fibre	45.0	49.9	52.9
Nitrogen-free extract	61.6 b	54.1 a	55.0
Total carbohydrate	56.0	52.5	54.1
Nutrient balance			
N retention (g/day)	34.4	36.6	31.41
N retention (% of intake)	28.0	30.1	24.8
Ca retention (g/day)	19.4	15.6	16.8
P retention (g/day)	10.2	9.6	9.9
Nutrient value of ration (%)			
DCP	7.9	8.1	8.3
TDN	52.6	49.4	51.2
Plane of nutrition (g/day)			
CP intake	776.8	774.7	792.3
DCP intake	494.6	478.9	504.4
TDN intake	3300	3000	3200

Notes: DCP = digestible crude protein; TDN = total digestible nutrients; $W^{0.75}$ = metabolic weight (size). a,b = means with different suffixes in a row differ significantly (P <0.05).
Source: Sastry, Katiyar and Agrawal, 1999.

TABLE 21
Effect of feeding alkali-treated neem seed kernel cake (ATNSKC) or urea-ammoniated neem seed kernel cake (UNSKC) on blood-biochemical parameters

Parameter	Control	ATNSKC	UNSKC
Haemoglobin (g, %)	11.0	12.1	13.8
Alkaline phosphatase (IU/L)	17.3	17.8	20.8
SGOT (IU/L)	25.1	24.3	29.7
SGPT (IU/L)	29.2	30.2	34.4
Urea (mg/100ml)	32.8	39.2	35.3

Notes: SGOT = serum glutamate oxaloacetate transaminase; SGPT = serum glutamate pyruvate transaminase.
Source: Sastry, Katiyar and Agrawal, 1999.

OM, total carbohydrate, NDF and ADF. While DCP intake was significantly reduced in the NSKC group, it was comparable in control and WWNSKC groups (Mondal, 1994). Cross-bred milch cows fed WWNSC replacing the GNC moiety of concentrate mixture at 10, 20 and 30 percent levels showed no significant difference in DM intake per 100 kg body weight (3.06–3.13 kg) nor per unit metabolic body weight (130.6–133.5 g). Similarly, average daily milk yield (6.60–6.88 kg), 4 percent FCM yield (6.52–6.81 kg), average fat (3.91–3.94 percent), non-fat solids (8.91–8.98 percent) and total solids (12.83–12.91 percent) did not differ significantly among different ration treatments (Kumar *et al.*, 1992). Though there was improvement in palatability of NSC and NSKC after laborious repeated water washing of the cakes, as soluble toxic bitters were removed to a great extent, still the process was not feasible for industrial application, besides being uneconomical due to loss of soluble nutrients. Therefore, WWNSC can only replace concentrate mixture fed to cross-bred cows up to 30 percent without undesirable effects.

Though water washing of cake converted it into a wholesome protein substitute (Nath *et al.*, 1989; Sastry and Agrawal, 1992), 22 percent DM was lost. To avoid such loss, processing the cake in alkaline medium without water washing was tried, either by soaking it in water (1:5 w/v) containing either NaOH (2 percent w/w) for 24 hours or by ensiling with 2.5 percent urea (w/w) for 5–6 days (Nagalakshmi *et al.*, 1996, 1999). The sun-dried and ground alkali-treated (ATNSKC) and urea-ammoniated (UNSKC) cakes were found suitable for feeding cattle and buffalo calves (Reddy, 1992; Sastry, Katiyar and Agrawal, 1999), growing lambs (Anandan *et al.*, 1999) and kids (Musalia *et al.*, 2000) without affecting their growth, nutrient utilization, blood profile, rumen fermentation pattern, physical and chemical carcass characteristics, including organoleptic sensory score and gross and histopathology of vital organs.

Alkali treatment or urea ammoniation therefore converts NSKC into a wholesome substitute for DGNC for feeding growing buffalo calves, and no discernable effect or clinical symptoms of ill health could be noticed due to feeding of processed NSKC (Tables 20 and 21) during the entire 270 days of the growth trial, thus confirming effective detoxification of NSKC by either of the processing methods.

Neem seed cake in poultry feeding

Feeding of de-oiled neem seed meal (DNSM) at or above 5 percent level for 8 weeks resulted in adverse effects on growth and feed efficiency in White Leghorn chicks (Subbarayudu and Reddy, 1975). Choudhary *et al.* (1981) also reported poor growth and nutrient utilization on feeding of either raw or water-soaked NSC at 30 percent level during 0 to 6 weeks of age in broiler chicks. Studies with Babcock cockerels fed DNSM for 4 months resulted in comparable feed intake, but the birds excreted reddish brown, fluidy faeces with gradual and progressive emaciation (Christopher, Ahmed and Sastry, 1976). Similarly, Sadagopan, Johri and Reddy (1981) observed significant reduction in weight gain of broiler chicks fed raw NSC at a 2.5 to 7.5 percent level. However, solvent extraction of the cake improved the growth rate. Undecorticated expeller neem cake at 10 percent of broiler chick diets depressed

(P <0.05) weight gain, while feed efficiency was reduced at the 20 percent inclusion level, and the growth inhibition was linearly correlated with increasing level of incorporation (Reddy and Rao, 1988a). In contrast, incorporation of undecorticated NSC improved its utilization (Reddy and Rao, 1988b). Furthermore, overnight acid (1 N HCl) followed by alkali (5 percent KOH w/v) soaking for 15 minutes, with water washing in between each treatment of solvent-extracted undecorticated neem cake, removed the bitterness, as indicated by comparable feed intake, growth and feed efficiency of chicks (Reddy and Rao, 1988c). Similarly, saponification of neem oil with 10 percent KOH completely detoxified the oil, as evident from comparable performance of broiler chicks fed saponified neem oil and grountnut oil (Reddy, Rao and Reedy, 1988).

Chand (1987) fed diets having either 10, 20 or 30 percent raw neem seed meal (NSM), or its equivalent as 0.9, 1.8 or 2.7 percent neem oil, to chicks and recorded poor growth and low feed efficiency. Inclusion of alcohol- and hexane-extracted NSM improved utilization, but inclusion beyond 30 percent decreased performance. Incorporation of alkali-treated and urea-ammoniated neem kernel meal (NKM) in broiler chick diets gave comparable performance at 50 percent replacement of groundnut meal, and no untoward effects were noted for carcass traits as well as sensory evaluation of meat (Nagalakshmi, 1993). Broiler chicks fed full fat NSM (FFNSM) at 2.5, 5, 7.5 or 10 percent of dietary levels showed a negative (P <0.05) correlation between the level of inclusion and the gain and feed conversion efficiency during the starter phase (0–5 weeks), whereas birds in the finisher phase (6–10 weeks) exhibited comparable performance, probably due to colonization by counteracting gut microbiota (Salawu, Adedeji and Hassan, 1994), and gross pathology of visceral organs were normal. During 8-week trials with cockerels, Odunsi *et al.* (2009) reported that water-soaked neem seed cake added with charcoal (0.4 percent, w/w) can replace 20 percent of SBM in the diet with no adverse effect on growth, feed efficiency or carcass traits.

Egg production

When groundnut meal was replaced with NSC at 25, 50, 75 and 100 percent levels in layer diet it caused a significant reduction in egg production, but egg weight and egg quality were not affected (Sadagopan, Johri and Reddy, 1981). Similarly, Verma, Gowda and Elngaovan (1998) recorded no adverse effect of feeding raw or pre-treated (2 percent NaOH) NKM at 10 percent of dietary inclusion on feed consumption, egg production, egg weight, internal egg quality (yolk colour, Haugh unit), shell thickness and organoleptic evaluation of boiled eggs during a 12-week period of laying. However, raw NKM at levels of 15 and 20 percent dietary inclusion adversely affected performance in layers (Gowda *et al*., 1998).

Blood biochemical profile

Feeding of 30 percent DNSM to Babcock cockerels resulted in significant depression in haemoglobin level and total erythrocyte count in the blood (Christopher, Ahmed and Sastry, 1976), with elevated total leucocyte count (TLC). Similarly, Chand (1987) reported a higher TLC when feeding 10–30 percent alkali-treated neem seed cake. However, no significant differences in the concentration of haemoglobin and total erythrocyte count (TEC) could be observed when feeding 2.5 percent urea-ammoniated neem kernel meal in broilers during a 6-week study. Similarly, no difference could be noticed in any blood parameters (haemoglobin, TEC, TLC) when feeding of water-soaked neem seed cake up to 3.6 percent in the diet of cockerels (Odunsi *et al*., 2009).

Carcass characteristics

No difference in the dressed meat yield of broilers could be observed due to feeding of 2.5 percent urea-ammoniated neem seed kernel cake (UNSKC) as reported by Nagalakshmi (1993). No untoward effect on appearance, odour, taste, texture, tenderness or juiciness could be observed when pressure-cooked meat, with or without salt (1.5 percent, w/w), was subjected to sensory evaluation on a 7 point hedonic scale by a panel of semi-trained judges. At the same time, greater breast and thigh meat yield was observed in cockerels fed 3.6 percent WWNSKC compared with the UNSKC group.

Harmful effects on health

Detailed patho-anatomical examination of vital organs of birds fed 30 percent DNSM by Christopher, Ahmed and Sastry (1976) revealed pale and shrunken muscles, pale visceral organs, with slightly smaller, light brown liver, spleen, kidneys and pale intestine. Histologically, liver, spleen kidneys and lungs showed extensive fatty changes, with catarrhal enteritis of varying degrees. Similarly, Vijjan and Parihar (1983) recorded mild degenerative changes even at a 10 percent level of feeding alcohol-treated NSC. Mild to severe nephritis, hepatitis and enteritis were noticed in birds on diets with 5–50 percent NSM. Birds fed a diet with 20 percent NKM inclusion showed degenerative ovarian changes, with loss of ovarian follicles, and histological changes in liver, kidney and intestine (Gowda and Sastry, 2000). Similarly, water-washed NKM (WWNKM) in the diet of cockerels at 20 percent level for a period of 12 weeks adversely affected spermatogenesis and fertility (Tyagi, Tyagi and Verma, 1996.), confirming the anti-fertility effect of neem. However, alkali- (1 and 2 percent, w/w) and urea- (1.5 and 2.5 percent, w/w) treated NSKC when replacing either 50 or 100 percent of GNC and fed to the broilers, produced no gross or histopathological abnormalities.

RECOMMENDATIONS

Based on the knowledge available, it appears that both neem and karanj oil cakes are rich in protein, which can be very good supplements for ruminants fed on roughage-based diets. They could also be incorporated in poultry diets for increasing productivity, but neither oilcake is suitable for feeding without pre-treatment because of the presence of toxic compounds and poor palatability.

The anti-nutritional factors of karanj cake are soluble in oil. Therefore, complete removal of oil from cake may be a more effective method than other chemical treatment methods. The chemical structure of toxic and bitter compounds of neem cake are a mixture of polar and non-polar groups. Some of these compounds might be toxic for the animals, since they are either soluble in water or fat. This is perhaps the reason why water-washing of neem seed cake has shown promising results with minimal toxicity.

Water-washed or de-oiled karanj cake and water-washed neem seed cake may be incorporated at up to 50 percent of the nitrogen part of conventional protein supplements like soyabean meal or groundnut cake without adverse effects on nutrient metabolism, growth and health of lambs.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Numerous experiments have been conducted on the detoxification of these two oil cakes to render them suitable for feeding to cattle, buffalo, sheep, goat and poultry, but not much information is available on the selective removal of the compounds with the most toxic effects.

Once the pre-treatment process for detoxification of these oil cakes has been standardized, there will be the need to develop an industrial process for detoxification so that treatment cost is minimized and their use becomes economically feasible.

Long-term feeding trials to examine the effects of feeding the treated oil cakes on the quality of livestock products (milk, meat and eggs) are needed before these cakes can be recommended for practical application by farmers or for use in commercial compound feeds.

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Chapter 23

Co-products of the United States biofuels industry as alternative feed ingredients for aquaculture

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ABSTRACT

The tremendous growth of the biofuels industry has made large amounts of co-products (i.e. distillers grain and crude glycerin) available for use in aquafeeds. This chapter reviews the prospects and challenges associated with their use in aquafeeds. Properties of each product as it pertains to fish nutrition and available research are described for different fish species. Despite the apparent deficiency in lysine and the high fibre content in DDGS, considerable amounts of DDGS can be fed to omnivorous fish species without impact on growth or product quality. Nutrient variability is, however, an issue that needs to be considered when feeding DDGS to fish. The use of crude glycerin in fish is less clear, and further research is necessary before nutritional recommendations can be made.

INTRODUCTION

High energy prices and government policies that encourage the use of biofuels have spurred a tremendous growth in the ethanol and biodiesel industries, both in the United States and internationally over the last decade. In 2005, United States total ethanol production was estimated at 15.8 billion litres, and by early 2010, 51 billion litres of ethanol were produced (RFA, 2011). Similarly, biodiesel production has increased dramatically from 284 million litres in 2005 to 1.7 billion litres in 2007 (NBB, 2007). The surge in biofuel production has been simultaneously accompanied by a growing supply of co-products such as distillers grain and crude glycerin (i.e. glycerin or glycerol). Total supply of United States distillers grain was estimated at 32.9 million tonne in 2010, an increase of more than 13 fold compared with 2000 (Figure 1). The United States biodiesel industry is expected to produce an estimated 640 000 tonne of crude glycerin between 2006 and 2015 (Nilles, 2006). Excess glycerin in the market creates enormous marketing challenges and requires finding new uses for this co-product. Competitive pricing of low value crude glycerin has created opportunities for this co-product to be used in livestock feeding.

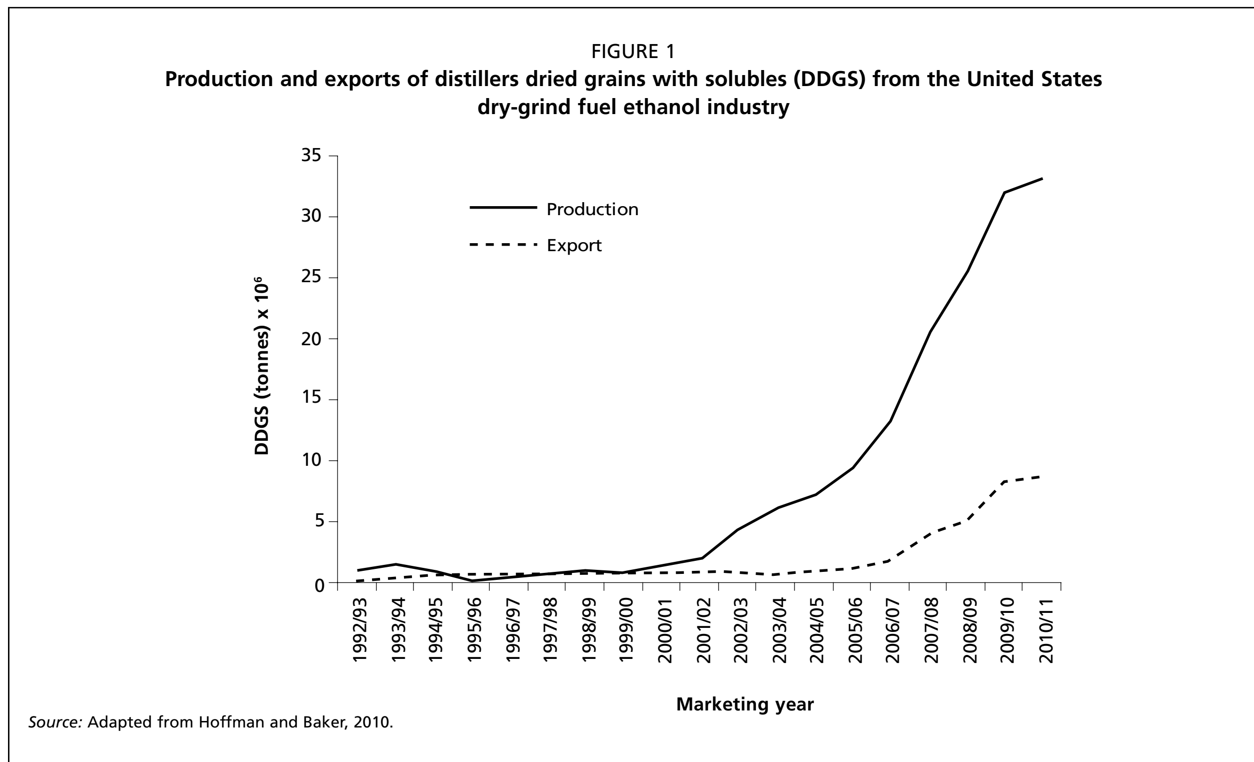
To date, distillers grain from the dry-grind ethanol industry have received considerable attention in animal feeds. In contrast, glycerol has been used more in industrial applications, although new research has shown that glycerol appears to be a promising energy source in animal diets. Distillers grain include traditional co-products, such as distillers wet grains, dried distillers grain with or without solubles (DDG and DDGS), and condensed distillers solubles (CDS). DDGS is the co-product that is most extensively produced in the ethanol industry. Recently, fractionation technologies used in ethanol production have resulted in new feeds with unique chemical compositions. Also, it is important to note that a small fraction of distillers grain is produced from beverage distilleries. However, the contribution of distillers grain from the beverage distilleries represented less than 2.7 percent of all the distillers grain produced in 2010/11 in the United States (Hoffman and Baker, 2010). In addition, maize (corn) is the primary feedstock grain used to make ethanol, accounting for more than 98 percent of all DDGS produced (Hoffman and Baker, 2010). Hereafter, the term "distillers grain" will refer to distillers dried grains with solubles (DDGS) (from maize) unless otherwise noted. Currently, DDGS is fed primarily to beef and dairy cattle, swine and poultry (Figure 2). No estimates on the current use of DDGS in aquafeeds could be found, but it is expected to be very small.

Another high growth sector in recent years has been aquaculture. Aquaculture has been growing at a rapid pace of approximately 6.2 percent per annum, from 38.9 million tonne in 2003 to 52.5 million tonne in 2008 (FAO, 2008), and currently accounts for over 50 percent of all food of aquatic origin consumed by humans worldwide. The value of aquaculture production was estimated at US$ 98.4 billion in 2008. However, concerns exist over the sustainability of aquaculture for a number of reasons, one of which is the increased pressure on feed ingredients, especially fishmeal

MAIN MESSAGES

- **DDGS from fuel ethanol production can be an effective protein ingredient in aquafeeds.**
- **DDGS serves to replace SBM and maize in the diet, but not fishmeal.**
- **For most fish species, a level of 20% DDGS appears to be the maximum inclusion if supplemental lysine is not added.**
- **If supplemental lysine is used, maximum DDGS levels greater than 20% can be used.**
- **Crude glycerine from biodiesel production appears to be a potential energy source.**
- **Much work needs to be conducted on use of glycerin in fish diets.**

FIGURE 1
Production and exports of distillers dried grains with solubles (DDGS) from the United States dry-grind fuel ethanol industry



Source: Adapted from Hoffman and Baker, 2010.

and fish oil. Fishmeal used in aquaculture represented 68.2 percent of total global fishmeal production in 2006 (Tacon and Metian, 2008), but increased pressure due to exploiting marine resources and rising prices could ultimately decrease the use of fishmeal, as it will inevitably be replaced by less expensive alternative proteins.

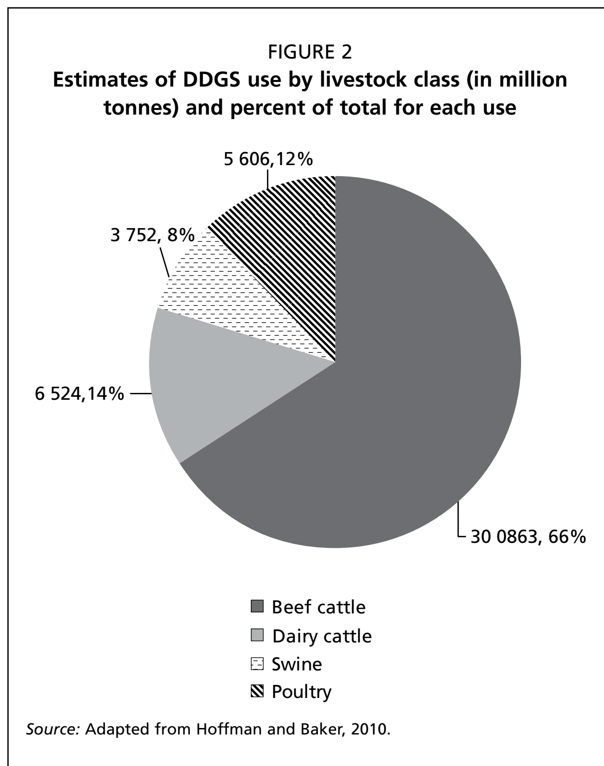
DDGS, a relatively cheap protein source (Figure 3) compared with fishmeal, is a candidate plant protein. During the last 10 years, DDGS market price has been generally between 5 percent and 20 percent that of fishmeal. While DDGS is not recommended as a direct, complete replacement for fishmeal, it can be used with, or in lieu of, other plant proteins (such as soybean meal – SBM) to reduce the use of fishmeal in aquafeeds. As shown in Figure 3, over the last decade the price of DDGS has ranged from approximately 20 percent to 70 percent that of SBM.

This chapter will review the nutrient composition of major biofuels (i.e. maize-based fuel ethanol and soy-based biodiesel) co-products (i.e. distillers grain and crude glycerin), will provide summaries of available nutritional studies for different fish species, and will conclude with final remarks on challenges associated with these co-products and areas of needed research.

Before proceeding, however, it is important to note a few key issues. First, maize is the primary feedstock for fuel ethanol production in the United States. Other starch-rich materials can theoretically also be used to produce ethanol, including barley, cassava, field peas, millet, triticale, oats, rice, rye, sorghum, sweet potato and wheat. Unfortunately, most of these alternative starch sources have only been investigated on a laboratory- or pilot-scale and are not readily commercially available. Not surprisingly, fish feeding trials are essentially non-existent for co-products from these substrates, and thus will not be discussed in this chapter.

In contrast, biodiesel can be produced from a variety of oilseeds and lipid-containing materials, including canola

FIGURE 2
Estimates of DDGS use by livestock class (in million tonnes) and percent of total for each use



Source: Adapted from Hoffman and Baker, 2010.

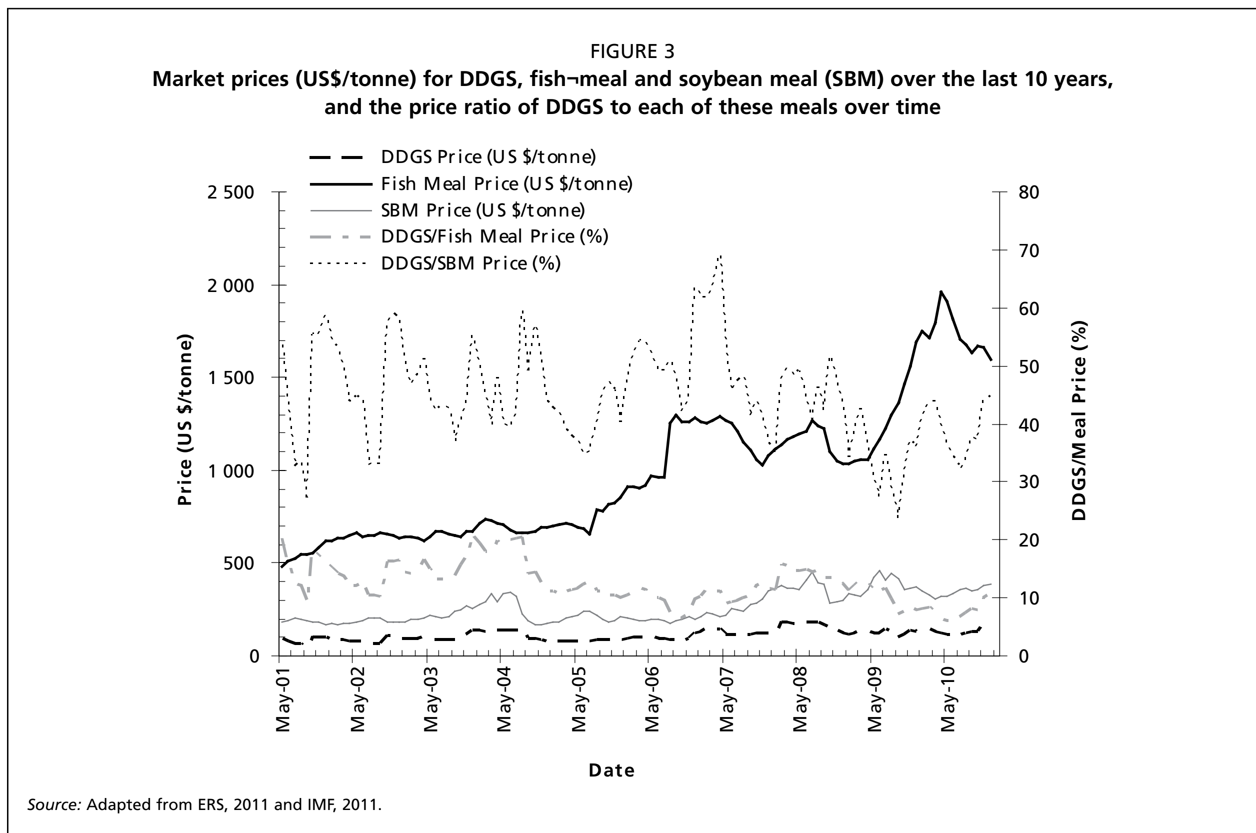
(rapeseed), castor beans, copra, cottonseed, flaxseed, jatropha, palm oil, poppy seed, safflower seed, sesame seed, shea nut, sunflower seed, animal rendering and others. The primary source for biodiesel in the United States, however, is soybean. Thus, our discussion will be focused on soy-based biodiesel co-products. But, our discussion will be limited to glycerin, and will not cover SBM or various soy protein concentrates or isolates. These topics have been covered in depth elsewhere (Gatlin *et al.*, 2007; Hertrampf and Piedad-Pascual, 2000; U.S. Soybean Export Council, 2011). Furthermore, algae-based biofuels have much promise for the future of the biofuels industry, but, to date, post-extraction algal residues use in any fish feeding trials has not been reported.

PROPERTIES OF DISTILLERS GRAIN

Physical properties

Some of the physical properties that are important to aquafeeds include particle size, bulk density and flowability. Because of the small size (<1.4 mm, on average) and variable size distribution of particles (Bhadra, Muthukumarappan and Rosentrater, 2009), handling DDGS can pose some logistical problems. Bulk density determines capacity of transport vessels and storage facilities. As with other properties, bulk density varies among DDGS sources and averages ~480 kg/m^3 (Rosentrater, 2006). For comparison, bulk densities of maize and SBM are 721 and 658 kg/m^3, respectively (Letsche, Lammers and Honeyman, 2009.). The low bulk density of DDGS translates into higher transportation costs. Variations in bulk density may be due to differences in particle size and to the amount of condensed distillers solubles (CDS) added back to distillers grain during manufacturing. Several factors affect the

FIGURE 3
Market prices (US$/tonne) for DDGS, fish¬meal and soybean meal (SBM) over the last 10 years, and the price ratio of DDGS to each of these meals over time



Source: Adapted from ERS, 2011 and IMF, 2011.

TABLE 1
Typical physical properties of distillers dried grains with solubles (DDGS)

Physical property	Mean (SD)	Range
Water activity (-)	0.53 (0.02)	
Bulk density (kg/m^3)	389.3 (24.1)	490–600
Angle of repose (°)	26.5 (1.8)	35.94–41.60
Colour Hunter L (-)	40.0 (1.6)	36.56–50.17
Colour Hunter a (-)	8.0 (0.4)	5.20–10.79
Colour Hunter b (-)	18.2 (0.9)	12.53–23.36

Notes: (-) denotes dimensionless quantities. *Sources*: Means (and Standard Deviations) from Rosentrater, 2006; Ranges from Bhadra, Muthukumarappan and Rosentrater, 2009, 2010.

flowability of DDGS, including particle size, soluble solid and fat contents (which are due to the CDS addition level), dryer temperature and moisture content at dryer exit (Ganesan, Muthukumarappan and Rosentrater, 2008). Manipulation of these properties and pelleting often improves the flowability of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Rosentrater, 2007).

The colour of DDGS can vary from golden yellow to dark brown. The amount of condensed distillers solubles (CDS) added to the distillers grain, the original colour of the feedstock grain and drying temperature are all factors that affect the colour of DDGS (Ganesan, Muthukumarappan and Rosentrater, 2008; Noll, Parsons and Walters, 2006). Colour can be a good indicator of heat damage (Maillard reaction) that occurs during the drying of distillers grain, especially to the most heat labile amino acid, lysine. In fact, the digestibility of lysine in swine has been shown to vary substantially among different DDGS sources (Stein *et al.*, 2006). In addition, a strong correlation has been determined between brightness/lightness (i.e. Hunter colour L) and digestible or bio-available lysine content of DDGS for poultry (Pahm *et al.,* 2009; Fastinger, Latshaw and Mahan, 2006) and swine (Pedersen, Pahm and Stein, 2005). Other physical characteristics of DDGS are summarized in Table 1.

Chemical properties

Nutrients in DDGS are concentrated nearly three times compared with those found in maize. This is because starch, which constitutes about two-thirds of the maize kernel, is removed during the fermentation process to produce ethanol. Predicting DDGS composition from that of maize, however, has to reflect multiple factors. Differences in processing within and among ethanol plants, especially drying conditions (temperature and time) and the amount of CDS added to the distillers grain and, to a lesser extent, the source and quality of maize, can create considerable variations in the nutrient composition of DDGS.

In fact, nutrient concentrations can vary substantially among DDGS sources (i.e. ethanol plants) (Table 2). Several papers on nutrient composition and influencing factors are available (Spiehs, Whitney and Shurson, 2002; Belyea, Rausch and Tumbleson, 2004; Belyea *et al.*, 2010). It is also important to recognize that nutrient composition of DDGS, as found in older publications (such as NRC, 1993), may no longer be applicable because DDGS from that generation was predominately made from alcohol beverage distilleries, not the newer fuel ethanol plants of today. In general, DDGS is a good source of energy and protein for various livestock animals. Fuel-based DDGS contains, on average, 11.0 percent moisture, 30.8 percent crude protein, 7.4 percent crude fibre, 11.2 percent crude fat and 5.5 percent residual starch (UMN, 2011).

Specifically, fish have requirements for amino acids rather than crude protein, per se. The amino acid profile of DDGS reflects that of maize, with lysine being the most

TABLE 2
Typical nutrient composition of distillers dried grains with solubles (DDGS)

Item	UMN	Spiehs, Whitney and Shurson, 2002	NRC
Dry matter (%, as is)	89.2 (1.6)	88.9 (1.7)	91.0
Crude fat	11.2 (14.3)	10.9 (7.8)	10.2
Crude fibre	7.4 (14.9)	8.8 (8.7)	10.0
Starch	5.5 (34.4)	-	-
Crude protein	30.8 (4.9)	30.2 (6.4)	29.7
Amino acids			
Arg	1.35 (9.6)	1.20 (9.1)	1.23
His	0.82 (9.1)	0.76 (7.8)	0.70
Ile	1.17 (7.1)	1.12 (8.7)	1.20
Leu	3.51 (8.8)	3.55 (6.4)	3.18
Lys	0.97 (12.6)	0.85 (17.3)	0.71
Met	0.60 (12.1)	0.55 (13.6)	0.55
Cys	0.61 (13.4)	-	0.51
Phe	1.49 (12.6)	1.47 (6.6)	1.53
Thr	1.12 (7.2)	1.13 (6.4)	1.08
Trp	0.23 (11.8)	0.25 (6.7)	0.11
Tyr	1.10 (12.6)	-	1.09
Val	1.55 (8.2)	1.50 (7.2)	1.65
Ash	5.7 (1.4)	5.8 (14.7)	7.0
Ca	0.05 (135.3)	0.06 (57.2)	0.15
P	0.79 (14.3)	0.89 (11.7)	0.73
K	1.02 (17.2)	0.94 (14.0)	0.44
Mg	0.31 (17.9)	0.33 (12.1)	0.18
S	0.69 (35.6)	0.47 (37.1)	0.38
Na	0.26 (188.8)	0.24 (70.5)	0.57
Cl	0.19 (25.5)	–	0.18
Zn (ppm)	58.80 (23.1)	97.5 (80.4)	87.91
Mn (ppm)	17.00 (26.1)	15.8 (32.7)	25.05
Cu (ppm)	6.00 (29.2)	5.9 (20.4)	58.02
Fe (ppm)	110.00 (39.1)	119.8 (41.1)	259.34
NFE	34.1	33.2	34.1

Notes: All nutrient values expressed as a percentage on a 100% dry matter basis (Coefficients of variation presented in parentheses when available); NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). *Sources*: UMN data are a compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n=62). Spiehs, Whitney and Shurson (2002) is a compilation of data from 1997 to 1999 (n=118). NRC data are from Nutrient Requirements of Fish (NRC, 1993).

TABLE 3
Typical nutrient composition of other distillers grain products

Item	Wheat DDGS[1]	Triticale DDGS[2]	Sorghum DDGS[3]	De-oiled DDGS[4]	Maize HPDDG[5]	Sorghum HPDDG[6]
DM (%, as is)	90.9	89.4	88.4	87.5	91.4	92.3
Crude fat	5.7	–	10.8	3.5	4.0	3.2
Crude fibre	8.1	–	8.0	–	–	–
Starch	2.1	–	–	5.6	8.3	–
Crude protein	40.3	32.4	34.2	34.0	43.6	48.2
Amino acids						
Arg	1.65	1.45	–	1.59	1.70	1.85
His	0.82	0.75	–	1.04	1.17	1.11
Ile	1.37	1.17	1.41	1.47	1.79	2.18
Leu	2.67	2.51	4.44	4.26	5.99	5.89
Lys	0.89	0.78	1.01	1.09	1.28	1.73
Met	0.64	0.55	0.61	0.68	0.91	0.85
Phe	1.83	1.50	–	1.61	2.35	2.47
Thr	1.21	1.07	1.20	0.95	1.58	1.79
Trp	0.39	0.14	0.22	0.18	0.28	0.39
Val	1.78	1.49	1.86	1.43	2.25	2.63
Ash	5.7	–	4.5	5.3	2.1	5.0
Ca	0.18	–	–	0.06	0.04	0.13
P	1.05	–	–	0.84	0.45	0.82
NFE	31.1	–	42.8	–	–	–

Notes: DDGS = distillers dried grains with solubles; HPDDG = high protein distillers dried grain. All nutrient values expressed as a percentage on a 100% dry matter basis. NFE= Nitrogen-free extract = 100 -(moisture + crude fibre + crude protein + crude fat + ash). *Sources:* (1) Avelara *et al.*, 2010; Cozannet *et al.*, 2010; Oryschak *et al.*, 2010a; Bandegan *et al.,* 2009. (2) Oba *et al.*, 2010; Oryschak *et al.,* 2010b. (3) Jones *et al.*, 2010; Urriola *et al.,* 2009. (4) Mjoun *et al.*, 2010. (5) Jacela *et al.*, 2010; Mjoun *et al.*, 2010; Applegate *et al.*, 2009; Widmer *et al.,* 2008. (6) Jacela *et al.*, 2010.

limiting. Compared with SBM and fishmeal, DDGS supplies (on a crude protein basis) higher amounts of Met and Leu, similar amounts of His, Phe, Thr, Trp and Val, but lower amounts of Arg, Ile and Lys. When comparing the amino acid profile of DDGS with the requirements of tilapia and rainbow trout, it can be concluded that DDGS is deficient in lysine for both tilapia and rainbow trout, and in tryptophan for tilapia (Table 4). The imbalance of amino acids in DDGS can limit its value for fish when used as a sole protein source, although, when economically viable, synthetic amino acids can be used to correct deficiencies. Combining DDGS with other protein meals is another option. In addition, low digestibility of amino acids in DDGS may further limit its nutritional value in fish diets. It is, however, important to note that improvements in the protein quality of DDGS in terms of concentration and digestibility of amino acids from DDGS produced in new generation ethanol plants may be an indication of improved and more controlled production processes.

DDGS is also a good source of the vitamins niacin, riboflavin and vitamin E, as well as various minerals. DDGS contains high levels of P (0.80%), with the majority of this P being inorganic, making DDGS a good source of digestible P in chicks (Martinez Amezcua, Parsons and Noll, 2004) and swine (Pedersen, Boersma and Stein, 2007)**.** In contrast, DDGS contains low concentrations of Ca, Cl and other trace minerals. In addition, unlike most plant proteins, DDGS does not contain anti-nutritional factors, which can prove to be very problematic for some proteins. Variability in nutrient composition is still, however, an issue when dealing with DDGS. For this reason, access to reliable nutrient composition information is necessary to minimize risks associated with nutrient variation when DDGS is used in fish feeds.

As mentioned, the majority of United States distillers grain currently comes from dry-grind processing of maize into fuel ethanol, with smaller amounts being derived from sorghum (milo) and wheat, as well as a small percentage from beverage distilleries. In Canada, wheat and triticale represent the major grains used in ethanol production. Barley is another grain that can be used for ethanol production; however, lower ethanol yield and higher costs of production limit the use of barley in ethanol production. Moreover, barley DDGS has limited value in aquaculture feeds due to its residual content of beta-glucans. Likewise, recent changes aimed at increasing the efficiency of maize-based fuel ethanol production have resulted in a variety of distillers grain of different compositions, which are becoming available to the marketplace. The compositions of these other co-products that may have potential in aquaculture feeds are presented in Table 3. Although the chemical composition of some of these co-products appears attractive for use in aquafeeds, their nutritive value is still unknown. Except for limited information for high protein distillers dried grains (HPDDG), none of these co-products has been evaluated in fish diets. Research in monogastric species

TABLE 4
Ratio of essential amino acid supplies from different ingredients to the dietary requirements of different fish species

Amino acid	Tilapia			Rainbow Trout		
	DDGS	SBM	Fishmeal	DDGS	SBM	Fishmeal
Arginine	1.19	2.05	1.61	1.11	1.92	1.50
Histidine	1.77	1.77	1.50	1.44	1.44	1.22
Isoleucine	1.40	1.67	1.52	1.60	1.91	1.74
Leucine	3.84	2.62	2.34	3.09	2.11	1.89
Lysine	0.70	1.42	1.64	0.66	1.34	1.54
Methionine/cystine	1.39	1.01	1.27	1.49	1.08	1.36
Phenylalanine/tyrosine	1.74	1.75	1.39	1.78	1.79	1.42
Threonine	1.11	1.21	1.18	1.73	1.89	1.84
Tryptophan	0.87	1.63	1.15	1.44	2.71	1.91
Valine	2.06	1.85	2.05	1.59	1.43	1.58

Notes: DDGS = dried distillers grain with solubles; SBM = soybean meal. *Sources*: amino acid composition of DDGS from compilation of data from 2008 and 2009 by University of Minnesota (UMN, 2011) (n = 62); amino acid composition of SBM and fishmeal from Nutrient Requirements of Fish (NRC, 1993). Dietary requirements from NRC, 1993.

indicates lower amino acid digestibility of wheat and sorghum DDGS compared with the parent grain (Bandegan *et al.*, 2009) or maize DDGS (Urriola *et al.*, 2009; Jacela *et al.*, 2010; Oryschak *et al.*, 2010a).

Feeding value of distillers grain to fish

Fish performance

DDGS had been fed to fish for some time. In fact, the use of DDGS as component in aquafeeds can be traced back to the late 1940s (Phillips, 1949). Formal evaluations of DDGS began in earnest during the last two decades, and can be divided into two phases: prior to the ethanol boom (before 2000–2001), where most research primarily involved the use of co-products from the beverage alcohol/distillery industry (see, for example, Wu *et al.,* 1994, 1996a, b, 1997; Tidwell *et al.*, 2000); and post 2000–2001, where the majority of evaluated DDGS came from the fuel ethanol industry (see, for example, Shelby *et al.,* 2008; Abo-state *et al*., 2009; Schaeffer, Brown and Rosentrater, 2009; Schaeffer *et al.,* 2010). The chemical composition of DDGS produced from these two processes reflects the composition of the feedstock grain used. The distillery process usually uses a mixture of grains, including barley, rye, wheat and maize, while the fuel ethanol process primarily uses maize as the substrate for fermentation. Also, protein quality from the two processes may differ. As discussed previously, protein quality of DDGS has improved over time, resulting in a feed ingredient that is relatively consistent and highly digestible compared with older generation DDGS. Several factors control the amount of DDGS that can be effectively included in diet formulations for cultured fish. Those factors are related to species requirements and limitations imposed by the nutrient composition of DDGS. High fibre and unbalanced profile of amino acids in DDGS are the main constraints to including greater amounts in aquafeeds.

A summary of available data on feeding DDGS to various freshwater species is presented in Tables 5 and 6. These studies were essentially designed to test the incremental inclusion rate of DDGS, with the goal of establishing an optimal feeding rate. Most studies included a control diet where no DDGS was fed, allowing for direct assessment of the effect of DDGS on fish performance. To date, DDGS has been evaluated in 8 freshwater species, namely Nile tilapia (*Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), yellow perch (*Perca flavescens*), common carp (*Cyprinus carpio*), freshwater prawn (*Macrobrachium rosenbergii*), red claw crayfish (*Cherax quadricarinatus*) and sunshine bass (*Morone chrysops* × *M. saxatilis*), and two saltwater fish species: milk fish (*Chanos chanos*) and Pacific white shrimp (*Litopenaeus vannamei*). Tilapia and catfish have been the most studied species (Table 5). In many cases, DDGS was used as source of protein and energy, replacing maize meal and SBM at different proportions. DDGS also replaced other feedstuffs such as fishmeal, rice bran, wheat middlings, sorghum meal and meat and bone meal. It appears from the dataset that DDGS is generally accepted by the aforementioned species, with some differences. Tilapia and catfish have been shown to tolerate higher amounts of DDGS in their diets. In fact, feeding DDGS at levels as high as 60 and 70 percent DDGS, supplemented with lysine, resulted in optimal growth and feed efficiency of tilapia (Shelby *et al.,* 2008) and channel catfish (Webster, Tidwell and Yancey, 1991), respectively. In those species, DDGS can be fed at up to 30 percent of the diet without the need for supplemental lysine. For most species, an inclusion rate of 20 percent DDGS seems readily acceptable. Although, the inclusion of DDGS was restricted at 10 percent in studies involving Pacific white shrimp, red claw crayfish and sunshine bass, feeding more than 10 percent may be possible. Another way to improve the utilization of DDGS in fish diets may be achieved through taurine supplementation. It has been shown that taurine is conditionally indispensable in several fish species fed all-plant-protein diets. In fact, replacing fishmeal (which is a

rich source of taurine), with plant proteins (which are usually very low in taurine), can result in taurine-deficient diets. Taurine supplementation has improved weight gain in several fish species, probably through enhanced voluntary feed intake (Takeuchi *et al.*, 2001; Park *et al.*, 2002; Gaylord, Teague and Barrows, 2006; Takagi *et al.*, 2006; Lunger *et al.*, 2007). It is expected that taurine supplementation for diets based on DDGS might improve feed utilization and growth of fish as well.

Tilapia

Published studies evaluating the use of DDGS in tilapia have involved a wide range of fish sizes (initial weight 0.5–190 g; final weight 6.1–907 g). In most studies, DDGS (0–100 percent) replaced maize and SBM in diets containing 0 to 8 percent fishmeal. Overall, feeding DDGS at levels between 15 and 30 percent appeared to maximize weight gain and feed efficiency. The addition of lysine allowed DDGS to be included at even higher levels of 40 to 60 percent. In general, feeding DDGS did not affect the flesh composition of tilapia.

Early studies on the use of DDGS in tilapia were conducted by Wu and colleagues using distillery-derived DDGS. Wu *et al.* (1994) reported that feeding 29 percent DDGS in combination with 6 percent fishmeal, or 22 percent DDGS in an all-plant-protein diet, to juvenile tilapia resulted in similar weight gain and feed conversion ratio as a control diet. Results from that study led Wu, Rosati and Brown (1996) to test whether higher inclusion rates of DDGS would sustain similar growth of tilapia compared with traditional diets. Two diets containing either 35 or 49 percent DDGS at dietary protein concentrations of 40 and 36 percent, respectively, were evaluated in tilapia fry. They found that the 35 percent DDGS diet resulted in similar weight gain and feed efficiency compared with the control diet, which was a 36 percent protein diet. Protein efficiency ratio was, however, higher in the control diet. At 49 percent DDGS, both weight gain and feed efficiency were depressed, indicating a lysine deficiency in diets containing the higher amounts of DDGS. Because lysine is the most limiting amino acid in DDGS-based diets, the addition of supplemental lysine may allow for greater DDGS inclusion levels. This question was investigated by Wu, Rosati and Brown (1997), who fed tilapia fry diets containing from 63 to 82 percent DDGS with added lysine. Overall, they found that, regardless of lysine supplementation, growth was negatively affected by high DDGS concentrations. In contrast, feed and protein efficiencies were similar for the 67 percent DDGS diet and the control diets.

In another study, Tidwell *et al.* (2000) evaluated the growth of juvenile tilapia fed diets consisting of pelleted or unpelleted DDGS (100 percent) in pond polyculture with freshwater prawn. Feeding either form of DDGS resulted in a 24 percent decrease in weight gain and 0.5 unit increase in feed conversion ratio compared with a commercial catfish diet. The economic efficiency (feed cost/weight gain), however, showed savings of US$ 0.29 and 0.40 per kg of fish produced, respectively, for pelleted and unpelleted DDGS, compared with the control diet.

To improve the dietary amino acid supply to the fish, one strategy is to feed DDGS as a blend with other proteins that are particularly rich in lysine. In this regard, Coyle *et al.* (2004) evaluated different protein blends in diets for juvenile hybrid tilapia. DDGS was included at 30 percent, with a combination of different protein sources, including fishmeal (8 percent), meat and bone meal (26 percent) and SBM (46 percent). They concluded that feeding DDGS with SBM resulted in lower weight gain and higher feed efficiency compared with the other protein combinations.

Additional studies (Lim *et al.*, 2007; Shelby *et al.,* 2008) evaluated the utilization of high levels of DDGS and whether supplementation with lysine would mitigate the associated negative effects on growth. Lim *et al.* (2007) found that optimal performance of juvenile tilapia was obtained at 20 percent DDGS without added lysine, while the addition of lysine to diets containing 40 percent DDGS improved feed utilization but not weight gain. Shelby *et al.* (2008), however, successfully included up to 60 percent DDGS with added lysine to diets containing 8 percent fishmeal, resulting in similar weight gain and feed efficiency compared with a control diet based on maize and SBM. These observations were confirmed by Abo-state, Tahoun and Hammouda (2009), who found that including up to 55 percent DDGS with added lysine in a 10 percent fishmeal diet resulted in even better weight gain and protein utilization by tilapia fingerlings compared with an SBM-based diet.

Recently, Schaeffer, Brown and Rosentrater (2009) found that weight gain, feed efficiency and fillet yield were adversely affected when DDGS was fed in excess of 30 percent of the diet, but their diets included no supplements. To more closely define the optimum inclusion rate for DDGS, Schaeffer *et al.* (2010) evaluated growth performance of juvenile tilapia fed diets with amounts of DDGS varying from 17.5 to 27.5 percent. They reported poorer growth of tilapia fed DDGS-based diets, and that feeding 20 percent DDGS resulted in maximum growth among the DDGS diets, although this corresponded to only 70 percent of that obtained with the commercial diet. The commercial diet contained 15 percent fishmeal, while the DDGS diets had 5 percent fishmeal.

It is clear that tilapia can effectively utilize DDGS; however, the large variability in the response of tilapia to feeding DDGS-based diets may indicate issues of consistency and quality of DDGS from different sources. Moreover, amino acid supplementation may be one way to improve the resulting performance of DDGS-based diets.

TABLE 5

Summary of studies evaluating the effects of feeding distillers grain products on growth performance, feed utilization and flesh composition in different fish species

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine[1] (%)	Optimum[2] (%)	Flesh composition	Reference[3]
Nile tilapia *Oreochromis niloticus*	34.9–67.7	0–27.5	Maize and SBM	55	5	no	17.5	–	Schaeffer *et al*., 2010.
	6.7–11	0–40	Maize and SBM	42	5	no	20	–	Schaeffer, Brown and Rosentrater, 2009.
	2–23	0–55	Maize and SBM	70	10	0–0.4	28/55	–	Abo-state, Tahoun and Hammouda, 2009.
	6.7–68.6	0–60	Maize and SBM	84	8	0.9	up to 60	–	Shelby *et al*., 2008.
	9.4–60.5	0–40	Maize and SBM	70	8	0–0.4	20/40	Whole body protein decreased at 40%	Lim *et al*., 2007.
	2.7–68.5	0–30	FM and SBM	70	0–8	no	30	No effect	Coyle *et al*., 2004.*
	26–120	0–100.00	–	84	0	no	–	No effect	Tidwell *et al*., 2000.
	0.5–11.4	0–82	CGF and SBM	56	0	0.25–0.75	none	–	Wu, Rosati and Brown, 1997.*
	0.4–20.9	0–49	Maize	56	0	no	35	–	Wu, Rosati and Brown, 1996.*
	30–122.4	19–29	Maize and SBM	103	0–6	no	29	–	Wu *et al*., 1994..
Hybrid tilapia *O. aureus × niloticus*	1.5–6.1	0–40	FM and wheat	90	3	0.4	Up to 40	–	US grains Council, 2007a.
Red tilapia	190–907	0–15	Maize and rice bran	120	0	no	Up to 15	No effect	US grains Council, 2006.
Channel Catfish, *Ictalurus punctatus*	9.1–80.4	0–30	Maize, SBM, wheat midds	56	5	0.3	30	Fillet protein decreased	Li, Oberle and Lucas, 2011.
	12.6–156.7	0–30	Maize and SBM	63	0	0.3–0.39	10/30[4]	Fillet fat increased, Protein decreased	Li *et al*, 2010a.
	86–491	0–30	Maize, SBM, wheat middlings	150	0	0.1–0.2	Up to 30	No effect	Zhou *et al*., 2010a.
	1.2–8.7	0–30	Maize, SBM, wheat middlings	56	0	0.2	30	–	Zhou *et al*., 2010b.
	13.3–67.1	0–40	Maize and SBM	84	8	0.4	40	Whole body fat increased	Lim, Yildirim-Aksoy and Klesius, 2009.
	48–1227	0–40	SBM and wheat midds	330	1	0.80–0.28	30/40	Fillet fat increased	Robinson and Li, 2008.
	33–226	0–30	Maize and SBM	110	8	no	30	No effect	Webster *et al*., 1993.*
	12.4–54.5	0–35[5]	FM and maize	84	0	0–0.4	35/35	–	Webster *et al*., 1992.*
	10–79.3	0–70	Maize and SBM	84	10	0–0.4	35/70	Whole body protein decreased and fat increased	Webster, Tidwell, and Yancey, 1991.*

TABLE 5 (Cont'd)

Species	Fish weight (initial – final; g)	DDGS (%)	Ingredient(s) replaced	Trial duration (days)	Fishmeal (%)	Lysine[1] (%)	Optimum[2] (%)	Flesh composition	Reference[3]
Rainbow trout *Oncorhynchus mykiss*	36.8–186.5	0–4[6]	SBM	84	31–33	no	4	–	Thiessen, Campbell and Tyler, 2003.
	49.8–96.2	0–22.5	In combination with CGM, replaced FM and wheat flour	42	7.5–22.5	0–1.23	15/22.5	Whole body fat decreased at 22.5% without Lys but not when Lys was added	Cheng and Hardy, 2004a.
	21–158.4	0–30	In combination with CGM, replaced FM and wheat flour	84	0	no	30[7]	Whole body protein decreased and fat increased	Stone *et al*., 2005.
Yellow perch *Perca flavescens*	19.1–54.3	0–50	SBM and Celufil	126	24	no	40	No effect	Schaeffer, Brown and Rosentrater, I2011..
Milkfish *Chanos chanos*	17.8–93.2	0–40	SBM, FM and wheat	–	2	0.3	20	–	US grains Council, 2007a.
Common carp *Cyprinus carpio*	41–168	0–15	SBM and rice bran	120	5	no	Up to 15	No effect	US grains Council, 2007b.
Freshwater prawn *Macrobrachium rosenbergii*	0.5–41.4	0–40	Maize, SBM, FM	105	0–7.5	no	40	–	Tidwell *et al*., 1993.*
Pacific white shrimp, *Litopenaeus vannamei*	0.45–25	0–10	Sorghum and FM	63	0	no	Up to 10	–	Roy *et al*, 2009.
Red claw crayfish *Cherax quadricarinatus*	0.12–4.2	0–10	FM	56	0	no	Up to 10	–	de Yta *et al*., 2012.
	5.75–62.3	0–30	In combination with other plant proteins, DDGS replaced FM	97	0	no	Up to 30	Tail muscle protein increased	Thompson *et al*., 2006.
Sunshine Bass *Morone chrysops* × *M. saxatilis*	15–69.7	0–10	Maize, SBM, MBM	56	0	no	Up to 10	No effect	Webster *et al*., 1999.*

Notes: DDGS = distillers dried grains with solubles; SBM = soybean meal; FM = fishmeal; CGM = maize gluten meal; MBM = meat and bone meal. (1) Lysine needed to achieve the optimal performance. (2) Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. When two optimum concentrations are given, the highest value corresponds to optimum concentration when lysine was added. (3) * indicates DDGS from alcohol distilleries, not fuel-based DDGS. (4) 10% for distillers solubles or distillers solubles from maize endosperm; 30% for DDGS. (5) Included at fixed rate with varying SBM levels, both replacing fishmeal and maize. (6) Thin distillers solubles. (7) Pellets containing DDGS processed either by cold pelleting or extrusion were tested: 20% inclusion of DDGS with cold pelleting resulted in similar gain weight and lower feed efficiency as control, but the inclusion of DDGS at all levels resulted in inferior performances when the diets were extruded at 130 °C.

TABLE 6
Summary of studies evaluating further aspects of feeding DDGS in different fish species

Species	Key findings	Reference[c]
Nile tilapia *Oreochromis niloticus*	The addition of up to 150 mg/kg of phytase to a 28% DDGS diet increased weight gain and feed utilization at 75 mg/kg	Tahoun, Abo-State and Hammouda, 2009.
	Dietary DDGS, at levels of 0, 10, 20 and 40% in diets, had no effect on haematology, immune responses, or resistance of Nile tilapia to *S. iniae* infection.	Lim *et al.,* 2007.
	DDGS had no effect on immune function or disease resistance.	Shelby *et al.*, 2008.
Channel catfish *Ictalurus punctatus*	Fish fed 20–40% DDGS diets had increased total serum immunoglobulin, and those fed the 30% DDGS diet had significantly increased antibody titres 21 days following *E. ictaluri* challenge.	Lim, Yildirim-Aksoy and Klesius, 2009.
	Organoleptic evaluation of fillets indicated higher intensity of fat complex flavour for fish fed graded amounts of DDGS.	Webster *et al.,* 1993.
Rainbow trout *Oncorhynchus mykiss*	Fractionation of wheat DDGS using sieving increased digestibility of DM and energy nutrient content in rainbow trout.	Randall and Drew, 2010.
	Phytase supplementation in diets containing 15% DDGS improved digestibility of dry matter, fat and some minerals.	Cheng and Hardy, 2004b.
	Replacing 50% of fishmeal with SBM and 1.65 g MHA/kg in a diet containing 18.5% of DDGS improved weight gain, FCR and apparent retention of crude protein and phosphorus.	Cheng, Hardy and Blair, 2003.
Sunshine Bass *Morone chrysops* × *M. saxatilis*	Digestibility of dry matter and organic matter, but not protein and lipid, with DDGS diets were less than those with diets consisting of fish and SBM.	Thompson *et al.*, 2008.

Notes: MHA is a feed supplement which contains methionine; FCR = feed conversion ratio.

Channel catfish

In most channel catfish studies, DDGS was included in place of a combination of SBM and maize. These studies agreed that DDGS is highly acceptable for channel catfish at levels in excess of 30 percent. Also, supplementation with lysine or the presence of fishmeal, or a combination, further increased the potential for inclusion rate of DDGS up to 40 percent or even higher. Fillets from fish fed DDGS appeared to be relatively low in protein and high in fat content, reflecting the composition of DDGS.

Early studies in catfish reared in recirculating systems and floating cages (Webster, Tidwell and Yancey, 1991; Webster *et al.*, 1992, 1993) showed successful feeding of DDGS up to 35 percent, which could be increased to 70 percent in a diet that contained 10 percent fishmeal and supplemental lysine. Webster, Tidwell and Yancey (1991) demonstrated that a blend of DDGS and SBM could be used to replace all of the fishmeal in the diet of juvenile channel catfish. The efficacy of feeding high amounts of DDGS in pond or recirculating systems was confirmed in recent studies (Li *et al.*, 2010a; Li, Oberle and Lucas, 2011; Zhou *et al.,* 2010a, b; Lim, Yildirim-Aksoy and Klesius, 2009; Robinson and Li, 2008). From these studies it can be concluded that feeding DDGS at levels up to 35 percent with supplemental lysine is feasible in an all-plant-protein diet.

New fractionation techniques being used in the ethanol industry offer the aquafeed industry new opportunities as well as challenges. Novel co-products often contain high crude protein concentration, which makes them more suitable for aquafeeds. Li *et al.* (2010a) showed that feeding HPDDG and distillers solubles at 20 and 10 percent, respectively, as part of an all-plant-protein diet resulted in improved weight gain and feed efficiency.

Rainbow trout

It is thought that DDGS has limited nutritional value for salmonids because of its high content of non-nutritive components, such as non-starch polysaccharides (NSP) and pigments. Conversely, the few available studies (Stone *et al.,* 2005; Cheng and Hardy, 2004a) showed some success in feeding DDGS to rainbow trout. These studies have demonstrated that DDGS can partially replace fishmeal when fed with maize gluten meal (CGM) and supplemental lysine. More specifically, Stone *et al.* (2005) evaluated the effects of feeding DDGS (0–30 percent) and pellet processing method on growth and feed efficiency of rainbow trout. They found that when cold pelleting was used, weight gain was maintained up to 30 percent DDGS, but feed efficiency was depressed at all DDGS inclusion levels. In contrast, feeding DDGS resulted in inferior performances when the diets were extruded at 130 °C.

In another study, Cheng and Hardy (2004a) reported that 50 percent of the fishmeal could be replaced by feeding 15 percent DDGS with appropriate amounts of CGM. The inclusion rate was increased to 30 percent when the diets were supplemented with lysine.

On a different tack, Thiessen, Campbell and Tyler (2003) investigated the use of thin distillers solubles as a palatability enhancer in rainbow trout fed different proteins. The inclusion of 4 percent thin distillers solubles did not promote any additional appetite or growth of rainbow trout.

Other species

The value of DDGS in other species cannot yet be firmly established, since for most species, only one study can be found in the literature. Furthermore, in most cases,

the use of DDGS was restricted to relatively low levels. In yellow perch, Schaeffer, Brown and Rosentrater (2011) reported that the inclusion of DDGS up 40 percent to partially replace SBM resulted in maximum growth and feed efficiency. Such performances were probably possible because of the high inclusion of fishmeal in those diets (24 percent). In sunshine bass, replacing maize meal with DDGS at 10 percent resulted in similar weight gain and feed efficiency (Webster *et al.,* 1999).

In its efforts to expand the use of DDGS in Asian aquaculture, the United States Grains Council led multiple experiments in major fish species grown in Asia, such as tilapia, common carp and milkfish. Farm studies have demonstrated that DDGS can be effectively fed at up to 15 and 20 percent, respectively, for common carp and for milkfish (U.S. Grains Council, 2006, 2007a, b).

Studies in crustacean species suggest that DDGS can be a viable source of protein to partially replace common protein sources such as fishmeal and SBM. Tidwell *et al.* (1993) evaluated the inclusion of 40 percent DDGS with SBM to partially or completely replace fishmeal for prawns grown in ponds. The fishmeal was reduced from 15 to 0 percent of the diet. Prawns fed DDGS diets had similar survival, yield per ha and feed conversion ratio compared with prawns fed the control diet with 15 percent fishmeal.

In Pacific white shrimp, Roy *et al.* (2009) reported similar weight gain, but lower biomass due to a tendency for higher mortalities for shrimps fed 10 percent DDGS compared with other feed alternatives, including poultry co-products, fishmeal and pea meal.

Two studies are available for red claw crayfish. Thompson *et al.* (2006) evaluated two levels of DDGS (18.3 and 30 percent) in diets with or without fishmeal. As DDGS increased in the diet, SBM increased and both sorghum and fishmeal decreased. They reported that feeding DDGS with SBM was equally effective in maintaining growth and feed efficiency as diets containing fishmeal. In another study, de Yta *et al.* (2012) fed the same dietary treatments previously evaluated for white Pacific shrimp by Roy *et al.* (2009) and found that similar to white shrimp, red crayfish can be fed a diet that contains 10 percent DDGS.

Flesh nutritional characteristics

Available data (Tables 5 and 6) suggest that feeding DDGS to various fish species is associated with alterations primarily in protein and fat contents of the final fish flesh. Feeding DDGS appears to increase fat content and decrease protein content, and these changes occurred either disjointedly or simultaneously (see, for example, Li, Oberle and Lucas, 2011; Li *et al*., 2010a; Lim, Yildirim-Aksoy and Klesius, 2009.; Robinson and Li, 2008; Lim *et al.,* 2007; Stone *et al.,* 2005; Cheng and Hardy, 2004a; Wu *et al*., 1996; Webster, Tidwell and Yancey, 1991). In other instances, the flesh composition remained unchanged (Schaeffer, Brown and Rosentrater, 2011; Tidwell *et al.*, 2000; Webster *et al.,* 1993, 1999). High fat concentrations and unbalanced amino acid profiles in the DDGS have been reflected in the flesh of fish fed DDGS-based diets. Thus, to mitigate some of these effects, dietary adjustments are necessary when feeding DDGS to various fish.

Organoleptic evaluations of fish fed DDGS-based diets are limited. Wu *et al.* (1996) found no differences in flavour characteristics of cooked tilapia, except a decline in "sweet" intensity for fish receiving 29 percent DDGS in their diets. Similarly, Webster *et al*. (1993) concluded that feeding DDGS to channel catfish had no adverse taste effects.

One of the concerns of feeding high amounts of DDGS is the negative impact on fillet pigmentation. DDGS contains, on average, 37 ppm of the xanthophyl pigments lutein and zeaxanthin, and this concentration varies among sources due to differences in heat treatment during drying of distillers grain (Salim, Kruk and Lee, 2010). Yellow pigments in DDGS will transfer to muscle tissues, which may render the final product less marketable. Li, Oberle and Lucas (2011) demonstrated that these pigments can be completely removed following the extraction of DDGS with ethanol. Feeding such products resulted in fillets with similar colouration to those from fish fed a SBM-based diet.

Digestibility

Digestibility coefficients are important for estimating the energy value and optimizing the use of ingredients in feeds. These are particularly important for co-products of the fuel ethanol industry, given the large variability associated with these materials. Evaluation of nutrient digestibility from DDGS in monogastric animals (swine) showed that digestibility of dry matter, energy, protein and lysine are low compared with traditional feedstuffs such as maize and SBM (Shurson, 2006; Stein *et al.*, 2006).

Information on DDGS nutrient digestibility in fish is rare. Thompson *et al.* (2008) compared nutrient digestibility from different feeds in sunshine bass. They reported very low digestibility coefficients for dry matter and organic matter (<15 percent) compared with SBM and fishmeal, which exceeded 40 and 60 percent, respectively. Protein and lipid digestibility for DDGS were 65 and 69 percent, respectively. Protein digestibility exceeded 84 percent for fishmeal and SBM, while lipid digestibility averaged 92 percent for fishmeal and 57 percent for SBM. Low digestibility of nutrients can increase faecal output and may deteriorate fish culture water quality. To date, there are no reports on nutrient digestibility from DDGS in omnivorous fish.

Fractionation techniques recently employed in ethanol production have resulted in new co-products, generally with higher protein, lower fibre and lower fat contents

compared with traditional DDGS. Thus such products may have improved digestibility and nutritive values compared with conventional DDGS. For example, Randall and Drew (2010) evaluated the digestibility of nutrients from different fractions obtained by sieving of wheat DDGS. Sieving increased crude protein and decreased fibre concentrations. In addition, sieving improved digestibility of dry matter and gross energy, whereas digestibility of ether extract and protein were unaffected and high, exceeding 90 and 100 percent, respectively. The use of enzymes such as phytase can improve the nutritive value of the feed. Studies in poultry showed that in addition to improvement in phosphorus availability, supplementation with phytase improved protein and amino acid digestibility and availability in poultry diets (Rutherford *et al.*, 2004). Similarly, Cheng and Hardy (2004b) evaluated different doses of microbial phytase in the diets of rainbow trout that contained 30 percent DDGS. Improvement in dry matter, ether extract, Ca, Mg, phytate-P, total P, Mn, Cu and Zn were observed when adding phytase as low as 300 FTU (phytase units)/kg of diet. Protein digestibility from these diets was high and similar to the reference diet, averaging 90 percent. Recently, Tahoun, Abo-State and Hammouda (2009) showed that feed utilization was improved by the addition of 75 mg/kg of phytase to a 28 percent DDGS diet fed to Nile tilapia.

Immune function

DDGS contains approximately 3.9 percent yeast cell biomass (Ingledew, 1999). Yeast components such as beta-glucans, mannan-oligosaccharides, chitin, proteins, nucleotides, vitamins and trace minerals are important in modulating immune function. The potential of DDGS to stimulate immune function in fish is unclear. For example, feeding DDGS had no effect on immune function or resistance to bacterial infection in Nile tilapia (Shelby *et al.,* 2008; Lim *et al.,* 2007). In channel catfish subjected to *Edwardsiella ictaluri* challenge, feeding DDGS increased immunoglobulin, antibody titre and days to first mortality. Mortality was decreased, suggesting improved resistance to pathogen infection (Lim, Yildirim-Aksoy and Klesius, 2009). The authors suggested further investigation of the immunostimulatory effect of DDGS and the identification of potential active components that may be present in DDGS.

DISTILLERS GRAIN: ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Overall, it appears that DDGS can be an effective source of energy and protein for fish. DDGS is not, however, recommended to be a direct, complete substitute for fishmeal or SBM. It is most effective when it replaces a combination of SBM and maize. Furthermore, the inclusion of DDGS is facilitated by the use of fishmeal and synthetic amino acids (primarily lysine) to improve the overall supply of amino acids to fish.

The use of DDGS in aquafeeds does present some challenges and limitations. Quality variation remains a major shortfall to using DDGS. Fish require high quality and dependable sources of nutrients to achieve high performance levels. DDGS can fill such requirements, provided the source is known, of consistent quality, and access to nutrient composition is available on a regular basis to nutritionists. DDGS also has some nutritional limitations when it is fed to fish. The high fibre content of DDGS, coupled with low digestibility of some nutrients, may limit its use in some fish species where nutrient-dense feeds are required. In addition, in recirculating aquaculture systems, DDGS use may also affect water quality because of potential increased faecal output.

When technical aspects are considered, handling of DDGS can pose some logistical problems because of the inherent physical properties of this granular bulk solid. Low bulk density and particle stickiness, which can lead to flowability problems, are the major challenges to the use of DDGS in animal feeds. These issues create transportation inefficiencies at all feed manufacturing levels, from transportation to feeding systems at the farm. These issues can be managed by implementing approaches such as manipulation of particle size and moisture content, or by the addition of flow agents. In aquaculture, feed is commonly manufactured using extrusion processing. Since DDGS contains high fibre and fat contents, coupled with a low starch level, extrusion of feeds containing DDGS can pose some difficulties. Once gelatinized (due to high processing temperatures), starch acts as a binder. Our research has shown that these limitations can be surmounted through the understanding of different interactions between process parameters and feed material. We have evaluated the extrusion of aquafeeds based on DDGS under a variety of processing conditions. Generally, as DDGS increases in the blend, decreases in pellet durability, expansion ratio, mass flow rate (throughput) and an increase in unit density and sinking velocity (i.e. no floatability) are observed. See, for example, Ayadi *et al.* (2011); Chevanan, Rosentrater and Muthukumarappan (2010); and Kannadhason *et al.,* (2010). Overall, it can be concluded that optimum pellets in terms of bulk density, durability and water stability can be obtained when DDGS is included at about 20 percent of the diet, which coincide with the optimum feeding level for most fish species. Improvements in pellet quality at high levels of DDGS are possible by the addition of different starches and binders.

Other challenges include mycotoxins, antibiotics and pigmentation. DDGS may contain mycotoxins if the parent grain is contaminated, although this risk is

minimal in the Midwest United States, where most ethanol production plants are located. In addition, more ethanol plants have implemented stricter standards for grain selection. Again, knowing the source of DDGS and test results, especially during growth seasons with most risk of mycotoxin development, are important for safe utilization of DDGS. Recently, concerns about antimicrobial residues in DDGS have surfaced. Antimicrobials, such as penicillin, virginiamycin, erythromycin and others, are commonly added to the fermentors to control bacterial infections with the goal to optimize ethanol production by yeasts. These antibiotics can end up in the DDGS; however, it is believed that they will be completely deactivated under the extreme temperatures and pH conditions applied during ethanol production. In addition, heat treatments associated with extrusion cooking could further inhibit such substances. Thus, the issue of antimicrobials in DDGS, although serious, is more speculation rather than a reality in animal feeds. Pigmentation of tissues is also of concern when feeding DDGS, especially to salmonids. Feeding DDGS to salmonids is believed to alter flesh pigmentation from the typical pink colour to a less desirable yellowish colour, but to date there are no published studies evaluating the effect of DDGS on the pigmentation of fish tissues.

Finally, as the ethanol industry increases the efficiency of producing ethanol, different distillers co-products will become available, creating more challenges and opportunities for the aquafeeds industry. These products are expected to be more nutrient dense, as the fibre fraction can be further fermented and the fat extracted, leading to products composed mainly of protein and ash. Such products may be more compatible with fish requirements, but will need research to characterize them and assess their nutritional value and efficacy for different fish species.

PROPERTIES OF CRUDE GLYCERINE

The principal co-product of biodiesel production is crude glycerin. Common feedstocks used in the biodiesel industry include pure or waste vegetable oils, or a mixture, and rendered animal fats. Refining of crude glycerin is often limited to large scale biodiesel producers, which make high purity glycerol for applications in the food, pharmaceutical and cosmetic industries. Small-scale producers generally limit the purification process to the removal of excess alcohol to yield a low value co-product with limited uses (Thompson and He, 2006).

Physical and chemical properties

Crude glycerine contains impurities, including spent catalysts, residual methanol, methyl esters, oils and fats, soaps, free fatty acids and various minerals such as Ca, Na, Cl, K, Mg, P and S (Thompson and He, 2006; Dasari, 2007). Some of the physiochemical properties of crude glycerine are presented in Table 7. Considerable variation exists among crude glycerine sources, largely because of differences in the biodiesel production processes and the parent feedstock used. Mader (2011) showed that crude glycerine derived from animal fats contained less glycerol and more impurities than that derived from vegetable oil feedstocks. Common glycerol content is between 75 and 85 percent; however, glycerol content as low as 38.4 and as high as 96.5 percent of the total crude glycerine can be found on the market (Hansen *et al.*, 2009). Other major constituents are moisture, fat and a variety of minerals. Residual methanol is usually found at low concentration (<100 ppm); however, samples containing higher concentration (>15 percent) can be found, creating some health concerns when crude glycerine is fed to livestock (Hansen *et al.,* 2009). The USDA Food and Drug Administration (FDA) limits methanol content

TABLE 7
Physiochemical properties of crude glycerine

Item	n	Average	Min.	Max.	Reference
Pure glycerol (%)	39	78.58	38.4	96.5	1, 2, 3, 4, 5, 6, 7, 8, 9
Moisture (%)	27	8.20	0	24.37	4, 5, 6, 7, 8, 9, 10
Protein (%)	10	0.26	0.05	0.82	1, 3, 4, 5, 9
Fat (%)	11	5.54	0.12	15	1, 3, 4, 5, 10
Ash (%)	31	4.15	0	29.4	1, 3, 4, 5, 6, 7, 9
Na (%)	2	1.23	1.2	1.26	5, 9
Cl (%)	2	1.78	1.7	1.86	5, 9
GE (KJ/kg)	9	18340	15119	20510	1, 5, 10
pH	25	6.20	2	10.8	4, 5, 6, 7, 10
Methanol (%)	31	1.72	0.0009	14.99	2, 3, 4, 5, 6, 7, 8, 10
Density (g/cm^3)	11	1.20	1.07	1.26	6
Viscosity (4.45 °C, cSt)	6	60.00	82	38	10
Viscosity (40 °C, cSt)	2	8.60	8.8	8.46	1
Colour (c.u.)	2	7.25	3.5	11	10

Key to references: 1. Thompson and He, 2006; 2. Dasari, 2007; 3. Groesbeck *et al.,* 2008; 4. Lammers *et al.*, 2008a; 5. Lammers *et al.*, 2008b; 6. Hansen *et al.*, 2009; 7. Kerr *et al.,* 2009; 8. Mach, Bach and Devant, 2009; 9. Gunn *et al.*, 2010; 10. Mader, 2011.

TABLE 8
Summary of studies evaluating the effects of feeding glycerol to different fish species

Fish species	Glycerol inclusion	Ingredient replaced	Optimum (%)[a]	Flesh composition	Reference
Channel catfish (*Ictalurus punctatus*)	0-20	Maize	10	Fillet fat decreased	Li *et al*., 2010b[(1)]
Rainbow trout (*Oncorhynchus mykiss*)	0-12	Wheat middlings	–	No difference	Menton, Slinger and Hilton, 1986[(2)]

Notes: Optimum determined based on growth gain and feed efficiency as similar or superior to a Control diet. (1) Glycerol from biodiesel production. (2) Free glycerol included in low energy diets compared with a diet with similar energy density.

to 0.015 percent (150 ppm) in the final animal feed (FDA, 2006). Because the boiling point of methanol is 64.4 °C (Lide, 2001), it is believed that extrusion processing, commonly used in preparing fish feed, could eliminate any residual methanol found in crude glycerine. The colour of crude glycerine can range from clear to dark, reflecting pigments and compounds found in the parent feedstock.

Feeding value of crude glycerine to fish

There has apparently been only one published study (Table 8) that has evaluated the use of crude glycerine from the biodiesel industry in fish (Li *et al.*, 2010b). This study used crude glycerine as a source of energy to replace maize meal in the diet of channel catfish. They determined that a level of 10 percent was optimal for weight gain and feed efficiency; fillet fat content decreased at levels in excess of 5 percent. Pure glycerol was evaluated in another study in rainbow trout (Menton, Slinger and Hilton, 1986). Replacing wheat middlings by free glycerol up to 12 percent of the diet resulted in comparable weight gain, feed efficiency and carcass composition as fish fed a diet with similar energy density. The authors also found that glycerol can be an effective precursor for gluconeogenesis, but not for lipogenesis; however, rainbow trout cannot efficiently utilize glucose as a source of energy.

CRUDE GLYCERINE ISSUES, CHALLENGES, KNOWLEDGE GAPS AND RESEARCH NEEDS

Studies in other monogastric species suggest that crude glycerin can be a viable energy source. However, considering the current level of research in fish nutrition, which is essentially non-existent, an optimum level can not be recommended at this time. More studies are required to determine the efficacy of crude glycerol in major species such as tilapia, channel catfish, rainbow trout and yellow perch. As with other co-products, variability is an issue that hinders the use of crude glycerin in aquafeeds. Residual methanol is a potential safety hazard that needs to be addressed as well. Considering the physical characteristics of crude glycerin, other issues that should be evaluated include extrusion processing behaviour, handling and storage characteristics, potential corrosive effects, and the effect of feeding glycerin on flesh quality and health of fish.

CONCLUSIONS

DDGS and glycerine, co-products from the fuel ethanol and biodiesel industries, respectively, appear to be viable alternative feed ingredients for aquafeeds. DDGS is best used to replace a portion of SBM and maize in the diet. Because of variability issues and inherent nutritional limitations of DDGS, an inclusion level of up to 20 percent appears to be safe for most omnivorous fish species, whereas 10–15 percent is recommended for carnivorous fish such as rainbow trout. Specifically, DDGS can effectively be included at concentrations of 20 to 40 percent for channel catfish, tilapia and yellow perch, but at lower concentrations (10–15 percent) for rainbow trout, bass and some crustacean species. Nonetheless, when economically viable, supplementation with lysine will allow for higher DDGS inclusion rates. It has also been shown that DDGS can be included at high concentrations (up to 40 to 60 percent) while maintaining feed quality in terms of water stability and pellet durability when DDGS is part of extruded aquafeeds. Interestingly, the optimal inclusion level of DDGS in aquafeeds for superior pellet quality appears to be around 20 percent, which coincides with optimal fish performance in most species. In some species, nutritional characteristics of the final products can be altered. Lower protein and higher fat contents are usually observed when feeding DDGS above optimal levels. The efficacy of crude glycerine in fish diets is less evident. Very limited information suggests that glycerine might be used as an energy source. However, comprehensive investigation is still needed to address the use of glycerol as a feed ingredient for major fish species. The effect of glycerol on feed processing, final product quality, metabolism and health of fish are some areas that needs further research before glycerine can be efficiently and safely used in aquafeeds. Biofuels will clearly continue to play a key role in the global energy portfolio over the coming years, and co-products such as DDGS, glycerine, as well as other new co-products yet to be developed, will continue to grow in quantity. Aquafeeds may be a viable opportunity for their utilization.

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Chapter 24

Cultivation of micro-algae for lipids and hydrocarbons, and utilization of spent biomass for livestock feed and for bio-active constituents

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ABSTRACT

The demand for energy is ever increasing, and concurrently the depletion of fossil fuels has been so rapid that it could lead to an energy crisis in the near future. At the same time, reducing the carbon footprint to mitigate global warming has been a subject of immediate attention. Production of energy through photosynthetic organisms such as micro-algae by harnessing solar energy might be a viable solution to some of these issues. In pursuit of renewable energy sources, efforts worldwide focus on identifying those organisms that can accumulate high quantities of biomass and produce molecules that can be converted to combustible materials. Economically viable processes for large-scale cultivation and downstream processing of biofuel precursors, such as lipids and hydrocarbons, have been a challenge, requiring adoption of technologies needing reduced inputs of energy and chemicals. Prudent energy audits to assess the viability of bio-energy processes are a necessity. The utilization of micro-algae for bio-energy production would be viable only when the whole process has a net energy gain, with complete utilization of algal biomass for biofuel and the co-products thereof used to produce food, feed and chemicals. The spent algal biomass—which is rich in proteins, carbohydrates, minerals and bio-active compounds—is ideal for feed applications. The paper outlines biorefinery approaches to integrated utilization of algal biomass for bio-energy, with co-production of valuable metabolites and nutrients as feed, with full utilization of all the fractions for economic viability of the process. These aspects are dealt with in detail in the various sections to provide a comprehensive overview of micro-algal technology for biofuel programmes *vis-à-vis* feed applications.

INTRODUCTION

Phytoplankton are the most important and major biomass producers in global aquatic ecosystems. Algae are the primary producers, generating approximately 52 000 000 000 tonne of organic carbon per year—almost 50 percent of the total organic carbon produced annually (Field *et al.,* 1998)—through photosynthesis utilizing solar energy and converting CO_2 and other simple inorganic compounds to myriad molecules (Chisti, 2007). These organisms populate the top layers of the oceans and freshwater habitats where they receive sufficient solar radiation for photosynthesis (Hader *et al.,* 1998). Micro-algae are unicellular to filamentous in form, whereas macro-algae or seaweeds are plant-like organisms. Micro-algae lack roots, vascular systems, leaves and stems, and are autotrophic in nature and photosynthetic. They are generally eukaryotic organisms, although cyanobacteria—a prokaryotic assemblage—are included under algae due to their similar photosynthetic and reproductive properties (Greenwell *et al.,* 2009). The thalli of algae display a wide range of organization, ranging from single cells (*Chlorella* spp.), through motile (*Chlamydomonas* spp., *Dunaliella* spp.), colonial (*Volvox* spp., *Botryococcus* spp.), filamentous (*Spirulina* spp., *Spirogyra* spp.) and plant like (*Chara* spp.) to giant seaweeds (*Postelsia* spp., *Fucus* spp.). (Fritsch, 1935).

Depleting fossil fuel reserves, with associated escalation in petroleum prices, has engendered huge demand for development of technology for renewable fuels from photosynthetic organisms with a smaller carbon foot print and an overall positive energy balance. In this context, micro-algae could be a possible solution. Their ubiquitous distribution and ecological adaptations give them certain advantage over other groups. The ability to cultivate algae under varied conditions, including using non-potable wastewater and operating in marginal areas, coupled with their photosynthetic efficiency and higher surface area

MAIN MESSAGES

- **Depletion of fossil fuels is leading to an energy crisis and highlighting the need for development of renewable energy sources**
- **Micro-algae, being photosynthetic with a high ability to produce hydrocarbons and lipids, offer multiple advantages as a source of bio-energy through harnessing solar energy, with the additional advantages of CO_2 sequestration and providing an eco-friendly alternative to meet energy requirements.**
- **In addition to providing bio-energy molecules, algae are good source of nutrients and health promoting substances, as well as valuable metabolites that are unique and of high commercial value.**
- **The algal biomass after extraction of bio-combustible materials can be used as feed for fish, poultry and animals.**
- **Development of eco-friendly and easily adaptable economic process for mass cultivation of biomass leading to net energy gain and their utilization towards various applications is discussed.**
- **A biorefinery approach to utilizing various fractions of algal biomass has been proposed to make processes more economical, with a bias towards energy production, biochemicals and feed in an eco-friendly manner utilizing algal biodiversity and harnessing solar energy.**

productivity than higher plants, make them a potential and economically viable resource for renewable fuel production (Gouveia and Oliveira, 2009). Large-scale algal cultivation has been developed for various purposes and the biomass can be harvested frequently as algae can have very short doubling times, ranging from 4 to several days. Further, some of the micro-algae accumulate lipids up to 50 percent of the dry weight in certain growth conditions. The lipid accumulation in micro-algae is influenced by light intensity, culture pH, availability of nutrients, dissolved oxygen concentrations and several other environmental factors. Apart from lipids, some micro-algae, such as *Botryococcus,* accumulate long-chain hydrocarbons that have properties similar to petroleum hydrocarbons (Metzger and Largeau, 2005; Dayananda *et al.,* 2007b). Also, certain micro-algae occur in extreme environmental conditions, like brackish and high saline waters, acidic or alkaline lakes, and at chilling temperatures. These extremophilic micro-algae can be exploited for the production of novel compounds of commercial and functional importance.

The preliminary step, and an important part of sustainable micro-algal technology, is extensive germplasm collection and biodiversity screening for the production of lipids and hydrocarbons. Furthermore certain parameters, like biomass productivity, lipid or hydro-carbon content and daily yield, and possibility of co-product generation must be considered for viable micro-algal technology applications (Subramaniam *et al.,* 2010). Micro-algae chosen after initial studies under controlled conditions must be evaluated for their performance in outdoor and scaled-up conditions. Large-scale cultivation methodologies involve optimization of media for high biomass and lipid yields, and adjusting physical parameters like light requirements, culture mixing, supply of CO_2, etc. (Pulz, 2001). Use of simple and inexpensive nutrient sources and re-usability of media should be considered in assessing potential for sustainable mass cultivation. Apart from mass cultivation, development of simple downstream processing must be critically evaluated, including harvesting procedures, processing the biomass for lipid or hydrocarbon extraction, and converting crude extracts to combustible fuels. Existing technology using energy-intensive methods, such as centrifugation and ultrafiltration for harvesting, and extraction methods like oil expelling or French pressing, has proven unviable for renewable energy production (Brennan and Owende, 2010). Nevertheless, it is increasingly evident that algae can be exploited for nutritionally and nutraceutically important metabolites for food and feed applications owing to their content of vitamins, proteins, pigments, fatty acids, sterols and polysaccharides. The potential of micro-algae as a source of antiviral, antitumour, antibacterial, anti-HIV agents and as food additives have also been well established (Cardozo *et al.,* 2007).

Production of bio-energy through photosynthetic systems is gaining strength and has a great future since it is renewable and eco-friendly. However, for any type of bio-energy production, systems need to be developed that operate in an economical manner in terms of total energy gain per unit area. A careful analysis of the present day technologies for utilizing algal biomass for energy production indicate that they do not take into account the total energy audit. One needs to look at the whole process from the point of view of net energy gain, addressing cultivation to utilization of the biomass for the production of lipids, hydrocarbons and other useful constituents. In addition to the target molecules for fuel generation, co-products could be of utility for food and feed, as well as a source of chemicals of importance to humankind. Utilization of sea water would address the water requirements for cultivation of biomass, which otherwise would compete with agriculture, potentially leading to water conflicts. Therefore, utilization

of marine forms and sea water has been advocated as a requirement for utilizing algal biotechnology for biofuels. Utilization of wastewater would also go a long way in not only producing algal biomass for energy but also providing wastewater treatment and bioremediation. Such technologies have the potential to be sustainable and eco-friendly, and could significantly help mitigation of pollution.

This review focuses on identification of suitable organisms for specific ecosystems, with a focus on cultivation in an economically viable manner and utilizing biomass in an eco-friendly approach, for the production of fuel, feed and chemicals. These aspects are dealt with highlighting developments in the respective areas and projecting trends in application technology under five headings: (1) algal biodiversity for the production of lipids and hydrocarbons; (2) large-scale cultivation of micro-algae; (3) downstream processing and conversion to biofuels; (4) use of micro-algae for food, feed and bio-actives; and (5) techno-economic analysis and bio-refinery concepts.

ALGAL BIODIVERSITY FOR THE PRODUCTION OF LIPIDS AND HYDROCARBONS

The ubiquitous occurrence of algae in marine, freshwater and terrestrial habitats with broad chemical diversity is the basis for their industrial and biotechnological applications (Figure 1). Several groups have been working on development of feasible systems for the production of lipids and other precursor molecules from micro-algae (Sheehan *et al.,* 1998; Illman, Scragg and Shales, 2000; Dayananda *et al.,* 2007a; Rodolfi *et al.,* 2009). The United States Department of Energy has initially invested more than US$ 20 million in an Aquatic Species Program to develop biofuels from micro-algae, with the project, mainly focusing on identification of oleaginous micro-algae and evaluation of different cultivation methods for the production of renewable fuels (Sheehan *et al.,* 1998). The lipid content of micro-algae varies between 5 and 80 percent on a dry weight basis, depending on the species, strain, growth phase and other environmental factors (Spolaore *et al*., 2006; Harwood and Guschina, 2009). Micro-algal distribution is influenced by various biotic and abiotic environmental factors, and so bio-prospecting for hyper-lipid-producing strains must recognize local climatic conditions. Some of the parameters important in screening biodiversity are fast growth and tolerance to environmental fluctuations (Mutanda *et al.,* 2011). In natural habitats, the four most important groups of algae in abundance are Green algae (Chlorophyceae), Diatoms (Bacillariophyceae), Blue-green algae (Cyanophyceae) and Golden algae (Chrysophyceae). Green algae and Diatoms are two important classes of micro-algae generally exhibiting high oil productivities on a culture volume basis (Table 1). Extensive review of the biodiversity of these important classes is beyond the scope of this chapter, but many reviews are available, such as Becker (2004) and Singh, Bhushan and Banerjee (2005).

Some of the extreme conditions under which algae thrive are highly acidic or alkaline, hypersaline, with high or freezing temperatures, in high radiation zones or in polluted environments. These extremophiles tolerate such conditions with the help of endogenously produced compounds called extremolytes. Some micro-algal forms known to exist in extreme conditions are *Dunaliella salina* in hypersaline waters (2–5 M NaCl) and *Spirulina* spp. in highly alkaline conditions (optimum around pH 10.5). *Cyanidium caldarium* and *Dunaliella acidophila* are acidophiles with an optimum pH of 2 to 3. Certain psychrotolerant micro-algae growing in polar glaciers have unique lipid compositions with commercial applications (Rajkumar *et al.*, 2010).

FIGURE 1
Schematic diagram showing various applications of micro-algae

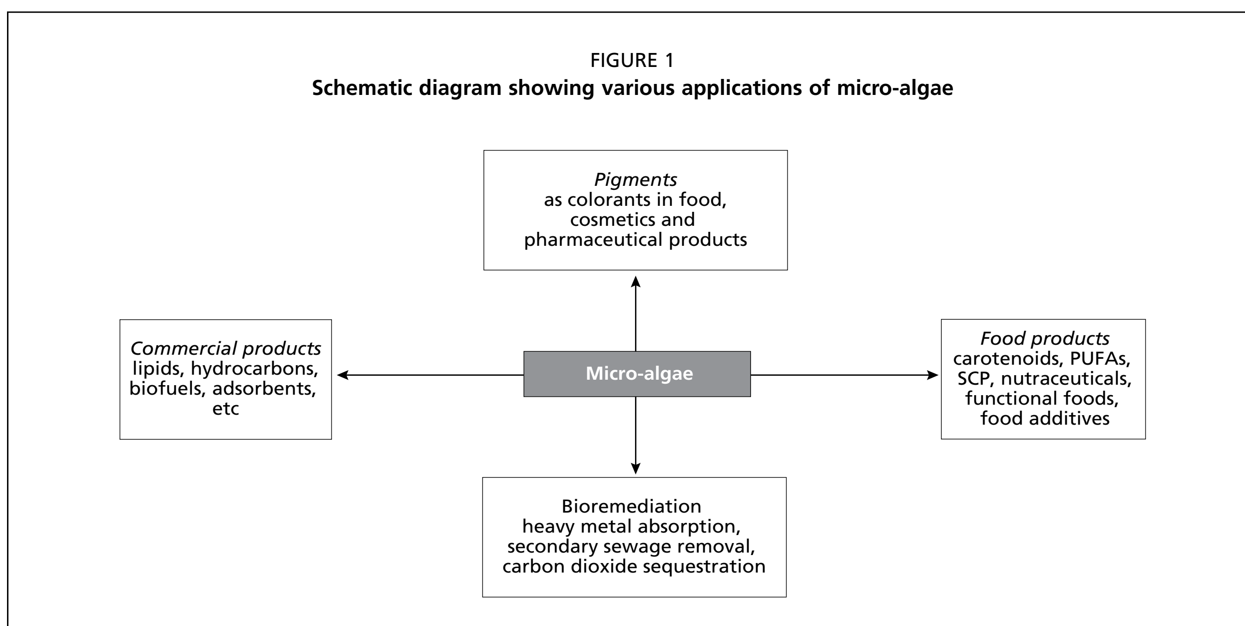


TABLE 1
Hyper-lipid-producing micro-algae

Micro-algae	Class	Lipid content (% dry weight)	Habitat
Botryococcus braunii	Chlorophyceae	25–75	Freshwater
Chlorella vulgaris	Chlorophyceae	30–35	Freshwater
Chlorococcum	Chlorophyceae	19.3	Freshwater
Scenedesmus spp.	Chlorophyceae	21.1	Freshwater
Neochloris oleoabundans	Chlorophyceae	35–54	Freshwater
Crypthecodinium cohnii	Dinoflagellate	20	Marine
Tetraselmis suecia	Chlorophyceae	36.4	Marine
Dunaliella primolecta	Chlorophyceae	23	Marine
Isochrysis spp.	Haptophytes	25–33	Marine
Skeletonema costatum	Bacillariophyceae	17.4	Marine
Chaetoceros muelleri	Bacillariophyceae	21.8	Marine
Chaetoceros calcitrans	Bacillariophyceae	17.6	Marine
Nitzschia spp.	Bacillariophyceae	45–47	Marine
Nannochloropsis spp.	Eumastigophytes	31–68	Marine
Schizochytrium spp.	Thraustochytriidae	50–77	Marine
Monodus subterraneus	Eumastigophytes	30.4	Marine
Thalsssiosira pseudonana	Bacillariophyceae	17.4	Marine

Sources: Chisti, 2007; Rodolfi *et al.*, 2009; Illman, Scragg and Shales, 2000; Mutanda *et al.*, 2011.

Dunaliella bardawil has been reported to survive high salt and high radiation levels by accumulating β-carotene (Ben-Amotz and Avron, 1990). Certain cyanobacteria, such as *Chroococcidiopsis* spp., exhibit resistance against desiccation and also high irradiation, which is attributed to the accumulation of radio-responsive pigments and efficient DNA repair mechanisms (Billi *et al.*, 2000). Extremophilic micro-algae offer certain advantages for large-scale cultivation as their growth requirements and conditions are unsuitable for other competing organisms and fight herbivore predation. In fact, the most commercially exploited micro-algae in outdoor cultivation are extremophiles such as *Spirulina* (tolerates pH 10–11) and *Dunaliella* (withstands salt concentrations several times that of sea water). Detailed studies on the use of algal extremophiles for sustainable energy production could help overcome some of the major hurdles in the mass cultivation of micro-algae, as discussed in later sections.

GREEN ALGAL LIPIDS AND HYDROCARBONS

Green algae have been extensively studied for their ability to accumulate lipids. Spoehr and Milner (1949) showed that *Chlorella pyrenoidosa* can accumulate lipid up to 85 percent in its biomass. Wood (1974) reviewed the lipid contents and fatty acid profiles of all the classes of green algae under different culture conditions. *Chlorella vulgaris, Chlorella sorokiana, Scenedesmus* sp.*, Chlorococcum* sp. and *Tetraselmis suecia* have been reported as potential micro-algae for lipid production (Illman, Scragg and Shales, 2000; Chisti, 2007; Rodolfi *et al.,* 2009; Huerlimann, De Nys and Heimann*.,* 2010). Green algae have wide occurrence, grow faster than other groups and grow on simple nutrient media. Major fatty acids present in green algae are palmitic acid (C16:0), oleic acid (C18:1) and alpha linolenic acid (ALA) (C18:3). The saturated fatty acids occur more in green algae, making them good sources for biodiesel production.

Botryococcus braunii, a colonial freshwater alga, has been extensively characterized for the production of hydrocarbons (Casadevall *et al.,* 1985; Dayananda *et al.,* 2007b). They accumulate long-chain hydrocarbons of >C30 chain length and are categorized into three races. Race A produces odd-numbered fatty acid-derived n-alkadiene type hydrocarbons ranging from C23 to C33. Race B produces unsaturated hydrocarbons called botryococcenes and methyl-branched squalenes. Race L produces tetra-terpenoid hydrocarbons known as lycopadiene (Metzger and Largeau 2005). Hydrocarbon production depends strongly upon the culture conditions, and it ranges from a minimum of 2 percent to a maximum of 86 percent (Dayananda *et al.,* 2006). Apart from hydrocarbons, *B. braunii* is of interest for the production of exopolysaccharides (Bailliez, Largeau and Casadevall, 1985). Our studies have demonstrated anti-oxidant properties of *Botryococcus* biomass through production of lutein (Rao *et al.,* 2006). Hydrocarbon obtained from *B. braunii* when hydrocracked produced a distillate with good fuel properties, comprising 67 percent gasoline fraction, 15 percent aviation fuel, 15 percent diesel fraction and remaining residual oil (Banerjee, Sharma and Chisti, 2002; Dayananda *et al.,* 2006). These fuels are reported to be free from N and S oxides (NO_X and SO_X) after combustion.

Dunaliella spp. (*D. tertiolecta, D. salina* and *D. bardawil*) are marine chlorophytes and have been the most studied strain for industrial exploitation, such as for β-carotene production. They accumulate β-carotene up to 14 percent on a dry weight basis (Ben-Amotz, 1995) and glycerol for

osmoprotection in hypersaline to brackish water environments. *D. tertiolecta* can be exploited for lipid production as it accumulates neutral lipids up to 50 percent under stress conditions, and has high CO_2 absorption capacity and fast growth (Oilgae, 2010). The β-carotene obtained from *Dunaliella* spp. can be used as natural food colorant and also in enhancing fish flesh colour and egg yolk colour (Becker 2004). Due to the rich content of β-carotene and lipid accumulation they can even be positioned as nutritional supplements. The carotenoid-rich fraction is composed of all-*trans* and the 9-*cis* isomer of β-carotene, which have high antioxidant activity and are known to prevent some forms of cancer. Carotenoids with their quenching action on reactive oxygen species have an intrinsic anti-inflammatory property, hence *Dunaliella* spp. can replace synthetic carotenoids (Murthy 2005).

DIATOMS AS SOURCES OF LIPIDS

Diatoms are a class of unicellular micro-algae belonging to Bacillariophyceae, and dominant phytoplankton in oceans, contributing up to 25 percent of global primary productivity (Ramachandra, Mahapatra and Karthick, 2009). The diatoms are a rich source of lipids, especially polyunsaturated fatty acids (Tan and Johns, 1996; Lebeau and Robert, 2003). The lipids are accumulated as oil droplets in marine diatoms, which could be explained as a physiological and biochemical adaptation providing cell buoyancy compensating for the heavy siliceous cell wall, and also as storage material against unfavourable conditions (Ramachandra, Mahapatra and Karthick, 2009). Silicon limitation in media is the major trigger for lipid accumulation in diatoms. Mysristic acid, palmitic acid and palmito-oleic acid are the dominant fatty acids in diatoms. Mixotrophic and heterotrophic cultivation of diatoms are being exploited for polyunsaturated fatty acid (PUFA) production. Apart from PUFA production, many diatoms, such as *Chaetoceros muelleri, Skeletonema costatum* and *Thalsssiosira pseudonana,* are used as a feed source in aquaculture in view of their good fatty acid profiles. The complete genome sequence for two diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum,* are available, and transgenic systems for many diatoms are well established, like *Navicula* spp. and *Cyclotella* spp., providing opportunities to improve lipid productivity by genetic engineering (Dunahay, Jarvis and Roessler*.,* 1995).

At present, the production of lipids in general and PUFA in particular by marine and freshwater micro-algae is the subject of intense research and commercial importance. Some of them are industrially exploited as potential sources of eicosapentaenoic acid (EPA), such as *Nitzschia laevis* and *Phaeodactylum tricornutum*. The annual worldwide demand for EPA is about 300 tonne, and fish oil is the major source of PUFAs (Singh, Bhushan and Banerjee, 2005.). However, the search for vegetarian sources of PUFAs and purified micro-algal PUFA as an alternative to fish oil, which is complex to purify and with intense odour, appears promising (Wen and Chen, 2003). Benefits of PUFA supplementation are well understood. One rare PUFA of micro-algal origin is gamma linolenic acid. Gamma linolenic acid (GLA; C18:3) is an isomer of ALA and is present in significant amounts in *Spirulina* spp. GLA has been identified as contibuting to prevention of skin diseases, diabetes and reproductive disorders (Gunstone, 1992). PUFAs from micro-algae are incorporated as supplements in infant formula and nutritional supplements (Table 2).

LARGE-SCALE CULTIVATION OF MICRO-ALGAE

The commercial cultivation of micro-algae began with the cultivation of *Chlorella* in Japan in the 1960s, followed by cultivation of *Spirulina* in Mexico and United States in the 1970s. In the last four decades, the industrial biotechnology of photosyntheic micro-organisms has grown tremendously and diversified. Large-scale cultivation systems of micro-algae takes two main forms: open ponds and closed reactors, reflecting the nature of the organism, culture media composition and other parameters, including culture pH, salinity and cultivation conditions.

The main goal of mass cultivation is to achieve higher productivity in terms of biomass for production of a metabolite. The three important factors affecting the mass cultivation of micro-algae are culture depth and related light levels, mixing or turbulence, and biomass density (Grobbelaar, 2009). The economics of large-scale cultivation are dictated by maximal yields and high rates of production. For industrial production systems, the micro-algae are generally grown

TABLE 2
Polyunsaturated fatty acids (PUFAs) produced from micro-algae

PUFA	Application	Micro-algal source
Gamma linolenic acid (GLA) C-18:3 – omega 3	Nutritional supplements and infant foods	*Spirulina* spp.
Arachidonic acid (AA) C-20:4 – omega 6	Nutritional supplements, immunomodulatory therapeutics	*Porphyridium creuntum, Parietochloris* spp.
Eicosapentanoic acid (EPA) C-20:5 – omega 3	Nutritional supplements and aquaculture	*Nannochoropsis* spp., *Phaeodactylum* spp., *Isochrysis pavlova*
Docosahexanoic acid (DHA) C-20:6 – omega 3	Nutritional supplements and Infant foods	*Schizochytrium* spp., *Crypthecodinium* spp.

Sources: Spolaore *et al*., 2006; Harwood and Guschina, 2009.

in open outdoor shallow ponds for simple maintenance and relatively low input costs.

Open cultivation systems

Most algae for commercial use are grown in the open air. The two most common open cultivation systems are circular and raceway ponds, in use for more than five decades. These systems can be developed using natural water bodies such as lagoons and ponds or artificial ponds such as raceways. Raceway ponds are generally oval shaped, closed loop channels of 0.2–0.5 m in depth where mixing is generally provided using paddle wheels. Raceway ponds are usually constructed from cement, or sometimes compacted soil channels with plastic liner (Brennan and Owende, 2010). Their shallow nature and continuous mixing meets the light requirements of the culture and prevents sedimentation. The open cultivation pond is cheap and they do not compete for agricultural land as they can be built on non-productive (marginal) land, or coastal regions for marine algal cultivation. The open system requires minimal investment in terms of light source and operations (Borowitzka, 1997; Chisti, 2008; Brennan and Owende, 2010; Mutanda *et al.,* 2011).

Open systems such as coastal shallow brackish-water ponds are extensively used for feed production in aquaculture and for other industrial applications. *Dunaliella* spp. are widely grown in these systems. These natural ponds are economical in terms of their operations, but only a limited number of species can be grown. Other physico-chemical parameters that affect productivity in open systems are evaporation losses, temperature fluctuations, inefficient mixing and light limitation. The evaporation losses increase the ionic concentration in the media causing severe osmolarity changes (Becker, 2004; Pulz, 2001; Lee, 2001). CO_2 requirements are more than can be met from the natural environment and thus constrain productivity. Hence carbonates and bicarbonates are used as carbon sources. Flue gases and CO_2 can be directly used as inorganic carbon for growth of cells in autotrophic mode. Due to the abovementioned limitations, the productivity of open ponds is low, and hence developing closed bioreactor systems for biomass production is preferred. One of the possible solutions to prevent contamination and severe evaporation losses are to cover the ponds with a greenhouse, which limits pond size considerably but gives a quasi-controlled environment.

Pond management

Since open ponds are highly susceptible to environmental fluctuations and contamination, certain measures are needed to keep the cultures healthy and productive. Contamination by other algae is very common in open ponds; this can be effectively managed by maintaining a critical cell concentration, preventing competing species growth. Contamination by rotifers can be controlled by reducing the culture pH, since they are sensitive to low pH, and later re-adjusting cultures to optimum pH. Mixing is essential as accumulation of biomass in one place leads to anaerobic decomposition and accumulation of bacteria. The most efficient way of maintaining cultures is through a batch system, with fresh unialgal inoculum as starting material for every batch. An initial optimal optical density of the culture is a key factor for health of culture and maintenance. Since the emphasis is on the production of biomass with the minimum of energy inputs, it is desirable to use windmills for agitation of cultures and also use marine forms to avoiding or minimize competition from contaminating organisms (Borowitzka*,* 2005).

Closed cultivation systems

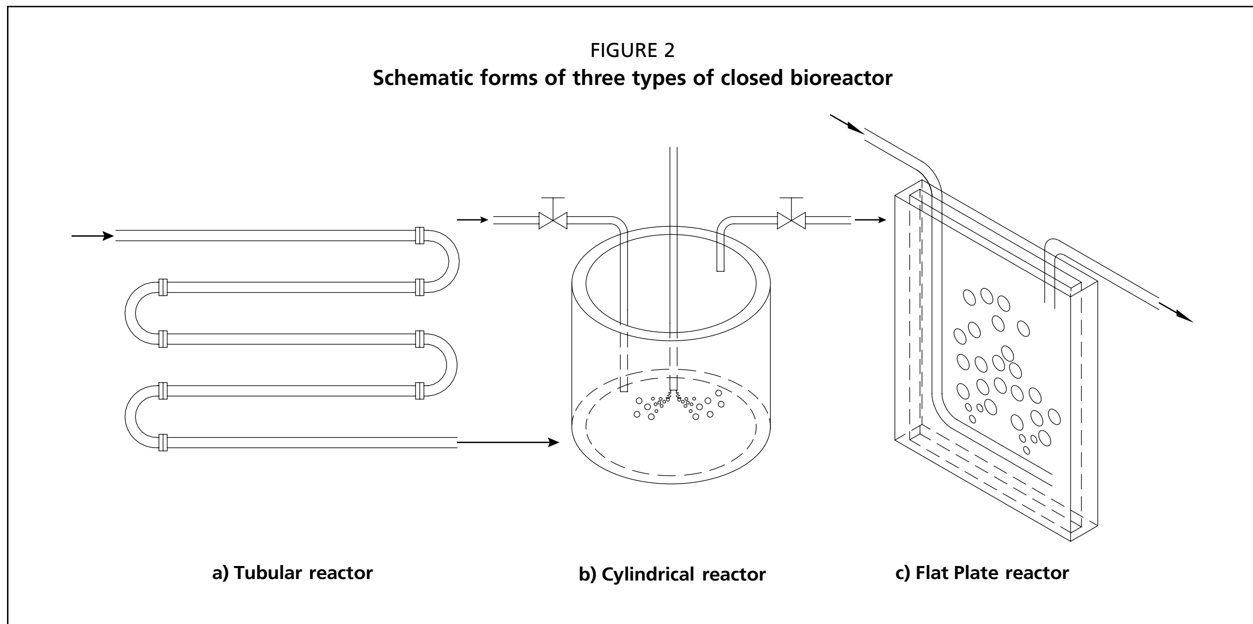
Maintenance of uni-algal culture in open ponds is very difficult, but can be achieved in closed bioreactor systems. In closed configurations, various culture parameters can be controlled and environment-sensitive strains growing in near-neutral pH conditions can be grown with higher productivities in closed photobioreactors. The closed reactor systems have high biomass productivity compared with open ponds since culture parameters such as illumination, turbulence and air exchange can be carefully regulated (Grobbelaar, 2009, 2010).

Six parameters or subsystems for photobioreactors are, light source, optical transmission system, reaction area, gas exchange system, filtration system (removal of biomass) and sensing system. Light source is an important design consideration, which includes variables like type of light source, intensity of light source, effect of light source on cell development in the algal culture, and dark period requirement of the algae (Anderson, Anil and Schipull, 2002). Several of these parameters interact, such as the optical transmission system and gas exchange system via the mixing that takes place in the reaction area.

Three types of closed reactors are commonly employed for mass cultivation: tubular; cylindrical or columnar; and flat plate (Lehr and Posten, 2009).

Tubular bioreactors consist of an array of glass or plastic transparent tubes connected by U bends to capture more sunlight (Tredici and Materassi, 1992). They can be aligned in a flat plane or as a coil around a vertical cylindrical support framework (Borowitzka, 1999). The tubes are generally 5–10 cm in diameter. The algal cultures are circulated in these narrow tubes using mechanical pumps or airlift systems (Brennan and Owende, 2010). Tubular bioreactors have high surface to volume ratio, hence light capture is higher and gives high productivities. *Spirulina platensis* and *Chlorella* spp. have been successfully grown in these systems. Combined airlift-tubular systems have been used in production of *Porphyridium cruentum, Phaeodactylum*

FIGURE 2
Schematic forms of three types of closed bioreactor



tricornutum and *Haematococcus pluvialis* (Garcia-Malea Lopez *et al.,* 2006; Converti *et al.,* 2006). A combined airlift-tubular system has two parts: an airlift system and a solar receiver. The airlift system provides gas transfer and means to harvest biomass, while the tubular solar receiver provides high surface to volume ratio.

Cylindrical or column bioreactors are generally vertically aligned with aeration provided at the bottom and illuminated through transparent walls. They offer efficient mixing, facilitating cell growth. Bubble column or airlift column type mix by a static mixer or baffles, or by air spargers for controlled agitation. Mixing increases the frequency of cell exposure to light and reduces the dark volume of the reactor. It also enhances mass transfer between nutrients, facilitates dissipation of heat, and prevents oxygen build up (Brennan and Owende, 2010; Kunjapur and Eldridge, 2010). Efficient illumination can be achieved by internal light guides that spatially distribute light into cultures (Kunjapur and Eldridge, 2010). Duration of light and dark cycles influences photosynthetic efficiency. Alternatively, improvement of productivity is possible by flashing light with optimal pulsing, although this will incur additional overall maintenance costs (Posten, 2009). LED photodiodes are being used for illumination instead of fluorescent lights, and continuous illumination can be achieved by using solar light during day and solar power driven LED lights at night to improve productivity (Briassoulis *et al.,* 2010). Light transmission into dense cultures is very difficult and this can be overcome by using light guides that direct the incoming light into the culture, increasing the reactor area exposed to light. Anderson, Anil and Schipull (2002) extensively studied the use of light guides in improving light penetration in photobioreactors. The simplest large-scale outdoor closed-cultivation system is use of bag reactors, which are easy to construct.

Flat plate bioreactors are highly robust and very high biomass yields can be achieved due to their high ratio of light illumination surface area to volume. They have a small light path – from a few to 70 mm at most. Mixing is achieved by sparging with CO_2-enriched air. Their simple configuration supports construction of multiple plates placed closed to each other and thereby efficiently utilizing land space (Posten, 2009).

Mixotrophic and heterotrophic production of biomass

Micro-algae being photosynthetic are hence predominantly cultivated in open ponds, exploiting their photoautotrophic nature. In heterotrophic growth, algae utilize organic substrates for metabolic process, while in mixotrophy both light and organic substrates are exploited. Under mixotrophic cultivation, the diurinal cycle can be efficiently utilized to prevent losses during aerobic dark respiration. *Spirulina platensis* and *Chlamydomonas reinhardtii* are efficiently adopted for mixotrophic growth (Chen, 1996; Andrade and Costa, 2007). In heterotrophic cultures, glucose or acetate are commonly used carbon sources, with glutamine or glutamate, or aspartate or aspargine, as common nitrogen sources.

Advanced fermentation technology and sophisticated reactors offer immense potential in growing micro-algae in heterotrophic conditions. Heterotrophic production has been studied extensively in *Chlorella protothecoides,* which can otherwise be grown photo-autotrophically (Miao and Wu, 2006; Chisti, 2007). Production of specialty chemicals like lutein from *C. protothecoides* or docosahexaenoic acid (DHA) from *Crypthecodinium cohnii* have been studied (Shi *et al.*, 1997; Shi and Chen, 2002; De Swaaf, Sijtsma and Pronk, 2003). The heterotrophic mode of growth can be

TABLE 3
Economic comparison of phototrophy and heterotrophy

Factors	Phototrophy	Heterotrophy
Energy source	Light	Glucose
Energy cost	US$ 0.07 per kWh	US$ 0.67/kg
Estimated cost/kg of dry weight	US$ 11.22	US$ 0.81
Actual cost/kg of dry biomass	Less than US$ 11.22	US$ 2.01
Productivity	0.4 g/L/day	5.8 g/L/day

Source: Adapted from Behrens, 2005.

a better option for cultivating strains that are susceptible to environmental fluctuations, such as *Haematococcus pluvialis*. An increase in biomass and astaxanthin content was observed when *H. pluvialis* was grown in heterotrophic media with acetate as carbon source (Tripathi *et al.,* 1999).

Utilizing heterotrophy for bio-energy production through micro-algae may not be a solution in the long term since it defeats the purpose of CO_2 sequestration. But it was observed that the specific growth rate of micro-algae grown in mixotrophic media supplemented with glucose or acetate was higher than in autotrophic cultivation (Lee, 2001).

Behrens (2005) compared the economics of algal biomass production in both phototrophic and heterotrophic modes of cultivation, with *Chlorella* spp. as the model organism. The major factors considered were construction costs for photobioreactors and fermenters, and associated energy costs: electricity in the case of photobioreactors and organic carbon for fermenters. Some of the assumptions were (1) cost of electricity was US$ 0.07/kWh; (2) 20 percent of the energy of the electricity is converted into visible light (based on the efficiency of fluorescent lamps); (3) all of the light energy is absorbed by the phototroph; (4) the photosynthetic efficiency of converting absorbed light into ATP and NADPH is 20 percent (theoretical efficiency for red light conversion into chemical energy); (5) the energy content of the algal biomass is 6.41 kWh per dry kilogram of algal biomass; (6) the carbon content of algae is 50 percent; and (7) all of the carbon of glucose is converted into algal biomass.

Therefore the heterotrophic mode of cultivation could also be an alternative for biofuel production, taking into consideration the high biomass productivity and promising results based on the trials conducted by Li, Xu and Wu, 2007.

CO_2 sequestration

CO_2 is one of the main gases responsible for greenhouse effects accelerated by human activities. The mitigation of CO_2 by biological methods is a very important strategy as this can give rise to biomass-derived energy options through photosynthesis. Among phototrophic organisms, micro-algae are the most efficient systems in absorbing CO_2 (Skjanes, Lindblad and Muller, 2007; Li *et al.,* 2008). Micro-algae can absorb CO_2 from a variety of sources, including atmospheric CO_2, emissions, flue gases from industries and CO_2 from soluble carbonates (Wang *et al.,* 2008). The rate of CO_2 removal is species dependent. Francisco *et al.* (2010) compared some of the micro-algal strains for CO_2 removal and observed that there is a wide range in the rates, ranging from 1.5 mg/L/minute in diatom *Phaeodactylum tricornutum* to 28.0 mg/L/minute in cyanobacterium *Aphanothece microscopica,* with higher ratios of CO_2 absorption and desorption rates indicating their greater efficiency. Based on their study, *Chorella vulgaris* with 11–13 hours of photoperiod and continuous cultivation in photobioreactors could achieve bioconversion of 3.07 kg CO_2/L/cycle. The CO_2 fixation rate (P) of the micro-algae can be calculated based on the biomass productivity, according to the equation P CO_2 = 1.88 × biomass productivity, which is derived from the approximate molecular formulae of micro-algal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007).

It is evident from Table 4 that chlorophycean micro-algae are efficient species in sequestering CO_2. Some strains of *Chlorella* spp. and *Scenedesmus* spp. can absorb up to 15 percent CO_2 (Huntley and Redalje, 2007; Francisco *et al.,* 2010). CO_2 abatement can be achieved by either directly passing the gaseous emission into the culture or by converting the gases chemically to soluble carbonates. Apart from use of flue gases as sources of CO_2, wastewater could also be used as a source of nutrients, especially for N and P. This combination of CO_2 uptake and wastewater treatment could render the large-scale cultivation process economically viable. *Botryococcus braunii* was shown to remove N and P from treated wastewaters; and *Chlorella vulgaris* was shown to remove ammonia from a steel manufacture plant's effluent and also to act as a sink for disharged flue gases. The CO_2 fixation and ammonia removal rates estimated for a *Chlorella vulgaris* strain was 26.0 g $CO_2/m^3/h$ (0.624 g/L/day) and 0.92 g $NH_3/m^3/h$, respectively (Yun *et al.*, 1997). The main problems in direct absorption of gaseous emissions are high temperatures and the presence of

TABLE 4
Average CO_2 absorption and fixation rates of micro-algae

Micro-alga	CO_2 (%)	Temperature (°C)	P CO_2 (g/L/day)
Chlorococcum littorale	40	30	1.0
Chlorella kessleri	18	30	0.163
Chlorella vulgaris	up to 15	25	0.045–0.624
Chlorella sp.	15–40	up to 42	up to 1.0
Dunaliella sp.	3	27	0.313
Haematococcus pluvialis	16–34	20	0.143
Scenedesmus obliqus	Upto 18	30	0.016–0.26
Botryococcus braunii	—	25–30	>1
Spirulina sp.	12	30	0.413

Notes: P - CO_2 fixation rate. *Source:* Adapted from Wang *et al.*, 2008.

other contaminants like NO_x and SO_x. Cooling of the gases is a costly option, but conversion of these gases into soluble carbonates such as Na_2CO_3 and $NaHCO_3$ is an easy option as some of the micro-algae can survive in extreme conditions of high pH, thereby minimizing invasive species and other biological contaminants (Benemann 2003; Wang *et al.,* 2008).

Wastewater utilization and cultivation of micro-algae

One of the economical means of cultivation of micro-algae for biofuel production is utilizing municipal wastewater and industrial effluent. The utilization of effluents provides a way for removal of chemical contaminants, such as heavy metals. *Scenedemus obliquus* has been extensively studied for utilization of wastewaters (Voltolina, Gomez-Villa and Correa, 2005; Hodaifa, Martinez and Sanchez, 2008). Sawayama, Minowa and Yokayama (1999) developed a cultivation strategy for uptake of nitrogen and phosphorous from sewage water and production of hydrocarbon rich biomass of *B. braunii*. Micro-algae are used in effluents from aquaculture, dairy farms and the food processing industry for removal of nutrients (nitrates, ammonia and phosphates) and odour, and to reduce acidity without chemicals. In oil drilling, micro-algae are used for reducing sludge and to remove metals and their precipitates. Effective utilization of algal biomass for waste treatment would ensure a net positive energy balance and economically viable algal cultivation technology.

An integrated approach of growing micro-algae in wastewater and utilization of biomass for biofuel is shown schematically in Figure 3. The spent biomass obtained after biofuel extraction would be utilized further for bio-energy production through bio-methanation, unlike freshwater-grown micro-algal spent biomass utilized for feed and other bio-active molecules.

Alternately the spent biomass can be converted to fuel products through pyrolysis, chemical catalysis or hydrocracking, and used in diesel, jet fuel or gasoline.

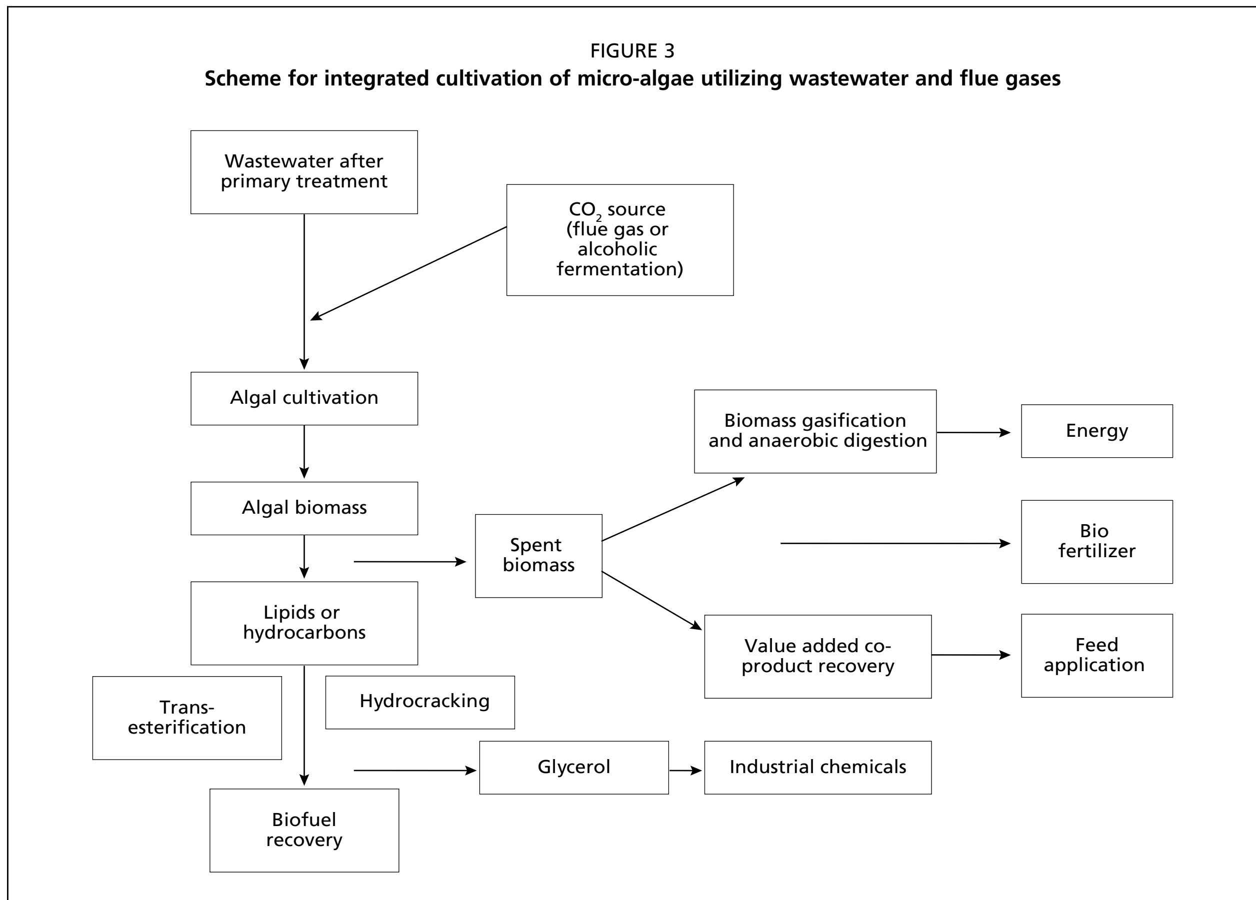
DOWNSTREAM PROCESSING AND CONVERSION TO BIOFUELS

Harvesting and dewatering of micro-algae

Mass production of micro-algae for metabolites requires efficient harvesting and extraction methods. Harvesting of biomass requires one or more solid-liquid separation techniques, which depend on the nature of the alga, such as size, density and metabolite to be obtained. The common techniques applied in the harvesting of biomass are centrifugation, flotation, flocculation and gravity sedimentation.

Centrifugation recovery is the most preferred method of harvesting for high value metabolites. Laboratory experi-

FIGURE 3
Scheme for integrated cultivation of micro-algae utilizing wastewater and flue gases



ments conducted on harvesting of biomass indicated centrifugal recovery is rapid and about 80–90 percent of algal biomass can be recovered with 1000–5000 *g* for 2–3 minutes (Chen *et al.,* 2010). Centrifugation is a preferred method of harvesting algal biomass for producing extended shelf-life concentrates for aquaculture hatcheries and nurseries (Knuckey *et al.,* 2006). The only limiting factor is the high capital and operating costs for harvesting of large quantities of water and algae (Grima *et al.,* 2003).

Gravity sedimentation is a type of solid-liquid separation which is commonly applied for separating micro-algae in water and wastewater treatment. Particles with higher density can be separated easily by gravity sedimentation, whereas particles with a diameter of a few microns require flocculants to form larger aggregates. Lamella separators and sedimentation tanks are used for enhanced micro-algal separation through sedimentation (Uduman *et al.,* 2010). Biomass-sediment is collected in a sump and recovered by pumping. Gravity sedimentation in sedimentation tank is time consuming and very few reports are available on this method; it relies on the autoflocculation principle. Addition of flocculants increases the efficiency of gravity sedimentation, which could be an inexpensive process.

Filtration is used effectively for the harvesting of larger micro-algae like *Coelastrum* spp. or *Spirulina* spp., but fails to recover micro-algae with smaller cell dimensions (Mohn, 1980). Filtration is theoretically simple, but very expensive in practice, and its understanding involves several issues, like filter pore size, filter material and also the design of the filters. Larger filter apertures allows smaller cells to pass through, whereas decreased pore size results in binding or blocking of filter pores, and reduced filtration rates. Filter pore size is dependent on the size of the algal species and algal aggregation rate. Filtration materials and filtration design are of primary importance, which determine the cost economics and efficiency of the process (Oswald, 1991).

Flocculation can be a preliminary step in the bulk harvesting process, which helps to aggregate the micro-algal cells in order to increase the effective particle size. Flocculation can be enhanced by addition of flocculants that can reduce or neutralize the surface negative charge of the cells thereby increasing the effective particle size for gravity settling. An ideal flocculant should be inexpensive, non-toxic and effective at low concentrations, and also should not affect further downstream processing (Grima *et al.,* 2003; Murthy, 2005). Flocculants generally coagulate algal cells by neutralizing the surface negative charge, as in the case of polycationic inorganic or organic compounds such as polyvalent metal salts, which are iron- or aluminum-based coagulants, like ferric chloride, aluminum sulphate and ferric sulphate. Coagulation efficacy of metal ions increases with increasing ionic charge (Brennan and Owende, 2010). Multivalent salts like alum are used effectively to harvest *Chlorella* and *Scenedesmus* in wastewater treatment processes (Grima *et al.,* 2003). Organic flocculants, apart from reducing or neutralizing the surface charge, can bring particles together by physical linkage through a process called bridging (Grima *et al.,* 2003). Chitosan is being used as a biodegradable organic flocculant that can be synthesized from natural sources (Divakaran and Pillai, 2002). Polymeric organic flocculants such as polyacrylamide, cationic starch, poly-ferric sulphate, etc., are commonly used for harvesting. Marine organisms cannot be effectively harvested through flocculation due to high ionic concentrations within cells (Bilanovic, Shelef and Sukenik, 1998). The main problems associated with flocculation are accumulation of the flocculant in the media and its effect on possible further recycling of media (Chen *et al.*, 2010).

Flotation relies on the attachment of air or gas bubbles to solid particles, which are then carried to the liquid surface and accumulate as float, which can be easily separated. Flotation is a more effective and beneficial harvesting method than flocculation since it obviates the use of chemicals. Solid particles can be separated by colliding air bubbles with the particles; for capturing particles smaller than 500 μm, flotation efficiency increases with decreasing particle size (Yoon and Luttrell, 1989; Uduman *et al.,* 2010). Although flotation could be a potential harvesting system, its efficacy has yet to be clearly evaluated (Brennan and Owende, 2010).

According to Uduman *et al.* (2010), any dewatering technology can be quantitatively evaluated by the following parameters: the rate of water removal of the dewatering technique; the solid content of the recovered micro-algae-water slurry (percent total suspended solids - TSS); and the yield of the processed micro-algae by the dewatering technique. They proposed a single-step simultaneous harvesting and dewatering process for micro-algae, from an initial 0.02 to 0.06 percent TSS to a primary harvest of 2 to 7 percent TSS, followed by dewatering to give 15 to 25 percent TSS.

The final step in harvesting is the complete dewatering and drying of the micro-algal slurry. This step is one of major economic importance. Selection of the drying method depends on the use for which the dried product is intended, and also the scale of operation. Some of the commonly available drying methods include sun drying, drum drying, freeze drying, air drying, oven drying and spray drying. Freeze drying and spray drying methods are found to be rapid and the most suitable drying method for algae, but is comparatively energy intensive. Spray drying is often used for algae, finding food applications such as with *Dunaliella* spp. and *Spirulina* spp. (Leach, Oleveira and Morais, 1998). Oven-type driers are also found to be effective and less energy intensive, but are not suitable for heat-sensitive metabolites (Mohn,

1978). Air drying and sun drying is the cheapest method for drying algal biomass, but it requires a large drying surface and has a long drying time (Prakash *et al.,* 1997). Lundquist *et al.* (2010) recommended online extraction of oil from wet biomass, avoiding the steps of drying and extraction, which could reduce operating costs by 20 to 25 percent.

Extraction of micro-algal lipids

Cell disruption is an important step in recovering intracellular products from micro-algae, and so properties of the cell wall play an important role in the extraction process. Some of the commonly used methods for cell disruption include mechanical disruption, like bead-beating, ultrasound and steam extraction (Mata*,* Martins and Caetano., 2010) and non-mechanical disruption, including application of organic solvents and addition of inorganic acids and alkali for pre-treatment processing. The most convenient method would be to efflux the metabolites or constituents of micro-algal cells using solvents, without disrupting cellular functions. Two-phase solvent mixtures, such as methanol-ethanol/hexane co-solvent systems, are advantageous, whereby more polar solvents are used to disrupt the membrane while the extracted lipids enter the non-polar solvent phase. This reduces the phase separation step during processing, thereby making solvent extraction more convenient and economical (Hejazi and Wijffels, 2004).

Green solvents such as ionic liquids and switchable polarity solvents can be exploited for extraction of lipids from micro-algae (Samori, Samori and Fabbri, 2010; Salvo *et al*., 2011). Ionic liquids are non-aqueous solutions of relatively large asymmetric organic cations coupled with a small inorganic or organic anion salt that remain liquid at moderate to room temperatures. The ionic solvents have hydrophilic ionic liquid and polar covalent molecules for both extraction and partitioning of lipids from micro-algal cells. Salvo *et al.* (2011) used a hydrophilic ionic liquid, 1-butyl-3-methylimidazolium, for a single-step extraction process involving lysis of micro-algal cell walls and separation of cellular lipids. After the auto-phase separation, the lower hydrophilic ionic phase can be re-used for extraction of micro-algal cells.

Switchable solvents have physical properties, such as polarity, solubilizing capacity, viscosity and conductivity, that can be converted from one form to other. The main advantages of switchable solvents is that many processes, such as extraction, phase separation and purification, can be achieved with one single agent (Phan, 2008). Hydrocarbon yields were higher when a switchable polarity solvent system containing 1,8-diazobicyclo-[5.4.0]-undec-7-ene and alcohol was used for extraction in *B. braunii* compared with conventional solvent extraction by n-hexane (Samori, Samori and Fabbri, 2010).

CONVERSION OF ALGAL LIPIDS AND BIOMASS TO BIO-ENERGY

Trans-esterification

The extracted micro-algal oil can be converted to biodiesel by trans-esterification. The trans-esterification process involves reaction of an alcohol with the triglycerides, forming fatty acid alkyl esters, in the presence of a catalyst. Based on the type of catalyst used, the trans-esterification process can be acid or base catalysed, and involve enzymatic conversion. In acid-catalysed reactions, HCl, H_2SO_4 or H_3PO_4 is used for trans-esterification, while in base catalysis strong bases like KOH or NaOH are commonly used. Base catalysis has many advantages over the acid-catalysed reaction since it is conducted at low temperature and pressure, and it has a high conversion yield and provides direct conversion to biodiesel without intermediate compounds. Balancing the advantages are several drawbacks, including being energy intensive, with problems associated with removal and treatment of alkaline catalyst from the final product. These problems could be solved by the use of bio-catalysts like lipases, but large-scale demonstration has not been reported (Svensson and Adlercreutz, 2008).

In situ acid-catalysed trans-esterification processes for biofuel production have been explored but the limiting factor is the high moisture content of algal biomass, affecting the conversion. The identification of lipid composition is an important criterion to assess the suitability of algal oil for high quality biodiesel production. Some of the important fuel properties considered for biodiesel include density, viscosity, flash point, ester value, cetane number and combustion heat (Mutanda *et al.,* 2011). In the study conducted by Francisco *et al.* (2010) on micro-algal strains of *Chlorella* spp.*, Dunaliella* spp.*, Phaeodactylum* spp.*, Aphonothece* spp.*, Phormidium* spp. and *Scenedesmus* spp.*,* it was found that the properties of biodiesel obtained from these strains were found to be similar to the American Society for Testing and Materials (ASTM) and European Union standards (Table 5).

Trans-esterification of algal lipids generates glycerol as the major co-product. Glycerol is an industrially important

TABLE 5
Characteristics of biodiesel

Properties	Biodiesel from micro-algal oil	Diesel fuel
Density (kg/L)	0.864	0.838
Viscosity (Pa/s)	5.2×10^{-4} (40°C)	$1.9 - 4.1 \times 10^{-4}$ (40°C)
Flash point (°C)	65–115	75
Solidifying point (°C)	-12	-50 – -10
Cold filter plugging point (°C)	-11	-3.0 (-6.7 max.)
Acid value (mg KOH/g)	0.374	0.5 max.
Heating value (MJ/kg)	41	40 – 45
HC (hydrogen to carbon) ratio	1.18	1.18

Source: Oilgae, 2010.

product that can be fermented to produce 1,3-propanediol, which is a precursor for many industrial products such as polymers, adhesives, paints, etc. (Yazdani and Gonzalez, 2007). The large-scale production of biodiesel by trans-esterification produces enormous quantities of glycerol, which poses a problem for its complete utilization and disposal. It has been estimated that for one billion gallons of biodiesel produced, approximately 400 000 tonne of glycerol will be generated (Fishman *et al.,* 2010). As the existing market for glycerol is currently saturated, a more efficient process for biodiesel production is required where there is a complete utilization of all the co-products generated in the large-scale production process.

Hydrocracking

Hydrocracking is another alternative for biofuel production from crude biomass extracts containing hydrocarbons. Although the acid and alkali catalysed reactions are faster and used extensively for biofuel production, these catalysts require additional treatment for their removal from the final product stream. A potential solution to this problem is the use of hydrocracking or catalytic cracking technology, where the conversion process involves two stages, combining catalytic cracking and hydrogenation. The hydrogenation step occurs at high temperatures (200–300 °C) and high pressures (35–200 bar), yielding low boiling, high quality hydrocarbons. The catalysts used for cracking are immobilized homogenous or heterogeneous metal-based catalysts derived from titanium or vanadate oxides, Al_2O_3, MgO and CaO. A wide range of feed stocks can be processed *via* hydrocracking, with removal of nitrogen and sulphur in the biomass feed stocks for gases yielding N- and S-free paraffinic hydrocarbons. Complex aromatic hydrocarbons and olefins are hydrogenated to simpler, lower boiling and lower molecular weight hydrocarbons. The challenges involved in this process are finding an ideal catalyst that operates at lower temperature and pressure, thereby reducing the energy inputs, and that also has resistance towards leaching by the active components of the feed stocks (Fishman *et al.,* 2010).

ETHANOL FROM ALGAL FEEDSTOCK

Ethanol is a clean burning fuel with high octane value. It is not used as 100 percent fuel but generally blended with gasoline in different proportions, thereby reducing dependence on gasoline and improve the octane rating of the blended fuel. Generally, ethanol is blended at 10 percent with gasoline—termed E-10—and is approved for use in United States. Another type of ethanol-based fuel is E-85, where the blend proportion is 85 percent ethanol and 15 percent unleaded gasoline, but used only in Flexible Fuel Vehicles (Oilgae, 2010). Algae could be the source of bioethanol due to their relatively high contents of carbohydrates in the form of polysaccharides. Ethanol can be produced either from algal biomass or from algal cake. Producing ethanol from algal cake is more economical than the use of biomass (Clarens *et al.,* 2010). Macroalgae are a better source for ethanol production because of their high polysaccharide content, such as *Sargassum* spp. (48%), *Gracilaria* spp. (45%), *Kappaphycus* spp. (35%) and *Euchema* (45%). Cell wall components of algae are the major sources of carbohydrates. Green algae mostly contain cellulose and hemicellulose; red algae contain cellulose and polysaccharides like agar and carrageenan; and brown algae contain cellulose and alginic acids. The cellulosic content in micro-algae is relatively less for production of ethanol. Certain algae accumulate starch in stress conditions, and this can be exploited for ethanol production by fermentation. The ethanol generated can be distilled. The following section discusses some methods of biomass conversion technologies for energy production, including biomass gasification and fermentation.

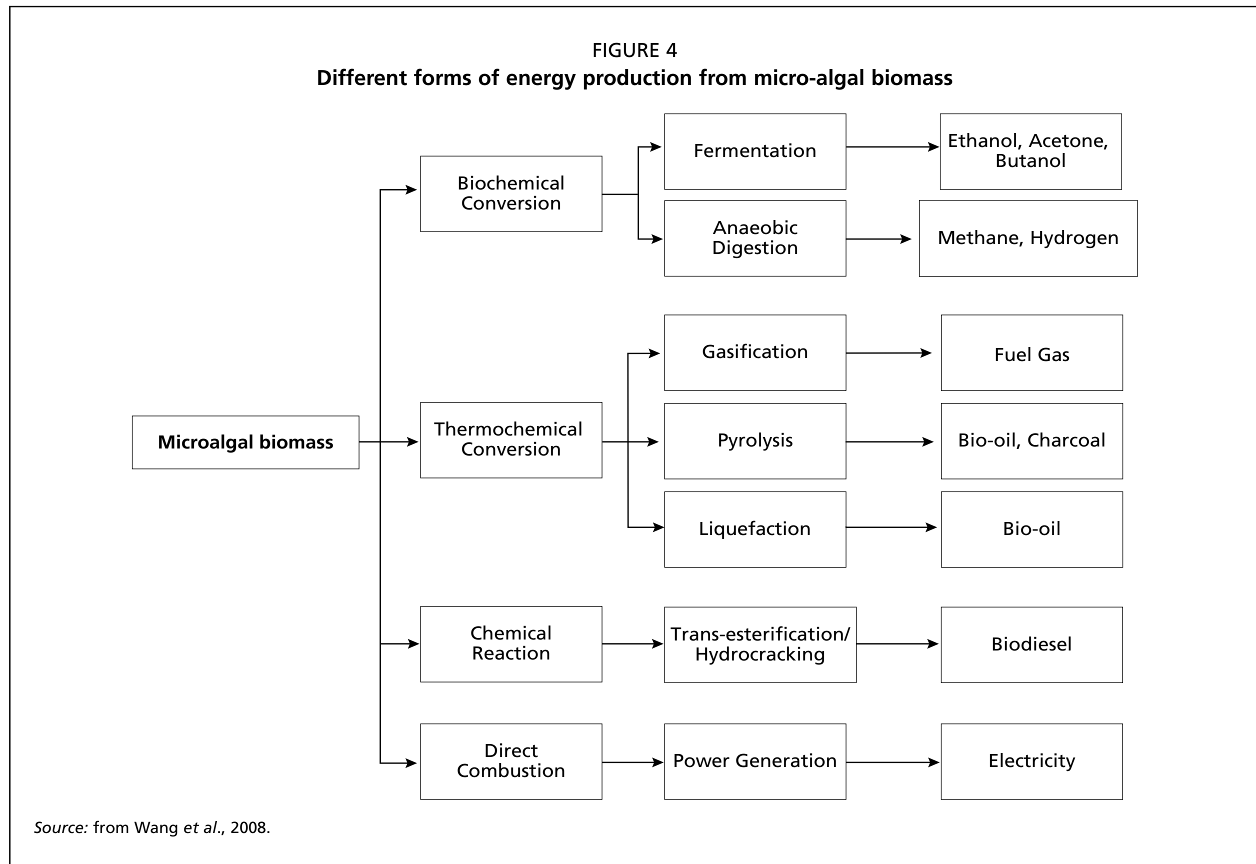
Biomass conversion technologies for energy production

Biomass-derived energy can be obtained from the de-oiled algal cake or spent biomass using various conversion technologies. Various processes, such as combustion (heat energy), pyrolysis (pyrolytic gas), gasification (syngas), thermochemical liquefication, and alcoholic fermentation (ethanol) are being explored to develop a sustainable technology for biofuel production from micro-algae (Figure 4).

Integrated biorefineries use residual biomass to produce biogas or other forms of energy to run the micro-algal production facility. Since micro-algal cultivation involves huge influxes of N & P, unutilized biomass would severely affect the environment and the economics of biodiesel production. Recycling the N & P from the residual biomass after oil extraction is important, and this can be achieved by anaerobic digestion of the algal waste to biogas-methane (Chisti, 2007; Sialve, Bernet and Bernard, 2009).

Thermal liquefaction of whole algal biomass by sub-critical water extraction can also be used for large-scale operations where the de-watering step can be eliminated. Sub-critical water extraction below critical temperatures and high pressure keeps water in the liquid phase, making the operation less polar and hence solubilising organic compounds from the cells. Cooling of water to room temperature creates phase immiscibility, leading to easy separation of metabolites. The operational advantage of sub-critical water extraction is shorter extraction time, environmental compatibility, and abundance and low cost of the extracting agent (Herrero, Cifuentes and Ibanez, 2006; Patil, Tran and Giselrod,, 2008). The main product obtained after liquefaction is 'bio-crude', which can be further upgraded to combustibles (Fishman *et al.,* 2010). Biomass gasification of

FIGURE 4
Different forms of energy production from micro-algal biomass



Source: from Wang *et al*., 2008.

the micro-algal concentrate can yield different liquid fuels; this process uses Fischer-Tropsch synthesis technology. The crude product obtained is called 'Syn-gas', which can be converted to various fuel derivatives by further processing, such as hydrogenation.

USE OF MICRO-ALGAE FOR FOOD, FEED AND BIO-ACTIVES

Food applications of micro-algae

The algal biomass remaining after extracting the hydrocarbons and lipids is a valuable co-product rich in nutritionally important metabolites for food and feed purposes. It is used for food applications, and also for feed applications in various industries, such as fish aquaculture and poultry, as well as in the nutraceutical market for both human and animal consumption. This section highlights the possibilities for utilization of algal biomass for feed and as a source of other valuable constituents. Such value addition would be of relevance not only to utilize all the co-products in a useful manner, but also as affording eco-friendly technology alternatives for the production of various nutrients and bio-actives apart from direct food applications.

Micro-algae with their immense chemical diversity provide an seemingly unlimited source for various applications in the food industry (Table 6). Algal products ranging from whole biomass to nutraceuticals like carotenoids and PUFAs are utilized in the food industry. The safety of these algae has been evaluated and commercial use approved in several countries. Many reviews are available detailing the potential uses of micro-algae as food sources (Venkataraman *et al.,* 1980; Becker, 2004; Pulz and Gross, 2004; Spolaore *et al.,* 2006; Ravishankar *et al.,* 2008; Plaza *et al.,* 2009; Milledge, 2010).

Micro-algae as a source of vitamins

Micro-algae also represent a valuable source of nearly all essential vitamins (e.g. A, B_1, B_2, B_6, B_{12}, C, E, nicotinate, biotin, folic acid and pantothenic acid). Vitamin B_{12} is mainly synthesized by certain bacteria that are associated with the gut flora of animals, contributing to the requirement of this vitamin. Since plants have no ability to synthesize vitamin B_{12} because of the absence of cobalamin-dependent enzymes, strict vegetarians (vegans) are at risk of developing vitamin B_{12} deficiency, and hence need to depend upon vitamin B_{12}-fortified foods or vitamin B_{12}-containing dietary supplements to meet the requirement. *Spirulina platensis* is one of the most widely consumed cyanophycean forms used as a food supplement and contains substantial amounts of vitamin B_{12}. Because the vegetarian diet does not contain vitamin B_{12}, *S. platensis*, along with other nutrients, helps in meeting the recommended daily requirement of vitamin B_{12} in the vegetarian diet, and also of meeting the requirement of needy individuals with varied food habits or health status (Kumudha *et al.,* 2010). Other water-soluble vitamins, including Vitamin C, riboflavin and

TABLE 6
Micro-algae commercially exploited for food applications

Micro-algae	Biomass or metabolite and commercial applications	Cultivation system	Location	References
Chlorella spp.	Whole biomass for aquaculture and single-cell protein Astaxanthin as Pigment agent Ferredoxin for laboratory use	Circular ponds with rotating arms; heterotrophic ferementers	Japan, Taiwan, Thailand and USA	Carvalho, Meireles and Malcata, 2006.
Dunaliella salina	β-carotene as pigment agent	Extensive open ponds; raceway ponds	Australia, China, India, Israel and USA	Murthy, 2005; Ravishankar *et al.,* 2008; Plaza *et al.,* 2009.
Haematococcus pluvialis	Astaxanthin, colouring agent, nutraceutical	Two stage systems; tubular reactors	India and USA	Gouveia and Oliveria, 2009; Kamath 2007; Ravishankar *et al.,* 2008; Plaza *et al.,* 2009.
Spirulina platensis	Whole biomass as food or feed; phycocyanin as colouring agent	Cicular ponds and open raceway ponds	China and India	Eriksen, 2008.
Isochrysis galbana	DHA - Food supplement and pharmaceuticals	Closed bioreactors		Day, Benson and Fleck, 1999.
Phaeodactylum tricornutum	EPA as food supplement and pharmaceuticals	Closed bioreactors		Spolaore *et al.,* 2006.
Crypthecodinium cohnii	DHA as food supplement and pharmaceuticals	Closed bioreactors (heterotrophic)	USA	Spolaore *et al.,* 2006.
Schizochytrium spp.	DHA as food supplement pharmaceuticals	Heterotrophic fermenters	USA	Sijtsma and Swaaf, 2004; Pyle, Garcia and Wen, 2008.

Notes: DHA = docosahexaenoic acid

TABLE 7
Comparison of vitamin content (mg/kg DM) of a few micro-algae with common foods and RDI

Source	Vitamin										
	A	B_1	B_2	B_6	B_{12}	C	E	Nicotinate	Biotin	Folic acid	Pantothenic acid
RDI (mg/day)	1.7	1.5	2.0	2.5	0.005	50.0	30.0	18.0	na	0.6	8.0
Liver	60.0	3.0	29.0	7.0	0.65	310.0	10.0	136.0	1.0	2.9	73.0
Spinach	130.0	0.9	1.8	1.8	na	470.0	Na	5.5	0.007	0.7	2.8
Baker's yeast	Trace	7.1	16.5	21.0	na	Trace	112.0	4.0	5.0	53.0	na
Spirulina platensis	840.0	44.0	37.0	3.0	7.0	80.0	120.0	na	0.3	0.4	13.0
Aphanizomenon flos-aquae	na	4.8	57.3	11.1	8.0	0.7	na	0.1	0.3	1.0	6.8
Chlorella pyrenoidosa	480.0	10.0	36.0	23.0	na	na	na	240.0	0.15	na	20.0
Scenedesmus quadricauda	554	11.5	27.0	na	1.1	396.0	na	108.0	na	na	46.0

Notes: na = not available; RDI = Recommended Daily Intake for an adult. *Source*: Adapted from Becker, 2004.

thiamine, are also present in certain marine micro-algae and in *Spirulina* spp. (Qasim and Barkati, 1985). Some marine algae, especially brown algae, contain α-tocopherol in significant quantities (Solibami and Kamat, 1985). Some of the lipid-soluble vitamins may be lost during oil extraction, but the spent biomass would be retained, containing proteins and other water-soluble vitamins that can be exploited for feed. Table 7 compares the vitamin composition of a few micro-algae with some common food stuffs and the recommended dietary intakes of vitamins.

MICRO-ALGAE AS SOURCES OF FEED

Micro-algae are commonly used in diets in the aquaculture industry, either as individual diets or as components of mixed diets. Some micro-algae rich in PUFAs, such as *Isochrysis galbana, Pavlova lutheri, Chaetoceros calcitrans* and *Thalassiosira pseudonana,* are used in culture of bivalve molluscs, crustacean larvae, and zooplanktons for crustacean and fish larvae. Many reviews are available on application of micro-algae in aquaculture (Borowitzka, 1997; Renaud, Thinh and Parry, 1999; Brown, 2002; Spolaore *et al.,* 2006).

One of the important applications of micro-algae in aquaculture is associated with its use as fish meal for colouring the flesh of salmonids and for inducing other biological activities. Several investigations have been carried out on the use of algae as additives, alongside fish meal. *Spirulina platensis* has been extensively used in rearing some fish species, including Red sea bream (*Pagrus major)*, Cherry salmon (*Oncorhynchus masou)*, Nibbler (*Girella punctata)*, Striped jack (*Pseudoceranx dentex),* Yellow tail (*Seriola quinqueradiata)* and Mozambique tilapia, to improve weight gain, muscle protein deposition, raw meat quality, flesh texture and taste (FAO, 2009). Supplementation of *Spirogyra* spp. for Carp (*Catla catla*) improved growth, muscle protein and fat content (Kumar,

Gajaria and Radha, 2004). The predominant source of carotenoids for salmonids has been synthetic carotenoids like astaxanthin, which has been used for pigmentation for the last 20 years (Ravishankar *et al.,* 2008).

Natural sources of astaxanthin for commercially raised salmonids include processed crustacean waste from krill, shrimp, crab and crawfish. However, crustacean waste products contain large amounts of moisture, ash and chitin, which limit their use in salmon feed. The efficiency of dietary astaxanthin using micro-algae for flesh pigmentation of Atlantic salmon and rainbow trout has been demonstrated by Torrissen, Hardy and Shearer (1989) and Storebakken (1988). Astaxanthin is even considered as a vitamin for salmon, as it is essential for the proper development and survival of juveniles. Choubert and Heinrich (1993) showed that feeding rainbow trout with algae up to 6 percent of the diet had no major effect on growth or mortality. Thus, the algae were concluded to be a safe and effective source of pigment. Astaxanthin has been used to enhance the immune response of fish and shrimp for maximum survival and growth. Natural micro-algal astaxanthin has shown superior bio-efficacy over the synthetic form. The skin colour of ornamental koi carp fish increased considerably when fed with diet containing astaxanthin enriched *Haematococcus pluvialis* cells at 25 mg/kg in the feed (Kamath, 2007).

Algae can be used in integrated livestock management in manure ponds for growing fish and removing of nutrients, thereby serving the feed requirement and avoiding use of fish meal. The good nutrient profiles of algae, carotenoids and PUFAs can improve fish quality considerably (Phang, 1992).

Micro-algae, with their good nutritional properties, can be exploited as poultry feed. Pigments must be supplemented in diets to enhance pigmentation in poultry meat and eggs (Bortolotti *et al.,* 2003), and *Spirulina,* with its high protein content and carotenoid levels, can be used to enrich yolk colour. Another micro-alga widely used for enriching yolk colour is *Haematococcus pluvialis*. Astaxanthin, with its broad nutraceutical properties, can replace artificial colorants and also improve poultry health and other egg properties. In poultry, astaxanthin has been shown to reduce chick mortality by 50 percent, and to reduce *Vibrio* spp. infections in eggs, thereby improving the nutritional value of eggs (Ravishankar *et al.,* 2008). *Chlorella vulgaris* is rich in lutein and can also be used a feed supplement to improve yolk properties.

The exact composition of algal meal depends on the algal species and the cultivation conditions, and also on the amount of oil that has been extracted. The approximate NPK value (by weight) for algae meal is 8 percent N, 4 percent P and 3 percent K (Oilgae, 2010). The algae meal can be directly used as agricultural fertilizer, as with many seaweeds that have been used as natural fertilizers, but the protein content in the micro-algae is thereby not utilized. Table 8 gives the biomass composition of few micro-algae commonly used in industries. Relatively high protein content of the algal biomass gives an indication of their utility as protein-rich animal feed. Subsequent to the oil extraction, the algae meal can be used as source of proteinacious feed. For example, protein digestibility of *Chlorella* was quite high in animals, up to 56 percent in the case of pigs (Oilgae, 2010). The protein quality is indicated by the amino acid profile and certain indices like chemical score and

TABLE 8
Nutrient composition of some micro-algae (% dry weight)

	Protein	Carbohydrate	Lipids	Nucleic acid
Anabaena cylindrical	43–56	25–30	4–7	na
Aphanizomenon flos-aquae	62	23	3	na
Scenedesmus obliqus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	na	1.9	na
Scenedusmus dimorphus	8–18	21–52	16–40	na
Chlamydomonas rheihardii	48	17	21	na
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	na
Spirogyra sp.	6–20	33–64	11–21	na
Dunaliella bioculata	49	4	8	na
Dunaliella salina	57	32	6	na
Euglena gracilis	39–61	14–18	14–20	na
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculate	52	15	3	na
Porphyridium cruentum	28–39	40–57	9–14	na
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Euglena gracilis	39–61	14–18	14–20	na

Notes: na = Not available. *Source*: Adapted from Becker, 1994.

TABLE 9
Amino acid profile of a few algae compared with some conventional protein sources (g/100 g protein)

Source	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser
Egg	6.6	8.8	7.2	5.3	5.8	4.2	3.2	2.3	1.7	5.0	na	6.2	11.0	12.6	4.2	2.4	4.2	6.9
Soybean	5.3	7.7	5.3	6.4	5.0	3.7	1.3	1.9	1.4	4.0	5.0	7.4	1.3	19	4.5	2.6	5.3	5.8
Chlorella vulgaris	3.2	9.5	7.0	6.4	5.5	2.8	1.3	na	na	5.3	9.4	6.9	9.3	13.7	6.3	2.0	5.0	5.8
Dunaliella bardawil	4.2	11.0	5.8	7.0	5.8	3.7	2.3	1.2	0.7	5.4	7.3	7.3	10.4	12.7	5.5	1.8	3.3	4.6
Spirulina platensis	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	10.3	5.7	2.2	4.2	5.1
Aphanizomenon flos-aquae	2.9	5.2	3.2	3.2	2.5	na	0.7	0.2	0.7	3.3	4.7	3.8	4.7	7.8	2.9	0.9	2.9	2.9

Notes: na = not available. *Source*: Adapted from Becker, 2004.

TABLE 10
Major constituents of four important micro-algae

Component	Algal biomass (% w/w)			
	B. braunii	*Chlorella* sp.	*Scenedesmus* sp.	*Spirulina* sp.
Moisture	5–6	5–8	5–7	5–8
Ash	10–35	8–10	6–8	10–12
Fat	6.9–15	8–12	8–14	2–3
Carbohydrates	9.75	12–16	10–15	15–20
Protein	20.8	40–50	50–55	50–60
Hydrocarbon	5–15	nd	nd	nd
Total chlorophyll	2.6	nd	nd	nd
Total carotenoids	0.7	nd	nd	nd
Phenolics	0.77	nd	nd	nd
Nucleic acids	nd	6–8	4–6	5–7
Fibre	nd	6–8	10–12	5–8

Notes: nd = not determined. *Sources*: Data for *B. braunii* from Sarada, 2007[unpublished], and for *Chlorella* sp., *Scenedesmus* sp. and *Spirulina* sp. from Ravishankar *et al*., 2008

essential amino acid (EAA) index. The amino acid profile of the micro-algae is comparable to that of standard protein sources, like milk or eggs. Table 9 gives the amino acid profile of few micro-algae. The amino acid profile is almost comparable with that of conventional protein sources, with some minor deficiencies in sulphur-containing amino acids such as methionine and cysteine.

Furthermore, micro-algae are good sources of carbohydrates, found in the form of starch, cellulose, sugars and other polysaccharides. The extractable micro-algal polysaccharides can be used as emulsifiers in the food industry. The available carbohydrates have good overall digestibility and are therefore suitable for feed applications. The spent biomass is rich in cellulosic polysaccharides and can also be utilized as a diet ingredient in ruminant feed mix as they have good cellulose digestibility. Addition of algae to the diet of cows resulted in a lower natural breakdown of unsaturated fatty acids and a higher concentration of beneficial compounds in meat and milk. It was observed that sewage-grown algae (supplemented at 5 percent) could replace 25 percent soybean meal used in broiler mash (Becker, 2004). In addition the crude fibre content of the spent algae could be used for therapeutic purposes. Many experiments with supplementation of whole algae biomass, from species such as *Spirulina*, *Scenedesmus* and *Chlorella,* showed hypo-cholesterolemic effect. In *Chlorella* species, an important compound of therapeutic value is β-1,3-glucan, which is immunostimulatory, with blood lipid reducing effects. Efficacy of this compound against gastric ulcers and hypercholesterolemia has also been reported, and there is some antitumour effect (Spolaore *et al*., 2006; Lee, Park and Kims, 2008).

Unconventional food and feed sources contain certain compounds, like nucleic acids, that are sources of purines, when consumed increase plasma uric acid concentrations, which are considered in humans to contribute to gout and uric acid stones in the kidney. The nucleic acid content of micro-algae varies normally between 4 and 6 percent (w/w), while other single-cell protein sources, like yeast and bacteria, are 8–12.5 and 20 percent, respectively (Becker, 2004). Compared with other sources, micro-algae as a source of feed is relatively safe, but it is recommended that intake of nucleic acids should not exceed 2.0 g from unconventional sources, indicating maximum intake of algal biomass not beyond 20 g/day or 0.3 g of algae per kg body weight. Extracts of the hydrocarbon-rich alga *B. braunii* showed significant antioxidant activity, and was non-toxic when whole biomass was supplemented as part of the diet for experimental animals. The antioxidant activity was attributed to carotenoids, especially lutein, which

constitutes 75 percent of the total carotenoid composition (Rao *et al.,* 2006; Dayananda, 2010).

MICRO-ALGAE AS SOURCES OF BIO-ACTIVE MOLECULES

The compounds of micro-algal origin that have found commercial application include fatty acids, steroids, carotenoids, phycocolloids, lectins, mycosporine-like amino acids, halogenated compounds, polyketides and toxins. Algal metabolites have exhibited a wide spectrum of activity, with the majority of them evaluated for properties such as herbicidal activity and cytotoxicity, and antibiotic, antitumour, antiviral, multi-drug resistance reversal and immunosuppressive effects (Burja *et al.,* 2001). Micro-algae have a cholesterol-lowering effect in animals and humans. *Aphanizomenon flos-aquae* also show a hypocholesterolemic effect that stimulates liver function and decreases blood cholesterol level (Vlad *et al.,* 1995).

Phycobiliproteins are one of most abundant proteins in many algae and cyanobacteria. It is used as a natural protein dye in the food and cosmetic industries. The major phycobiliproteins include phycoerythrin, phycocyanin, allophycocyanin and phycoerythrocyanin. Phycobiliprotein derived from *Spirulina* sp. is used as a natural pigment in foods such as chewing gum, dairy products and jellies (Santos *et al.,* 2004). Phycobiliproteins serve as labels for antibodies, receptors and other biological molecules in fluorescence-activated cell sorters, and are used in immunolabelling experiments and fluorescence microscopy for diagnostics (Roman *et al.,* 2002). Pharmacological properties attributed to phycocyanin include antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, antiviral and antitumor activity, treatment in atherosclerosis, lipase activity inhibition, and serum lipid reduction.

Polysaccharides from micro-algae include carbohydrates found in the form of starch, glucose and sugars, with good overall digestibility, making the dried whole micro-algal mass a source for foods or feeds (Becker, 2004). Micro-algal (cyanobacteria and diatoms) extracellular polymeric substances that are polysaccharidic in nature present unique biochemical properties that make them interesting biotechnologically (Singh, Bhushan and Banerjee, 2005). The hydrocarbon-rich micro-alga *Botryococcus* sp. has been exploited for exopolysaccharide production (Bailliez, Largeau and Casadevall, 1985; Dayananda *et al.,* 2007b). Some of these exopolysaccharides have biological activity attributes, such as cytotoxic and anti-tumour properties (Li *et al.,* 2011).

TECHNO-ECONOMIC ANALYSIS OF MICRO-ALGAL BIOMASS PRODUCTION FOR BIOFUELS, AND CO-PRODUCTS

For successful realization and utilization of algal biomass for fuel and feed purpose, it is important to produce algal biomass at costs lower than US$ 1/kg, with a high content of oil for producing biocombustibles, and subsequently utilize the spent biomass for feed purposes. The production cost of micro-algal biomass of *Spirulina* sp. or *Chlorella* sp. is around US$ 4/kg. Strain selection to improve quality for bio-energy and feed use is a crucial determining factor in the economics, with US$ 1/kg as the cost of biomass, and with lipids, carotenoids and other valuable as co-products, it would be economical to utilize the biomass for adoption for bio-refinery purposes.

In large-scale production, availability of water sources and their usage are the major factors determining the production costs, which reach the proportions of large-scale agriculture. Supply of nutrients like N, P and K, and use of commercial fertilizers at the large-scale industry level have potential negative impacts on energy balances. Therefore, use of agricultural and municipal waste streams is one possible option for reducing operational costs and also for achieving positive balance and reducing the carbon footprint. Freshwater-based cultivation is a costly process, so re-use of water and an integrated approach utilizing wastewater or industrial effluents could significantly reduce the cost of production. Further, the economic yield can be improved if the photosynthetic efficiencies of micro-algae can be pushed to achieve the theoretical limit, which is about 11 percent. However, under natural conditions (during summer), the photosynthetic efficiencies are about 2–3 percent, with an average biomass yield of 3.97 g DM/m^2/day (Grobbelaar, 2009; Larkum, 2010). Various options to improve photosynthetic efficiencies are being considered, such as adjusting the frequency of light and dark cycles, development of short-light-path reactors with high turbulence to achieve up to 8 percent photosynthetic efficiencies and biomass yield of about 200 g DM/m^2/day (Grobbelaar, 2009).

Commercialization needs thorough techno-economic modelling and analysis, life-cycle analysis (LCA) and resource assessment (Fishman *et al.,* 2010). LCA is an approach to assess the resource use and environmental impacts of industrial processes, mainly the green house gas emissions and carbon footprint. Yang *et al.* (2011) examined the LCA of biofuel production from micro-algae with respect to water footprint and nutrient balance. They reiterated the necessity of recycling water or using of marine or wastewater for making micro-algae-based biofuel production an economically competitive technology. According to them, to generate 1 kg of biodiesel, about 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate are required if freshwater is used without recycling. Recycling of water after the harvest of biomass, or use of sea or wastewater, decrease water requirement by 90 percent and eliminates nutrient requirements, except for phosphates. Gerbens-Leenes, Hoekstra and Van der Meer (2009) compared the

water footprint of bio-energy from some of the agricultural crops and concluded that the water footprint is high and not competitive enough.

Norsker *et al.* (2011) calculated the micro-algal biomass production costs for three different production systems operating at commercial scale: open ponds, horizontal tubular photobioreactors and flat-panel photobioreactors. The resulting biomass production costs for these systems were € 4.95, € 4.15 and € 5.96 per kilogram, respectively. The parameters included for the costing were irradiation conditions, mixing, photosynthetic efficiency of systems, medium, carbon dioxide costs and dewatering. Optimizing production with respect to these factors resulted in a price of € 0.68/kg. They conclude that at this cost, micro-agal-based biofuel production is promising and competitive. Lundquist *et al.* (2010) thoroughly assessed the technical and engineering systems involved in biofuel production from micro-algae. The biomass production systems considered are those for biofuel production or for wastewater treatment, with outputs as biogas, or biogas and oil, and a farm size of 100 or 400 ha. The major technical assumptions were 25 percent recoverable lipid content, biomass yield of 22 g DM/m^2/day (80 t/ha/year) and a resulting oil yield of 20 000 L/ha/year. CO_2 was supplied from a flue gas source. According to their report, the overall production costs for biofuel production as a co-product of wastewater treatment was of high economic feasibility, at US$ 28/barrel of oil or US$ 0.17/kwh electricity produced through biogas generation. However, in the case of biofuel or biogas production as the major objective based on wastewater treatment, the system was very costly, at US$ 332/barrel of oil and US$ 0.72/kwh electricity generated through biogas. Their recommendations include use of bio-flocculation for harvesting biomass, online extraction of oil from wet biomass, and emulsification of oil, which together would reduce operating costs by 20–25 percent, avoiding the steps of drying and extraction.

BIOREFINERY APPROACH IN MICRO-ALGAL UTILIZATION

A bio refinery is an integrated approach to biomass conversion processes to produce fuels, power and value-added chemicals from biomass. The biorefinery is analogous to today's petroleum refinery, which produces multiple fuels and products. The first step in the biorefinery concept is cultivation of micro-algae, with limited inputs and avoiding use of nutrient chemicals like fertilizers. If the production systems are intended for biofuel production, nutrient-rich sources of wastewater can be utilized. This system is advantageous in terms of the natural treatment of the wastewater, which could be recycled for algal cultivation or used for irrigation. Industrial effluents like distillery wastes or sewage ponds are good nutrient sources. Since micro-algae are better CO_2 sinks than higher plants, flue gases from industry can be used as a source of CO_2. Alternatively, if biomass production systems involve generation of valuable co-products, then cultivation systems utilizing marine ecosystems like coastal and estuarine areas are more economical in operational terms. Solar generated power can be effectively utilized in operating raceway ponds and pumping the culture for further downstream processing. The spent media can be utilized as a source of exopolysaccharides that have many potential bio-active properties. Lipid-rich diatoms like *Cylindrotheca closterium, Thalassiosira pseudonana* and *Skeletonema costatum* produce extracellular polysaccharides that can be harvested for further applications (Urbani *et al.,* 2005; Li *et al.,* 2011), and also recycled for algal cultivation.

There are different ways of realizing economic value from micro-algal spent biomass after oil extraction. The best option would be to achieve complete utilization of the biomass for maximum energy recovery by various conversion technologies, such as biomass gasification and thermochemical processes that generate syngas, which can be combusted or can be converted to chemicals like alcohols, ethers, etc. Pyrolysis can be employed where an oil-like liquid is produced that can be processed to fuels. Anaerobic fermentation of spent biomass yields methane, and power generation from these co-processes enhances the sustainability of micro-algal derived biofuels.

The second option is utilization of glycerol generated from the trans-esterification process of conversion of crude algal lipids to biodiesel. Glycerol can be used as feed for generation of biomass. Pyle, Garcia and Wen (2008) used a biodiesel-derived crude glycerol as a source of carbon for heterotrophic production of DHA by *Schizochytrium limacinum,* with comparable yields to other feeds. Glycerol is a highly reduced carbon source that can be converted to many industrially important compounds, including 1,3-propanediol, dihydroxyacetone, succinic acid, propionic acid, ethanol, citric acid, pigments, polyhydroxyalconate, squalene and biosurfactants by use of bacterial genera like *Klebsiella*, *Citrobacter*, *Enterobacter*, *Clostridium*, *Propionibacterium*, *Anaerobiospirillum* and *Escherichia.* (Yazdani and Gonzalez, 2007; Silva, Mack and Contiero, 2009).

Another option is recovery of polysaccharides and proteins from mono-algal cultures grown in clean environments for use as animal feeds. Animal feeds can use spent biomass with low lipids but with high content of micronutrients such as minerals, anti-oxidants, proteins and vitamins, in supplementation of rations for fish, poultry and other livestock. Some enhancement of the spent biomass might be required for greater efficacy as feed supplement. The residual biomass could be used as soil

fertilizer and conditioner. Thus production and utilization of algal biomass with net energy gain in the process without waste generation would be desirable for the sustainable exploitation of the technology for energy needs.

Several companies worldwide are working on algae-based biofuels. Though cost effective, viable technology has not yet been developed, the approaches suggested in this review for utilizing spent biomass for feed and all other different fractions in a biorefinery manner would go a long way in making the process economic.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

- There is a continuing need to screen the vast biodiversity of micro-organisms to identify high yielding strains.
- Further genetic improvement of potential strains for achieving higher biomass and metabolite yield and subsequent scale-up..
- It is important to devise culture methodologies based on the nature of the alga.
- Efficient utilization of seawater, wastewater, flue gases and various carbon dioxide sources for enhanced production of biomass.
- Utilization of wind and solar power for culture agitation and harvesting.
- Drying of biomass employing solar drying systems.
- Achieving the least energy losses for net energy gain.
- Developing bio-refinery approaches with minimal energy inputs and effective utilization of all by-products and co-products.
- To develop integrated systems of feed and food production for use of biomass in a meaningful manner.
- Algal biomass derived feeds and feed supplements need to be developed for augmenting animal products, aquaculture and the poultry industry, thus adding value to existing technologies.
- Utilization of marginal land for algal biomass cultivation.
- Exploring setting up of production plants at the sea surface or in coastal areas.

Table 11 lists some of the potential process developments for utilization coupled to integration of technology with renewable energy inputs for net energy gain.

CONCLUSIONS

The energy demands of the world coupled with deteriorating environmental conditions have highlighted the need for eco-friendly measures for sustainable solutions. In this regard, algal biotechnology utilizing the vast biodiversity available for production of bio-energy molecules is already recognized as a promising area for meeting the dual demands for energy and respect for the environment. Having realized the importance of photosynthetic carbon fixation for the production of energy-rich molecules, the development of technologies to produce biomass on a massive scale would need research and developmental inputs for evaluating viable technology alternatives. The identification of algal forms, their cultivation and utilization would go a long way to realize the desired objectives. Approaches to achieving net energy gain in a sustainable and eco-friendly manner need to be developed and adopted. The current trends and future prospects touched on in this review could provide directions for advances in effective utilization of algal biotechnology for fuel, food, feed and chemicals.

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TABLE 11
Process innovation and renewable energy utilization for net energy gain and utilization of co-products

Process steps	Adoption of innovative measures and use of renewable energy
Employing organisms with high growth rate in addition to high product or metabolite production	Responsiveness to environmental conditions such as stress factors for increase yields needs to be explored.
Adoption of open bioreactors	Media optimization, culture conditions.
Mixing of culture	Windmill driven.
Harvesting of the culture	Through sedimentation. Adoption of proper gradient for separation of the biomass.
Recycling of the water back to the inoculum ponds.	Windmill driven.
Adoption of closed bioreactors	Use of solar radiation for providing the light source and mixing of culture through windmill-driven peristaltic pumps,
Drying of algal biomass	Solar drying system.
Extraction of constituents (lipids and hydrocarbons) and utilization of spent biomass	Biorefinery approach.

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