Chapter 17

Camelina sativa in poultry diets: opportunities and challenges

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ABSTRACT

Feed represents over 65 percent of the cost for poultry production. Fast-growing and high-producing poultry are fed high calorie and high protein maize-soybean-based diets. Considering the high demand for maize and other oil crops for biofuel production, finding alternative sources of energy could reduce production costs. *Camelina sativa* is an oilseed crop of the Brassica family that is emerging as an important biofuel crop. Nutrient composition of camelina meal indicates that the meal has 36–40 percent crude protein, 11–12 percent fat, and 4600 Kcal/kg gross energy. The fat in camelina meal is rich in α-linolenic acid (~30 percent), the parent fatty acid of the health-promoting omega-3 family, and γ-tocopherol, an antioxidant vitamin. In addition, camelina contains other bio-active compounds such as flavonoids and phenolic products. Therefore, incorporating camelina in poultry diets will: (1) provide energy and protein to the birds, (2) provide health-promoting omega-3 fatty acids and tocopherol-rich-foods to humans, (3) improve the antioxidant activity and lipid stability of poultry products, and (4) increase the market value of the crop. Feeding trials aimed at evaluating the optimum amounts of camelina meal in feed for meat-type broilers and egg laying hens were conducted. Special emphasis was given to omega-3 fatty acid and tocopherol incorporation in meat and eggs, and thus developing value-added functional poultry foods. The results obtained were: (1) camelina meal could be incorporated into broiler and layer rations at 10 percent without affecting bird performance and meat or egg quality; (2) feeding camelina meal led to over 3-fold increase in omega-3 fatty acids in chicken meat and 8-fold increase in eggs; (3) incorporation of 10 percent camelina meal led to 2.5- to 3.2-fold reduction in the omega-6:omega-3 ratio in meat and eggs; and (4) inclusion of camelina meal at 5 and 10 percent led to significant reduction in lipid oxidation products and an enhancement in γ-tocopherol and antioxidant activity in the dark meat. Investigating factors that can enhance the feeding value as well as the health-promoting and antioxidant properties of camelina will provide greater potential for developing camelina-based functional feeds and value-added wholesome poultry foods for human consumption.

INTRODUCTION

Camelina sativa or false flax ("gold of pleasure") is an oilseed crop of the Brassica (Cruciferae) family. The crop can be grown on marginal farmland, with relatively low inputs and no irrigation. Although camelina has been cultivated in Europe for over 2000 years for oil and livestock fodder, the crop has gained increased popularity recently as a biofuel source due to its oil content (Putnam *et al.*, 1993). Camelina is not a food crop; however, co-product (i.e. meal) obtained after oil extraction from the seed is valuable as animal feed (Pilgeram *et al.*, 2007). To use camelina meal as a potential animal feed requires information on its chemical composition, nutritive value, digestibility and product quality aspects. In this context, studies on using camelina in the diet of beef heifers (Moriel *et al.*, 2011), dairy cows (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau *et al.*, 2011) and ewes (Szumacher-Strabel *et al.*, 2011) have been reported. In the current chapter, opportunities and challenges associated with using camelina meal in the diets of meat-type broilers and egg-laying birds are discussed.

CAMELINA SATIVA MEAL: CHEMICAL COMPOSITION AND NUTRITIONAL VALUE

Camelina sativa is an oilseed producing plant. Camelina seeds contain over 38.9 percent fat, 30 percent α-linolenic acid (18:3 n-3) (an omega-3 fatty acid), and 25.8 percent crude protein. Due to the high oil content, omega-3 fatty acid and crude protein, finding alternative use of camelina meal (a co-product obtained from camelina seed after oil extraction) in animal diets will increase the market value of the crop. The nutrient profile of camelina meal is shown in Table 1. Cold-pressed camelina meal contains 35–40 percent crude protein, 6–12 percent fat, 6–7 percent ash, and 41 percent neutral-detergent fibre. The gross energy is 4600–4800 kcal/kg. The oil content, fatty acid composi-

MAIN MESSAGES

- **Camelina meal is rich in protein, fat and essential n-3 and n-6 fatty acids, and could be incorporated into poultry rations as a source of energy, protein and essential n-3 and n-6 fatty acids.**
- **Feeding camelina meal up to 10 percent of the diet did not affect growth performance and feed consumption, nor meat and egg quality.**
- **Feeding 10 percent camelina meal led to increases in health-promoting omega-3 fatty acids of over 3-fold in chicken meat and 8-fold eggs.**
- **Camelina meal at 10 percent led to significant reduction in lipid oxidation products and an improvement in antioxidant activity in the dark meat.**
- **Consuming two large eggs from hens fed 10 percent camelina meal could provide over 300 mg omega-3 fatty acids to the average human diet.**

tion and other nutrient profiles of the meal can vary due to cultivar, season, processing method and other agronomic factors (e.g. soil type).

The fatty acid composition of the camelina meal has received considerable attention due to its high content of essential fatty acids. The meal is rich in omega-3 and omega-6 essential fatty acids (also known as n-3 and n-6 fatty acids). The omega-6:omega-3 fatty acid ratio is 0.90 to 0.70. α-Linolenic acid (18:3 n-3) is the major omega-3 fatty acid, constituting over 29 percent, with linoleic acid (18:2 n-6) constituting up to 23 percent. Oleic acid is the major mono-unsaturated fatty acid, followed by eicosenoic acid (20:1). Other mono-unsaturated fatty acids include palmitoleic (16:1) and erucic acids (22:1, <2 percent). Altogether, total mono-unsaturated fatty acid constitutes over 32 percent. Saturated fatty acids in the meal include palmitic acid (16:0, 9 percent) and stearic acid (18:0, 2.5 percent). Fat-soluble vitamin (tocopherols) content of the meal is over 200 µg/g. The protein of camelina meal contains several essential amino acids, such as threonine, glycine, methionine, valine, isoleucine, leucine, lysine and phenylalanine. Lysine and methionine are usually the first-limiting acids in poultry nutrition, which makes camelina meal a potential source of protein for poultry. Among the minerals in camelina meal, potassium is the major mineral, followed by sulphur, phosphorus, magnesium and calcium. Camelina meal also contains other phenolic compounds and glucosinolates. The high protein, energy, omega-3 and omega-6 fatty acid and essential amino acid content of camelina meal makes it a potentially suitable source of plant protein, essential fatty acids and amino acids for use in poultry rations.

FEEDING CAMELINA MEAL TO POULTRY

Feed represents the major cost for food animal production. Therefore, development of non-traditional, low-cost feed sources may reduce production costs. High producing and fast growing poultry (egg layers and meat-type) are fed high calorie (2800–3200 kcal/kg/day) and high protein diets (16–22 percent crude protein). In the United States, poultry diets are based on maize and soybean meal. Considering the high demand for maize and other oil crops for biofuel production, there has been great interest in finding alternative sources of energy and protein to reduce poultry production costs. In this respect, camelina meal has attracted attention from nutritionists due to its gross energy and crude protein content. Recently, studies were conducted with meat-type broiler chickens, egg layers or turkeys on feeding camelina meal, and results are summarized in Table 2. These studies were aimed at (1) finding the optimum levels that can be incorporated into the ration without affecting production performances, (2) assessing product quality, and (3) testing the efficacy of camelina meal in enriching the meat or eggs with omega-3 fatty acids.

Effect on production performance of feeding *Camelina sativa* meal to broiler chickens

The effect of feeding camelina meal to broiler birds led to conflicting results in growth performance (Table 2). Aziza, Quezada and Cherian (2010a) fed 2.5, 5 and 10 percent camelina meal to broiler birds and these authors reported no difference in 42-day body weight gain, carcass weight or feed efficiency (gain:weight) when compared with maize-soybean-based control diet-fed birds. However, Ryhanen *et al*. (2007) and Pekel *et al*. (2009) reported that feeding 10 percent camelina meal or expeller cake to broiler chickens impaired the growth between 15 and 37 days of age, decreased feed intake during the starter phase, impaired feed efficiency and reduced the final body weight by 7–10 percent compared with the control group. Studies on feeding camelina meal to turkey hens by Frame, and Petersen (2007) reported no significant differences in final weight, weight gain or feed conversion when 10 percent camelina meal was included in the diet from 9 weeks through to 13.5 weeks of age. The discrepancies in growth performance of birds fed diets containing camelina meal

TABLE 1
The nutrient profile of camelina meal

Parameter	Value
Gross energy	4755 kcal/kg
Crude protein	36.2 %
Crude fibre	8.4 %
Ash	6.5 %
Neutral-detergent fibre	41.8 %
α-Tocopherol	5.2 µg/g
γ- Tocopherol	201.7 µg/g
Phenolics	4006 µg/g
Flavonoids	21.2 mg/g
Glucosinolates	21.4 µmol/g
Minerals	**ppm**
P	10 214
K	13 204
Ca	2 703
Mg	4 696
S	9 122
Na	15.4
Fe	151
Mn	25.1
Zn	61.1
Cu	9.18
Al	5.37
Amino Acid	**%**
Aspartic acid	2.84
Threonine	1.34
Serine	1.36
Glutamic acid	5.50
Glycine	1.82
Alanine	1.60
Valine	1.89
Isolecucine	1.38
Leucine	2.32
Tyrosine	0.82
Phenylalanine	1.47
Lysine	1.77
Histidine	0.84
Arginine	2.16
Tryptophan	0.43
Methionine	0.92
Cystine	0.95
Fatty Acid	**%**
Palmitic (16:0)	8.29
Palmitoleic (16:1)	0.25
Stearic (18:0)	2.38
Oleic acid (18:1)	20.33
Linoleic (18:2n-6)	23.87
α-Linolenic (18:3n-3)	29.48
Eicosenoic (20:1)	10.67
Eicosadienoic (20:2n-6)	1.52
Eicosatrienoic (20:3n-6)	1.05
Erucic (22:1)	1.75

Notes: Values are indicative, subject to variation due to differences in batch, cultivar, soil type or processing method used. Amino acid values are expressed as g per 100 g sample (as-is basis). Fatty acid values are reported as percent of fatty acid methyl esters.
Sources: Adapted from Aziza, Quezada and Cherian, 2010a, b; Cherian, Campbell and Parker, 2009.

could be due to the availability of nutrients in the meal. Camelina belongs to the Brassica family, which is high in non-starch polysaccharides and glucosinolates that can affect feed consumption and growth performance of broiler chickens (Budin, Breene and Putnam, 1995). In addition, phenolic compounds such as phenolic acids and tannins that are present in the Brassica family, soil type, bird age and meal preparation methods can affect digestibility, leading to discrepancies in reported results. Pekel *et al*. (2009) reported that addition of copper (150 mg/kg) enhanced feed consumption and body weight of birds fed camelina meal. The beneficial effect of Cu may be due to its ability to alleviate the negative effects of glucosinolates present in the meal. Although glucosinolates themselves show no toxic effects on animals, the breakdown products of glucosinolates can form toxins by the endogenous plant enzyme myrosinase or can influence gut microflora, affecting growth and feed efficiency (Schuster and Friedt, 1998). These factors should be taken into consideration when evaluating results from feeding camelina meal to broiler birds.

Effect on production performance of feeding *Camelina sativa* meal to egg laying hens

Oil seeds and oilseed meals are incorporated into laying hen rations as a source of energy, crude protein and essential omega-3 fatty acids. In this respect, much work on feeding flax seed to laying birds for omega-3 egg production has been well documented (Cherian, 2008). Typically, oil seeds or their meals are restricted to less than 10 percent of the rations (Bean and Leeson, 2003). Lipid quantity and type of fatty acids in oil seeds in the laying hen diet can significantly affect the content of fatty acids, fat soluble vitamins and pigments in the egg yolk. The alteration of egg lipid nutrient profile is due to the fact that chickens are monogastric (single stomach) animals and that there is a high turnover of lipids in laying hens, causing egg lipid to mirror dietary fats. This has led to the successful production and marketing of omega-3 fatty acid- and vitamin-modified specialty eggs worldwide (Cherian, 2009). Considering the high content of α-linolenic acid in camelina meal, studies were conducted to test the efficacy of the meal in enriching eggs with omega-3 fatty acids. Feeding trials conducted in our laboratory showed that inclusion of camelina meal at over 10 percent of the ration can affect egg production, feed consumption and egg yolk weight. When the meal was included at 5, 10 and 15 percent of the ration, it was observed that hen-day egg production ([total number of eggs produced/total number of hens × number of days on test diet] × 100), was lowest for the 15 percent inclusion level (Cherian, Campbell and Parker, 2009) (Table 2). Yolk weight as a percentage of egg weight was lower for the 10 and 15 percent inclusion levels. However, decrease in

TABLE 2
Summary of studies investigating the effect of camelina meal in poultry

References	Bird type	Salient findings
Acamovic *et al.*, 1999.	Broiler	Nutritional evaluation of the meal done in broiler birds by precision method (tube feeding) reported reduced digestibility coefficient for nitrogen and dry matter.
Aziza, Quezada and Cherian, 2010a.	Broiler	Feeding camelina meal at 10% led to over 2.5-fold increases in omega-3 fatty acid of white and dark meat. No difference in final body weight at 10% inclusion.
Aziza, Quezada and Cherian, 2010b.	Broiler	Feeding 10% camelina meal led to: (1) a >1.5-fold increase in γ-tocopherols in the thigh meat; (2) increase in thigh meat antioxidant activity; and (3) reductions in thiobarbituric acid reactive substances in the meat during storage and cooking.
Cherian, Campbell and Parker, 2009.	Layer	Feeding camelina meal at 10% led to over 8-fold increase in omega-3 egg fatty acids. Camelina meal at low levels (5 and 10%) did not lead to any changes in egg production or egg quality. Addition of camelina at higher levels (15%) led to reductions in egg production, yolk fat and yolk size. No effect on egg weight.
Frame and Petersen, 2007.	Turkey	Camelina meal could be included in turkey diets up to 5%.
Pekel *et al.*, 2009.	Broiler	10% camelina meal reduced body weight when fed during the first 3 weeks of life.
Pilgeram *et al.*, 2007.	Layer	Fed 15% camelina meal containing diets to layer birds. No adverse effect on bird health or egg production. Over 140 mg of α-linolenic acid reported in eggs.
Rokka *et al.,* 2002.	Layer	No effect of feeding camelina seed oil on the sensory attributes of chicken eggs.
Ryhanen *et al.*, 2007.	Broiler	Inclusion of 5 or 10% *Camelina sativa* expeller cake reduced feed intake and feed conversion ratio during starter phase. Feeding the meal had no effect on meat sensory quality aspects.

TABLE 3
The systematic and trivial names of different omega-6 and omega-3 fatty acids in white meat and egg and their shorthand notation and concentrations

Systematic name	Common name	Shorthand notation	Content (%)	
			White Meat	Egg
Omega-6 Fatty Acids				
all-cis-9,12-octadecadienoic	Linoleic acid	18:2 n-6	17.5	9.3
all-cis-6,9,12-octadecatrienoic	γ-Linolenic acid	18:3 n-6	0.7	0.1
all-cis-8,11,14-eicosatrienoic	Dihomo-γ-linolenic acid	20:3 n-6	0.9	0.1
all-cis-5,8,11,14-eicosatetraenoic	Arachidonic acid	20:4 n-6	3.2	1.5
all-cis-7,10,13,16-docosatetraenoic	Adrenic acid	22:4 n-6	0.7	0.1
all-cis-7,10,13,16,19-docosapentaenoic	Docosapentaenoic acid	22:5 n-6	0.3	0.2
Omega-3 Fatty Acids				
all-cis-9,12,15-octadecatrienoic	α-Linolenic acid	18:3 n-3	1.1	0.2
all-cis-5,8,11,14,17-eicosapentaenoic	Eicosapentaenoic acid	20:5 n-3	0.2	0.0
all-cis-7,10,13,16,19-docosapentaenoic	Docosapentaenoic acid	22:5 n-3	0.6	0.1
all-cis-4,7,10,13,16,19-docosahexaenoic	Docosahexaenoic acid	22:6 n-3	0.7	0.6

Notes: Values are reported as a percentage of fatty acid methyl esters and are subject to variation reflecting bird diet, age or strain.

yolk weight was not associated with egg weight. No difference was found in egg weight, albumen weight, albumen height, shell weight or shell thickness due to camelina meal feeding at any level. Minimal effects were noted to yolk colour due to feeding camelina meal to laying hens.

Effect on meat and egg lipid composition of feeding *Camelina sativa* meal

Birds have a high capacity for lipid biosynthesis and most of the accumulated fat is of dietary origin. Lipids constitute over 30 percent in egg and 10 percent in broiler carcass. Fatty acids are the major components of egg and meat lipids. Among the different fatty acids present in animal products, omega-3 fatty acids have received considerable attention in the past decade due to their several health-promoting effects (Barceló-Coblijn and Murphy, 2009; Palmquist, 2009). Some of the common omega-6 and omega-3 fatty acids present in chicken meat and egg, their systematic and trivial names and concentrations are shown in Table 3. It should be noted that the concentrations of fatty acids are highly dependant on the dietary lipid source.

Effect on changes in meat fatty acid composition of feeding *Camelina sativa* meal

The use of feeds containing omega-3 fatty acids in poultry diets provides a straightforward and well-adapted, successful approach to fortifying poultry food lipids with health promoting omega-3 fatty acids (Cherian, 2002, 2008). In this respect camelina meal has attained interest due to its high (>29 percent) α-linolenic acid content (Table 1). One of the major goals of feeding camelina meal to broiler birds is to test its efficacy in enriching meat with α-linolenic acid and other long-chain omega-3 fatty acids. Studies in our laboratory investigated changes in white (breast) and dark (thigh) meat lipid charac-

TABLE 4
Omega-3 fatty acid content and Omega-6:Omega-3 ratio in white and dark meat from birds fed diets containing 10 percent camelina meal

Diet	α-Linolenic (18:3) (mg/100 g)	Total long chain omega-3 (mg/100 g)	Total omega-3 (mg/100 g)	Omega-6: Omega-3
Camelina Meal				
Dark Meat	0.56	0.32	0.88	7.54
White Meat	0.26	0.19	0.45	6.84
Control Diet				
Dark Meat	0.22	0.07	0.29	21.45
White Meat	0.05	0.09	0.14	17.28

Notes: Adapted from Aziza, Quezada and Cherian, 2010a. Control is maize-soybean meal basal diet.

teristics of birds fed different levels of camelina meal. In birds fed diets containing 10 percent camelina meal, 2- and 3-fold or greater increases in α-linolenic acid were observed. In addition to α-linolenic acid, other 20- and 22-carbon omega-3 fatty acids, such as eicosapentaenoic (20:5 n-3), docosapentaenoic (22:5 n-3) and docosahexaenoic (22:6 n-3) acids, were also enhanced upon feeding camelina meal. The total omega-3 fatty acids (>18C) in the dark and white meat was 2- to 2.5-fold greater than from birds fed a maize-soybean-based control diet (Aziza, Quezada and Cherian, 2010a). The incorporation of 10 percent camelina meal led to 2.5- to 2.8-fold reduction in the omega-6:omega-3 ratio. The total omega-3 fatty acid content and omega-6:omega-3 ratio in the dark and white meat from birds fed 10 percent camelina meal are shown in Table 4.

Thus, consuming a 100 g portion of dark or white meat from birds fed 10 percent camelina meal could provide 0.88 and 0.45 mg/100 g of omega-3 fatty acids when compared with 0.29 and 0.14 mg/100g from birds fed a maize-soybean-based diet. Traditionally, flaxseed has been the major terrestrial source of omega-3 fatty acid used in animal feeding for omega-3 fatty acid enrichment purposes (Gonzalez and Leeson, 2001; Cherian, 2008). Feeding broilers diets with 10 percent flaxseed could provide more than 300 to 500 mg of n-3 fatty acids in 100-g servings of dark meat (Cherian, 2008).

Effect on egg total fat and fatty acids of feeding *Camelina sativa* meal

Feeding high levels of camelina meal (>10 percent) led to over 6 percent reduction in egg total fat content (Cherian, Campbell and Parker, 2009). Feeding 10 percent camelina meal led to an 8-fold increase in total omega-3 fatty acids, a 10-fold increase in α-linolenic acid and an 8.5-fold increase in docosahexaenoic acid compared to control birds fed a maize-soybean diet (Cherian, Campbell and Parker, 2009). The total omega-3 fatty acid content in eggs was 0.3 vs 2.69 for eggs from birds fed a control or 10 percent camelina meal diet. The omega-6:omega-3 fatty acid ratio was 14.8 vs 4.6 for eggs from birds fed the control diet and 10 percent camelina meal. Thus, consuming two large eggs from hens fed camelina meal could provide over 300 mg omega-3 fatty acids to the human diet.

Effect on meat and egg oxidative stability and quality aspects of feeding *Camelina sativa* meal

The oxidative stability of food lipids is inversely related to the degree of unsaturation or number of double bonds present in the carbon chain. Introduction of a double bond in the carbon chain introduces a kink in the molecule and changes the biochemical reactivity of the fatty acid, ultimately affecting food lipid quality. Thus, highly unsaturated structures in the food lipid matrix are less stable because unsaturated fatty acids will favour the abstraction of a hydrogen atom and will initiate the oxidation process. In addition, factors such as total lipid content, types and amount of iron present, reducing compounds (e.g. ascorbic acid), concentration of natural antioxidants (e.g. carnosine, anserine and tocopherol), antioxidant enzymes (catalase, superoxide dismutase) and others (oxygen, heating, cooking, salt, temperature, storage) can also affect oxidative stability and meat quality aspects (Min and Ahn, 2005). Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde which is a reactive aldehyde and forms lipid oxidation products. Malondialdehyde and other "thiobarbituric reactive substances" condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative that can be assayed spectrophotometrically and is commonly used to measure lipid oxidation products in food lipids.

Limited studies have been reported on meat and egg oxidative stability and quality upon including camelina meal in broiler diets. In a recent study, when camelina meal was incorporated at either 5 or 10 percent in broiler diets, 49 and 36 percent reductions in thiobarbituric reactive substances were observed during short-term (2 day) and long-term (90 day) storage, respectively, in the dark meat (Aziza, Quezada and Cherian, 2010b). Similarly, upon cooking, thiobarbituric reactive substances were reduced over 48 percent in dark meat from birds fed a 10 percent camelina meal diet compared with birds fed the control diet (Aziza, Quezada and Cherian, 2010b). The improvement

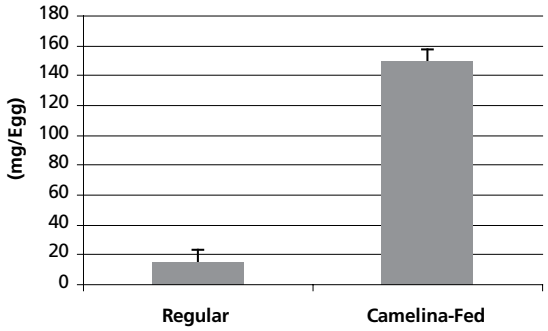
in meat stability may be due to the tocopherols and other flavonoids supplied through the diet. This is justified by the 1.7-fold increase in γ-tocopherols and antioxidant activity in the thigh meat of camelina meal-fed birds (Aziza, Quezada and Cherian, 2010b). While assessing sensory qualities of meat from birds fed camelina meal, Ryhanen *et al.* (2007) reported that inclusion of meal had no adverse effect on meat taste, juiciness or tenderness. Therefore, inclusion of camelina meal rich in bio-active compounds may prove to be beneficial for providing omega-3 fatty acids while reducing oxidative stress associated with omega-3 poly-unsaturated fatty acid enrichment. However, the observed beneficial effect of thiobarbituric reactive substances noted in meat was not observed in eggs from hens fed >10 percent camelina meal. Very few studies have reported sensory aspects of eggs from hens fed camelina. Rokka *et al*. (2002) fed camelina seed oil to hens and these researchers reported that inclusion of camelina oil had no effect on the sensory attributes of chicken eggs.

Using *Camelina sativa* to increase human supply of functional nutrients

Major advancement has been made in the past two decades in our understanding of the mechanisms whereby diet can influence health. As a result, functional nutrients have been introduced as a new concept for nutrients that provide health benefits beyond basic nutrition. The health-promoting effects of such nutrients have led to the development of "functional foods" or "nutraceuticals". Increased awareness of such "functional foods" has led consumer to seeking these nutrients from food or supplements. Among the different nutrients, several animal food lipid components (e.g. omega-3 fatty acids, fat-soluble vitamins and pigments, conjugated linoleic acid, antioxidants, phospholipids) have been widely researched. As animal food lipids contribute a major portion of fat in the western diet, much work has been done to enrich poultry food lipid portions (egg, white and dark meat) with n-3 fatty acids (Rymer and Givens, 2005; Cherian, 2009). Table 4 and Figure 1 show the omega-3 content of meat and eggs from hens fed camelina meal.

Consuming 1 egg could provide over 140 mg of omega-3 fatty acids and 100 g of thigh meat could provide 0.9 mg of omega-3 fatty acids. In addition, the meal could also enrich food products with tocopherols and other phenolic compounds (Aziza, Quezada and Cherian, 2010b). Feeding flax to broiler birds is associated with negative effects on performance (Ajuyah *et al*., 1991; Gonzalez and Leeson, 2001). Flax is also approximately twice the price of wheat and maize. Therefore, for reducing feeding costs while increasing omega-3 fatty acids and other functional nutrients in animal food lipids without affecting bird growth, use of biofuel-based co-products should be investigated.

FIGURE 1
Total omega-3 in eggs from hens fed camelina meal



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

DEVELOPING *CAMELINA SATIVA* AS A FUNCTIONAL FEED: CHALLENGES

The nutritional value of feedstuffs is largely determined by their content of available nutrients. To use camelina meal effectively in poultry feed, further information is needed on its metabolizable energy, and protein and amino acid digestibility and availability in different age groups and strains of meat- or egg-type birds. Accurate determination of dietary amino acid digestibility and availability is essential for balancing feed for optimum growth as well as limiting N excretion to the environment. The effects of processing or use of enzymes in enhancing nutrient digestibility need to be investigated. Such research may provide answers to the reduction in growth observed in young birds fed camelina meal. In addition, effects of the meal on product organoleptic quality during storage and cooking, along with long-term effects on bird health aspects, need to be further investigated. Camelina varieties typically have low levels of glucosinolates (20–24 µmol/kg) and erucic acid (22:1) (<2 percent) compared with other mustard species. Investigating factors that can enhance the nutritive value as well as the health-promoting effects of camelina will provide greater potential for developing camelina-based functional feeds and value-added poultry foods for human consumption. Such results may also lead to gaining approval for unrestricted use of camelina meal as a feed ingredient.

CONCLUSIONS

Consumer demand for animal protein has made poultry production one of the fastest growing livestock industries around the world. Livestock feeds are potentially the highest value applications for camelina meal. Being a new co-product, farmers and researchers have not established optimal use of camelina meal and many questions remain about how to best use it effectively. Efforts to minimize nutrient variation among cultivars and processing methods may help to produce a stable feed product, and will help

in comparing results obtained from different locations and laboratories. Feed represents over 65 percent of the cost of poultry production. Using co-products from biofuel production, such as camelina meal, can reduce feed cost while promoting environmental equilibrium and sustainability. Studies conducted on feeding camelina meal to broilers and egg laying hens show that the meal can be included in broiler and layer diets up to 10 percent without compromising bird performance, while potentially increasing the omega-3 fatty acid content 3-fold in the meat and 8-fold in eggs. In addition, dietary camelina meal at 10 percent led to significant reduction in lipid oxidation products and an improvement in γ-tocopherol content and antioxidant activity in the dark meat. The increase in omega-3 fatty acids and tocopherols of eggs and meat will be beneficial to human nutrition as poultry products are the major source of animal protein around the world. The research results obtained will increase the market value of the crop because meal is the by-product of oil extraction and accounts for 70 to 80 percent of the oilseed harvest. Therefore, finding the optimum level of camelina meal in poultry diets without affecting bird performance, health, product quality and sensory characteristics will reduce food production cost while achieving greater independence of food supply.

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Chapter 18

Utilization of lipid co-products of the biofuel industry in livestock feed

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ABSTRACT

Biofuels, which are new and renewable alternative fuels of biological-origin, have been receiving more attention globally due to energy needs and environmental consciousness. The biofuels industry owes its feasibility not only to high petroleum prices and governmental support, but also to the added-value co-products suitable for use as animal feed and supplements.

The objective of this review chapter is to collate and describe the lipid co-products derived from the biofuel industry and to further discuss their potential role as feeds or supplements in the diet of ruminants, their effects on animal performance and public health, and possible risks.

INTRODUCTION TO BIOFUELS

The oil crisis and the constant search for environmentally friendly, acceptable and relatively safe alternatives for fossil fuels have encouraged mass production of biofuels in a number of countries. The growing concern over greenhouse gas (GHG) emissions, as well as fluctuations in gasoline and diesel fuel prices, has prompted several governments to encourage the introduction of renewable biofuels into the market. Promotional measures, such as economic and local regulations, subsidies, tax exemptions and penalization of all fuel not including the required amount of biofuel fixed by law, have been implemented in order to foster the development of the biofuel industries (Behzadi and Farid, 2007: Bloch *et al.,* 2008; Colibar, Korodi and Popovici, 2010).

In addition to the reduction of hazardous pollutants emitted from the transportation sector, increased rural development and national energy security are other important outcomes that benefit the communities. The increment in intellectual and profitable management of unutilized energy sources that potentially exist in urban and agricultural waste fractions, to produce the so called second-generation biofuels, is another advantage influenced by the economic incentives (Najafi *et al*., 2009; Willson, Wiesman and Brenner, 2010).

Currently, the two main types of first-generation biofuels produced and commercialized around the world are biodiesel and bio-ethanol, both derived from plant parts and generally recognized as clean sources. Biodiesel is obtained by the esterification of vegetable oils to alkyl mono-esters that can be used directly as combustible fuels (Van Gerpen, 2005). The most common crops used for this purpose are feedstocks rich in oil content, like rapeseed (canola oil), sunflower and soybean. Bio-ethanol is obtained from the fermentation of hydrocarbons, such as those concentrated in cereal grains, sugar cane and sugar beet.

In recent years the expansion of the biofuel industry was in part responsible for a sharp rise in the prices of grains and oilseeds destined for livestock. Fortunately, the solution for that threat to the farm feed sector was found in the utilization of co-products derived from the manufacturing process of biofuels as low cost feed alternatives (Willson, Wiesman and Brenner, 2010).

SOAPSTOCK

The increased need for intestinal absorption of unsaturated fatty acids (FA) in cattle is driven mainly by nutritional guidelines that promote reduced intake of saturated fatty acids by humans and the observed enhanced animal performance (Jenkins and Bridges, 2007). Rumen-protected fat sources provide essential and non-essential fatty acids that otherwise would have been transformed by micro-organisms in the rumen to yield other end products. For example, dietary unsaturated fatty acids are processed by an array of bacterial enzymes and form trans- fatty acid intermediates and stearic acid (Wallace, 2002). Hence, although essential linoleic and α-linolenic acids comprise the majority of fatty acids consumed by cattle, stearic acid comprises most of the fatty acids leaving the rumen and reaching body tissues. This results in a loss of specific essential and non-essential fatty acids that are provided in the diet not only in order to supply the nutritional requirements of man but also to achieve certain benefits to animals. Even though some fatty acids can be synthesized from stearic acid in ruminant body tissue, the economic loss is great also due to unnecessar-

MAIN MESSAGES

- **Lipid co-products from the bio-ethanol and biodiesel processing industries can be excellent sources of nutrients for ruminants.**
- **With the growth of biofuel production from various feedstocks, livestock producers will have many nutrient-dense co-product feed resources readily available at economical prices.**
- **As more novel extraction and refining technologies are developed, better quality co-products destined for livestock feed will be achieved.**
- **Potential risks should be taken into consideration, so adequate risk assessments should be conducted in order to avoid adverse effects in animals and to safeguard public health.**

ily wasted metabolizable energy. Moreover, fatty acids are known to inhibit fibre digestion in the rumen, whereas the pre-formed calcium soaps of FA have little or no such effects (Enjalbert *et al*., 1997).

Soapstocks, from a nutritional point of view, are saponified fatty acids formed when free fatty acids and divalent cations (usually calcium) are combined. They were originally developed as a form of rumen-inert fat to avoid ruminal fermentation and are commercially available today for enhancing the tissue supply of unsaturated fatty acids in cattle (Palmquist, 1994; Brown, 2006).

COMPOSITION

The refining process for biodiesel generates a distillate rich in free fatty acids and other lipid components (Haas, 2005). It comprises an aqueous phase and an oily phase (also termed acid oil). The acid oil consists of acylglycerols, phosphoacylglycerols, free fatty acids (FFA), triacylglycerides (TG), di-acylglycerides, mono-acylglycerides, pigments and other lipophilic materials (Haas *et al*., 2003; Wang *et al*., 2007). When the distillate is reacted with calcium oxide, calcium salts of the fatty acids present in the distillate are formed and separate further from the unsaponifiable matter.

Fatty acid content of soapstock is a reflection of the parent oil composition (Table 1). The glycerides that will probably be detected have their origin in the partial hydrolysis of the remained TGs, during refining of the biodiesel (Dumont, Suresh and Narin, 2007). For instance, the major TG in canola oil is triolein; hence, a relatively high concentration of mono-olein and di-olein glycerides will be present in the soapstock (Durant *et al.,* 2006).

Effect on ruminants

Palm fatty acids distillate reacted with calcium oxide to develop a rumen-protected fat (commercialized as Megalac®) (Gardner and Rudden, 2004) was proven to be effective in the protection of fatty acids against ruminal biohydrogenation (Scollan *et al*., 2001; Palmquist, 1994). It was also found to significantly increase the digestibility of feed neutral-detergent fibre (NDF) compared with unprotected fatty acids (Palmquist, 1994). Fatty acids from palm oil were the source of choice due to the reliability and consistency of the fatty acid profile, in addition to their stability at the average and optimal rumen pH (Gardner and Rudden, 2004). (Tables 2 and 3)

Later, calcium salts of fatty acids from other sources of vegetable oils (such as rapeseed and soybean oils) were developed and their effectiveness was investigated. For example, after studying the response of dairy cows to Ca salts of fatty acids, it has been observed that rapeseed oil fatty acids were not as inert as palm oil Ca salts in the rumen (Ferlay, Chilliard and Doreau, 1992.). Thus, it was concluded that saponification of polyunsaturated FAs was probably not an efficient way to protect them against ruminal biohydrogenation and to increase their secretion in the milk (Ferlay *et al*., 1993). At the same time, calcium salts of fatty acids from rapeseed distillate (commercialized as Energol) were observed to augment the oleic, linoleic,

TABLE 1
Fatty acid content of different soapstock sources according to Dumont, Suresh and Narine, 2007

Fatty acids	Concentration (g/kg) reported on dry basis			
	Cottonseed	Maize grain	Groundnut seed	Canola seed
Myristic acid	2.4	0.31	6.6	Not detected
Palmitic acid	93.1	8.62	86.2	46.3
Palmitoleic acid	1.8	Trace	Trace	1.5
Stearic acid	9.6	5.07	4.58	Not detected
Oleic acid	60.7	93.6	11.6	155.7
Linoleic acid	165	178	77.5	33.1
α-linolenic acid	Not detected	Not detected	Not detected	0.024
Arachidic acid	0.77	0.76	Traces	1.2

TABLE 2
Chemical composition of MEGALAC®

Component	Concentration (g/kg)
Oil (Werner-Schmidt)	84
Protein	0
Fibre	0
Ash	12
Calcium	9
Moisture	5

Source: Gardner and Rudden, 2004.

TABLE 3
Fatty acid profile of MEGALAC®

Fatty Acid	g/100 g of total fatty acids
C14:0	1.5
C16:0	48.0
C18:0	5.0
C18:1	36.0
C18:2	9.0

Source: Gardner and Rudden, 2004.

α-linolenic and stearic acid content in the milk of dairy cows and reduce that of palmitic acid (Komprda *et al*., 2005).

In general, a reduction in milk fat total saturated FAs (including palmitic acid, which imposes a negative effect on cardiovascular disease risk to dairy cows, was observed (Givens *et al*., 2009). The concentrations of cis-mono-unsaturated FAs were enhanced probably due to their escaping rumen biohydrogenation.

Regarding the ability to manipulate the fatty acids profile in meat, evidence suggests that feeding linseed soapstock to finishing steers raised the total amount of omega-3 fatty acids in the longissimus muscle (Quinn *et al*., 2008). Brandt and Anderson (1990) reported the same when they compared supplementing finishing steers with tallow or soybean soapstock as fat sources. In another study, although the FA composition of the muscle tissue was not altered, subcutaneous adipose concentrations of 9-cis,12-cis-linoleic acid (LA) and liver tissue concentrations of (all-cis)-5,8,11,14,17-eicosapentaenoic acid (EPA) were the highest in lambs fed 4 percent calcium salts of palmitic and oleic acid (Seabrook, Peel and Engle, 2011).

PHYTONUTRIENTS

Extensive research with palm biodiesel (palm oil methyl esters) revealed that phytochemicals such as carotenes (pro-vitamin A, lycopene, phytoene), tocopherols and tocotrienols, sterols, squalene, a mixture of phospholipids (better known as lecithin), polyphenols and co-enzyme Q_{10}, remain intact in the biodiesel after the trans-esterification reaction (Wattanapenpaiboon and Wahlqvist, 2003). Data about palm phytonutrients concentrations and percentage of the components in crude palm oil are presented in Table 4.

TABLE 4
Palm phytonutrient concentrations and percentage of the components in crude palm oil

Palm phytonutrient	Concentration (mg/kg)
Vitamin E	600–1000
Carotenoids	500–700
Phytosterols	300–620
Squalene	250–540
Phospholipids	20–100
Co-enzyme Q_{10}	10–80
Polyphenolics	40–70
Components in palm oil	**g/kg**
Tryglycerides	>900
Dyglycerides	20–70
Monoglycerides	<10
Free fatty acids	30–50
Phytonutrients	10

TABLE 5
Concentration of selected phytonutrients in crude palm oil (CPO) and palm phytonutrients concentrate (PPC)

Component	Concentration (mg/kg)	
	CPO	PPC
Carotenes	530	23710
Vitamin E	1020	14640
Sterols	910	15780
Squalene	44	320

Source: Chandrasekaram *et al*., 2009.

TABLE 6
Concentration of selected phytonutrients in unsaponified crude palm oil (Unsap CPO) and unsaponified palm phytonutrients concentrate (Unsap PPC)

Component	Concentration (mg/kg)	
	Unsap CPO	Unsap PPC
Carotenes	19570	209880
Vitamin E	39290	78660
Sterols	32430	30500
Squalene	2770	8110

Source: Chandrasekaram *et al*., 2009.

These high value phytochemicals are recovered with the aid of several processes incorporating short-path distillation, supercritical fluid technology, saponification, crystallation and solvent treatment (Choo *et al*., 2006, 2009). The presence of phytonutrients in the palm phytochemicals concentrate (also termed palm fatty acid distillate – PFAD) obtained after the distillation of the palm biodiesel was found to be ten times higher than that in crude palm oil (Chandrasekaram *et al*.,2009) (Tables 5 and 6). A saponification process is necessary to further separate the unsaponifiable matter (the phytonutrients) from the small amount of free FAs and esters remaining in the PFAD, to reach a purity of more than 90 percent (Lin and Yoo, 2009).

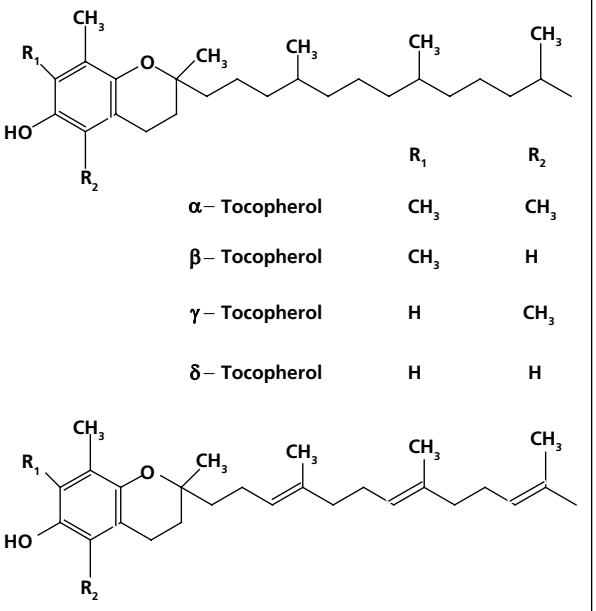
Rapeseed, soybean and sunflower are also a good sources of phytochemicals, especially tocopherols and

sterols (Ooma and Mazza, 1999; Buczenko, De Oliveira and Von Meien, 2003). Extraction of these valuable compounds from the co-products (for example fatty acid distillate) obtained during the biodiesel production will therefore be interesting for their trade as value-added co-products of this industry.

Dried distillers grain (DDGS), a co-product from the bio-ethanol industry (comprising principally bran, protein and germ fractions of the grain used in the fermentation process, together with remnants of yeast cells), also contains significant amounts of phytonutrients. The main constituents are tocopherols and phytosterols. The oil extracted from DDGS may be further processed to yield a distillate rich in these various bio-actives (Winkler *et al*., 2007; Winkler-Moser and Vaughn, 2009; Leguizamon *et al*., 2009; Winkler-Moser and Breyer, 2011).

The above-mentioned bio-active components are much appreciated for application as standard reference materials, functional food, nutraceuticals and cosmeceuticals for human well-being. With novel technologies for the production of biodiesel and the consequent increased quantities of the relevant co-products in the future, a greater proportion of them can be shifted for use as feed additives and vitamins destined for livestock, at more appealing prices.

FIGURE 1
Chemical structure of vitamin E



Source: Christie, 2010.

EFFECT ON RUMINANTS

Vitamin E

'Vitamin E' is the generic name for a group of eight natural compounds: α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol, (Figure 1) which differ in the location of methyl groups on their chromanol ring structure (Williams *et al*.,1993; Röhrle *et al*., 2011). The principal and most investigated vitamin E form, with antioxidant and immune functions, is α-tocopherol. However, important or more effective, or both, functions of non-α-tocopherol like γ-tocopherol and tocotrienols are being revealed (McDowell *et al*., 2007).

Concentrations and distribution of tocopherols significantly depend on kind of oil analysed (Tables 7 and 8), but α- and γ- tocopherols are usually dominant (Ooma and Mazza, 1999).

Vitamin E is essential for body functions such as growth, reproduction, prevention of various diseases (white muscle disease in young ruminants, foetal death and resorption, retinal degeneration) and protection of the integrity of tissues (McDowell *et al*., 1996; Rooke, Robinson and Arthur, 2004). Supplementation of domestic animals with vitamin E has potentiated their antibody responses to a variety of pathogens and their adaptability to stressful situations (Finch and Turner, 1996; Rajeesh *et al*., 2008; Cusack *et al*., 2009).

In addition, feeding levels of vitamin E that are considerably higher than NRC requirements is required to improve animal product quality (Liu, Lanari and Schaefer, 1995) such as extending beef colour stability and minimizing off-flavors in milk due to lipid oxidation. Higher levels of vitamin E in the ruminant diet increases α-tocopherol concentrations in the tissues and, owing to its antioxidant properties, it protects not only membranal lipids but also myoglobin from oxidation. This results in delayed onset of discoloration in fresh, ground and frozen beef, and in suppression of lipid rancidity (Liu, Lanari and Schaefer, 1995).

TABLE 7
Tocopherol contents (mg/kg) in selected vegetable oils (possible raw materials for biodiesel production)

Oil	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol
Rapeseed	268	–	426	–
Canola	272	0.1	423	–
Soybean	116	34	737	275
Maize	134	18	412	39

Source: Ooma and Mazza, 1999.

TABLE 8
Tocopherol content (mg/kg) in plant oils

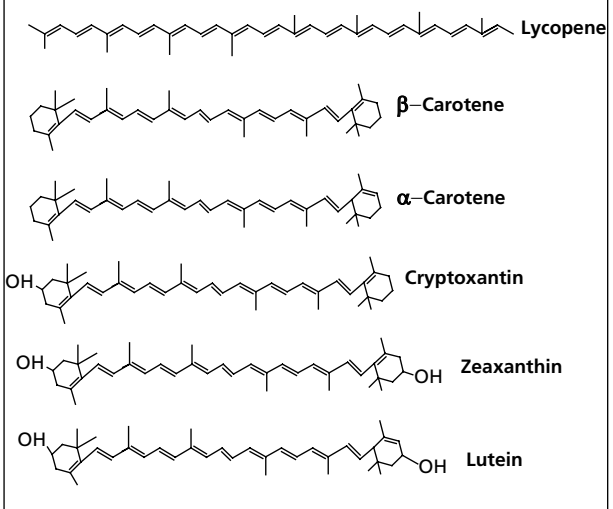
Oil	Total tocopherol content (mg/kg)
Grapeseed	121 ± 6
Linseed	367 ± 8
Olive	177 ± 3
Groundnut	226 ± 4
Sunflower	535 ± 8

Note: Values are mean + SD. *Source*: Gryszczyska-Swiglo *et al*., 2007.

Carotenes

Carotenes belong to the carotenoids family, a group of natural pigments that encompasses more than 600 molecules synthesized by higher plants and algae. These compounds

FIGURE 2
Chemical structure of various carotenoids



Source: Arab, Steck-Scott and Bowen, 2001.

TABLE 9
Various types and composition of carotenes in palm oil

Carotene type	Part in general carotenes (g/100 g)
Phytoene	1.27
Cis-β-carotene	0.68
Phytofluene	0.06
β-carotene	56.02
α-carotene	35.16
Cis-α-carotene	2.49
ζ-carotene	0.69
γ-carotene	0.33
δ-carotene	0.83
Neurosporene	0.29
β-zeacarotene	0.74
α-zeacarotene	0.23
Lycopene	1.30

Source: Puah *et al*., 2005.

are characterized by a linear poly-isoprene structure with conjugated double bonds either *per se* (as in lycopene, $C_{40}H_{56}$) (Figure 2) or as derived by cyclization of the two extremities, with oxidation (as in xanthophylls such as lutein and zeaxanthin, $C_{40}H_{56}O_2$) or without oxidation (carotenes, $C_{40}H_{56}$) (Calderon *et al*., 2006; Noziere *et al*., 2006).

Concentration of carotenes in crude palm oil is approximately 640–700 ppm (Choo, 1994) and 0.25–3.6 ppm in virgin olive oil (Tanouti *et al*., 2011).

Non-oxidized carotenes are known as general components of the carotenes fraction (Table 9).

The β-carotene content of forages is reduced by sun-curing, ensiling and storage, and is quite variable. Hence, green pasture is the most abundant natural source of carotenes for ruminants (Miller, 1968; Kalac and Mcdonald, 1981).

Ruminants depend entirely on feed as their source of carotenoids, not being able to synthesize them *de novo*, but metabolize or convert them into other carotenoids.

In sheep and goats, absorbed β-carotene is assumed to be almost entirely transformed into retinol (vitamin A) in the enterocytes. In contrast, in cattle, not all absorbed β-carotene is transformed into retinol and thus β-carotene is the main carotenoid present in their plasma, stored in tissues and secreted in milk fat (Mora *et al*., 1999; Cardinault *et al*., 2006; Lucas *et al*., 2008).

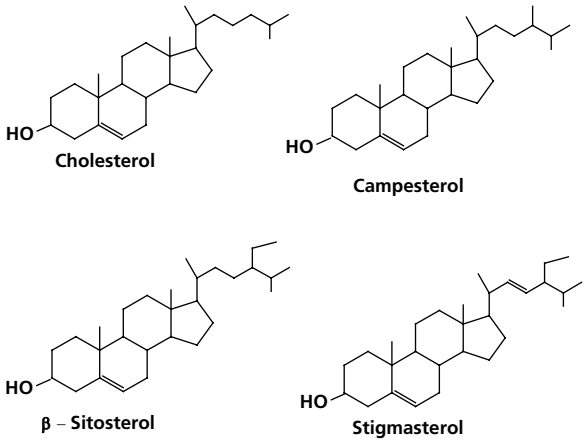
A deficiency in retinol may cause xeropthalmia (a night blindness disease) and reduce reproductive efficiency in dairy cows, through impaired ovarian function and increased incidence of abortion (Wang *et al*., 1987; Haliloglu *et al*., 2002). Apart from having pro-vitamin A properties, β-carotene *per se* also plays an important role as antioxidant. Some positive effects of β-carotene on mammary gland health, rumen function, milk yield and immunity have been reported (Hino, Andoha and Ohgi, 1993; De Ondarza and Engstorm, 2009a, b).

Certain changes in the organoleptic characteristics of meat and milk from ruminants fed on diets rich in β-carotene were reported (Ellis *et al.,* 2007). Some of them are most desired from the point of view of public health, consumer acceptability or preference on the one hand, and animal producers and food manufacturers on the other. The augmented levels of β-carotene and vitamin A in milk as a consequence of supplying them in the ruminant diet, could be beneficial for the production of functional foods (i.e. butter, margarine) (Ellis *et al.,* 2007). Additionally, their abundancy in meat and milk can supply the nutritional requirements recommended for humans (Simmone, Green and Bransby, 1996; De Ondarza, Wilson and Engstrom, 2009.). It should be noted, though, that high levels of β-carotene and vitamin A were found to adversely affect the fatty acid profile in intermuscular fat tissue and marbling deposition (Siebert *et al*., 2000, 2006; Pyatt and Berger, 2005; Dikeman, 2007).

Phytosterols

Plant sterols and stanols (their reduced form), also called phytosterols and phytostanols, are natural constituents of plants and are part of the triterpene family (Moreau, Whitaker and Hicks, 2002). They are non-nutritive compounds whose chemical structure resembles that of cholesterol, a predominant sterol in animals (Figure 3). Phytosterol content ranges from 0.14 percent in olive oil to 1.6 percent in maize oil (Gul and Amar, 2006). In plants they are responsible for the regulation of the fluidity and permeability of cell membranes, serve as substrates for the synthesis of numerous secondary plant metabolites, and act as biogenic precursors of plant growth hormones and hormonal precursors.

FIGURE 3
Chemical structure of sterols



Source: Awad and Fink, 2000.

The best dietary sources of phytosterols are unrefined plant oils, seeds, nuts and legumes; in certain plants, such as *Amaranthus* spp. or *Butyrospermum parkii* (shea butter tree), it can reach more than 10 percent. The predominant forms being β-sitosterol, campesterol and stigmasterol, followed by brassicasterol, avenasterols and ergosterol (the latter is a known precursor of vitamin D_3, that is also formed in fungi) (Tapiero, Townsend and Tew, 2003; Milovanovic, Banjac and Vucelic Radovic, 2009).

Studies with animals and humans show that phytosterols reduce the absorption of cholesterol, thus lowering its serum level and leading to a reduction in the risk of cardiovascular diseases (Kamal-Eldin and Moazzami, 2009; Weingartner, Bohm and Laufs, 2009). In addition, they are considered to have anti-inflammatory, anti-bacterial, anti-ulcerative and anti-tumor properties (Awad and Fink, 2000).

Phytosterols supplied as immuno-modulators (commercialized as Inmunicin Maymoin, a product consisting primarily of β-sitosterol) in the diet of pigs during the nursery and finishing periods have been shown to fortify the immune system (decrease mortality and percentage of culls) and improve average daily gain and feed efficiency (Fraile *et al*., 2009). Hence, it will be interesting to conduct trials aiming to prove the same utility in ruminants.

Polyphenols

Polyphenols are secondary metabolites of plants, known to be involved in defence mechanisms and the survival of the plant in its environment (Manach *et al*., 2004). These compounds possess characteristic aromatic rings (single, as in simple phenols, to several, as in flavonoides and condensed tannins) (Figure 4) attached to a hydroxyl group, which confers on the molecule part of its diverse biological activities (Singh, Bhat and Singh, 2003).

Polyphenols are present in a variety of plants utilized as important components of both human and animal diets. Polyphenols in vegetable oils are a complex mixture of compounds that include derivatives of hydroxybenzoic and hydroxycinnamic acids, as well as oleuropeins, coumarins, flavonoids and lignins (Kozlowska *et al*., 1990; Valavanidis *et al*., 2004).

Polyphenols are usually soluble in basic media and alcohols, but they can present in plant oils at low concentrations. Concentration of polyphenols in virgin olive oil may be from 63 mg/kg to 406.5 mg/kg (Tanouti *et al.,* 2011). As a rule, they are dissolved in the dispersed water phase. This phase is stable due to presence in oils of such substances like lecithin and other phospholipids.

The presence of polyphenols in the diet of ruminants improves the efficiency of protein degradability and digestibility (except when the level of tannins is not monitored correctly and reaches high levels), thus ameliorating feed conversion. It also reduces the concentration of urea excreted in cattle manure (Reed, 1995; Frutos *et al*., 2004). Additionally, polyphenols augment ruminant performance by inhibiting bloat and reducing the incidence of subclinical helminth infections (O'Connell and Fox, 2001).

As they possess potent antioxidant activity, their deposition in animal tissues and secretion in milk is mostly desired, because it protects the lipid components in meat and milk products as well as providing dietary antioxidants for human consumption. In this manner, functional-healthy products are achieved (Weisburger *et al*., 2002; Priolo and Vasta, 2007; Moñino *et al*., 2008; Cuchillo Hilario *et al*., 2010; Jordan *et al*., 2010).

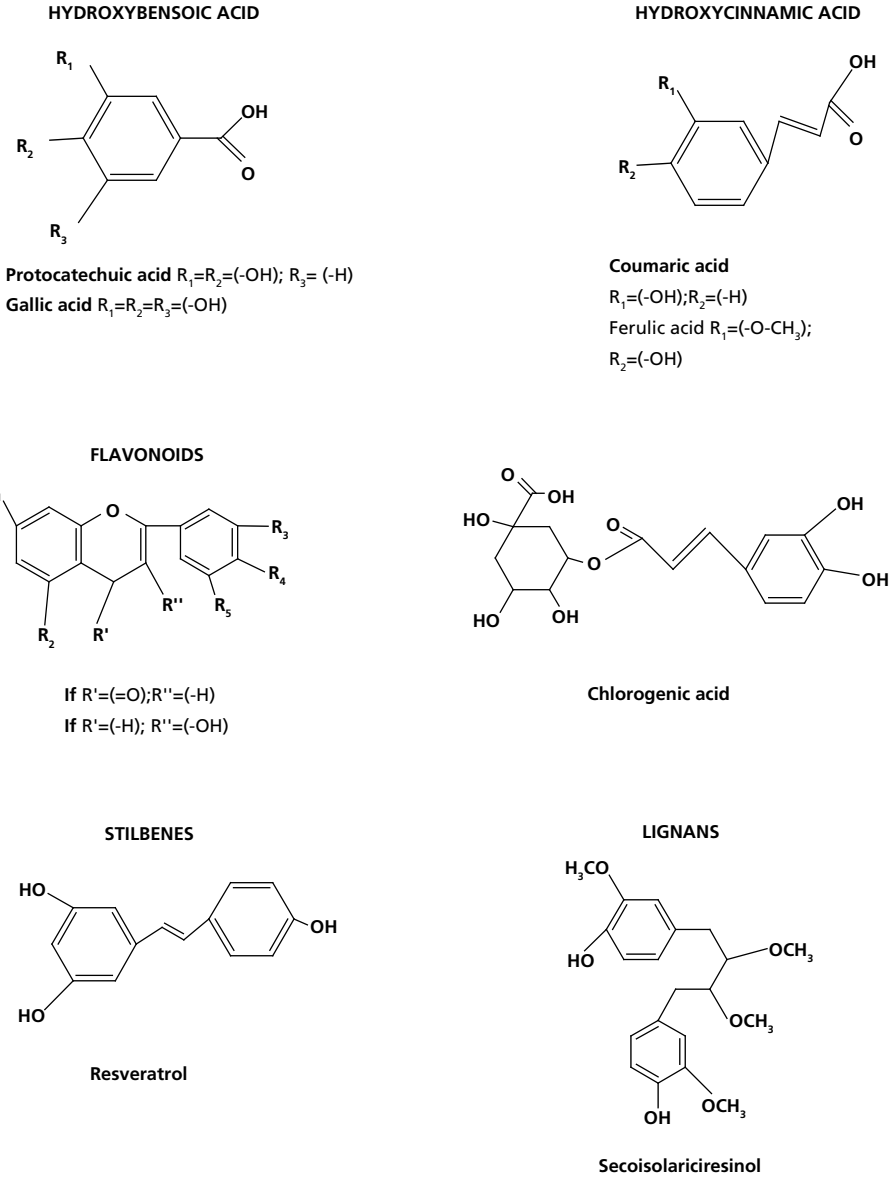
The prohibition on use of growth-promoting antibiotics in animal feeds (EU, 2003) and the constantly increasing demand for organically produced milk and meat, have prompted livestock producers to look for more acceptable alternatives (Wallace, 2004). In addition, some phenolic extracts have been demonstrated to inhibit hyper-ammonia-producing bacteria in the rumen and exert beneficial effects on rumen fermentation (Flythe and Kagan, 2010). They have also been shown to inhibit certain pathogens, hence their potential role as natural and less hazardous replacements for antibiotics (Wells, Berry and Varel, 2005).

Lecithin

Lecithin is primarily a natural mixture of phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidic acid (PA) (Figure 5), and which contains minor quantities of other water-soluble or hydratable components (glycolipids and oligosaccharides) (Pickard, 2005).

Soybean is the predominant vegetable source of lecithin due to its availability, and the lecithin has outstanding func-

FIGURE 4
Chemical structure of polyphenols



tional characteristics, mainly as a surfactant and emulsifier (Wilson, 2003). However, lecithin products from seeds of rape, sunflower, glandless cotton and maize are also potential commercial sources. Seed of glanded cotton contains more phospholipids than any other oilseed (with the exception of soybean), but has the disadvantage that gossypol (a toxic compound normally present in the cotton seed) tends to bind to the phospholipids during the solvent extraction process (Pickard, 2005).

Information about the chemical structure of lecithins from different oils are presented in Tables 10 and 11.

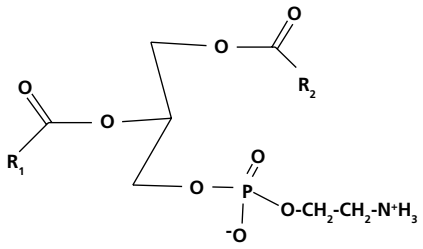
Lecithins are used in animal feed recipes as dust suppressors, economic emulsifiers (e.g. stabilization of milk replacers for feeding calves) and essential FA sources (Van Nieuwenhuyzen and Tomas, 2008). Feeding soy lecithin to ruminants was found to favourably change the FA profile in longissimus muscle and subcutaneous adipose tissue in lambs (Lough *et al*., 1992). It also increased FAs and

TABLE 10
Fatty acid composition of vegetable lecithins (g/100 g)

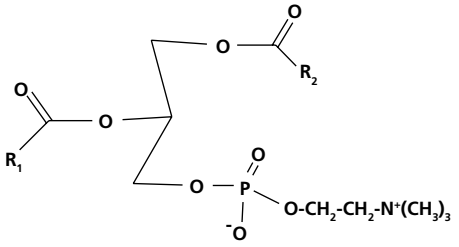
Fatty acid	Soybean	Sunflower seed	Rapeseed
16:0	16	11	7
18:0	4	4	1
18:1	17	18	56
18:2	55	63	25
18:3	7	0	6
Others fatty acids	1	4	5

Source: Van Nieuwenhuyzen and Tomas, 2008.

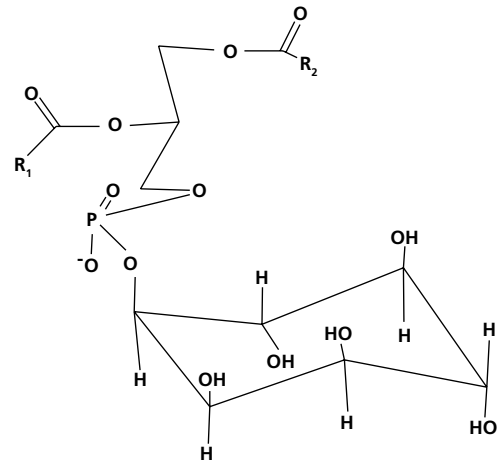
FIGURE 5
Structures of the main phospholipids in vegetable lecithins



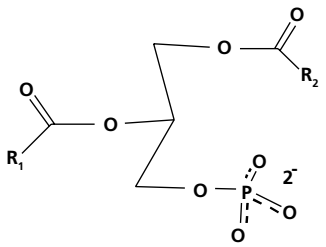
3 - sn - Phosphatidylcholine (PS)



3 - sn - Phosphatidylethanolamine (PE)



3 - sn - Phosphatidylinisositol (PI)



Phosphatidic acid (PA)

Note: R_1 and R_2 indicate $-C_{15}$ and $-C_{17}$ hydrocarbon chains.

Source: Joshi, Paratkar and Thorat, 2006.

TABLE 11
Phospholipid composition of liquid vegetable lecithins (g/100 g of lecithins fraction) by ^{31}P-NMR

Phospholipid	Soybean	Sunflower seed	Rapeseed
PC	15	16	17
PE	11	8	9
PI	10	14	10
PA	4	3	4
Other phospholipids	7	6	6

Notes: ^{31}P-NMR indicates analysis of P isotpe-marked lecithins using nuclear magnetic resonance techniques. For PC, PE, PI and PA see Figure 5. *Source*: Van Nieuwenhuyzen and Tomas, 2008.

protein digestion in the hindgut both *in vitro* and *in vivo* (Jenkins and Fotouhi, 1990; Wettstein, Machmuller and Kreuzer, 2000; Hristov, Neill and McAllister, 2003; Pivoda *et al.*, 2010). Both methane production and ammonia concentration in the rumen were significantly reduced, implying that efficiency of feed conversion was increased.

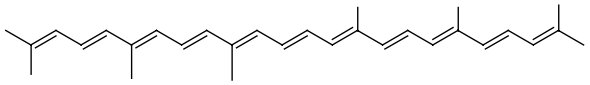
Squalene

Squalene – an isoprenoid compound with 6 isoprene units (triterpene) – is an intermediate metabolite in the synthesis of cholesterol and phytosterols (Figure 6). This unsaturated, thermally unstable and light-sensitive hydrocarbon appears in high concentrations (50–90 percent by weight) in the liver oils of certain species of deepsea sharks (Bakes and Nichols, 1995; Wetherbee and Nichols, 2000). It is also present in lower concentrations in foods such as avocado, aubergine, poultry and tuna, as well as in some common edible oils such as olive, palm, groundnut, and rapeseed (Catchpole and von Kamp, 1997; Catchpole, Von Kamp and Grey, 1997; Chua *et al.*, 2007).

Concentration of squalene in olive oil can be from 136 to 708 ppm (Kiritsakis, 1990).

Squalene has been demonstrated to be effective in decreasing total cholesterol, low-density lipoprotein-cholesterol and triglyceride levels. It is also used extensively as a strong antioxidant in the food and cosmetic industries

FIGURE 6
Chemical structure of squalene



Source: Fox, 2009.

(Fan *et al*., 2010). Dietary supplementation with squalene enhanced the reproductivity of boars and improved semen count and quality in meat-type male chicken (Zhang *et al*., 2008; Li *et al*., 2010). Therefore, the administration of squalene with other vitamins and feed additives is expected to strengthen the immune system and to improve livestock productivity.

A surprising revelation regarding the accumulation of squalene in the intermuscular fat in reindeers (northern-most freely ranging ruminants in Scandinavia) fed pellets that contained squalene, was made by Sampels, Pickova and Wiklund (2005). The levels of squalene found in the reindeer meat (0.5–1 percent) were above the recommended values for common human dietary fats and oils (0.002–0.3 percent squalene in total fat) (Sampels, Pickova and Wiklund, 2005.). This discovery may encourage research regarding the deposition of squelene in the tissues of ruminants and its secretion in milk, in order to promote the creation of functional foods.

POTENTIAL RISKS FROM FRACTIONS CONTAINING SUCH PHYTOCHEMICALS

Deodourizer distillates, by-products of the refinement of vegetable oils, are a known repository for hazardous substances such as dioxins, furans, PCBs (polychlorinated biphenyls) and pesticides. They have been banned from direct use in animal feeds in the United States, due to the elevated levels of these contaminants that may accumulate in livestock tissues (biomagnification) (Halbert and Archer, 2007).

Therefore, although the new biodiesel production plants aim to minimize the presence of harmful impurities by utilizing novel advanced technologies, this crucial issue must be supervised by the corresponding authorities (Brambilla and De Filippis, 2005). It is advised that a thorough examination of the biodiesel lipid co-products should be carried out in order to assess possible presence of other possible toxic compound that can harm the health of both animals and humans (EU, 2003).

CONCLUSIONS

When used correctly and with prudence, the lipid co-products from the biofuel industries could offer significant benefits to agriculturalists, animal producers and consumers of functional-healthy products. However, adequate risk assessments should be conducted in order to avoid adverse effects in animals and on public health.

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Chapter 19

Potential and constraints in utilizing co-products of the non-edible oils-based biodiesel industry – an overview

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ABSTRACT

The biofuel industry is undergoing exponential growth, fuelled by the high demand for renewable sources of energy and advancing technology. With the increasing production of biofuels, the volume of co-products has, in parallel, grown dramatically. During the last few years, many non-edible oil feedstocks were suggested that resulted in new co-products to supplement those resulting from conventional feedstocks and that are accepted by the livestock feed industry. These earlier co-products are also used in applications ranging from soil fertilizers to pharmaceuticals, which is not the case with the emerging co-products from non-edible oil feedstocks, many of which contain either toxic or antinutritional compounds. Sustainability of the biofuel industry hinges on the use of feedstocks that are not competitive with human and animal nutrition and that are produced from plants that grow in poor and marginal soils. Another important criterion that ensures sustainability is the use of the resulting co-products as value-added products. Since the biofuel-derived cakes and meals constitute a rich source of crude protein, ranging from 11 percent (*Mesua ferrea*) to 58 percent (*Crambe abyssinica*), these have the potential to be used as animal feeds. In this chapter, current knowledge on the potential and constraints of using oil cake or meals from the emerging biodiesel industry based on non-edible oil for livestock feed is examined. This information will assist in enlarging the feed resource base by identifying promising novel feed resources and in identifying potential detoxification treatments where necessary.

INTRODUCTION

The worldwide production of renewable fuel is expected to grow quickly and its share in global energy production is expected to increase. Biodiesel production, which started on a small scale in the early 1990s, quadrupled between 2000 and 2005 (Brown, 2009). Conversion of vegetable oils into biodiesel has undergone several new developments (Meher, Sagar and Naik, 2006). This has resulted in some of the feedstocks taditionally used as animal feed, e.g. soybean and rapeseed, becoming feedstocks for the biofuel industry. Europe, the leader in biodiesel production processed from vegetable oils, is largely dependent on these two crops to sustain production. Biofuel production, like any agriculture-based industry, will absorb agricultural products, but will also result in co-products, including protein-rich oilcakes and meals, which can be used as animal feed.

Unlike other agro-industrial activities, biofuel production should not compete for oil and other natural resources needed for human food production. A convenient way to avoid competition with food production is to promote the use of plant species with products that are non-edible and that can grow on poor soil and under harsh climatic conditions. Based on this concept, biodiesel production from non-edible oils presents a promising option. However, concerns have been raised about the sustainability of using non-edible oils for this purpose as the resulting co-products are often toxic if fed directly to livestock. This would limit complementarity among the sectors of agriculture, the biofuel industry and the animal feed industry. The toxic co-products obtained during biodiesel production can also pose risks to the environment.

The toxicity of non-edible oil feedstock originates from the plant secondary metabolites they contain. These secondary metabolites are present in plants for their protection, including acting as antioxidants, thus enabling the plants to grow in harsh environments. However, their anti-nutritional and toxic factors result in the resultant oil and co-products being non-edible.

The multiple and widespread use of biofuel co-products from edible oil resources, including the use of cakes and meals for livestock feed, is well documented. Literature is scarce and isolated on the use of biofuel co-products from

MAIN MESSAGES

- **Many potential feedstocks have shown promising results in feeding trials after detoxification. Studies on oil cakes and meals of *Ricinus communis, Crambe abyssinica, Azadirachta indica* and *Pongamia pinnata* show possibilities for feeding to farm animals after subjecting them to appropriate detoxification treatments.**
- **Further studies are needed to fill the gaps in knowledge for the possible detoxification and further use of *Hevea brasiliensis, Thevetia peruviana, Mesua ferrea, Calophyllum inophyllum* and *Croton tiglium*.**
- **Scaling up of promising detoxification processes is needed. Implementation of positive results can be successful only if large quantities of the derived meals can be treated and used for animal feeding.**
- **The development, use and scaling up of the detoxification processes should be accompanied by socio-economic analysis.**
- **Preparation of high-value protein isolates and peptides for use in livestock feeds could be an alternative approach to use of otherwise non-edible cakes and meals – an area that so far has received little attention**.

non-edible oils as animal feed. Also, data on nutritional value, intake, digestibility and toxicity are scattered and not systematically collated. This presents a challenge in estimating the potential of these products for animal feed. The present chapter synthesizes information on the nutritional composition of the co-products, their toxicity and the attempts made to enhance by detoxification their utilization as animal feed.

PROMISING NON-EDIBLE OIL PLANT SPECIES

Many plant species are known for their oil seeds, but exploitation of the oil cakes from non-edible oil species originates from the fact that many of these plants are non-ubiquitous in distribution, their production is seasonal and their co-products are usually non-edible for livestock (Sivaramakrishnan and Gangadharan, 2009). The list of such non-edible oil seed plants is long, but this chapter only considers nine promising oleaginous species, whose oil is potentially suitable for use as biodiesel (Azam, Waris and Nahar, 2005) and their co-products are reported to be toxic when used as animal feed. *Jatropha curcas*, another promising non-edible oil plant that is being extensively promoted, is not discussed here. The utilization of jatropha seed meal, cake and protein isolate is presented elsewhere in this publication.

Castor (*Ricinus communis* L.)

Commonly known as castor, *Ricinus communis* is a wild plant growing in large quantities in most tropical and sub-tropical countries. The plant requires air temperatures ranging between 20 and 26 °C, with low relative humidity. However, its extreme toxicity limits its cultivation in many countries. Castor is grown mainly for its oil. On average, castor seeds contain 46 to 55 percent oil by weight (Ogunniyi, 2006). The oil is used in production of viscous lubricants, important oleochemicals, surface coatings, soaps, cosmetics and pharmaceuticals (Ghandi, Cherian and Mulky, 1994).

Rubber (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.)

Para rubber is a perennial tree, indigenous to South America. It has been cultivated as an industrial plantation crop since its introduction to Southeast Asia (Abdullah and Salimon, 2009). The important contribution of para rubber trees is the latex used for natural rubber production (Ravindran and Ravindran, 1988). Rubber tree seed yields are between 100 and 150 kg/ha (Stosic and Kaykay, 1981). The seed has a high content (43 percent) of semi-drying oil, which can be used in the paint industry (Lauw Tjin Giok *et al.*, 1967).

Although the seeds contain cyanogenic glycosides, they are locally used as an ingredient in human nutrition after appropriate treatment (soaking and cooking). This procedure reduces the cyanide content from 330 mg to 8.9 mg/100 g in seeds (Lauw Tjin Giok *et al.*, 1967).

Crambe (*Crambe abyssinica* Hochst)

Crambe is an oil plant of the cruciferous family, a native to the Mediterranean region that adapts well to the cold weather of much of Europe (Falasca *et al.,* 2010). A low water requirement, a short crop cycle of about 90 days, hardiness and the possibility of using it as a catch-crop between main cropping seasons has attracted attention for its use as a feedstock for biodiesel production (Macagnan, Chaves and Café-Filho, 2010). Yields vary widely: from 1125–1622 kg/ha in Russia to 450–2522 kg/ha in the United States (Falasca *et al.,* 2010). The thousand-seed weight is about 6–10 g, with the hull representing 25 to 30 percent of the total weight (Carlson and Tookey, 1983).

Thevetia (*Thevetia peruviana* K.Schum.)

Thevetia is a native of tropical America, but has been naturalized in tropical regions worldwide. It is grown as an ornamental shrub, generally as hedges, despite the high oil (61 percent) and protein (37 percent) content of the seed

(Ibiyemi *et al.,* 2002). Seeds, leaves, fruits and roots are used in traditional medicine as a purgative, as an emetic and for intermittent fever treatment (Gata-Gonçaves *et al.,* 2003). However, latterly it has been regarded as a potential source of biologically active compounds, including insecticides (Reed, Freedman and Ladd, 1982), rodenticides (Oji *et al.,* 1994; Oji and Okafor, 2000), fungicides (Gata-Gonçaves *et al.,* 2003) and bactericides (Saxena and Jain, 1990; Obasi and Igboechi, 1991).

Polanga (*Calophyllum inophyllum* L.)

Commonly called Alexandrian laurel, *Calophyllum inophyllum* is a tree found mainly in the tropics. It grows on rocky and sandy seashores, requires moderate temperatures and an annual rainfall ranging from 750 to 5000 mm. It is planted up to 1200 m altitude (Louppe, Oteng-Amoako and Brink, 2008). A mature tree may yield 50 kg of dry fruits (45 percent kernel). According to Ajayi *et al.* (2008), the seed contains 49.2 percent oil, an oil that has long been used for lighting in India and across the Pacific. The purified oil is used in cosmetics and also to treat glandular swellings in the neck and jaws (Louppe, Oteng-Amoako and Brink, 2008).

Nahar (*Mesua ferrea* L.)

Mesua ferrea is an evergreen tree growing naturally in the sub-canopy of moist tropical and subtropical forests in India. It grows at 100 to 1000 m altitude, but does not coppice well (Khan *et al.*, 1999).

It is used as firewood. The tree yields a timber used for heavy construction. The flowers are used in dyeing. In traditional medicine, the flowers are used to terminate pregnancy. The kernels and the seed oil are used for dressing wounds (Orwa *et al.*, 2009).

Neem (*Azadirachta indica* A.Juss.)

Commonly known as neem, *Azadirachta indica* is one of the most important native trees of India. It grows also in South and South East Asia and other tropical regions. Neem survives at annual average temperatures ranging between 21 and 32 °C with an annual rainfall between 120 and 1120 mm. It is usually found on plains and low-lying hilly areas, and altitudes between 700 and 800 m. Its resistance to drought and its ability to grow in poor soils leads to its incorporation in forestation programmes (Yakkundi, 1997; Uko *et al.,* 2006). All the tree parts (roots, trunk, bark, leaves and fruits) have been used in industry and folk medicine. Neem oil is considered as non-edible because it is rich in sulphur compounds (acyclic di-, tri- and tetra-sulphides with di-n-propyl disulphide being the major component). These sulphur compounds and limonoids (tetranortriterpenoids) give the oil seed cake a bitter taste (Yakkundi, 1997).

Karanj (*Pongamia pinnata* (L.) Pierre)

Karanj, as it is commonly called, is native to the Asian subcontinent, is found naturally along coasts and riverbanks as it is tolerant of water-logging, and both saline and alkaline soils. It can withstand harsh climates and is suitable for degraded lands (Wani and Sreedevi, 2011). The seeds yield non-edible karanj oil, which has medicinal properties (Wani and Sreedevi, 2011).

Croton (*Croton tiglium* L.)

Croton is native to tropical Asia. It grows in subtropical humid to tropical dry conditions up to an altitude of 1500 m, with an annual rainfall from 700 to 4300 mm, temperatures from 21.0 to 27.5 °C and a soil pH from 4.5 to 7.5 (Duke, 1983).

Croton oil is a very strong laxative and is highly toxic when used as such. The oil has also been used in preparations as a counterirritant on the skin (Alexander *et al.,* 2008a), although other studies resulted in the oil being deemed unsafe for either use due to its carcinogenic activity (Hecker, 1968) and the presence of phorbol esters, known for their tumour promoting activity (Goel *et al.,* 2007).

CHEMICAL COMPOSITION OF CO-PRODUCTS OF THE NON-EDIBLE OIL-BASED BIODIESEL INDUSTRY

Oil cakes or oil meals are solid residues obtained after oil extraction from the seeds. Their composition varies depending on plant species, growing conditions and extraction methods used (Kolesárová *et al.*, 2011). The oil cake is the co-product obtained after oil extraction by mechanical pressing and usually contains residual oil. However, in order to maximize oil extraction, the cake can be exhaustively extracted by organic solvents. The resulting co-product, called oil meal, is low in residual oil but it contains more crude protein (CP) than oil cake. One common feature of oil cakes and meals is their high protein content (Ramachandran *et al.*, 2007).

The chemical compositions of the different non-edible oil cakes and meals are summarized in Table 1. Traditionally oil cakes and meals from edible plants are used in livestock feeding because of their high protein content. Non-edible oil cakes and meals are also rich in protein. CP content is highest for crambe meal and lowest for mesua meal.

Castor meal contains 27 percent CP and its fibre content is higher than the other non-edible cakes and meals. Castor meal is deficient in the essential amino acids methionine, lysine and tryptophan (Table 2). Rubber seed meal contains 22 percent CP, but its ash content is lower than many other oil cakes and meals. The amino acid composition of rubber seed meal shows a well balanced profile with high levels of glutamic acid, aspartic acid and leucine. Lysine and sulphur amino acids are deficient (Oyewusi, Akintayo and Olaofe,

TABLE 1
Nutritional and antinutritional components (g/100 g DM) of different oil cakes and meals

	Co-product	Ether extract	Crude protein	Crude fibre	Minerals	Toxic compounds	Reference
Ricinus communis	Meal	0.3	27.1	41.1	7.5	Ricin (thermolabile protein), ricinine (alkaloid), CB-1A (stable allergen)	Ghandi, Cherian and Mulky, 1994.
Hevea brasiliensis	Meal	15.8	21.9	ND	2.3	Cyanogenic glycosides (linamarin and lotaustralin), phytohaemagglutinin (antifertility factor)	Oyewusi, Akintayo and Olaofe, 2007.
Crambe abyssinica	Meal	0.9	46 – 58	6.7	8.6	Epi-progoitrin (thioglucoside)	Carlson and Tookey, 1983.
Thevetia peruviana	Meal	0.5	42.8 – 47.5	5.20	ND	Cardiac glycosides (thevetin A, thevebioside, glucoperuvoside and acetylated monoside)	Atteh, Ibiyemi and Ojo, 1995; Usman *et al.,* 2009.
Calophyllum inophyllum	Cake	ND	24	ND	ND	Calaustralin (phenylcoumarin derivative)	Mohapatra and Samal, 2002.
Mesua ferrea Linn	Meal	0.96–14.0	11.3–15.7	4.5–9.2	5.3 – 5.4	Unknown apolar toxic factor(s)	Baruah, Kalita and Saikia, 1997; Konwar, Ahmed and Medhi, 1999.
Azadirachta indica	Cake	3.6–9.1	45.0–49.4	5.5–8.6	7.6 – 9.5	Azadirachtin (tetranortriterpenoid antifeedant), isoprenoids and nimbidin (sulphurous compound)	Rao, 1987.
Pongamia pinnata	Cake	14.4	24.2	3.9	5.2	Karanjinin (furano-flavonoid), antinutritional factors (phytates, tannins and protease inhibitors and glabrin)	Natanam, Kadirvel and Viswanathan, 1989.

Notes: ND = not determined.

TABLE 2
Amino acid composition of different oil cakes and meals (g/16 g N)

	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Lys	Leu	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	Reference
Ricinus communis	4.1	7.9	9.1	1.4	18.3	4.2	1.6	4.7	2.8	6.1	1.6	3.7	3.6	5.4	3.3	0.3	2.3	5.5	Mottola *et al.*, 1968; Vilhjalmsdottir and Fisher, 1971.
Hevea brasiliensis	2.4	5.1	8.0	1.5	11.2	4.0	2.4	3.5	5.0	7.2	1.5	4.9	1.8	3.0	2.3		3.4	3.8	Oyewusi, Akintayo and Olaofe, 2007.
Crambe abyssinica	4.0	6.5	6.8	2.7	15.6	5.0	2.4	3.9	5.3	6.3	1.7	3.7	5.9	3.8	3.8	1.5		5.0	Carlson and Tookey, 1983; Lazzeri *et al.,* 1994.
Thevetia peruviana	10.1	10.5	44.6	2.2	31.9	9.0	3.5	4.0	9.1	9.2	1.4	5.8	8.5	8.8	4.5		3.9	8.5	Atteh, Ibiyemi and Ojo, 1995; Usman *et al.,* 2009.
Calophyllum inophyllum		9.2		1.3			1.2	2.3	3.3	4.9	0.6	3.5			2.3	0.9		3.1	Venkatesan and Rege, 1973.
Azadirachta indica	3.7	8.5	10.6	0.3	24.2	4.3	1.9	2.6	3.3	6.5	1.1	3.8	3.9	4.9	3.2	1.2	2.1	3.6	Rao, 1987.
Pongamia pinnata	4.1	6.2	13.3	0.1	19.1	4.7	2.8	4.2	8.4	10.1	0.4	6.6	5.1	4.5	2.7		2.0	5.4	Vinay and Sindhu Kanya, 2008.

2007). In addition to a significant amount of CP in crambe meal, protein efficiency tests showed that its protein is of good nutritional quality, with a well balanced amino acid profile (Ghandi, Cherian and Mulky, 1994).

Thevetia meal has a protein content comparable to that of soybean meal (Atteh, Ibiyemi and Ojo, 1995). Thevetia meal protein is rich in lysine but deficient in methionine, cysteine and isoleucine.

Calophyllum meal has medium protein content (24 percent). The amino acid composition shows very low methionine, appreciably lower than that of ricinus and crambe meals (Venkatesan and Rege, 1973). Mesua meal has the lowest protein content among the species discussed here, with only 11 to 16 percent CP (Baruah, Kalita and Saikia, 1997). Azadirachta cake is similar in composition to crambe cake. Unlike crambe, azadirachta protein is limited in sulphur-containing amino acids, although it is rich in lysine (Rao, 1987). Meal from pongamia is a good source of protein, rich in lysine, leucine, tyrosine and phenylalanine, and in sulphur-containing amino acids (Vinay and Sindhu Kanya, 2008).

TOXICITY OF NON-EDIBLE CAKES AND MEALS

Non-edible oil cakes and meals are characterized by the presence of anti-nutritional and toxic factors (Table 1) which preclude the utilization of these co-products directly as animal feed (Sivaramakrishnan and Gangadharan, 2009).

Castor cake is poisonous and allergenic to animals because of the presence of three antinutritional compounds: ricin (a heat labile toxic protein), ricinine (a toxic alkaloid) and a stable allergen known as CB-1A (Gardner *et al.*, 1960; Ogunniyi, 2006; Gowda *et al.*, 2009). For details on the detoxification of castor meal and its utilization in animal diets, see Anandan and Sampath (this volume).

Rubber cake is toxic because of the presence of linamarin and lotaustralin, cyanogenic glycosides which after enzymic hydrolysis by linamarinase (an endogenous glucosidase) liberate HCN (Ukpebor *et al.*, 2007). Raw rubber meal is suspected of containing an unknown anti-fertility factor and phytohaemagglutinin. Feeding raw rubber meal caused a decline in semen volume and sperm count when fed up to 20 percent of the diet for white leghorn cockerels (Ravindran, Rajaguru and De Silva, 1987). It caused a depression in plasma protein and albumin when fed at more than 10 percent in the diet of growing swine (Babatunde, Pond and Peo, 1990).

Crambe meal contains epi-progoitrin, a thioglucoside, which undergoes a hydrolysis reaction sequence, initiated by the thioglucosidase enzyme system, leading to any of four major products: two diastereomeric (2S)-l-cyano-2-hydroxy-3,4-epithiobutanes and (S)-1-cyano-2-hydroxy-3-butene (Daxenbichler, Van Etten and Wolff 1968); and 5-vinyloxazolidine-2-thione (goitrin), which suppresses thyroidal iodine uptake and causes thyroid hyperplasia and hypertrophy (Gould and Gumbmann, 1980). Thus feeding raw crambe meal with intact glucosinolates and active thioglucosidase can reduce palatability and cause growth inhibition and pathological changes in body organs (Carlson and Tookey, 1983).

The most important active constituents of thevetia responsible for exerting cardiotonic effects are the cardiac glycosides (Langford and Boor, 1996), among which are thevetin A, thevebioside, gluco-peruvoside, acetylated monoside and other cerebrosides (Bisset and Bogor, 1962). Raw thevetia cake was extremely toxic when fed up to 15 percent in the starter and finishing diets for broilers (Atteh, Ibiyemi and Ojo, 1995).

Mesua cake is toxic when oil extraction is not complete, due to the presence of unknown deleterious substances in the residual oil (Konwar, Ahmad and Medhi, 1999).

One of the toxic compounds of calophyllum cake has been identified as calaustralin (Dash *et al.*, 1990).

Toxicity of azadirachta cake is caused by the presence of azadirachtin, tetranortriterpenoid (an antifeedant), isoprenoids and nimbidin, a sulphurous compound (Yakkundi, 1997; Usman *et al.*, 2005; Saxena *et al.*, 2010). Uko *et al.* (2006) incorporated up to 30 percent raw full fat azadirachta kernels into cockerel chick diets. Feed intake and body weight gain were depressed independently of the inclusion level, and starting from 15 percent in the diet, anaemia and leucocytosis occurred. Defatted azadirachta meal included up to 10 percent in the diet of in-lay Japanese quails reduced feed efficiency (but intake, egg production and quality were not affected) and caused adverse effects in liver and kidney tissues with long-term feeding (Elangovan *et al.*, 2000).

Pongamia cake contains anti-nutritional factors such as phytates, tannins, protease inhibitors, glabrin and a fat-soluble constituent karanjinin (a furano-flavonoid) (Vinay and Sindhu Kanya, 2008). When fed untreated to chicks, karanj expeller cake depressed weight gain when included at 10 percent of the diet, and elicited 100 percent mortality at 40 percent inclusion rate (Natanam, Kadirvel and Ravi, 1989). At 10 percent, untreated karanj cake and meal fed to 18-week-old white leghorn pullets decreased feed efficiency, egg production and quality (Natanam, Kadirvel and Viswanathan, 1989). Long-term feeding at 20 and 24 percent cake or meal in lamb concentrate had deleterious effects on lamb performance, especially spermatogenesis (Singh *et al.*, 2006).

Croton meal, in addition to containing carcinogenic phorbol esters, contains a toxic glycoprotein belonging to the type II group of ribosome inactivating proteins, crotin, similar to ricin (Stirpe *et al.*, 1976). Crotin showed LD_{50} of 20 mg/kg body weight when administered intraperitoneal in mice (Alexander *et al.*, 2008a). Non-toxic lectin, with effects on agglutination and haemolytic abilities

of erythrocytes in sheep and rabbits, was isolated from croton seeds (Banerjee and Sen, 1981).

POSSIBILITY OF FEEDING SOME UNTREATED NON-EDIBLE CAKES AND MEALS FROM SEEDS THAT GIVE NON-EDIBLE OILS

Feeding non-edible cakes and meals is not recommended before the appropriate treatment. However, feeding trials with untreated non-edible cakes and meals have been carried out based on two principles: (1) feeding up to, but not beyond, the threshold level of toxicity; and (2) the apolar toxic compounds get extracted with the oil, making the oil non-edible and the residual meal edible.

Untreated mesua cake feeding is reported to be possible when the oil extraction is complete, because the toxic compounds are soluble in the oil (Konwar, Ahmad and Medhi, 1999). Raw mesua meal containing 14 percent residual oil could be included up to 15 percent in the starter diet of chicks without any effect on body weight, but at higher levels feed efficiency was reduced (Baruah, Kalita and Saikia, 1997). When included in the diet of white leghorn layers at 15 percent of the ration, egg production and weight were significantly depressed (Baruah, Kalita and Saikia, 1997).

Calaustralin, a phenylcoumarin derivative present in *Calophyllum inophyllum* (Bhushan, Rangaswani and Seshadri, 1975), is not polar and thus can be extracted with the oil. De-fatted calophyllum cake can be fed at up to 15 percent in the diet of chicks, but with slight growth depression (Dash *et al.*, 1990). Mohapatra and Samal (2002) reported that an amino acid deficiency was the cause of the decline in weight gain of laying hens when offered calophyllum cake at 37 percent of their diet.

POSSIBILITY OF FEEDING SOME TREATED NON-EDIBLE CAKES AND MEALS FROM SEEDS THAT GIVE EDIBLE OILS

There are some non-edible meals and cakes that originate from seeds whose oils are edible. Examples are: *Balanites aegyptica*, *Terminalia bellirica*, *Putranjiva roxburghii*, *Perilla frutescens*, *Madhuca indica* and *Moringa oleifera*. *Camelina sativa*, which belongs to this group, is not discussed here. The utilization of its meal and cake in animal feeding is discussed in Chapter 17 of this document.

Balanites aegyptica cake is regarded as unsuitable for livestock feeding because it contains steroidal sapogenins (Chapagain and Wiesman, 2007). Sapogenin content was reduced from 3.2 g/100 g protein in the cake to 2.4 g/100 g in the fine fraction (Mohamed, Wolf and Spiess, 2000). Protein extraction by wet sieving using methanol reduced sapogenin to 1.7 g/100 g protein in the protein extract (Mohamed, Wolf and Spiess, 2000). Either fraction, obtained from air classification or wet sieving, has lower *in vitro* protein digestibility (82.0 and 86.4 percent, respectively) compared with the balanites cake (93.7 percent), due probably to their (fractions) enrichment in phytic acid (Mohamed, Wolf and Spiess, 2000).

Due to the high content of total phenols and tannins (Alexander *et al.*, 2008b), *Terminalia bellirica* seeds are used traditionally for tanning purposes (Rukmini and Rao, 1986). Terminalia meal contains unidentified heat stable antinutritional factors that result in lower feed intake and death in rats, mice and chicks (Rukmini and Rao, 1986).

Putranjiva roxburghii kernels contain phenyl, isopropyl and sec-butyl iso-thiocyanates of glucosides (Puntambekar, 1950). Chaudhary *et al.* (2008) isolated a trypsin inhibitor from the putranjiva seeds, active over a broad range of pH (2–12) and temperature (20–80 °C). Raghavendra *et al.* (2010) found that the methanol extracts of the seeds, which contain phenols, alkaloids, steroids, flavonoids and glycosides, showed cytotoxicity with an LC_{50} of 427.7 µg/ml in the brine shrimp lethality assay.

Although perilla seed oil is edible, perilla seed may be a source of a food allergen. Two cases were reported and studied by Jeong *et al.* (2006), where perilla seed caused anaphylaxis in two patients.

Mahua cake contains sapoglucosides that are bitter and toxic to livestock (Varma and Singh, 1979). Because of the harvesting time (at peak rainfall), the occurrence of aflatoxins constitute an additional problem when feeding mahua cake (Sidhu, Chandra and Behl, 2009). Mahua meal can be fed raw, up to 22 percent of the concentrate to rams, without any differences in slaughter weight and carcass characteristics (Kesava Rao *et al.*, 1998). Feeding mahua meal up to 15 percent in broiler chick rations induced lower feed intake, lower body weight gain and poor feed conversion ratio (Kumar, Vaishnava and Sajjan, 2000). Hot water and isopropanol (60 to 80 percent) treatment resulted in reducing the saponins content by 74 and 90 percent, respectively (Varma and Singh, 1979).

Moringa seeds contain glucosinolates that yield 4-(α-L-rhamnosyloxy)-benzyl isothiocynate after crushing (Makkar and Becker, 1997; Bosch, 2004). The glucosinolates present can be removed by water treatment (Makkar and Becker, 1997). However, the seeds also contain cationic peptides that have antibiotic properties and at high levels could decrease productivity (Ben Salem and Makkar, 2009).

A summary of feeding trials with these non-edible cakes and meals, either raw at low inclusion rates or after appropriate treatment, is reported in Table 3.

DETOXIFICATION METHODS

The risk of toxicity can be less serious with decreasing contents of the toxic compounds and anti-nutritional factors following appropriate treatments. Methods of detoxification can be classified into chemical, physical, biochemical and a combination of these processes.

TABLE 3
Effects on animal performance of feeding non-edible cakes and meals (from seeds that give edible oil) after different detoxification treatments

	Toxic compound	Detoxification methods and animal response	Reference
Balanites aegyptica	Steroidal sapogenins, diosgenin as the aglycon.	Up to 20% in the sheep diet. No significant difference in feed intake, liveweight gain and carcass analysis with the control group fed cotton-seed meal. A distinct black mucous membrane of the rumen was observed.	El Khidir *et al.,* 1983.
		At up to 12.5%, the diet fed to laying hens induced diarrhoea and retarded growth and led to cessation of egg laying.	
Terminalia bellirica	Heat stable factors	Fed up to 10% either raw or cooked to rats, mice and chicks. Feed intake was 1 g/animal/day. In two weeks, all the animals receiving raw as well as cooked kernel meal died.	Rukmini and Rao, 1986.
Perilla frutescens	Not reported	Raw meal up to 28% in rat diet when fed for 4 weeks did not affect significantly the feed intake but because of its deficiency in valine, body weight gain was less.	Longvah and Deosthale, 1998.
		De-hulled and cooked meal fed up to 28% in diet resulted in comparable body weight gain in rats fed casein.	
Madhuca indica	Sapoglucosides	Fed treated cake (first with 2.5% ferrous sulphate, then cooked or treated with 2.3% HCHO) up to 22% of the concentrate in rams' diet did not affect the slaughter weight, carcass characteristics or meat quality attributes.	Kesava Rao *et al.,* 1998.
		Washed meal (repeated cold water washing) replaced up to 100% of groundnut cake in buffalo diet. No significant difference in feed intake, nutrient digestibility or milk yield and composition.	Tiwari and Patle, 1983.
Moringa oleifera	Glucosinolate, cationic peptides (anti-fermentative)	Raw cake could be fed up to 6 g daily to growing lambs. At 4 g inclusion per day of raw cake the growth rate of the lambs improved.	Ben Salem and Makkar, 2009.

Chemical treatments

Chemical treatments include additives, alkaline and acidic treatments and solvent extraction. Although chemical treatment can reduce substantially the content of toxic compounds, the resulting meal or the protein extract has lower protein and amino acid content. Sodium hydroxide treatment reduced up to 98 percent the allergen content in castor meal (Gardner *et al.*, 1960) and reduced the toxicity of pongamia meal by converting karanjinin to less toxic intermediates (Panda, Sastry and Mandal, 2008).

Ammoniation of crambe meal resulted in the disappearance of glucosinolates (Kirk, Mustakas and Griffin, 1966), but this treatment decreased lysine levels (Liu, Steg and Hindle, 1993) and formed undesirable cyanobutane and other aglucon products in the meal which were still toxic (Carlson and Tookey, 1983). Ammoniation of azadirachta cake was found to result in a detoxified product suitable for animal feeding (Nagalakshmi *et al.*, 1999).

Hydrochloric acid treatment (soaking the meal in 2 percent HCl for 1 hour at room temperature, bringing up the pH to iso-electric point by diluted alkali and washing the residue) of pongamia meal resulted in the removal of up to 54 percent of the tannins, up to 72 percent of the phytates and up to 74 percent of trypsin inhibitor activity. This had the corollary of reduction of the protein content from 33 percent in the raw meal to 23 percent in the treated meal, but without affecting available lysine (3.6 percent to 3.5 percent) (Vinay and Sindhu Kanya, 2008).

Other chemical additives have also been used for inactivation of the toxic compounds. Calcium hydroxide was less effective than HCl for the detoxification of pongamia meal. Although 0.5 percent $Ca(OH)_2$ reduced the content of tannins and phytates substantially, it also led to a significant decrease in nutritive value of proteins and destruction of lysine, with the production of toxic constituents such as lysino-alanine (Vinay and Sindhu Kanya, 2008). Sodium carbonate left 1.7 percent epi-progoitrin in crambe meal, thus reducing its content by 82 percent (Mustakas *et al.*, 1976), while only 0.6 percent remained in ferrous sulphate-treated meal (Kirk *et al.*, 1971).

Solvent extractions are used depending on the polarity of the toxic compounds. Water washing is one of the successful methods of detoxification carried out on crambe meal (Baker *et al.*, 1977), azadirachta meal (Agrawal, Garg and Nath, 1987) and pongamia meal (Vinay and Sindhu Kanya, 2008), despite the loss of water-soluble nutrients. Water washing of crambe meal after inactivation of thioglucosidase resulted in 20 to 25 percent DM loss, but the resulting meal contained 50 percent CP, a balanced amino acid composition and 0.6 percent residual epi-progoitrin (Baker *et al.*, 1977). Rubber meal soaked in water (1:3) for 24 hours resulted in a substantial reduction in HCN content after one month of storage (from 120 to 2.6 mg/kg) (Narahari and Kothandaraman, 1983). Acetone extraction of crambe meal resulted in total removal of thioglucosides and epi-progoitrin from the meal, with good residual biological value protein (Van Etten *et al.*, 1969). Alcohol extraction of thevetia meal by a mixture of ethanol+methanol (80:20) resulted in 98 percent reduction in the glycoside content, with 18 percent DM loss and 25 percent CP increase (Oluwaniyi, Ibiyemi and Usman, 2007). Extraction of azadirachta meal with 80 percent methanol resulted in

TABLE 4
Effects on animal performance of feeding non-edible cakes or meals after different detoxification treatments

	Treatment	Animal response	Reference
Ricinus communis	Roasted meal	Adverse effect when fed up to 10% to ducks for four weeks.	Okoye *et al.*, 1987.
	Two-stage cooked dehulled meal (100 °C for 50 minutes)	Up to 10% in the diet of six week-old broiler birds for optimum performance.	Ani and Okorie, 2009.
	Meal supplemented with $Ca(OH)_2$ at 4 to 6%	Up to 10% and 15% in the diet of sheep and beef cattle, respectively, without adverse effects on feed intake or daily body weight gain.	De Oliveira *et al.*, 2010. Diniz *et al.*, 2010.
	Meal mixed with *Shorea robusta* seed meal (1:1), followed by treatment with ammonia and heat.	The mixture was fed at 20% of the diet to rats. The rats survived and had weight gain and feed intake comparable to the control group (15% casein).	Ghandi, Cherian and Mulky, 1994.
Hevea brasiliensis	Meal prepared from soaked-cooked-dried seeds	Fed up to 60% in the diet of rats (approximately 20% of protein in the diet). No evidence of toxicity. Feed intake, protein efficiency and growth rate comparable to casein-fed group at the same incorporation level.	Lauw Tjin Giok *et al.*, 1967.
Crambe abyssinica	Meal extracted with aqueous acetone	Replaced 20% of casein in the rat diet. Normal growth and equivalent protein efficiency compared with the casein.	Van Etten *et al.*, 1969.
	Meal supplemented with Na_2CO_3	When fed at dietary levels of 20 to 30% to chicks, there was a growth limitation (70–80% of the control). Some adverse side effect on organs.	Carlson and Tookey, 1983.
	Water-washed meal	Up to 10% in broiler chicken diet but with a decrease in feed intake.	Kloss *et al.*, 1994.
	Heat-Carbonate-treated meal	Up to 70% in beef cattle diet. Lower feed intake and daily weight gains, without significant differences in feed efficiency. Increased palatability with dehulling. Dehulled crambe meal so prepared, can replace up to two-thirds of soybean meal in the supplement.	Lambert *et al.*, 1970.
		Up to 30% in the pig diet. Crambe meal so treated has higher energy digestibility but lower protein utilization than rapeseed meal.	Liu *et al.*, 1995.
Thevetia peruviana	Alkali and acid treatment of the cake	Reduced feed intake and weight gain at up to 15% in the chick diet. Alkaline and acid hydrolysis was not efficient.	Usman *et al.*, 2009.
	Protein concentrate from the cake	Fed up to 30% to replace soybean in the chick diet with satisfactory performance of 90% of the animals and 10% mortality.	Odetokun, Akindumila and Ibukun, 1999.
	Autoclaved cake	Up to 10% in rabbit diet, no mortality, reduced feed intake, diarrhoea and rough and dry coat observed. Autoclaved cake could not support productive growth.	Taiwo, Afolabi and Adegbuyi, 2004.
Calophyllum inophyllum	Protein extract from the meal	Diets with 25 and 50% were able to support normal growth in rats when adequately supplemented with deficient amino acids.	Venkatesan and Rege, 1973.
Azadirachta indica	Water-washed cake	Up to 45% of concentrate fed to growing calves with no adverse effects on intake, digestibility and weight gain.	Nath, Rajagopal and Garg, 1983.
		Fed up to 40% of concentrate to buffalo calves. Led to higher weight gain, higher nitrogen balance and reduced urinary N. No significant difference in intake and CP digestibility.	Agrawal, Garg and Nath, 1987.
		Could be fed up to 25% of concentrate to growing goats without significant difference in feed intake, body weight gain and feed conversion efficiency.	Verma, Sastry and Agrawal, 1995.
	Organic solvent-extracted cake	Ethanol-hexane extracted cake fed up to 84% of the diet to rats without toxic effect. Protein use efficiency was comparable to the conventional cake.	Rao, 1987.
		Methanol extracted cake fed to rats at a rate of 25% in diet was promising in terms of feed efficiency and weight gain.	James, Ameh and Agbaji, 2009.
	Urea-ammoniated meal	Replaced groundnut meal totally (22.5% of concentrate) in the diet of goats, without significant effect on feed intake and body gain weight.	Anandan *et al.*, 1999.
		Lower digestibility parameters in lambs when included at 33% of concentrate. Similar average daily weight gain but enlarged kidneys observed.	Musalia *et al.*, 2000.
	Alkali-treated meal	Up to 10% inclusion in the diet could support the overall productive performance of white leghorn, without any obvious adverse effect.	Verma, Gowda and Elangovan, 1998.
Pongamia pinnata	Water-washed cake	Fed at 13.5% of the concentrate to lambs, it did not affect the feed intake, body weight gain and nutrient digestibility.	Soren and Sastry, 2009.
	Toxin-bonded cake	Fed at 13.5% of the concentrate to lambs; it decreased feed intake and body weight gain.	Soren and Sastry, 2009.
	Alkali-treated meal	NaOH-treated meal could replace up to 12.5% of the soybean meal in the starter diet of the broiler chicken without significant difference in body weight gain and feed efficiency.	Panda, Sastry and Mandal, 2008
	2% hydrochloric acid-treated meal	At 30% in the rat diet, no deleterious effects.	Mandal, Ghosh Majumdar and Maity, 1985.

a spent meal free from the antinutritional factors (Saxena *et al.*, 2010).

Protein extraction is another procedure to obtain pure protein isolates for use as animal feed and additives. The method consists of protein solubilization in alkaline solution followed by protein precipitation by acid at the iso-electric pH (Saetae and Suntornsuk, 2011). Usman *et al.* (2005) isolated proteins from azadirachta meal using 0.5 M NaCl at pH 7.5.

Physical treatments

Physical treatments lead to denaturation of the active toxic compounds and include thermal treatments (autoclaving, moist heat and microwave) (Liu, Steg and Hindle, 1993). Dry heating was effective in the de-allergenization of alkali-treated ricinus meal at 205 °C for 95 minutes (Gardner *et al.*, 1960). Cooking ricinus meal for 10 minutes destroyed its ricin content (Barnes, Baldwin and Braasch, 2009). Steam cooking of crambe meal reduced its content of epi-progoitrin by 30 percent, but increased the toxic nitrile content and decreased the level of available lysine (Liu, Steg and Hindle, 1993).

Biochemical treatments

Biochemical detoxification treatments are based on enzymic and fermentative reactions. Trypsin (6 percent by weight of the meal) digestion of ricinus meal resulted in a complete de-allergenization of the treated meal (Gardner *et al.*, 1960). The binding properties of tannins were used by Ghandi, Cherian and Mulky (1994). In their work, the toxic factor of the castor cake was neutralized by the tannins present in sal (*Shorea robusta*) seed meal.

Fermenting rubber cake and meal with the mycelium of the edible mushroom *Pleurotus tuberregium* for 96 hours at room temperature resulted in a decrease of its total cyanogens content from 500 to 5 ppm for the cake and from 300 to 4 ppm for the meal. The treatment resulted additionally in an increase in CP content from 29 to 39 percent (Ukpebor *et al.*, 2007).

A study realized by Hundsdoerfer *et al.* (2005) indicated that the larvae of *Hyles euphorbiae* could metabolize synthetic phorbol esters (12-tetradecanoyl-phorbol-13-acetate) either injected or fed. Phorbol esters occur in different species of the Euphorbiaceae, e.g. croton and jatropha.

EFFECTS OF FEEDING TREATED NON-EDIBLE CAKES OR MEALS ON ANIMAL RESPONSE AND PERFORMANCE

The usefulness of treated non-edible oil cakes and meals as animal feed depends on the efficiency and economic viability of detoxification methods and the possibility of using the treated co-products. The results of the feeding trials conducted on laboratory and farm animals after appropriate detoxification treatments of the non-edible cakes and meals are summarized in Table 4. Some of the detoxification attempts have shown promising results, and, at low levels of their inclusion in diets, many can be adopted without adverse effects on animal welfare and performance. Although many treatments are promising, the challenge lies in developing cost-effective and simple processes that can be adopted by farmers.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Studies on the possible use of co-products of the biofuel industry based on non-edible oils for animal feeding show that a lot still needs to be done. Main knowledge gaps are:

- The nature of some toxic compounds in these co-products is unknown. The current methodologies for analysing many of the toxic and antinutritional factors need improvement. For developing an effective detoxification process, it is necessary to define the chemical nature of the toxic compound(s) and their mode of action. This information is not available for many of the co-products.
- There is a need to further improve the detoxification processes for *Thevetia peruviana, Hevea brasiliensis, Calophyllum inopyllum, Mesua ferrea* and *Croton tiglium*. Studies on the utilization of the detoxified material by various farm animal species should also be conducted.
- Scaling up is needed for promising detoxification processes. The implementation of positive results can be successful only if large amounts of the co-products can be treated and used for animal feeding.
- The development, use and scaling up of the detoxification processes should be accompanied by socio-economic analysis.
- Preparation of high-value protein isolates and peptides for use in livestock feeds could be an alternative approach for use of otherwise non-edible cakes and meals, an approach that so far has received little attention. Processing for preparation of protein isolates and peptides could eliminate the toxic and antinutritional factors. Future work is warranted on this topic.

CONCLUSIONS

To make the biofuel industry more profitable and sustainable, use of the co-products-derived cakes and meals, generally rich in protein is of utmost importance. Detoxification has been successful for some of these products:

- *Ricinus communis* meal cooked at 100 °C for 50 minutes could be considered for addition at up to 15 percent in chick diets. The addition of lime at 4 percent was also promising when fed at up to 10 and 15 percent in the diet of sheep and beef cattle, respectively.
- *Hevea brasiliensis* meal soaked in water and left to ferment, or meal obtained from originally soaked seeds,

contains less HCN (reduced from 120 to 2.6 mg/kg DM after one month of storage). However feeding trials on farm animals need to be conducted to confirm the safety of feeding.

- Heat-carbonate-treated dehulled-meal from *Crambe abyssinica* has been shown to have acceptable palatability and can replace up to two-thirds of soybean meal in the supplement for beef cattle.
- Water washing, methanol extraction, urea and alkali treatments of *Azadirachta indica* meal gave promising results when fed to farm animals. Water-washed neem cake could be fed at up to 45 percent of concentrate for calves.
- Water-washed *Pongamia pinnata* meal can be incorporated at up to 13.5 percent of the concentrate in lamb diet. Alkali treatment was also effective.

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Chapter 20

Status of biofuels in India and scope of utilizing castor (*Ricinus communis*) cake – a biofuel co-product – as livestock feed

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ABSTRACT

Biofuel policy in India is unique in that it has been clearly spelt out that feedstock must be based on non-food sources, thus avoiding a possible food vs fuel conflict. Further, the policy views biofuels as a potential means to stimulate rural development and generate employment opportunities by using vast areas of land that are otherwise unfit for agriculture. Although the policy has the ambitious target of achieving 20 percent biofuel blending by 2017, currently less than 5 percent blending of petrol has been achieved. Based on current production levels, it is unlikely that India will fulfil the set targets. Major reasons include slow progress in establishing the area under jatropha (*Jatropha curcas*) cultivation; low productivity and poor market infrastructure for jatropha; land availability constrained by sugar cane expansion; a plateau in productivity of sugar cane; the price structure for biofuels; and import policy. Among the various co-products of biofuel, castor (*Ricinus communis*) cake is one of the potential resources that could be used for feeding livestock. Although castor cake has high protein, its use in livestock feeding is restricted due to the presence of toxic factors and it is currently being used as organic fertilizer, leading to under-utilization of a precious resource. Substantial research has been carried out to identify the nature of the toxins, their toxicity, susceptibility to various treatments and production response of different livestock to feeding processed cake. In spite of all the efforts, castor cake has not found a place as a feed resource and continues to be used as organic fertilizer, leading to its under-utilization. All the major castor producing countries – Brazil, China and India – have large livestock populations and big demand for protein supplements, so an appropriate detoxification technology to make use of castor cake could add great value to the castor, to the benefit of livestock producers and processing industries.

INTRODUCTION

India is one of the fastest growing economies in the world and energy is a critical input for socio-economic development. Fossil fuels will continue to play a dominant role in fulfilling the energy needs of India in the next few decades. Provisional estimates have indicated that domestic crude fossil fuel oil is able to meet only about 25–30 percent of demand, while the rest is met from imported crude. Biofuels are globally considered sustainable and an eco-friendly source of energy, and these also enhance national energy security and decrease dependence on imported fossil fuels. The growing interest in and demand for biofuels have resulted in diversion of grains, oilseeds, land and water resources to biofuels which otherwise could have potentially contributed to food and feed resources. For a large country like India, with a billion-plus human population and limited land mass, the role of biofuels has to go beyond the objective of achieving energy security and sustainability, towards addressing food and feed security. Choice of feedstock for biofuel production and efficient utilization of biofuel co-products can to a great extent address these issues. In the light of the above, an attempt has been made here to review the present status of biofuels in India, and the available technologies for utilizing castor cake – a potential biofuel co-product – as livestock feed.

STATUS OF BIOFUELS IN INDIA

India is one of the largest users of hydrocarbons and it is imperative that the country has a biofuel policy in place to address the issues of the economy (import expenditure), environment and energy security. The Government of India is seriously looking for use of alternative fuels to meet energy demand in a technically efficient, economically viable and environmentally sustainable manner. There are many concerns and challenges to be overcome if biofuels are to contribute positively to an improved environment as well as to agricultural and rural development (FAO, 2008). The 'National Policy on Biofuels' of India, released in 2009,

MAIN MESSAGES

- **Biofuel policy in India is based on non-food feedstock to minimize food-fuel conflict, and aims at utilizing the vast wastelands otherwise unfit for agriculture to stimulate rural development and employment.**
- **Castor cake – a protein rich, castor oil industry co-product – continues to be used as organic fertilizer, leading to under-utilization of a precious protein resource.**
- **The toxic principles in castor cake limit the direct use of castor cake as livestock feed, and research efforts to evolve suitable detoxification procedures have yielded variable results.**
- **Research conducted so far has shown that processed castor cake can certainly be incorporated at low levels in ruminant feeds, and with better processing methods higher levels of incorporation are possible.**
- **A concerted effort by the castor processing industry, researchers, feed industry and livestock farmers could lead to evolving efficient and commercially viable technologies for utilizing castor meal as livestock feed.**
- **Utilizing castor meal as livestock feed will have great relevance for countries like India, China and Brazil, which are not only the largest producers of castor cake but have large livestock populations and high demand for protein supplements.**

foresees biofuels as a potential means to stimulate rural development and generate employment opportunities, as well as aspiring to reap environmental and economic benefits arising out of their large-scale use. The Policy aims at mainstreaming biofuels by setting an indicative target for their blending up to 20 percent with petrol and diesel in the transport sector by 2017 (GOI, 2009). It is categorically mentioned in the Policy that the programme is to be carried out based solely on the non-food feedstocks that are raised on degraded or wastelands not otherwise suitable for agriculture, thus avoiding a possible conflict between food and fuel security. Bio-ethanol produced from sugar cane molasses and biodiesel produced from non-edible oilseed crops like jatropha (*Jatropha curcas*) and pongamia (*Millettia pinnata*) are currently being promoted for commercial use.

The biofuel industry in India is still in its infancy and biofuel production in India accounts for around 1 percent of global production. Of the 2.15 billion litres of ethanol produced in 2008, only 280 million litres were used for blending with petrol, and the target of blending petrol with 5 percent ethanol has yet to be achieved. The major reason for this has been the competing demand for ethanol for potable purposes and the chemical and pharmaceutical industries. To address this issue, the Government has recently increased the minimum purchase price of ethanol from Rs 21.50 to Rs 27.00 per litre of ethanol, hoping that this would increase the availability for blending. Large-scale blending of biodiesel with conventional diesel has not yet started in India. Around 20 biodiesel plants annually produce 140–300 million litres of biodiesel, which is mostly utilized by the informal sector locally for irrigation and electricity generation, and by the automobile and transportation companies for running their experimental projects (USDA, 2010).

The Planning Commission launched the National Biodiesel Mission to promote jatropha, and the first phase (2003–2007) was mostly a demonstration phase. The second phase involved the expansion of the activities of the first phase to make the programme self-sustaining by producing enough biodiesel to meet the 20 percent blending target (NCAER, 2007). Efforts by the different state governments and the federal government to boost the production of feedstocks for biofuels include the announcement of a minimum purchase price for jatropha seed and for biodiesel, subsidy programmes and tax incentives.

Shinoj *et al*. (2011) projected the demand for ethanol and biodiesel (Table 1) for varying levels of blending of biofuels, considering that the annual demand for petrol is increasing at 8.5 percent and diesel at 7.5 percent.

Based on their projections, Shinoj *et al.* (2011), concluded that to achieve its 20 percent blending target

TABLE 1
Projected demand for biofuels in India (million tonne)

Year	Petrol demand	Diesel demand	5% blending		10% blending		20% blending	
			Bio-ethanol	Biodiesel	Bio-ethanol	Biodiesel	Bio-ethanol	Biodiesel
2011–12	14.37	64.19	2.08	3.21	2.80	6.85	4.23	12.84
2016–17	21.61	92.15	2.68	4.61	3.76	9.83	5.92	18.43
2020–21	29.94	123.06	3.31	6.15	4.80	13.13	7.80	24.61

Source: Shinoj *et al.,* 2011.

India has to triple ethanol production or has to go for massive imports, both of which are unlikely due to the plateau in the productivity of sugar cane, demand for land and water for staple crops, import policy and high price of ethanol in international markets. Similarly for biodiesel, the jatropha-based biodiesel production programme is bogged down because of obstacles like slow progress in planting (current plantation area is 0.5 million hectares against the requirement of 26.25 million hectares for 20 percent blending), sub-optimal processing and marketing infrastructure, and under-developed distribution channels (Shinoj *et al*., 2011).

BIOFUELS FEEDSTOCK AND CO-PRODUCTS

Globally, the major feedstocks for biofuels are maize, sugar cane and oilseeds, as shown in Table 2.

Unlike other countries, which rely heavily on food crops like maize and oilseeds for their biofuel production, India's major biofuel feedstocks are molasses for ethanol, and non-edible oilseed such as jatropha and pongamia for biodiesel. Other minor feedstock include sugar cane juice, sweet sorghum, tropical sugar beet, edible oil wastage and animal fats. Although India is the largest producer of castor, the possibility of using castor oil for biodiesel production has not been explored intensively. In contrast, in Brazil, the third-largest producer of castor, the Brazilian Ministry of Agrarian Development has revived castor production as raw material for biodiesel (Lago, 2009). The co-products of feedstocks, such as bagasse (fibrous residue of sugar cane after juice extraction), oilseed cakes and glycerol, can be used for feeding livestock as sources of roughage, protein or energy. The scope and limitations of biofuel feedstock co-products from castor for livestock feed is discussed briefly here.

CASTOR CAKE PRODUCTION AND UTILIZATION

India is the largest producer of castor seed, followed by China and Brazil, accounting for around 73, 12 and 7 percent of global production, respectively (FAOSTAT data, 2009). Globally, the area under castor bean has not changed significantly over the last two decades, with little change in production (Table 3). The production of castor seed in India, largest producer of castor, has shown a consistent increase. Much of the castor oil produced in India is exported after meeting local demand. Currently castor oil is not being used for biodiesel production, and in the event of its use as biodiesel the local demand for castor oil in India would go up. This is likely to stimulate castor production, as the castor crop has several advantages over other biodiesel crops in terms of availability of high yielding varieties, short production cycle and consistent, superior yields. Castor oil is one of the world's most useful and economically important natural plant oils, with wide applications. Castor is a high-yield oilseed crop producing around 50 percent oil by weight in the seed, out-yielding conventional oilseeds like soybean, rapeseed, groundnut, sunflower and cottonseed. Castor oil obtained from castor seeds has high viscosity, heat and pressure stability; low freezing point; and the ability to form waxy substances after chemical treatments (Conceic *et al*., 2005), making it a potential candidate for biodiesel. There are different cultivars of castor, and oil content varies from 46 to 55 percent by weight (Ogunniyi, 2006). The residual castor cake obtained after oil extraction is approximately half of the seed weight. Whole seed contains 29 to 31 percent hulls, which are high in fibre and lignin, and de-hulling improves the oil extraction yield by 15–20 percent, besides improving the oil quality (Shashikala and Singh, 1992). De-cortication machines capable of de-shelling castor seeds are used in Brazil with an efficiency

TABLE 2
Distribution of feedstock in major biofuel producing countries

Country or region	Feedstock		Co-products
	Bio-ethanol	Biodiesel	
USA	Maize	Soy (40%), tallow (20%), canola (20%), palm (20%)	Distillers grain, oilseed cake and glycerol
Brazil	Sugar cane	Soy (80%), tallow (10%), other vegetable oils (10%)	Bagasse, oilseed cake and glycerol
EU	Beet/grain	Rapeseed (50%), soybean oil (40%), palm (5%) and tallow (5%)	Distillers grain, oilseed cake
China	Maize	Waste vegetable oils	Distillers grain, Glycerol
Canada	Maize	Tallow	Distillers grain, Glycerol

Source: Anon., 2009.

TABLE 3
Production of castor seed and cropped area

	1995		2000		2005		2009	
	Area	Production	Area	Production	Area	Production	Area	Production
India	789	780	1080	883	864	991	840	1098
China	190	170	290	300	240	220	210	190
Brazil	76	33	195	101	231	169	159	91
World	1237	1083	1769	1373	1586	1497	1481	1484

Notes: Area in thousand hectare; production in thousand tonne.

of 85 percent and an output of 650 kg/hour (Lago, 2009). De-cortication not only helps in improving the protein content and improve the efficiency of extraction but also reduces the fibre and lignin content in the hulls, which adversely effects the quality of the cake.

The composition of castor cake from different countries as reported by different researchers is presented in Table 4. The protein content of residual cake varies from 29 to 60 percent depending upon whether decorticated or corticated seeds are used for extraction (Mottola *et al.*, 1968; Okorie and Anugwa, 1987; Anandan, Anil and Ramachnadra, 2005). Alongside its high protein content, castor seed contains highly toxic and allergenic compounds, which severely limit or prevent its use as feed after oil extraction (Thorpe *et al.,* 1988; Audi *et al.*, 2005). The rumen degradability of castor bean meal protein was estimated to be 61.9 percent (Diniz *et al.,* 2011). Furthermore, castor bean meal protein was analysed for its amino acid composition and was found to be deficient in the essential amino acids lysine, tryptophan and methionine (Table 5) (Vilhjálmsdóttir and Fisher, 1971). Currently, it is being used as manure in India due to its high nitrogen, potassium and phosphorus content (Parnerkar *et al.*, 2001). Besides toxic principles, castor cake has high levels of fibre and lignin due to the presence of the seed hulls. High fibre and lignin content of the castor cake in monogastric animals such as poultry and pigs can be an issue, as they have limited ability to digest fibre.

Much research has been carried out to develop detoxification and de-allergenation methods so as to be able to use castor cake as livestock feed, with varying degrees of success. These technologies have not been very successful, as can be judged from the fact that in spite of the huge availability and low cost of castor cake in comparison with high costs of conventional protein supplements in India, castor cake has not been accepted as a feed resource, and it continues to be used as organic fertilizer.

TOXIC PRINCIPLES

Castor cake contains three undesirable constituents: a highly toxic, heat labile protein called ricin; a toxic alkaloid, ricinine; and a powerful and very stable allergen known as Castor bean 1 allergen (CB-1A) (Coulson, Spies and Stevens, 1960; Horton and Williams, 1989). The ricin is easily destroyed by heat and can be inactivated during the de-solventization step following solvent extraction. Ricin is reported to be present to the extent of 1.5 percent in the castor cake (Ambekar and Dole, 1957). The ricinine is present at very low levels, 0.23 percent of cake (Hinkson, Ellinger and Fuller, 1972) and presents no problem in animal feeds provided the feeds do not contain high levels of castor meal. Ricinine is also reported to have goitrogenic activity (Pahuja *et al.*, 1978) but ricinine or its hydrolysates even up to 100 mg/kg body weight were found to be harmless (Rao, 1970). The CB-1A allergen, however, requires a special processing step to de-activate it. CB-1A is a non-toxic, unusually stable protein that exhibits an extraordinary capacity to sensitize individuals exposed to small concentrations of the dust from castor beans or the castor cake. Alilaire was the first to describe human hypersensitivity to

TABLE 4
Chemical composition as percentage of castor cake

Country	DM	CP	CF	EE	Ash	NFE	NDF	ADF	Lignin	Ca	P	References
India	–	39.4	–	1.4	7.6	–	40.0	30.6	–	0.9	0.95	Gowda *et al*., 2009.
Nigeria	90.2	29.4	32.0	8.5	6.8	13.5	38.3	21.3	2.1	–	–	Babalola, Apata and Atteh, 2006.
Brazil	88.1	37.8	–	3.1	–	–	46.5	41.1	4.5	0.78	0.68	de Oliveira *et al*., 2010a.
Nigeria	93.1	36.4	37.7	2.2	5.4	11.4	–	–	–	–	–	Okoye *et al*., 1987.
Brazil	90.7	35.8	–	1.7	–	–	47.2	35.1	5.1	–	–	Diniz *et al*., 2010.
India	–	41.6	26.7	1.6	5.7	24.4	56.6	46.6	7.2	–	–	Anandan, Anil and Ramachandra, 2005.

Notes: DM = dry matter; CP = crude protein; CF = crude fibre; EE = ether extract; NFE = nitrogen-free extract; NDF = neutral-detergent fibre; ADF = acid-detergent fibre; Ca = calcium; P = phosphorus.

TABLE 5
Amino acid composition of castor bean meal protein

Amino acid	as % of protein	Amino acid	as % of protein	Amino acid	as % of protein
Valine	5.44	Methionine	1.51	Tryptophan	0.31
Isoleucine	4.68	Phenylalanine	4.02	Tyrosine	2.82
Leucine	6.42	Lysine	2.68	Cysteine	1.68
Threonine	3.44	Histidine	1.25	Proline	3.74
Glycine	4.31	Alanine	4.26	Asparatic acid	9.67
Serine	5.44	Hydroxyproline	0.28		
Glutamic acid	18.87	Arginine	8.61		

Source: Vilhjálmsdóttir and Fisher, 1971.

castor bean (Jones, 1947). CB-1A is the principal allergen of the castor bean and is a polysaccharidic protein factor. The allergen contents of de-corticated, de-fatted castor beans ranged from 6.1 to 9.0 percent, while the commercial castor cake contained 0.09–4.2 percent of the same (Coulson, Spies and Stevens, 1960).

Ricin is a 62–66 kDa protein consisting of two polypeptide chains, approximately 32 kDa and 34 kDa in size, linked by a disulphide bond (Audi *et al*., 2005). Ricin (RCA60) is a class II ribosome-inactivating protein; a heterodimeric protein. The A-chain of the ricin molecule is the effective toxin. It works by depurinating specific residues on the rRNA of the 28 S subunit of the ribosome, halting translation (Endo *et al*., 1987). The B-chain of the ricin molecule is responsible for cell entry. The disulphide link between the chains is not essential for the enzymatic activity of the A-chain, but it is necessary for toxicity, since the A-chain cannot enter the cell without the B-chain (Harley and Beevers, 1982; Lord *et al*., 2003). Ricin has relatively low toxicity when orally consumed, but when injected or inhaled, the LD_{50} can be as little as 3–5 µg/kg body weight (Audi *et al*., 2005). Ricin is also reported to inhibit rumen microbial growth (de Oliveira *et al.,* 2010b). The allergen consists of ricin agglutinin (RCA120), a potentially harmful allergen. Ricin and ricin agglutinin share around 90 percent homology within the A chain of the proteins, meaning that detection of the ricin A-chain is directly linked to detection of the agglutinin when using A-chain-specific antibodies (Pinkerton *et al*., 1999). The allergens set is composed of albumins 2S, formed by heavy and light subunits with molecular mass of 9 and 4 kDa, respectively (Thoyts, Napier and Millichip, 1996). Biochemical and immunological data relative to nine different fractions of albumins 2S resulted in identification of seven fractions exhibiting allergenic potential (Machado *et al.,* 2003). Allergen is a matter of concern for the people handling the cake, while the animals are unaffected by the allergen (UNIDO, 1989).

DETOXIFICATION AND DE-ALLERGENATION OF CASTOR CAKE

Growing demand for feed resources and the high cost of conventional feed resources in developing countries have prompted researchers to seek alternative feed resources. Although there are claims (Kim, 2001) that the normal extraction and de-solventization processes for meals are capable of total destruction of ricin, the presence of ricin in the solvent-extracted castor cake indicates that the normal processing methods are not capable of destroying the toxin totally. There is therefore a need for proper detoxification before any further use as livestock feed. Current oil extraction procedures utilize solvent extraction, which does not involve heating the meal, leaving the ricin and agglutinin mostly intact (Ogunniyi, 2006). The United Nations Industrial Development Organization (UNIDO) sponsored a research programme to investigate methods to detoxify castor meal in an economically feasible way to enable utilization of castor meal by the feed industry. The UNIDO work was carried out by Rhee in 1987 and published by UNIDO (1989). Similarly the International Castor Oil Association published a technical bulletin (ICOA, 1989) on detoxification and de-allergenation of castor meal. In addition, a lot of research has been carried out in the past and efforts to develop a suitable processing method for effective utilization of castor cake as livestock feed continue.

A number of different approaches – physical, chemical and biological, alone or in combination – have been tried by different workers. The efficacy has generally been tested based on the actual reduction in the toxic principles before and after, indirect quantification of toxins (using precipitin, neutralization and agglutination) or animal experiments. Autoclaving at various pressures (10–20 psi) and duration (15–60 minutes) was earlier tried to detoxify castor meal (Jaki, 1940; Ambekar and Dole, 1957; Okamato *et al*., 1965; Mottola, Mackey and Herring, 1971). Autoclaving highly toxic castor pomace for periods of 15 minutes or more resulted in essentially complete destruction of the toxin, with minimal changes in the physical character of the substrate (Kodras, Robert and MacVicar, 1949). Autoclaving at 125 °C for 15 minutes or at 20 psi for 60 minutes almost completely destroyed ricin with minimum physical changes in oil cake properties (Purushotham, Rao and Raghavan, 1986). Dry heat does not seem to have much effect on reducing toxin levels in the castor cake (Heller, 1932). Ambekar and Dole (1957) reported that the heating of castor bean meal to 150 °C for 3 hours did not reduce the toxin levels, and feeding the heat-treated cake resulted in rat mortality. However, a few reports also exist (Tangl, 1938; Okorie *et al*., 1985) showing the beneficial effects of heat treatment in various time and temperature combinations in removal of the toxin. Earlier attempts to detoxify castor bean meal by steaming at different temperature and time combinations were not successful (Borchers, 1949; Okorie *et al*., 1985). The absence of toxic symptoms in chicks fed hot-water-extracted castor cake indicated that the water treatment was more effective (Vilhjálmsdóttir and Fisher, 1971). Dry heating (200 °C [400 °F]) and moist cooking with different chemicals (1–2 percent NaOH, 3–10 percent formaldehyde, 0.9 percent HCl) was effective in reducing the toxin by 98–100 percent as determined by the precipitinin test (Gardener *et al*., 1960). In studies with rats, tannins have been successfully used to neutralize the toxic effect of castor meal extract in rats. This is based on the ability of the tannins to react with proteins to form tannin protein complexes that interfere with digestibility and absorption of the proteins (Gandhi and Mulky, 1994). Of the various chemicals tried, treatment with NaCl at 1 per-

TABLE 6
Effect of different detoxification methods and their efficacy in toxin reduction

Technology	Process	Response	Source
Solid state fermentation (SSF)	Biological detoxification using SSF of castor bean waste by fungus *Penicillium simplicissimum*	Ricin reduction to undetectable levels. Reduction in allergic activity by 16%	Godoy, Gutarra and Maciel, 2009.
Thermoplastic extrusion	1 or 2% CaO, followed by extrusion	2% was more efficient than 1% CaO. Simultaneous detoxification and de-allergenation	Ascheri *et al*., 2007.
Two-stage cooking	Cooking at 100 °C for 20, 30, 40, 50 or 60 minutes	Cooking at 50 and 60 minutes resulted in reduction of ricin by 70 and 77%, respectively	Ani and Okorie, 2006.
Lime treatment of castor cake (feed grade @ 4% w/w)	Cake wetted with water containing feed-grade lime (4% of cake weight)	Lime treatment reduced ricin by 58%	Gowda *et al*., 2009.
Boiling or autoclaving	Boiling or autoclaving of seeds for 20 minutes before solvent extraction	Promising reduction of chain A (ricin) detected by antibody reaction	Daniel *et al*., 2009.
Hot press	Heating the crushed meal to high temperature resulting in meal expelled at 130 °C	No reactivity with antibody, implying effective destruction of ricin	Daniel *et al*., 2009.
Physicochemical treatments	Physical: soaking, steaming, autoclaving (15 psi for 30 minutes), heating Chemical: ammonia, formaldehyde, lime (10 & 20%) and tannic acid	Less than 90% (varying from 27 to 90%) reduction in ricin	Anandan *et al.,* 2005.
Physicochemical treatments	Boiling (30 & 60 minutes), Autoclaving (15 psi 30 minutes), Lime 4%, Sodium hydroxide 10%	Above 91% (varying from 91 to 100%) reduction in ricin	Anandan *et al*., 2005.

cent was found to be most effective (Kiran, 1998; Agarwal, 2001) in detoxifying castor cake.

Some of the recent approaches for detoxification and their efficacy as reported by different researchers are summarized in Table 6.

As far as approaches by crop scientists are concerned, there are basically two ways of toxin reduction. The first is a conventional one based on selection and breeding, whereby the varietal differences in toxin levels are exploited. This involves screening and identifying lines with low toxin levels and promoting the low-toxin lines as commercial cultivars. The second one is the biotechnological approach, whereby efforts are made to suppress or knock out the genes involved in toxin production. Work is underway by the crop scientists at the Directorate of Oilseeds Research, Hyderabad, India, to reduce the toxic endosperm proteins, ricin and *Ricinius communis* agglutinin in the seed through post-transcriptional gene silencing approaches (DOR, 2010).

FEEDING STUDIES USING CASTOR CAKE

Feeding studies using laboratory animals or domestic animals to assess efficacy, although costly and time consuming, is always preferred over the chemical quantification of the toxins. Animal response to the processed cakes would be influenced by the efficiency of the detoxification; the level of the cake in the diet; duration of feeding; and the animal species. Studies have been carried out since early 1940 by different workers using differently processed cake at varying levels in different species. Studies in fattening cattle at 10 percent of castor bean meal (CBM) did not have any ill effects. In growing cattle, at 10 percent level, feed intake and growth were reduced in comparison with the cottonseed fed group (Bris and Algee, 1970). Butter from cattle fed CBM showed slightly increased viscosity and lower iodine value (Popvic, 1968), and it was concluded that incorporation of CBM at a 10 percent level was not economical. Reddy, Reddy and Reddy (1986) observed optimum feed intake with a comparable plane of nutrition in experimental buffaloes fed 30 percent CBM ration compared with those on the control ration. The growth rate and efficiency were depressed in lambs when autoclaved CBM replaced groundnut cake nitrogen beyond 33.3 percent without affecting nutrient intake and digestibility (Purushotham, Rao and Raghavan, 1986). Kiran (1998) noticed a significant depression in the digestibilities of dry matter in sheep fed 26 percent raw castor bean meal, while processed castor bean meal (1 percent NaCl and 0.2 percent NaOH, w/w in 1:2 w/v) resulted in comparable nutrient digestibility. No vital organs revealed any gross or histopathological changes due to feeding of NaCl-treated castor cake at 21 percent of the diet in rabbits (Agarwal, 2001).

The results of the studies using detoxified cakes in different animals as reported by other researchers are summarized in Table 7.

From the animal experiments conducted it shows that ruminants are relatively more tolerant than monogastrics and can withstand higher levels. Interestingly, a few of the studies using untreated cake showed no deleterious effects in ruminants, and this needs to be further investigated. In economic terms, a few of the studies involving the feeding cost of production revealed that feeding treated castor cake in place of conventional oilcakes resulted in either

TABLE 7
Effect of different processing methods of castor cake on production responses in livestock

Processing method	Feeding details			Animal response	Reference
	Species	Level	Duration		
Non ruminants					
Two-stage cooking: Cooking at 100 °C for 50 minutes	Broiler finisher birds	0, 10, 15 and 20% of the diet	4 weeks	Birds fed 10% cake had similar feed intake and weight gain as control, At higher levels there was depression in feed intake and weight gain,	Ani and Okorie, 2009.
Roasting of seeds at 140 °C for 20 minutes	Broiler birds	0, 10, 15, 20 and 25% of the diet	6 weeks	Inclusion of cake reduced the feed intake and weight gains at all levels. Watery faeces, salivation, drooping of the wings, poor feathering, emaciation and death were observed at 20 and 25% levels. Severe congestion of the internal organs and haemorrhages, degeneration of the renal epithelial cells, hepatocytes, bile duct proliferation and lymphocytic depletion in the lymphoid organs were observed at all levels of inclusion, although at 10% level there was no mortality.	Okoye *et al.,* 1987.
Fermented cake. Water soaked (1:4 ratio) and fermented for 5 days in airtight conditions	Broiler chicks	0, 5, 10 and 15% of the diet	56 days	No deleterious effect on growth response, nutrient digestibility, blood cell counts, serum enzymes and carcass yield in 5% fed groups.	Oso *et al.*, 2011.
Boiled castor bean meal supplemented with β-xylanase	Broiler birds	0, 10, 15, 20 and 25% of the diet	49 days	Weight gains up to 15% level were comparable to control. At higher levels weight gain reduced and there were changes in haematological values and serum constituents.	Babalola, Apata and Atteh, 2006.
Roasting of seeds at 140 °C for 20 minutes	Ducks			Reduced feed intake and weight gain in ducks when fed at 10% of the diet	Okoye *et al.,* 1987.
Hot water extraction 4 times 10 minutes each (1:5 times water)	Chicks	40% of the diet	21 days	Hot-water-extracted cake was satisfactory, while supplementing with lysine and tryptophan gave comparable results to soybean protein in terms of growth and feed conversion ratio.	Vilhjálmsdóttir and Fisher, 1971.
Boiled castor meal	Growing rabbits	0, 10, 15, 20 and 25% of the diet	14 days	Daily gain and feed conversion ratio was comparable up to 15% inclusion level. Animals in 10 and 15% groups had 33% mortality, while 20 and 25% groups had 100% mortality	Adedeji *et al*., 2006.
Ruminants					
CaO treatment at 6% of cake (soaking cake in CaO solution followed by drying)	Crossbred cattle	0, 33, 66 and 100% replacement of soybean meal	14 days	Did not affect the digestive and physiological variables at different levels and treated cake can partially or totally replace soybean meal. The feed cost for unit carcass gain at 66% level was found to be comparable. Even the untreated castor cake gave satisfactory results with regard to digestive and physiological variables.	Diniz *et al.,* 2010.
Ricin detoxified meal	Dairy cows	10 and 20% meal 0.5% castor oil	14 months	Transfers of ricinine, ricin, hydroxy fatty acids and antigens were at or below detection limits. Milk from cows on long-term castor meal and castor oil intake was not apparently detrimental when fed to calves and rats. No residue accumulation in muscle was observed, and no abnormal conditions were associated with feeding castor meal to the cows.	Robb *et al*., 1974.
(i) Expeller cake, (ii) Solvent extracted cake (i) and (ii) treated with 4%lime	Sheep	0 and 15% of the diet	21 days	There were no changes in blood enzyme profile with treated and untreated cakes. Lime treatment improved the efficiency of microbial protein synthesis.	de Oliveira *et al*., 2010a.
Alkali treatment (4% lime treatment – technical grade) followed by extrusion	Growing sheep	28% of concentrate mixture (13% of diet)	7 months	Feed intake and weight gain were not affected. Animals were healthy throughout the experiment and there were no pathological changes in visceral organs.	NATP, 2004.
	Milking buffaloes	10% of the concentrate mixture	75 days	Feed intake, nutrient utilization, milk yield and composition were comparable to control group, and the castor-fed group gave greater profit.	
(i) Solvent extracted (ii) Lime treatment (feed grade lime @ 4% of cake, soaked overnight and dried)	Adult sheep	0 and 12.3% of the total mixed diet	150 days	Feeding solvent extracted or lime treated cake had no effect on body weight changes, nutrient digestibility, rumen fermentation pattern and histopathology of visceral organs compared with control group fed soybean meal.	Gowda *et al*., 2009.

comparable or lower feeding costs. Differences in the digestive physiology of ruminants versus non-ruminants mean that the high fibre and lignin content of castor cake are likely to affect the performance of monogastric animals. Although at low levels of inclusion the performance was comparable to controls, feed industry and farmers are not yet accepting the technology. Before the technology can be accepted there is probably a need for more focused studies involving the feed industry and farmers on a large scale, with better interaction among stakeholders, namely castor processing industries, researchers, feed industries and livestock farmers.

KNOWLEDGE GAPS AND FUTURE RESEARCH NEEDS

Ricin is one of the most potent naturally occurring plant toxins, and all care has to be ensured that the detoxification process is foolproof. This will ensure that the detoxified cake can be safely fed to any category of livestock irrespective of species or age. Different researchers have used various approaches to quantifying the toxin and as a result, though some of the methods were effective in neutralizing the toxins completely, this was not necessarily reflected in animal experiments. This is a major limitation: lack of a sensitive and commonly accepted approach. Further, few of the processing methods were limited to neutralization of toxins, and the subsequent animal experiments were not carried out to ascertain the efficacy of the same. There is a need to identify the most reliable and acceptable method that would have high correlation between the chemical quantification and animal response. This would ensure that the selection of an appropriate detoxification method based on toxin quantification would correlate well with the response in animal studies. In addition, most of the studies have been carried out at a laboratory scale, where the conditions are comparatively easy to control. Up-scaling to a commercial level while retaining the same efficiency always presents a problem. Involving the industrial partners in evolving appropriate processing methods at an earlier stage of technology development would facilitate easier adoption of technology. The technology needs to be practical, industry adaptable and inexpensive for detoxifying the ricin and completely inactivating the allergens without affecting the quality of the product. Crop scientists using recent advances in plant breeding and biotechnological approaches could contribute significantly by evolving new varieties with low or negligible toxin levels.

CONCLUSIONS

An increased demand for biofuels based on castor seed would result in availability of large quantities of castor cake, and utilizing this feed resource would add great value to the castor processing industry and livestock production. At present, the cost of untreated castor cake is 40 to 60 percent cheaper than the conventional protein supplements used in livestock feed in India, and adding the processing cost would not change the price structure drastically, thus making castor seed cake competitive and commercially viable. Incidentally, the major producers of castor – countries like Brazil, China and India – have large livestock populations and a shortage of protein supplements. The technology could have great relevance for regional development. Although a lot of research has been carried out and the research continues to develop an appropriate technology for detoxification, the current technologies have not been adopted by industry or otherwise commercialized. There is a strong need to address this issue, involving the researchers and the industries concerned in successfully translating the knowledge generated into commercially viable technologies. This would only be possible by bringing together all the stakeholders: crop breeders, livestock nutritionists, castor processing industries, feed industries and farmers. A collaborative approach is required all the way from selection of superior cultivars with low anti-nutrients; through selection of effective and economical detoxification method; to scaling up methods for commercial-scale operations; together with large-scale field trials involving plant breeders, livestock nutritionists, feed technologists, castor processing industries, feed mills and farmers. This offers a way forward for successful commercialization and popularization of castor cake as livestock feed.

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