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Implications for the Feed Industry

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1. Introduction

The animal feed industry relies on cereal grains and pulses to supply energy and protein, respectively. Increasing amounts of both groups of ingredients, but in particular, cereal grains, are being used for the production of ethanol for biofuel. Currently, about a third of the maize crop produced in the United States is used for ethanol production and will rise to about 43 % by 2015 (van der Aar and Doppenberg, 2009). Although limited in impact, a considerable amount of oils produced from oilseeds such as canola, soybean, peanut and sunflower is being processed into biodiesel. This is causing a major strain in the supply of edible oil for feed manufacturing. An indirect effect of the increased use of maize for ethanol production is the change in land use, whereby, farmers in North America are converting land previously used for soybean production into maize production (Anon., 2011a). Although maize is the main cereal grain used by the ethanol industry, it is by no means the only grain used but plants in Canada and Europe tend to use more wheat while the two main plants currently in production in Australia and a few in the USA rely on sorghum.

Regardless of the grain type used, the ethanol industry generates a large amount of waste, principally in form of distillers' dried grains with soluble (DDGS). It is estimated that a kilogram of maize grain generates three equal parts of ethanol, DDGS and carbon dioxide (Saunders and Rosentrater, 2009). Although the two by-products, DDGS and CO₂, represent value-adding to the primary grain resource, the ethanol industry is faced with need to dispose of large volumes of DDGS. However, as more grains are used for producing ethanol, less is becoming available to the feed industry. Distillers' dried grains have become a feed resource, first explored for ruminant animal and pig feeding but lately for poultry too.

The composition of DDGS is highly variable, depending on such factors as the base grain used, the age of the manufacturing plant, the distillation process, and the preparation of the final product, especially drying and packaging (Cozannet et al., 2009; Meyer et al., 2010; Cozannet et al., 2010c). Variability in quality and nutrient composition is a major problem militating against the use of DDGS for animal feeding. Although the ethanol and feed industries both agree to the terminology of DDGS, as the residue resulting from fermentation and distillation, there are many types of DDGS.

Ethanol is also made from sugarcane and similar ingredients with readily fermentable sugars. This yields a fibrous residue known as bagasse that can be fed to ruminant animals. As with grain residue, there is great variability in the quality of bagasse according to the raw material (crop) used, age of crop and method of processing during juice extraction. Biodiesel

production is the other side of the biofuel industry, resulting into the production of glycerol and press cakes as by-products. This chapter examines the range of by-products of the biofuel industry that can be used for animal feeding; response of animals to diets containing these products; limitations to their use, and potential for increased utilization of such products.

2. By-products of the biofuel industries

Distillers’ dried grains are the most important by-product of the biofuel industry for the feed industry. Distillers’ grains arise from fermentation of cereal grains, followed by distillation of ethanol. The wet product could be marketed as such (wet distillers’ grains, WDG) or is dried to different levels of moisture content. Some soluble material may be returned to the waste grain prior to drying, giving rise to the term, DDGS. There are plants, which use grains to produce glucose syrup rather than ethanol. The process also yields DDGS but there may also be low quality syrup, which can be mixed with the waste residue prior to drying, to form a cake. The biodiesel industry yields only one main by-product, glycerol, which is a mixture of glycerine, impurities and any remaining alcohol plus the catalyst, usually sodium hydroxide, that is used in the process. The press cake that is derived from the oil extraction process is also different from regular meals, as has been borne out by tests conducted on pigs (McKinnon and Walker, 2009). The biofuel industry in Brazil and Australia relies more on sugarcane than on cereal grains. The fermentation of cane sugar to ethanol yields a fibrous material, bagasse, which is mainly used for feeding ruminant animals, and will be further discussed in this Chapter. The composition of key by-products of the biofuel industry that may be useful as feed is shown in Table 1.

Item (% as-fed basis)	DDGS	WDGS	HP-DDGS	Rapeseed cake
Dry matter	86.7	45	91.7	90.4
Crude protein	26.9	30.2	39.6	32.1
Ether extract	13.3	14.2	3.6	18
Starch	7.65	3.65	11.2	0
NDF	30.2	30.8	22.2	27.7
ADF	13.1	20.2	11.2	19.7
Calcium	0.01	0.03	0.02	NI
Phosphorous	1.18	0.87	0.44	NI
Sulfur	0.15	0.72	0.81	NI
Reference	(Kelzer et al., 2010)	(Kelzer et al., 2010)	(Babcock et al., 2008)	(Schöne et al., 1996)

NI: Not indicated; HP-DDGS: high-protein DDGS.

Table 1. Nutrients composition of some biofuel by-products

2.1 Cereal by-products

As has been highlighted above, DDGS are the main by-products from the fermentation of cereal grains and distillation of ethanol. In terms of yield, one kg of grain would yield about

a third of its weight in ethanol, another third as DDGS and a third will be lost as carbon dioxide (Saunders and Rosentrater, 2009). Generally about 0.84 kg maize DDGS would result from the production of one litre of ethanol (Kim *et al.*, 2010b). The residue would be dried as such or some of the soluble material that is obtained is put back into it, resulting in changes in colour, moisture and nutrient composition. The drying process greatly influences the quality of the final product. Heating induces the Maillard reaction, which results in binding of sugars to lysine, with the result that the latter becomes unavailable. Several feeding trials have shown that lysine is the least available amino acid in DDGS (Jacela *et al.*, 2010b; Cozannet *et al.*, 2010; Yang *et al.*, 2010). Drying also changes the colour of DDGS, creating a range of light to dark products. Some DDGS are overheated and become dark as a result. Generally, light DDGS have a higher nutritive value than dark ones (Cozannet *et al.*, 2009; Cozannet *et al.*, 2010b) but Seabolt *et al.* (2010) have shown that pigs prefer the dark DDGS to light products and tended to lose weight when placed on diets containing light DDGS. For poultry Cozannet *et al.* (2010c) have reported a strong relationship between lightness (luminance) score and lysine:CP ratio and also lysine digestibility.

Production plants may also influence the physical quality of the DDGS. Major differences have been observed in the quality of maize DDGS from old and new plants in North America. In general, the technology involved in ethanol production continues to improve, so that the quality of DDGS is continuously improving. This will be the driver to increased utilisation of DDGS by the poultry and pig industries. In a comparative trial on DDGS from two plants, one old and the other new, Hastad *et al.* (2003) reported metabolizable energy values of 13.1 and 14.6 MJ/kg, respectively for the two products when fed to pigs. In another study involving ten plants that were less than 5 years old, Spiehs *et al.* (2002) reported wide variations in the composition of DDGS originating from the plants. The mean content of crude protein was 30.2 %, with a coefficient of variation (CV) of 6.4 %; while crude fat and crude fibre contents were (mean, CV): 10.9, 7.8 and 8.8, 8.7 %, respectively. Lysine was the most variable of the amino acids, with a CV of 17.3 % while methionine had a CV of 13.6 %. There may also be differences in composition of DDGS from different crops although Yang *et al.* (2010) reported that there were no differences in ileal digestibility of CP between DDGS from maize or wheat or combinations of these two grains when fed to pigs but values were smaller for the wheat DDGS than the other two products. Lysine and threonine were the least digestible. Ileal digesta viscosity was greater on the diet containing the mixed DDGS than single grain DDGS. Distillers' dried grains are known to compact during storage, creating a flow problem. The degree of compaction is related to moisture content after drying, handling, container filling method, filling height and container size (Clementson *et al.*, 2010; Clementson and Ileleji, 2010).

2.2 Sugarcane by-products

Bagasse is the main by-product of the biofuel industry when sugarcane or similar ingredients are used as source of carbohydrates for ethanol. It is the fibrous material that is left after the stalks are crushed and the juice is extracted (Anon., 2011b). Bagasse contains 45-55 % cellulose, making it suitable for further processing for the extraction of ethanol. It also contains 20-25 % hemicellulose, 18-24 % lignin, 1-4 % ash and about 1 % wax. In Brazil, the canes are milled rather than crushed, to maximise juice extraction and reduce the moisture content of the resulting bagasse, and to increase its value as a fuel. The juice is filtered, pasteurised and evaporated to produce a syrup. The syrup is then centrifuged to form sugar

crystals and molasses, another by-product of the sugar-from-sugarcane industries. Molasses is also rich in carbohydrates and can be used as a source of energy and a sweetener in animal feeding.

2.3 Biodiesel by-products

Biodiesel production begins with the production of oil from oil-rich ingredients such as oilseeds, certain pulses and cereal grains. Oilseeds like canola, peanuts, sunflower and soybean are the ingredients, which when used will create the most strain in feed supply. The oil extraction process yields a press cake that can be used for animal feeding (McKinnon and Walker, 2009). The oil is subsequently used to produce biodiesel, yielding glycerol as the main by-product (Anon, undated). The production of biodiesel therefore creates a strain to feed supply at two points – first at the point of supply of full-fat protein meals and the second at the point following the catalytic conversion of edible oil into biodiesel and glycerol. The last process creates a shortage of oil for animal feeding, oil being essential in most non-ruminant diets as a source of energy, particle binder and sweetener.

3. Use of biofuel by-products for animal feeding

There is no doubt that the biofuel industry will create some feed shortage for the animal industry. Some of the effects are direct while there are also indirect effects. More grains are being used for ethanol production, resulting in a reduction in volumes available for animal feeding. Large areas of land that were used for soybean production in the United States are also being re-directed into maize production as maize prices rise, in response to shortage and subsidies to the biofuel industry. The requirement for EU states to increase the biofuel share in fossil fuels to at least 10 % required 19 % of the annual cereal consumption and the full output of rapeseed (Popp, 2008). To fully substitute fossil fuels, the EU would require at least twice the current output of cereals and 25 times the current rapeseed and sunflower seed production. Ileleji (2010) was of the view that the real success of the maize ethanol industry will not depend on how well maize ethanol does as a fuel but on how well its by-product, DDGS, does as a feed. The feed industry is being compelled to use the by-products arising from the biofuel industry for animal feeding. It is speculated that the proportion of these by-products in the diet will continue to rise as less conventional ingredients become available. In an assessment of the potential for use of DDGS by the Chinese livestock sector, Fabiosa *et al.* (2009) estimated that producers could make a saving of one dollar per hundredweight through the use of DDGS in diets. The report cautioned that mycotoxin contamination and nutrient profile variability must be watched. Batal (2009) has also cautioned that DDGS cannot completely replace maize in poultry diets due to their inherent limitations in terms of price, handling and logistics in the mill and nutrient variability. These limitations are further discussed below.

The increased inclusion of biofuel by-products in diets may negatively impact on animal productivity, as will be highlighted later in this chapter. However, nutritional studies are intensifying into the identification of ways by which the nutritive value of diets that are high in these by-products can be improved. Ultimately, many of the by-products are more suitable for ruminant animal feeding due to the high fibre content and nutrient deficiencies. However, producers of non-ruminant animals such as pigs and poultry are being forced to use the by-products in response to shortage and cost of grains. The use of biofuel by-

products for non-ruminant animal feeding entails more diligent feed management and will be examined further.

3.1 Ruminant animal production

By their anatomical and physiological nature, ruminant animals would use by-products of the biofuel industry more efficiently than non-ruminants. Many of the by-products are fibrous and deficient in fermentable carbohydrates while being higher in non-carbohydrate constituents, particularly protein. Ruminant animals are endowed with the rumen ecosystem, which aids in the digestion of fibre. Rumen microbes are also able to utilise low quality protein to generate higher quality protein and also synthesise nutrients such as vitamins from other sources. Therefore, ruminant animal production is not generally as negatively affected as non-ruminant animal production when by-products of the biofuel industry are included in the diet. The efficiency of production may, however, be compromised since DDGS, especially that made from wheat, contains significant amounts of rumen degradable protein (Belyea *et al.*, 2010). The results of studies on the use of some by-products for ruminant animal feeding are summarised in Table 2.

Species	By-product	Optimum inclusion rate or proportion (%)	Reference
Beef cattle	WDGS	40	(Larson <i>et al.</i> , 1993)
		40	(Corrigan <i>et al.</i> , 2009)
	DDGS	20	(Vander Pol <i>et al.</i> , 2006)
		20	(Huls <i>et al.</i> , 2008)
Dairy cattle	DDGS	30	(Kalscheur, 2005)
		21	(Ranathunga <i>et al.</i> , 2010)
		30	(Janicek <i>et al.</i> , 2008)
	Glycerol	430 g/day (DM basis)	(DeFraain <i>et al.</i> , 2004)
Sheep	DDGS	40	(Held, 2006)
	Rapeseed meal	30	(Mandiki <i>et al.</i> , 1999)

Table 2. Summary of selective studies using biofuel by-products in ruminant animal diets

The use of DDGS may actually be of advantage to the ruminant animal industry as the product has been shown to reduce methane emission by nearly 20 % when included in beef cattle diets (McGinn *et al.*, 2009). Distillers’ dried grains have been successfully used to replace compound feed in the feeding of breeding ewes, with the material marginally improving milk yield, wool production and flock fertility (Dimova *et al.*, 2009). This is similar to the findings of Franke *et al.* (2009) who reported no effects of DDGS or rapeseed meal on milk yield of dairy cows, although milk protein content was reduced. Another study by Kleinschmit *et al.* (2006) actually reported an increase in milk yield, milk fat yield and feed efficiency on dairy cattle diets containing 20 % DDGS. The DDGS tended to increase the proportion of butyrate in the rumen although the total concentrations of volatile fatty acids were higher on the diet without DDGS.

The feed intake of lambs in response to DDGS inclusion does not appear to depend on the source grain of the DDGS, with similar effects being observed on diets containing maize, barley or wheat DDGS at 20 % of a grower diet (McKeown *et al.*, 2010). However, feed

efficiency was poorer on the wheat DDGS than on the maize DDGS, due to greater production of ruminal ammonia and lower digestibility of the former. These researchers concluded that maize, wheat or triticale DDGS can replace a mixture of barley grain and canola meal at up to 20 % in diets for lambs. Mckinnon *et al.* (2008) established that wheat DDGS could replace barley grain at up to 50 % in diets for cattle.

In another study, Aldai *et al.* (2010) compared the meat quality of cattle raised on diets supplemented with barley or maize DDGS. Meat obtained from the diet containing the barley DDGS was darker at 24h and less tender than meat from animals raised on the diet containing maize DDGS.

Sugarcane bagasse is used extensively to feed cattle in many sugar-producing countries such as Brazil and Cuba (Dhore *et al.*, 2006; Murta *et al.*, 2009; Nagalakshmi and Reddy, 2010). Although such bagasse may be from the sugar industry rather than the biofuel subsidiary, the products resulting from the two industries are similar. Most of the reports point to a positive effect of the inclusion of bagasse in terms of dry matter intake and changes in rumen function, including nutrient digestibility. Cassava bagasse has been observed to alter the rumination and eating patterns of cattle when included in a silage mix at between 5 and 15 % (Silva *et al.*, 2005). The times spent ruminating and eating were reduced while inactive time increased linearly with increase in the level of the product in the diet. The efficiency of eating was not affected but the efficiency of dry matter use increased linearly with increasing levels of bagasse in the silage.

3.2 Non-ruminant animal production

Most of the by-products of the biofuel industries would naturally not be ideal ingredients for non-ruminant animals, particularly poultry, in view of their chemical composition and sometimes physical properties. However, the quality of DDGS, for example, has continued to improve with improvement in fermentation, distillation and drying processes. This will re-position the product as a useful feed for non-ruminant animals, especially poultry (Hastad *et al.*, 2003; Swiatkiewicz and Koreleski, 2008). These authors recommended that DDGS could be safely included in starter diets for broilers and turkeys at 5-8 % and 12-15 %, respectively in the grower-finisher phase. A summary of some of the studies on the use of by-products for non-ruminant animal feeding is shown in Table 3.

Ganesan *et al.* (2008) reported that more than 13 million tonnes of DDGS were produced in 2007 and the output keeps rising as new ethanol plants come into production. As has been pointed out, these products will be increasingly used for non-ruminant feeding due to feed shortages. In a trial on pigs, Avelar *et al.* (2010) reported a reduction in body weight gain when wheat DDGS was included in diets of weaned pigs at between 5 and 20 %. This contrasts with the findings of Jones *et al.* (2010) who did not observe any negative effects of maize DDGS in the diet of weaner pigs in terms of feed consumption, weight gain or feed efficiency. Sorghum DDGS, on the other hand, reduced FCE. Feoli *et al.* (2007), however, observed a reduction in weight gain as a result of a reduction in DM, protein and energy digestibility in finishing pigs on diets containing 40 % maize DDGS. Skiba *et al.* (2009) reported a similar reduction in FCE in broiler chickens with only 10 % DDGS in the diet but this was attributed mainly to feed particle selection and wastage rather than reduction in nutritive value. The relative economic efficiency of broiler chickens on 6 % DDGS was found to be approximately equal to that of chicks on a maize control diet (Shalash *et al.*, 2009).

Species	Type of by-product	Inclusion rate (%)	Reference
Swine (gestating sows) (Weanling pigs)	DDGS	50	(Wilson <i>et al.</i> , 2003)
	DDGS	30	(Burkey <i>et al.</i> , 2008)
	Glycerol	10	(Lammers <i>et al.</i> , 2007)
(Growing finishing pigs)	DDGS	30	(Xu <i>et al.</i> , 2007)
Broilers	DDGS	6-15	(Lumpkins <i>et al.</i> , 2004)
	DDGS	30	(Barekatain <i>et al.</i> , 2011)
	Glycerol	10	(Simon <i>et al.</i> , 1997)
	Rapeseed meal	10	(McNeill <i>et al.</i> , 2004)
Layers	Rapeseed meal	10	(Mawson <i>et al.</i> , 1995)
	DDGS	15	(Lumpkins <i>et al.</i> , 2005)
	DDGS	15	(Roberson <i>et al.</i> , 2005)

Table 3. Summary of selective studies using biofuel by-products in non-ruminant animal diets

In a trial to determine the optimal level of inclusion of DDGS in diets for pigs, Linneen *et al.* (2008) observed a reduction in daily gain and feed consumption as DDGS rose in the diet, particularly beyond 10 %. There was, however, a linear improvement in these measurements with the inclusion of white grease at between 0 and 6 %, along with the DDGS. The feed efficiency of the pigs was increased due to inclusion of DDGS in the diet. Min *et al.* (2008) have established that DDGS can be used at up to 30 % in broiler chicken diets without detrimental effects on feed intake but feed efficiency was reduced, a result that was linked to the reduced pellet quality. Further supplementation with glycerine, at 5 %, had no effect.

There are not many reports on the use of glycerol for non-ruminant feeding, although the product could be useful as an alternative to lipids, particularly as a source of energy. Schieck *et al.* (2010) evaluated the effect of long- or short-term feeding of glycerol to growing pigs, in diets containing 8 % of the product. Pigs that were fed on such diets for 14 weeks ate more than animals on the control diet while those that were fed only in the last 8 weeks of trial consumed similar amounts of feed to the control. Long-term feeding also resulted in higher daily gain although short-term fed pigs grew faster than the control. Hot carcass weight was greater in the long-term fed pigs than in the other groups and pork quality based on a taste panel assessment was not affected by treatment. The response to glycerol may be dependent on the quality of the product. In another study on pigs, Hanczakowska *et al.* (2010) observed a reduction in weight gain when crude glycerol was included in the diet at 10 %, but such an effect was absent when refined glycerol was used, the latter generally improved fibre digestibility. It was concluded that crude glycerol had limited value as a feed supplement for pigs. In tests with pigs, crude glycerol has been found to be adequate in digestible energy, therefore the poor response to it cannot be ascribed to poor energy supply (Lammers *et al.*, 2008).

A comparative trial has been conducted on biodiesel press cakes obtained from canola or mustard when fed to broiler chickens (Thacker and Petri, 2009). Ether extract digestibility and nitrogen retention were higher in the canola press cake group than in the canola meal or mustard press cake groups. Feed efficiency on the two press cakes was superior to that on canola meal. Mustard press cake was recommended mainly due to its lower price.

3.3 Miscellaneous animal feeding

There are not many reports on the feeding of by-products of the biofuel industry to animals other than farm animals. Bonoma *et al.* (2008) reported that weanling horses were not adversely affected by DDGS replacing up to 30 % of the concentrate portion or 15 % of diet. Juvenile hybrid tilapia have been raised on diets containing 30 % DDGS and further supplemented with fish meal, meat meal or soybean meal (Coyle *et al.*, 2004). It was found that the fish meal could be excluded from the diet without detriment to growth rate or protein efficiency ratio, thus reducing feed costs. Nagalakshmi and Reddy (2010) described a study in which sugarcane bagasse was used as sole roughage source in a complete diet that was expanded/extruded before feeding to lactating buffaloes. This was compared to a traditional diet of concentrate mixture, chopped sorghum straw and small amounts of green Napier grass. Feed intake on the bagasse-containing diet was reduced but protein digestibility was increased. There were no differences in milk yield, fat-corrected milk yield and non-fat solids. Feeding cost per unit of milk produced was substantially (>31 %) reduced with the bagasse-supplemented diet.

4. Limitations of biofuel by-products for animal feeding

The impact of biofuel by-products in animal feeding is generally more positive than negative. However, there are limitations to the use of these by-products for animal feeding, some of which would have become obvious from the discussion of animal response to their inclusion, variously described above. In this section, we will collate these constraints and discuss them in greater detail.

4.1 Availability and cost

Generally, feed cost is often regarded as the highest variable cost of production, with a strong bearing on profitability of the animal industry. Therefore, supply of any by-products seems to be a fundamental issue limiting its usage in animal diets. Currently, most biofuel plants are located in a limited number of countries around the world, including the USA and Brazil, and there is uncertainty over the number of plants being built in other parts of the world and also the extent of material being imported into developing countries, which are unable to construct local biofuel plants. In this regard, location of biofuel plant may be fundamentally crucial to the ability to supply and distribute biofuel by-products to the feed industry as transportation cost will be added to final price and hence unfavourably affect the ability of the livestock producer to use such materials. Furthermore, feed prices and their fluctuations will negatively influence the use of by-products in animal feeds, which can be noticeable more in the regions that import by-products. Besides, the demand from other markets may affect the availability of by-products for animal feeding. As a prime example, glycerol is an acceptable energy source for both ruminant and non-ruminant animals but high demand by the pharmaceutical and polymer industries as well as its use by the food industry may restrict its availability for animal feeding.

4.2 Fibre

Generally, during the fermentation process, most of the components present in cereal grains become concentrated in distillers' by-products except starch, which is converted to ethanol. Therefore, the starch content of the final by-products is much lower than that of the main grain. A large proportion of the remaining carbohydrates is regarded as fibre, also called

non-starch polysaccharides (NSP). The fibre content of by-products varies according to processing methods. High-protein DDGS, for instance, are produced when the germ is removed from the main grain. Such material, therefore, contains less fibre and higher protein compared to conventional DDGS (Jacela *et al.*, 2010b). Nevertheless, neutral detergent fibre (NDF), acid detergent fibre (ADF) and total dietary fibre are approximately three times higher than those in the main grain. While in ruminant animals these fibre fractions can be readily digested due to high fibrolytic activities of rumen microbes, non-ruminants are unable to break down NSP because of the absence of such activities in their small intestine. In addition, the presence of NSP in non-ruminant gastrointestinal tract has been shown to contribute to poor performance by creating a viscous environment and subsequent impediment to nutrient digestibility. However, there are limited data on the nature of NSP in biofuel by-products. Choct and Peterson (2009) analysed 6 DDGS samples from different ethanol plants across the Midwest, USA and reported an average of 40 % total carbohydrates, most of which were identified as insoluble NSP with less than 10 % soluble NSP. Regarding the composition of NSP, they identified the main sugars as glucose and xylose followed by arabinose, galactose and mannose in descending order of concentration. This would explain the possible adverse effect of arabinoxylans and xylans in birds on DDGS-rich diets.

Although most by-products of the biofuel industry with high fibre content are regarded as desirable feed ingredients for ruminant animals, there might be a limitation to how much fibre-rich materials can be fed to lactating dairy cattle. High-fibre supplements in lactating dairy cattle diets can result to significant differences in milk composition, nutrient intake as well as nutrient apparent digestibility (Bernard and McNeil, 1991).

4.3 Bulk and storage

Basically, storage and space allocation will be primary issues for the new ingredients introduced to the feed mills as there is always a limitation for designation of space for ingredients other than the common ones being used by feed manufacturers. A wide range of factors including physical characteristics, flowability and also the time that ingredients are used will determine bin space allocation. Notably, not only space allocation for the bulky ingredients such as DDGS can be problematic due to being fairly light at around 480.6 kg/m³ but also take considerably more time and cost to be delivered and stored, compared to the conventional ingredients and meals. Producers, therefore, have sometimes been reluctant to accept biofuel by-products, in particular, DDGS owing to inconvenience and cost of handling. There are limited reports related to bulk density and particle size distribution of DDGS. In an experiment conducted by Knott *et al.* (2003) on 16 samples from different ethanol plants in Minnesota, South Dakota to investigate average particle size and bulk density of DDGS, they reported average particle size to be 1282 microns (SD= 305, CV= 24%) and also an average of 572.4 kg/m³ for bulk density. In the same experiment, there was moderate positive correlation between bulk density and moisture content, indicating that with a decrease in moisture content of DDGS, bulk density tends to decrease.

Generally, the ability of the solid particles and powders to flow during unloading from storage containment or transporting vehicles is defined as flowability (Babcock *et al.*, 2008). Flowability of biofuel by-products, in particular, DDGS is regarded to be an issue for transportation and storage which can be affected by a wide range of factors including cooling and drying practices, particle size and the amount of residual sugars (Shurson,

2005). It also seems that humidity greater than 60 % has an adverse effect on flowability of DDGS due to a tendency of this by-product to absorb moisture (Babcock *et al.*, 2008).

Shipment concerns arising from moisture content and bulk density can also be an issue for the other by-products such as wet dried grain with solubles (WDGS) with high moisture content (65 to 70%). It has been voiced by some feed manufacturers that bagging of WDGS is sometimes a problem as the material ends up compacting (Babcock *et al.*, 2008).

4.4 Nutrient imbalances and variability

Variations and deficiency in some nutrients are regarded to be the most important issues limiting the usage of biofuel by-products in animal feeding. Generally by-products result from several steps inherent to biofuel or ethanol production, including different temperatures and drying practices, enzymes used as well as rate of soluble incorporated, in the case of DDGS. Additionally, live yeast added during the fermentation process may interact with the factors mentioned above to influence the nutritive value of final by-products substantially. These discrepancies predominantly are reflected on protein, metabolizable energy and mineral contents of final products. Numerous studies have been conducted to investigate nutrient characteristics of DDGS. The study by Spiehs *et al.* (2002), which examined the nutrient variability of DDGS from 10 ethanol plants in Minnesota and South Dakota has been highlighted. In another study conducted by Batal and Dale (2006), TME_n evaluation of seventeen samples from different plants showed a wide range from 10.4 to 13.3 MJ/kg with a mean of 11.8 MJ/kg. Pedersen *et al.* (2007) also showed a higher average energy content (22.7 MJ/kg) of ten DDGS samples compared to corn grain. The high energy content of DDGS is associated with high level of fat in the residue (Swiatkiewicz and Koreleski, 2008).

The protein content of DDGS has been reported in several experiments to vary from 23 to 32 % (Cromwell *et al.*, 1993, Belyea *et al.*, 2004, Pedersen *et al.*, 2006, Fathi and Afifi, 2008). Several factors regulate the protein content of DDGS, as described previously, distillers' soluble and wet grain are two main components from which DDGS are formed. Substantial variations were shown by Belyea *et al.* (2004) regarding soluble composition from different batches. They postulated possible contribution of proportional rate of components to variation in protein content of DDGS. More recently, Kingsly *et al.* (2010) observed variable nutritive value of DDGS when different ratio of wet distiller grains and solubles were blended together. Additionally, yeast protein and its amino acid composition may also have an effect on protein content and composition of DDGS since yeast protein constitutes approximately 50 % of the protein in DDGS (Belyea *et al.* 2004). Furthermore, the drying process can have crucial influence not only on variability of nutrients but also on concentrations and availability of amino acids in different samples (Bandegan *et al.*, 2009, Martinez-Amezcuca *et al.*, 2007). Recently, Bandegan *et al.* (2009) reported average Lys and Met concentration of 5 different samples of wheat DDGS to be 0.74 and 0.61 %, respectively. More variable data for Lys content was observed by Fastinger *et al.* (2006), ranging from 0.48 to 0.76 % in 5 DDGS samples from different sources. Amongst the most limiting amino acids, Lys and Met appeared to be the most variable in DDGS, the reason being that the heating of wet distiller's grains may adversely affect availability of heat-sensitive amino acids. Excessive heat during the drying process may accelerate reactions between reducing sugars and Lys (the Maillard reaction), which leads to unavailability of Lys in DDGS (Batal and Dale, 2006). In poultry, due to lack of enzyme to breakdown bonds between Lys and carbohydrates, this form of Lys becomes unavailable (Fastinger *et al.*, 2006).

As mentioned before, overheating during DDGS production is mainly responsible for losses in nutritional value of final by-products (Cromwell *et al.*, 1993). It has also been shown that subsequent by-products resulting from excess heat appear to be darker compared to those samples treated by less severe drying practices (Kim *et al.*, 2008). Therefore, the colour of DDGS can be predominantly used as a general guide of nutritive value and amino acid availability of DDGS samples. Cromwell *et al.* (1993) observed the lowest Lys concentration in the darkest DDGS sample, about 0.62 % and the highest amount of 0.86 % in the lightest sample. They also obtained a correlation as high as 0.67 between Hunterlab L score and Lys content for the same samples.

Nutritionists regard nutrient imbalances as the main concern when biofuel by-products are to be incorporated in animal diets. Depending on type of animals and species, different nutrients may be more important. Vander Pol *et al.* (2006) postulated that incorporation of WDGS in the diet containing more than 8 % fat for cattle may lead to a reduction in feed intake. These nutrient imbalances can be considered for a variety of nutrients such as minerals and amino acids. Batal and Dale (2003) reported a severe deficiency of sodium in an experiment conducted with laying hens using NRC values for DDGS, indicating the necessity of analysing DDGS sample prior to incorporation into diets. There is also a possibility of increase in phosphorus and sulphur in ethanol by-products which may occur with greater rate of solubles added to distillers' grain. Large amounts of sulphur (more than 0.4 %) can cause polioencephalomalacia in cattle (Babcock *et al.*, 2008).

Finally, the presence of mycotoxins in by-products of biofuel can be a potential threat for animal health and performance if the product is not monitored and becomes contaminated. Noteworthy, the process of fermentation and production of biofuels is usually incapable of destroying mycotoxins, therefore they will be reflected in by-products if the source grains were contaminated. Schaafsma *et al.* (2009) reported the mean concentration of deoxynivalenol, a toxin from *Fusarium graminearum*, in condensed distillers' solubles to be 7.11 mg/kg, which was four times higher than that in corn as main grain (1.80 mg/kg). In the same study, the concentration of deoxynivalenol was found to be 5.24 mg/kg. Nevertheless, care should be taken by regular monitoring and measurement of mycotoxins in grain and by-products to keep the concentration of mycotoxins close to the recommended level in the main grain otherwise such an antinutritive factor will be present in the final by-products, posing a risk to animal and possibly human health.

5. Scope for increased use of biofuel by-products

As the proportion of by-products produced by the biofuel industry is increasing rapidly, attempts have been made to maximise their utilisation in livestock diets. Depending upon different biofuel processes, species and the type of by-product, there has been a wide range of applications to enhance the nutritive value of by-products to facilitate their wider inclusion in animal diets, especially for swine and poultry. In this section existing applications employed to enhance the nutritional quality of by-products are discussed mainly for DDGS and WDGS, which are the most widely used by-products for non-ruminant and ruminant animals.

5.1 Further processing and management

It has been widely accepted that any alteration in the process of biofuel and in particular ethanol production will lead to changes in finished by-products (Belyea *et al.*, 2004; Gibson

et al., 2005). Therefore, depending upon the species that by-products are fed to a number of new technologies may be taken into consideration prior to and after the completion of by-product production. In modern bioethanol plants enzymatic milling (EM) is a new procedure in which hemicellulases and protease are used. Those enzymes facilitate separation of non-fermentable fibre and germ prior to fermentation and this leads to an increase in fat and protein content of DDGS, which makes it a more desirable ingredient for non-ruminant animals (Kim *et al.*, 2010). Further processing can be applied to the recovered germ and the pericarp and endosperm fibre to produce corn germ oil and phytosterols, respectively. In this regard, a combined separation method (Elusieve process) was developed by Srinivasan *et al.* (2005) in which sieving and elutriation were applied to separate fibre from DDGS. In the described process, an upward stream of air was created by a blower followed by sieving to separate particles based on density, size and physical form of DDGS components. Srinivasan *et al.* (2005) also evaluated the nutrient characteristics of different factions (light and heavy) obtained from the Elusieve method showing 13 to 41 % and 4 to 127 % increase in protein and fat content of the heavier fraction, respectively. Fibre content of lighter fraction of DDGS was also reduced.

As discussed previously, the presence of NSP in DDGS could impede nutrient digestibility and therefore performance in non-ruminants, in particular, broiler chickens. There are some reports on the beneficial role of extrusion in enhancement of nutrient digestibility through physical disruption of cell wall, and hence breakdown of NSP to the smaller factions (Oryschak *et al.*, 2010b, Oryschak *et al.*, 2010a, Camire, 1991). Oryschak *et al.* (2010b) found a 10-34 % increase in apparent ileal digestibility of amino acids in both extruded corn and wheat DDGS. High temperature and pressures through the extrusion process are believed to act as effective eliminators of microbial contamination and also making cell content more accessible and susceptible to enzymatic hydrolysis (Oryschak *et al.*, 2010b). Camire (1991) ascribed the improvement in protein digestibility as a result of extrusion to denaturation of protein via heat and pressure applied through extruder, which presumably increases the exposure of peptide bonds to enzymatic digestion. However, Al-Marzooqi and Wiseman (2009) recommended mild conditions of extrusion in order to avoid adverse effect of high temperature on amino acid availability.

Pelleting as a further process on DDGS may also improve flowability, a constraint that was highlighted in a previous section. It has been demonstrated that any level of agglomeration during the pelleting process can improve flowability of DDGS but this must be undertaken at the ethanol plants which would probably increase the cost of production and hence the final price of DDGS (Behnke, 2007).

The usage of WDGS for ruminants and in particular dairy cows faces some constraints largely due to high moisture, content which causes handling problems and also limits the time of storage. This can be minimized by ensiling and addition of organic acids, to prevent spoilage and improve handling as well as extended shelf life. Wet DGS has a relatively low pH, which can be an advantage for preservation, confirming that WDGS can be ensiled with other suitable companions such as soybean hulls and beet pulp that are low in protein, fat and phosphorus (Kalscheur *et al.*, 2004; Anderson *et al.*, 2009). The nutrient profile of WDGS can even be improved by ensiling as was recently demonstrated by Mjoun *et al.* (2011). They showed that a mixture of 50 % WDGS with 50 % whole plant corn can result to an optimization in nutrient and fermentation profile, therefore, maximizing aerobic stability that may ensure higher inclusion of WDGS in dairy cattle diets.

Ammoniation is a method generally used to improve the nutritive value of fibrous feed materials (Dean *et al.*, 2008). Zade *et al.* (2009) employed the technique to improve the value of rising levels of pith bagasse for beef cattle, but found no improvement in feed consumption, daily weight gain or feed efficiency although feed costs were reduced. In an earlier study, urea treatment of sugarcane bagasse was found to increase its nitrogen content and *in vitro* dry matter digestibility (Hassoun *et al.*, 1990). When demethylated, glycerine would be a useful energy source for broiler chickens but issues such as residual methanol, sodium or potassium, feed flow and handling would need to be better understood (Batal, 2009).

Meyer *et al.* (2010) have reported a study in which DDGS was fed to growing cattle in combination with rapeseed meal (RBM) and compared to soybean meal or separate feeding of RSM and DDGS. Combining the two products led to an increase in voluntary feed intake and weight gain.

5.2 Supplementation with microbial enzymes

Among the key approaches to improve nutrient utilization in animal diets, application of various exogenous enzymes has drawn enormous attention over the past decade. It has been consistently shown that exogenous enzymes may enhance animal performance and nutrient utilization by animals, in particular non-ruminants. In this regard, diets containing high concentrations of NSP have shown considerable response to exogenous NSP-degrading enzymes. In addition, the concentration of NSP in biofuel by-products is relatively high due to fermentation process, which removes most carbohydrates and almost triples the amount of NSP compared to that in the original grain. Specifically, this is a constraint to the use of this by-product by non-ruminant animals that are unable to digest NSP. Therefore, there may be a potential for the use of exogenous enzymes in the diets containing biofuel by-products.

For poultry, there are a few reports concerning the use of xylanase to break down NSP, to improve the growth performance of birds on DDGS-rich diets. Recently, Barekatin *et al.* (2011) showed significant improvement in FCE of broiler chickens fed 300 g/kg sorghum DDGS as a result of xylanase supplementation. Similarly, results of an experiment conducted by Liu *et al.* (2011) with broilers demonstrated that xylanase addition to a diet containing corn DDGS could increase hemicellulose and dry matter digestibility by 5 and 20 %, respectively. Furthermore, the combination of exogenous enzymes such as xylanase, amylase, protease and phytase could have a sub-additive effect in growth performance of birds, as has been shown by Olukosi *et al.* (2010).

Exogenous multi-enzyme complexes also appear to be beneficial for amino acid digestibility of broilers fed diets containing DDGS. Oryschak *et al.* (2010a) found between 6 and 19 % improvement in lysine, tryptophan, methionine, isoleucine, histidine and phenylalanine digestibility when a multi-enzyme complex was included in a diet with 15 % DDGS and fed to broiler chickens. The effect of enzymes on the biodiesel by-product, rapeseed press cake, is also promising. Significant improvement in feed conversion ratio (1.9 to 1.84) was found by Jozefiak *et al.* (2010) in broiler chickens on such diets.

The influence of enzymes, however, is inconsistent for other non-ruminant animals. In a series of experiments conducted by Jacela *et al.* (2010a) no effect of microbial enzymes was observed when finishing pig diets contained DDGS. It seems that variation in carbohydrate composition, difference in age and animal species as well as availability of substrate for

specific enzymes to act on may be logical explanations for inconsistencies in response to exogenous enzymes with by-products in the diets.

There are conflicting reports on the effectiveness of microbial enzymes in diets containing DDGS for pigs. Feoli *et al.* (2008) reported an improvement in nutrient digestibility and growth as a result of supplementation with microbial enzymes possessing β -glucanase, protease, amylase and xylanase activities. In contrast, Jones *et al.* (2008) did not observe any positive effect of microbial enzymes on diets containing sorghum DDGS when fed to pigs. Pigs on the diets with sorghum DDGS were also less efficient than those on maize DDGS (Jones *et al.*, 2008).

5.3 Probiotics

Currently, probiotics are used by the animal industries to alter the profiles of microbial populations in the digestive tract, to improve nutrient utilization and health of the animal. They are not used as such to increase the digestion of feed components although such targeted introduction of species is a possibility in ruminant animal nutrition. The relatively high level of fibre in DDGS and bagasse, for example, has been highlighted. Such material will benefit from pre-digestion with microbes and this can be achieved through ensiling, which generally enables preferred microbial species to pre-digest feed material, particularly forage. Direct fermentation of bagasse or DDGS may also be effective although such material would be more useful for ruminant animals and pigs than poultry. Ramli *et al.* (2005) have reported on such a product; bagasse subjected to solid-state fermentation with *Aspergillus sojae* prior to feeding to goats. Such a diet was found to improve the flavour, aroma and overall quality of loin meat of goats compared to animals on basal lucerne hay. A mutant strain of *Trichoderma viride* has been similarly used on sugarcane bagasse, to improve the digestion of the material through the cellulase enzymes secreted (Valino *et al.*, 2004).

6. Recommendations and conclusion

There is no doubt that by-products of the biofuel industry will continue to become increasingly important components of the animal diet. It is most likely that less and less grains will be available for animal feeding until such a time that DDGS will become the primary product utilised in animal feeding. The biodiesel industry also produces enormous amounts of glycerol, which need to be discarded and this could be useful as a source of energy provided there is improvement in technology to refine it. The volume of biodiesel press cakes relative to conventional meals will also increase as more oilseeds are used for the production of biodiesel rather than edible oil. In areas where sugarcane and similar crops are used for ethanol production, bagasse will become available and while most of it will be used for energy production, some of it can be valuable feed resource, following further processing.

The limitations that have been identified in the use of by-products of the biofuel industry for animal feeding will wane as technology becomes available for further processing or feed management. This will place the by-products in stronger position to be used at higher levels in the diet.

To initiate or improve the utilization of by-products of biofuel industry, the following recommendations are worth considering:

1. There is a need to develop rapid methods of product testing, in order to determine the nutrient composition of the products prior to feed formulation. This will reduce the effect of variability from batch to batch and between plants producing the same material.
2. More research should be conducted to determine the optimum inclusion levels of the various products. Currently very few such research have been reported and commercial use of the products is limited by lack of knowledge of how much can be included without further tests.
3. Many of the products will benefit from supplementation with other feed additives including microbial enzymes or combinations of the by-products themselves, for example, glycerine used with DDGS. This may increase their acceptability by animals and improve nutritive value.
4. A single by-product may not be useful for all classes of animals. By-products should be used for the kind of animal for which they are most suitable. While there is a strong drive for minimising feed costs, animal welfare and overall productivity should also be considered in the application of by-products of the biofuel industry.

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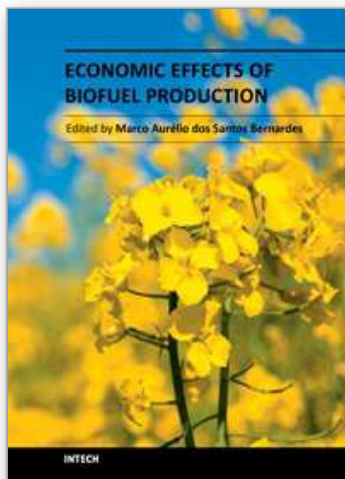
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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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