doi:10.1017/S0043933913000792

Reviews

Functional patterns of exogenous enzymes in different feed ingredients

Y.M. BAO[1], L.F. ROMERO[2] and A.J. COWIESON[1]*

[1]Poultry Research Foundation, Veterinary Science Faculty, University of Sydney, Camden, NSW 2570, Australia; [2]Danisco Animal Nutrition – DuPont Industrial Biosciences, Marlborough, Wiltshire, SN81XN, United Kingdom
***Corresponding author: Aaron.Cowieson@sydney.edu.au**

The use of carbohydrases and proteases in poultry production occasionally results in inconsistent physiological responses that are difficult to explain. However, it has previously been demonstrated that a substantial portion of the variation in enzyme efficacy is associated with the inherent ileal digestibility of starch, protein and lipid, and so an appreciation for the concentration of the undigested fractions of these nutrients in a diet may help to determine the magnitude and consistency of feed enzyme responses. Considering that the expense of *in vivo* physiological assays for the assessment of enzyme functional pattern is high, it is crucial to develop suitable *in vitro* methods to predict the magnitude of enzyme response. The purpose of the current paper is to describe inherent characteristics of poultry diets and feed ingredients in response to carbohydrases and proteases in order to aid the development of *in vitro* assays to predict feed enzyme functional patterns.

Keywords: carbohydrases; in vitro study; ileal digestibility; broilers

Introduction

The supplementation of animal feed with exogenous enzymes can improve the nutritional value of feed ingredients, increasing the efficiency of digestion. Since the mid 1980s, feed enzymes have dramatically improved the profitability of commercial poultry production. The current feed enzyme market is worth an estimated $700-800 million USD. The use of phytase has been extensively reviewed by Selle and Ravindran (2007), Adeola and Cowieson (2011) and Woyengo and Nyachoti (2011), and its use is unequivocally valuable in poultry production, although phytate digestibility with such supplementation is far from complete (Slominski, 2011).

Though the use of phytase has been shown to be advantageous in the majority of cases, the use of carbohydrases and proteases occasionally results in apparently inconsistent responses. There are many potential reasons for the inconsistency of an enzyme product

© World's Poultry Science Association 2013

Received for publication November 27, 2012
Accepted for publication February 26, 2013

to elicit a beneficial economic response including the application of inappropriate matrix values, nutrient imbalances, and variance in underlying ingredient quality, changes in ingredients proportions with reformulation, and others. One key reason however is the 'responsiveness' of the diet to enzyme modification. 'Responsiveness' of a diet or an ingredient to exogenous enzymes is elusive and difficult to accurately define but it includes factors such as substrate concentration and 'accessibility', inherent digestibility, nutrient interactions, anti-nutrients, and solubility in water. A greater understanding of the likely responsiveness of a diet to carbohydrase and protease addition would allow maximising the economic benefits of these enzymes.

Rosen (2010) concluded that the inclusion of specific feed ingredients contributed to the magnitude of feed enzyme response. Choct (2006) summarised that the so-called 'viscous' feed ingredients such as wheat, barley, rye, triticale and oats responded to carbohydrases due to a higher concentration of water soluble non-starch polysaccharides (SNSP). Interestingly, in maize and sorghum based diets, there are substantially lower concentrations of SNSP, these grains also have been reported to respond reasonably consistently to supplemental carbohydrases. Cowieson and Ravindran (2008) assumed that exogenous carbohydrases and protease increase nutrient digestibility not only via a reduction in digesta viscosity but also via a reduction in cell wall integrity, generation of fermentable disaccharides, low-molecular weight polysaccharides and oligosaccharides, improving protein solubility, decreasing endogenous losses and overcoming anti-nutritional factors. It is now recognized that the concentration of undigested nutrients including protein, starch and fat in the ileum can be used to predict the functional pattern *i.e.* the magnitude and consistency of carbohydrases and proteases in response to the control diet with different inherent characteristics. The assumed functional pattern of carbohydrases and proteases is that feed ingredients with high ileal digestibility may respond less readily to exogenous enzymes than that with a poor digestibility; therefore, as Cowieson and Bedford (2009) indicated, the evaluation of the inherent digestibility of the control diet to which enzymes will be added appears to be important regarding the likely effect on productivity.

Considering that the expense of *in vivo* ileal digestibility assays for the assessment of enzyme functional pattern is high, it is crucial to develop suitable *in vitro* methods to predict the magnitude of enzyme response. Currently, the *in vitro* analysis of diets in the poultry industry is generally limited to the determination of gross chemical composition (*i.e.* dry matter, ash, crude protein, crude fat, crude fibre and starch) or to crude indices of 'quality' such as bushel weight and broken grains. Near-infrared reflectance spectroscopy (NIRS) assessments have successfully estimated the energy value of starch and fibre concentrated feed ingredients for broilers (Losada *et al.*, 2010). Gross chemical measurements provide little useful information on the characteristics of the diet in relation to its inherent digestibility and, although useful, NIRS does not consistently predict the nutritional value of most feed ingredients (Losada *et al.*, 2010). Therefore, appropriate *in vitro* assays that are able to quantify the inherent digestibility of nutrients in a diet at the ileal level may help to develop multiple regression models for feed enzyme functional patterns.

The current paper describes the drivers of the inherent digestibility of different poultry diets or feed ingredients, and identifies characteristics that explain variance in digestibility.

Inherent characteristics of feed ingredients for broiler chickens

Dietary protein and energy yielding substrates such as starch and fat are the most costly nutrients in poultry diets and are required in adequate quantities and proportions for optimal performance (Moosavi *et al.*, 2011). It is generally accepted that improvements in AME in response to exogenous enzymes in lower energy diets are greater than that of higher energy diets. Francesch and Geraert (2009) indicated that enzyme supplementation often decreased feed intake due to the increased energy availability. Accordingly, lower energy density diets often result in increased feed intake due to the bulk limit, and in practice, the contribution of feed enzymes on the AME values may not be always sufficient to reduce feed intake and improve the feed conversion ratio (FCR). Furthermore, broiler chickens fed diets that are essentially adequate in all nutrients often still respond to exogenous enzyme addition, suggesting that enzyme benefits may result from changes in less tangible metrics such as appetite control, digestive physiology, immunology, or microbiology *i.e.* 'net effects'. Modern broilers have been genetically selected to efficient converters of plant-based nutrients into animal protein, but Klasing (2007) ascribed their rapid growth rate to high feed intake and not necessarily increased nutrient digestibility. Indeed, as Cowieson (2010) indicated that, for various reasons, a significant proportion of nutrients (especially those of endogenous origin) does escape digestion and leave the ileum. Weurding *et al.* (2003) suggested that the extent of starch digestion in the small intestine was positively correlated to the AME content of poultry diets, and Cowieson and Bedford (2009) showed clear relationships between the inherent digestibility of macro nutrients and enzyme activity.

STARCH

It is well known that starch is the major energy-yielding component of poultry diets and is presumed to be almost completely digestible by the terminal ileum (Wiseman, 2006). However, Zelenka and Čerešňaková (2005) and Jenkins *et al.* (1987), suggested that the variation in starch concentration among different cereals and varieties, and, in particular, the starch/protein interface, might affect the inherent digestibility of the diet. Recently, in an *in vitro* study, Wong *et al.* (2010), reported that in sorghum the content of amylose and total starch, together with protein digestibility, accounted for 94% of variation in starch digestibility.

Approximately 60 to 70% of the organic matter (OM) of cereals is starch, which is composed of two polymers of glucose: amylose and amylopectin (Wong *et al.*, 2010). Further, more than half of the apparent metabolisable energy content of diets for broiler chickens is provided by starch (Weurding *et al.*, 2003; Wong *et al.*, 2010). Cowieson (2005) suggested that even relatively small differences in starch digestibility among ingredients could have a substantial impact on dietary variation in the AME content. Amylopectin makes up 70-80% of most starch sources and is the major component, strongly influencing the physiochemical characteristics of starch (Dona *et al.*, 2010). The amylose: amylopectin ratio is negatively correlated with starch digestion in pigs (Svihus *et al.*, 2005) and chickens (Meng and Slosminski, 2005), likely due to steric hindrance associated with tightly packed amylose. In human nutrition, Englyst *et al.* (1992) showed that the extent of starch digestion within small intestine is variable and a substantial amount of starch escapes digestion by the end of the small intestine. In broiler chickens, Meng and Slominski (2005) and Weurding *et al.* (2001) summarised incomplete starch digestion in corn, wheat, barley, pea, legumes and some complete diets (*Table 1*).

Table 1 Undigested starch fraction in cereals, legumes and corn-soy based diets (%) (Adapted from Weurding *et al.*, 2001 and Meng and Slominski, 2005).

Starch source	Starch content	Ileal digestion coefficient	Undigested fraction	Gross energy loss[4]
	g/kg diet	27-28 d of age		kcal/kg
Corn[1]	332.6	97.0%	3.0%	41.43
Wheat[1]	329.2	94.4%	5.6%	76.69
Sorghum[1]	334.0	95.3%	4.7%	65.30
Barley[1]	272.0	98.1%	1.9%	21.50
Peas[2]	133.8	80.4%	19.6%	92.79
Horse beans[2]		81.5%	18.5%	
Common beans (heated)[2]		72.3%	27.7%	
		19-20 d of age		
Corn[3]	440.3	92.7%	7.3%	133.6
Corn-soy based diets	375.0	93.2%	6.8%	106.1
Corn-canola based diets	347.4	93.4%	6.6%	95.4
Corn-peas based diets	464.6	87.3%	12.7%	245.5

[1]Grains mixed with soybeans and meat meal; [2]Legumes mixed with corn; [3]Corn mixed with casein, fish meal and isolated soy. The gross energy value in starch was calculated as 17.39 MJ/kg or 4160 kcal/kg (Carré *et al.,* 1995).

Cereal grains have an undigested starch fraction between 2%-8% at the terminal ileum of chickens (*Table 1*). In legumes, this fraction varies from 19-28%, though the total starch content is considerably lower than is the case for cereals. Although corn-soybean meal diets are regarded as essentially ideal for poultry, the digestibility of corn starch is about 93% at 20 d of age, much lower than that at 28 d of age. Wiseman (2006) suggested that poor digestibility values for starch in young broilers are attributable to the physico-chemical structure of starch and interactions between starch and other components. Thus, the interaction between starch and other components may be more important than starch *per se* in some ingredients. However, Jiang *et al.* (2008) indicated that the exogenous amylase improves productive performance for young chickens by compensating the digestive system of the birds and Zanella *et al.* (1999) suggested that starch digestion will be virtually completed by the hindgut for older birds, implying that exogenous carbohydrases, notably amylases and glucoamylases, may be more effective in younger broiler chickens.

Although chicks have been considered to adapt rapidly to starch digestion when fed at hatch, Thomas *et al.* (2008) and Svihus (2011) have shown that the total tract starch digestibility in modern fast-growing broiler chickens dropped from five to seven days of age and was restored to normal high level at 14 days of age in wheat, sorghum and corn based diets. Further, Meng and Slominski (2005) reported that a higher corn inclusion level resulted in the relative lower starch digestibility due to the higher NSP contents, suggesting that NSP-degrading enzymes potentially improve starch digestibility in corn-based diets. Unexpectedly, Weurding *et al.* (2001) reported the higher starch digestibility in barley than that in corn, implying that exogenous enzymes improved the performance of birds fed on high viscous grains such as barley and wheat for reasons other than starch digestibility *per se,* possibly due to lower feed intake and/or fat and protein digestion constraints. Surprisingly, corn-canola meal based diets have similar starch digestibility, but poorer performance than corn-soy based diets possibly due to the higher concentration of NSP (Meng and Slominski, 2005), indicating an interaction between starch, protein, and water insoluble NSP. Weurding *et al.* (2001; 2003) reported that broiler chickens grew faster and more efficiently on a diet containing slowly digestible starch than on a diet containing rapidly digestible starch, possibly leading to a more efficient protein deposition. Although Weurding *et al.* (2003) suggested four physiological mechanisms responsible for the better performance of broiler chickens to slowly digestible starch, it is majorly believed that the diet containing slowly digested starch could supply the ileum with glucose, thereby sparing amino acids from being oxidized and be used for muscle growth. Recently, Del Alamo *et al.* (2009) confirmed that starch digestion rate may be affected by protein digestion rate and other components apart from cereal starch. Thus, the synchronized supply of digestible starch and protein along the small intestine may be more important than starch or protein digestion rate considered in isolation. D' Alfonso (2005) reported that faster *in vitro* starch digestion rate of corn resulted in improved ileal starch digestibility *in vivo*. In practice, the interaction between starch digestion rate and protein digestion rate may result in conflicting results among different cereal ingredients and complete diets. In isolation, higher starch digestibility or faster starch digestion rate may not always lead to better performance.

PROTEIN

Rubio and Clemente (2010) have demonstrated that the degree of protein and peptides hydrolysis strongly affect the digestibility of amino acids in rats. In poultry, Cowieson (2010) has extensively reviewed the effect of carbohydrases on inherent digestibility of amino acids in corn and wheat based diets, indicating that methionine is typically very readily digested and cysteine very poorly. Therefore, carbohydrases have more opportunity to improve the digestibility of cysteine compared with that of methionine in complete broiler diets though it is not clear why cystein in corn and wheat based diets is poorly digested.

Table 2 Undigested protein fraction in different ingredients.

Undigested portion	Sorghum	Wheat	Corn	Soybean	Canola	Wheat bran	Rice bran	Barley
Protein	22.0%	23.0%	27.3%	20.7%	24.9%	25.5%	28.8%	29.6%
Cystein	20.0%	13.0%	29.0%	33.0%	24.4%	43.0%	36.2%	35.0%
Threonine	31.0%	39.0%	38.0%	23.0%	35.0%	33.0%	36.2%	40.0%
Glycine	28.0%	30.0%	12.1%	20.7%	23.7%	34.0%	21.3%	32.0%
Asptic acid	22.0%	37.0%	24.0%	19.0%	30.0%	44.0%	26.0%	29.0%
Serine	22.0%	24.0%	25.0.0%	19.0%	33.0%	39.0%	30.1%	33.0%
Alanine	16.0%	31.0%	21.0%	17.0%	22.0%	46.0%	23.0%	24.0%
Valine	21.0%	28.0%	18.0%	16.0%	27.0%	27.0%	25.5%	21.0%
Isolucine	19.0%	24.0%	16.0%	14.0%	25.0%	38.0%	22.6%	23.0%
Histindine	26.0%	27.0%	17.7%	16.1%	19.1%	47.0%	29.5%	22.0%
Tyrosine	25.0%	31.0%	22.0%	14.0%	25.0%	44.0%	18.2%	27.0%
Phenylalanine	17.0%	21.0%	13.0%	14.0%	21.0%	47.0%	28.7%	20.0%
Lysine	28.0%	35.0%	26.0%	14.0%	24.0%	26.0%	19.2%	20.0%
Leucine	16.0%	21.0%	8.0%	15.0%	22.0%	35.0%	12.0%	19.0%
Glutamic acid	17.0%	17.0%	10.0%	13.0%	16.0%	22.0%	20.0%	12.0%
Arginine	20.0%	28.0%	14.0%	11.0%	17.0%	33.0%	19.1%	20.0%
Methionine	16.0%	26.0%	12.0%	11.0%	9.0%	50.0%	30.0%	7.0%

Adapted from Ravindran and Bryden *et al.*, 1999, Ravindran *et al.*, 2005, Donkoh and Attoh-Kotoku (2009), and Al-Marzooqi *et al.,* 2010. All data were calculated on the basis of apparent ileal amino acid digestibility.

From *Table 2*, it is clear that the inherent digestibility of methionine in rice bran and wheat bran is much lower than that in other feed ingredients. Therefore, the magnitude of enzyme effect on diets including rice bran and wheat bran may be expected to be higher than on typical corn-soybean diets. Cowieson (2010) summarised that the apparent digestibility of threonine in most feed ingredients including wheat bran and rice bran is relatively poor. It is not clear if the addition of synthetic threonine may reduce carbohydrases effects on the inherent digestibility of threonine in broiler diets but this is likely to be the case.

Widyaratne and Drew (2011) reported that highly digestible proteins and low-protein diets maximized the efficiency of utilisation of dietary protein. However, in low-protein diets, there was no significant difference in protein digestibility between highly digestible and low digestible protein sources. It was noticed that poorly digestible protein strongly decreased feed intake and therefore body weight gain of broiler chickens (Widyaratne and Drew, 2011). Thus, it may be difficult to investigate the response of enzymes in low protein diets based on protein digestibility due to lack of divergence in digestibility for various protein sources. On the other hand, higher concentrations of poorly digestible proteins in poultry diets can lead to poorer nutrient digestion and absorption owing to the impact of anti-nutrients and indigestible carbohydrates present in legumes.

The protein content, the location of the protein in the ingredient, the presence of anti-nutrients such as trypsin inhibitors, the quality of industrial processes of protein ingredients, and the accessibility of the proteins to endogenous and exogenous proteases may affect feed enzyme functional patterns in different ingredients. Although cereals are usually used primarily as an energy source, they contribute about 25-30% of dietary protein in poultry diets. Accordingly, the quality of cereal proteins may also influences the functional patterns of carbohydrases or proteases.

Allee and Baker (1970) indicated that corn protein is deficient in lysine and tryptophan. Black *et al.* (2005) reported that a small portion of sorghum proteins, the λ-kafirins, was difficult to access by digestive proteases; while Choct (1997) ascribed reduced protein digestibility to increased soluble NSP concentrations in wheat, multi-carbohydrase enzymes failed to improve protein digestibility in a corn diet probably due to the much lower water soluble NSP concentration in corn (Meng and Slominski, 2005). Recently, Cowieson (2010) reviewed the effect of multi-carbohydrase enzymes on corn soybean meal based diets and suggested a consistent 16% improvement in undigested protein portion, assuming it resulted from the release of structure protein (glycoprotein) in soybean meal, rather than from the elimination of encapsulating effect of the cell wall (Meng and Slominski, 2005). Surprisingly, Kocher *et al.* (2000) and Meng and Slominski (2005) reported a negative protein digestibility response in canola meal-based diets in response to an carbohydrases admixture including xylanase, glucanase and cellulase, indicating that the supplementation of carbohydrases in the higher inclusion level of canola meal diets might result in more NSP hydrolysis products, which might influence the protein digestibility by broiler chickens. Therefore, the interaction between water soluble, insoluble NSP and protein, both in cereals and other protein sources, may complicate the effect of xylanase on NSP and protein digestibility and the overall value needs to be further investigated. In addition, as described previously, the interaction between protein and starch might also affect the effect of carbohydrases on protein digestibility. Unfortunately, as Svihus *et al.* (2005) indicated, interactions between starch and protein have usually been ignored due to the fact that protein digestion precedes starch digestion spatially. Further work needs to be done in this area. In particular, the ileum may not be the ideal site to investigate the interaction between starch and protein digestion, or indeed for exploration of exogenous enzyme value. More proximal regions of the small intestine may be more useful to study feed enzyme functional patterns.

FAT

There is scarce information about inherent fat digestibility in feed ingredients or complete diets although it is well-known that the degree of saturation of the constituent fatty acids, their chain length and proportion of free fatty acids present within a supplemental blend affects dietary energy values (Wiseman *et al.*, 1992). For young broiler chickens, increased supplemental free fatty acid content and degree of saturation depress the fat digestibility (Wiseman and Salvador, 1991). Dänicke *et al.* (2000) demonstrated that increasing the ratio of dietary unsaturated and saturated fatty acids (U:S ratio) up to 1.5, improved fat digestibility and accordingly, lower U:S ratio resulted in pronounced xylanase responses in a rye-based diet. *Table 3* shows the compositions of saturated and unsaturated fatty acids in major feed ingredients. Considering that most vegetable fat sources have a high content of unsaturated fatty acids and animal fat or some vegetable (*e.g.* palm oil) are rich in long-chain saturated fatty acids (Smink *et al.*, 2008), in practice, utilisation of vegetable oil in poultry diets may be reflected in increased fat digestibility. However, Jørgensen *et al.* (2008) suggested that unsaturated vegetable fat has a positive effect on digestibility of dietary fat and protein but a negative effect on starch digestibility. It is of interest to note that although lipid digestibility is generally assumed to be independent of the composition of the diet, there is the evidence that unsaturated fatty acids inherent in the grain portion of a diet enhance the utilisation of the supplemental fat (Mateos and Sell, 1981).

Table 3 The compositions of saturated and unsaturated fatty acids in major feed ingredients.

Ingredients	Unsaturated (g/kg)	Saturated (g/kg)	U:S ratio
Corn	26.7	4.1	6.5
Soybean meal	11.4	2.0	5.7
Wheat	8.8	2.1	4.2
Canola meal	17.4	1.1	15.8
Wheat bran	22.6	5.3	4.3
Rice bran	102.3	27.6	3.7
Sorghum	19.1	4.2	4.5

Adapted from Sauvant *et al.*, 2004.

On the other hand, it appears that the fat digestibility is significantly decreased in diets with higher NSP concentrations (*Table 4*) due to the oil-encapsulating effect of NSP (Slominski *et al.*, 2006) or alterations in viscosity (diffusion of high molecular weight compounds) due to water soluble NSPs. Further, the deficiency in bile acid secretion in young birds resulted in lower lipid digestibility (Carré *et al.*, 1995), either more added fat or high NSP contents in starter diets for young broilers may depress total feed utilisation (Moore *et al.*, 2008). However, in iso-caloric diets, a reduced dietary fat concentration, can depress the performance of young broilers and Cowieson *et al.* (2010) suggested minimum fat concentrations could maximize bioefficacy of NSP enzymes in corn-soy based diets because the reduction in fat level might compromise protein digestibility due to an increased rate of gastric emptying.

Table 4 Undigested fat fraction in different diets.

Fat sources	Dietary concentration g/kg	Ileal digestion coefficient %	Undigested fraction %	Gross energy loss kcal/kg[1]
Corn-soy with flaxseed	80.0 g/kg	56.4%	43.6%	325.8
Wheat-fish meal	–	66.1%	33.9%	–
Barley - soy diets	56.0 g/kg	76.4%	23.6%	123.4
Corn-soy with cottonseed	35.5 g/kg	82.7%	17.3%	57.4
Corn- soy diets	80.0 g/kg	85.1%	14.9%	111.3

Adapted from Cowieson (2010), Liu *et al.*, 2010, Slominski *et al.*, 2006, Almirall *et al.*, 1995.
[1] The gross energy value in fat was calculated as 39.04 MJ/kg or 5450 kcal/kg (Carré *et al.,* 1995).

It is noticed that for young broiler chickens in a typical corn-soybean diet, the energetic loss contributed by undigested fat and starch is about 210 kcal/kg. Because the energetic loss associated with the undigested protein is estimated to be around 190 kcal/kg (Cowieson, 2010), there is a loss of around 400 kcal of energy from undigested starch, protein and lipid.

Interactions between the inherent nutrient digestibility and phytate or NSP

Cowieson (2005) and Thorne *et al.* (1983) have respectively reviewed factors that affect the nutritional value of corn and legumes, suggesting phytate, enzyme inhibitors and resistant starch primarily affect the nutritional value of corn and legumes. White *et al.* (2008) indicated that cereal-based feeds for newly weaned piglets, the hydration and structural characteristics affect the rate and extent of starch hydrolysis, therefore, the digestibility that can be monitored using *in vitro* digestibility tests.

PHYTATE P

Extensive reviews about the effect of phytate on the digestibility of carbohydrates, amino acids, proteins and minerals can be found in previously described reviews (Selle and Ravindran (2007), Adeola and Cowieson (2011) and Woyengo and Nyachoti (2011), and will not be covered in detail herein. Briefly, Thompson and Yoon (1984) suggested that phytate might impair the digestibility of wheat starch probably due to the formation of mineral/phytate/starch complexes in the intestines (Angel *et al.*, 2002). Cowieson *et al.* (2006) and Yu *et al.* (2012) have suggested that phytate can bind protein in the early part of the digested part and make it less accessible to attack from endogenous proteases Additionally, the existence of complexes of phytate and fatty acids and reduced fat digestibility has also been reported (Singh, 2008). Thus, phytate may not directly influence the bioefficacy of exogenous carbohydrases or protease *per se*, but the solubility of those substrates and accessibility for enzymatic digestion. Nonetheless, an inhibitory effect of phytate on the activity of other endogenous protelytic enzymes has been reported to reduce protein digestibility (Woyengo and Nyachoti, 2011).

An appreciation for the concentration of phytate in feed ingredients in the context of carbohydrase biochemistry may be also instructive due to the potential for a carbohydrase-mediated reduction in the integrity of cell wall architecture. Bedford (2000) previously reported that xylanases and glucanases hydrolyse cell wall material, releasing previously encapsulated cell contents. Therefore, it is theoretically possible for a carbohydrase or protease to increase the concentration of soluble or reactive phytate in the lumen of poultry or swine. So, although phytate is not a focus of research for carbohydrase efficacy studies, it may be an obscure contributor to observed effect. The solubility of phytate with and without carbohydrase treatment may be measured *in vitro* and this may shed light on risk associated with these mechanisms.

WATER-SOLUBLE AND INSOLUBLE NSP

It is well-known that vegetable protein sources contain higher water soluble and insoluble NSP than cereal feed ingredients and cereal by-products contain little water soluble NSP but substantial concentrations of insoluble NSP. Higher fibre diets have a low metabolisable energy value and may cause abrasion of the intestinal mucosa and increase the amino acids requirements for synthesis of mucosa (Mushtaq *et al.*, 2009). Fernandez *et al.* (2000) indicated that adding xylanase to a wheat based diet could reduce intestinal viscosity and therefore decrease the numbers of *Campylobacter jejuni*. Furthermore, Janssens and Gaethofs (2007) suggested that xylanase could break down the arabinoxylan chains into smaller fragments, which may slow proliferation of gram-positive cocci and presumptive enterobacteria (Vahjen *et al.*, 1998). It is possible that shifting microbial populations towards commensal populations with the use of xylanase may reduce the energy and amino acid requirements of the host to maintain pathogenic populations under control in the intestine.

Angkanaporn *et al.* (1994) indicated that even 15-35 g/kg diet arabinoxylans can either increase endogenous losses of amino acids or reduce protein digestibility, which is a relatively low concentration compared to the range of dietary crude fibre in commercial diets of 4.5% to 5.5%, even those for young broilers contain less than 3% crude fibre. Although the amount and solubility in water of arabinoxylan in corn appears insufficient to influence intestinal viscosity and consequently nutrient availability, the multi-carbohydrases preparation still significantly improved ileal starch digestibility in corn diet and protein digestibility in corn-soy based diets (Slominski, 2011). JØrgensen *et al.* (1996) reported that water-insoluble NSP could be partially degraded in the small intestine but may be poorly fermented in the chicken ceca and remains almost completely undigested. However, significant disaccharides, low-molecular weight polysaccharides and oligosaccharides derived from either water-soluble or insoluble NSP due to the use of exogenous enzymes may be fermented in ceca, although the net efficiency of utilisation of dietary energy via hind gut fermentation is estimated to 65% and 50% of that of glucose absorbed in adult cockerels and broiler chickens (Choct and Kocher, 2000). Bedford and Cowieson (2012) suggested that oligomers produced from enzyme action on corn is quite different from those produced from wheat and therefore influence the enzyme response. Interestingly, arabinoxylo-oligosaccharides derived from wheat bran significantly improved FCR in both wheat and corn based diets due to decreased feed intake (Courtin *et al.*, 2008). Jamroz *et al.* (2002) indicated that a proportion of the total NSP in barley was degraded in the ceca of chickens (46%), ducks (27%), and geese (90%), respectively. Negative values for the degradation of water soluble NSP were observed in chicken and duck but geese fermented soluble NSP to a much greater extent. Hetland *et al*. (2003) suggested that the inclusion of water-insoluble NSP might improve gizzard functioning and increase the reflux of digesta from duodenum to the gizzard, facilitating the contact between nutrients and endogenous enzymes. Jimenez-Moreno (2009) concluded that even young broiler chickens needed a minimal amount of water-insoluble NSP for optimal performance.

Pettersson and Aman (1989) and Choct *et al.* (1995) have shown that xylanase significantly increased water-insoluble NSP digestibility in rye and wheat. In contrast, it is well-documented that in cereal grains with high concentrations of NSP, such as wheat and barley, water soluble arabinoxylans and ß-glucans result in increased intestinal viscosity and depressed starch, fat, and protein digestibility. As indicated previously, while water-soluble NSP results in the increased viscosity, insoluble NSP actually shorten the residency time of digesta, possibly leading to changes in starch digestion rate depending on the ratio of water-soluble NSP and insoluble NSP. Further work needs to be done in this area.

In proteinaceous feed ingredients, Daveby *et al.* (1998) indicated that α-galacto-oligosaccharides (*e.g.*, raffinose, stachyose and verbascose), containing α-galactosidic linkages, are undegradable by digestive enzymes of humans and monogastric animals. Pectic polysaccharides, comprising mainly arabinose, uronic acids and rhamnose residues, are the dominant cell wall components in dehulled legumes. The nutritive value in soybean meal and canola meal is limited by the presence of pectic polysaccharides (Choct, 1997). Although oligosaccharides in soybean meal may be extensively fermented in ceca, leading to a positive physiological response (*e.g.*, acidition of digesta, decreased ammonia concentration, and increased short chain fatty acids), these oligosaccharides may also increase accumulation of cecal digesta composed of undigested constuents and increase bacterial mass (Jankowski *et al.*, 2009). It is of importance to notice that canola meal contains on average 2.5% α-galacto-oligosaccharides (Kocher *et al.,* 2000) but the sum of oligosaccharides averaged 5.3% for soybean meal (Jankowski *et al.,* 2009), suggesting that the displacement of soybean meal with canola meal in diets might significantly reduce oligosaccharides concentrations in broiler diets. It is expected that α-galacto-oligosaccharides are subjected to bacterial degradation due to lack of endogenous α-galactosidase activity in the host (Carré *et al.*, 1995). Interestingly, there is no direct evidence that increased water insoluble NSP contents depress starch digestibility but may depress apparent protein digestibility in peas (Gabriel *et al.*, 2008) and canola meal (Fang *et al.*, 2009) due to the increase in bacterial protein losses (Carré *et al.*, 1995) and protein and amino acid flows at the terminal ileum (Mateos *et al.*, 2012). Thus, the effects of enzymes on the NSP component of the diet may affect patterns of ileal digestibility of protein, starch and lipids. At least, Cowieson and Bedford (2009) indicated that for ingredients with high concentrations of water insoluble NSP, the improvement of the undigested amino acids is low and for ingredients with high concentrations of water soluble NSP, the improvement of the undigested fraction is higher.

Prediction of feed enzyme functional pattern based on feed ingredient characteristics

Recently Cowieson and Bedford (2009) and Cowieson (2010) have demonstrated that the inherent digestibility of nutrients in the diet prior to enzyme addition is a good indicator of the magnitude of enzyme response. So, in diets where ileal digestibility coefficients for starch, amino acids or fat are low, the response to exogenous enzymes is typically higher. However, there are a range of reasons why a particular feed ingredient or diet may have a relatively poor digestibility and it may be overly simplistic to conclude that all such ingredients will be responsive to feed enzymes. The concentration of substrate for the enzyme is of course relevant and the dominant nutritional fraction that is influenced by the enzyme *i.e.* fat, starch, fibre or protein will dictate how the enzyme-modified diet is perceived by the bird. It is expected that this enzyme response is multi-factorial and may fit multiple regression models. Previous mechanistic work to explore correlations between chemical characteristics of feedstuffs and the *in vivo* digestibility and/or enzyme response has focussed largely on single ingredients. Though this approach is logical, an inherent problem is that within any one feed ingredient there is relatively little variation in chemical or nutritional characteristics. A more robust approach is to geometrically generate ingredient mixtures with radically different characteristics which will possibly set up multiple regression models to predict feed enzyme response magnitudes.

Due to the limitation of gross chemical measurements, some *in vitro* methods need to be developed to provide useful information on the characteristics of the diet in relation to its inherent digestibility. Wong *et al.* (2010) summarised *in vitro* methods that have been developed to assess inherent protein and starch digestibility in sorghum, indicating that *in vitro* pepsin and *in vitro* amylase assays are commonly used to assess protein and starch digestibility, respectively but they do not address effects of interactions between feed ingredients in the control diets. Recently, Doucet *et al.* (2010) established a swine model by using *in vitro* assessment to predict *in vivo* starch digestibility, suggesting that starch characteristics such as hydration, structure and amylolytic digestion are fundamentally associated with the inherent digestibility. Dona *et al.* (2010) summarised starch characteristics affecting digestion, indicating that *in vitro* water solubility index (WSI) and amylolytic released glucose are strongly correlated to the *in vivo* starch digestibility. Thus, water absorption index (WAI), WSI and dietary physical density representing the combination of different factors affecting the nutrients digestibility, have been used to characterise the hydration properties and potential *in vivo* digestion of feed ingredients (Janas *et al.*, 2010; Al-Rabadi *et al.,* 2012).

It is well-known that starch digestion rate in the small intestine of broiler chickens varies considerably among feed ingredients and Weurding *et al.* (2001) has demonstrated that digestion rate of the digestible starch and the resistant starch fraction will affect the extent of starch digestion. Although numerous *in vitro* starch digestibility methods and *in vitro* organic matter (OM) assays are available, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst *et al.*, 1992) have been used most commonly to predict human (Woolnough *et al.*, 2008) and chicken (Weurding *et al.*, 2003) *in vivo* nutritional responses, which indicates that the correlation between starch hydrolysis *in vitro* and *in vivo* may better defined by the pattern of hydrolysis over time rather than hydrolysis at a specific point in time. The *in vitro* starch digestion rate in feed ingredients and complete diets may be better measured by the glucose release method described by Sopade and Gidley (2009).

One important physical property of diets is their intrinsic solubility. Based on their solubility in water, diluted salt solutions, alcohol solutions and alkali solutions, proteins in feed ingredients are usually classified into four main fractions (albumin, globulin, prolamin and glutelin) (Byers *et al.*, 1983). The major soybean proteins are globulins which are soluble in diluted salt solutions (Wolf, 1970). The salt-soluble proteins in corn, sorghum and wheat are 20, 30 and 33%, respectively (Byers *et al.*, 1983; Wall and Paulis, 1983). Accordingly, 40, 28 and 50% of storage proteins in corn (Zein), sorghum (Kafirin), and wheat (Gladin), respectively, are prolamins. These different fractions have different properties, hydrophobicities and molecular weights and are likely to have different susceptibilities to exogenous enzymes. Indeed, the salt-solubility of protein in different corn samples has been demonstrated to correlate with ileal digestible energy values, indicating that salt-soluble protein may represent some ileal digestible characteristics in feed ingredients (Gehring *et al.,* 2012). Therefore, it is possible to develop multiple regression equations by using gross chemical measurements, *in vitro* starch digestion rate, water absorption characteristics, physical density or other *in vitro* parameters, to predict ileal protein, starch and fat digestibility or feed enzyme functional patterns.

Conclusions

A reduction in feed cost is a major goal for feed producers and poultry integrators. Supplementation of diets with microbial enzymes is one way by which feed input costs can be controlled. Historically, the value of carbohydrases and proteases for poultry diets has been limited by equivocal animal data and apparent inconsistency at an end user level. A substantial part of this inconsistency may have been mediated via a lack of appreciation for the inherent nutritional value of the control diet to which the enzymes will be added, and an incomplete understanding of the physiological factors determining the link between digestion and absorption. Genesis of predictive equations based on multiple regression models of *in vitro* data and *in vivo* ileal digestibility values may shed light on these factors and improve the consistency of response. Though single-ingredient models can be helpful, especially mechanistically, a more empirical proportionality approach is likely to result in more robust models. The use of feed ingredient mixtures, generated systematically, with radically different inherent characteristics to explore enzyme effects in a multi-nutrient space will be instructive and allow primary drivers for feed enzyme effect to become apparent.

References

ADEOLA, O. and COWIESON, A.J. (2011) Opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. *Journal of Animal Science* **89**: 3189-3218.

ALLEE, G.L. and BAKER, D.H. (1970) Limiting nitrogenous factors in corn protein for adult female swine. *Journal of Animal Science* **30**: 748-752.

AL-MARZOOQI, W., KADIM, I.T., MAHGOUB, O., Al-BUSAID, M., Al-LAWATI, S.M., AlMAQBALY, R., Al-WHEEBI, S. and Al-BAKERY, A.N. (2010) Apparent ileal amino acids digestibility of four varieties of barley for two strains of chickens. *International Journal of Poultry Science* **9**: 527-532.

ALMIRALL, M., FRANCESCH, M., PEREZ-VENDRELL, A.M., BRUFAU, J. and ESTEVE-GARCIA, E. (1995) The differences in intestinal viscosity produced by barley and ß-glucanase alter digesta enzyme activities and ileal nutrient digestibility more in broiler chicks than in cocks. *The Journal of Nutrition* **125**: 947-955.

AL-RABADI, G.L., TORLEY, P.J., WILLIAMS, B.A., BRYDEN, W.L. and GIDLEY, M.J. (2012) Particle size heterogeneity in milled barley and sorghum grains: effects on physio-chemical properties and starch digestibility. *Journal of Cereal Science* **56**: 396-403.

ANGEL, R., TAMIM, N.M., APPLEGATE, T.J., DHANDU, A.S. and ELLESTAD, L.E. (2002) Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficiency. *Journal of Applied Poultry Research* **11**: 471-480.

ANGKANAPORN, K., CHOCT, M., BRYDEN, W.L. and ANNISON, E.F. (1994) Effects of wheat pentosans on endogenous amino acid losses in chickens. *Journal of the Science of Food and Agriculture* **66**: 399-404.

BEDFORD, M.R. (2000) Exogenous enzymes in monogastric nutrition-their current value and future benefits. *Animal Feed Science and Technology* **86**: 1-13.

BEDFORD, M.R. and COWIESON, A.J. (2012) Exogenous enzymes and their effects on intestinal microbiology. *Animal Feed Science and Technology* **173**: 76-85.

BLACK, J.L., HUGHES, R.J., NIELSEN, S.G., TREDEA, A.M., MACALPINE, R. and BAREVELD, V. (2005) The energy value of cereal grains, particularly wheat and sorghum, for poultry. *Proceedings of Australian Poultry Science Symposium* **17**: 21-29.

BYERS, M., MIFLIN, J. and SMITH, S.J. (1983) A quantitative composition of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. *Journal of the Science of the Food and Agriculture* **34**: 447-462.

CARRÉ, B., GOMEZ, J. and CHAGENEAU, A.M. (1995) Contribution of oligosaccharide and polysaccharide digestion, and excreta loss of lactic acid and short chain fatty acids, to dietary metabolisable energy valuses in broiler chickens and adult cockerels**.** *British Poultry Science* **36**: 611-629.

CHOCT, M. (2006) Enzmes for the feed industry: past,present and future. *World's Poultry Science Journal* **62**: 5-16.

CHOCT, M. (1997) Feed non-starch polysaccharides: chemical and nutritional significance. *Feed Milling International* **June**: 13-26.

CHOCT, M. and KOCHER, A. (2000) Non-starch carbohydrates: Digestion and its secondary effects in monagastrics. *Proceedings of the Nutrition Society of Australia* **24**: 31-38.

CHOCT, M., HUGHES, R.J., TRIMBLE, R.P., ANGKANAPORN, K. and ANNISON, G. (1995) Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition* **125**: 485-492.

COURTIN, C.M., BROEKAERT, W.F., SWENNEN, K., LESCROART, O., ONAGBESAN, O., BUYSE, J., DECUYPERE, E., VAN DE WIELE, T., MARZORATI, M. and VERSTRAETE, W. (2008) Dietary inclusion of wheat bran arabinoxylooligosaccharides induces beneficial nutritional effects in chickens. *Cereal Chemistry* **85**: 607-613.

COWIESON, A.J. (2010) Strategic selection of exogenous enzymes for corn/soy-based diets. *Journal of Poultry Science* **47**: 1-7.

COWIESON, A.J. (2005) Factors that affect the nutritional value of maize for broilers. *Animal Feed Science and Technology* **119**: 293-305.

COWIESON, A.J., ACAMOVIC, T. and BEDFORD, M.R. (2006) Phytic acid and phytase: implications for protein utilisation by poultry. *Poultry Science* **85**: 878-885.

COWIESON, A.J., BEDFORD, M.R. and RAVINDRAN, V. (2010) Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *British Poultry Science* **51**: 246-257.

COWIESON, A.J. and BEDFORD, M.R. (2009) The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complilmentary mode of action. *World's Poultry Science Journal* **65**: 609-624.

COWIESON, A.J. and RAVINDRAN, V. (2008) Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy,minerals and amino acids. *British Poultry Science* **49**: 37-44.

D' ALFONSO, T.H. (2005) Sources of variances of energy digestibility in corn-soy poultry diets and effect on performance: starch, protein, oil and fibre. *Krmiva* **2**: 83-86.

DÄNICKE, S., JEROCHA, H., BOÈTTCHERA, W. and SIMON, O. (2000) Interactions between dietary fat type and enzyme supplementation in broiler diets with high pentosan contents: effects on precaecal and total digestibility of fatty acids, metabolizability of gross energy, digesta viscosity and weights of small intestine. *Feed Animal Feed Science and Technology* **84**: 279-294.

DAVEBY, D.Y., RAZDAN, A. and ÅMAN, P. (1998) Effect of particle size and enzyme supplementation of diets based on dehulled peas on the nutritive value for broiler chickens. *Animal Feed Science and Technology* **74**: 229-239.

DEL ALAMO, G.A., VERSTEGEN, M.W.A., DEN HARTOG, L.A., PEREZ, D.E., AYALA, P. and VILLAMIDE, M.J. (2009) Wheat starch digestion rate affects broiler perfpormance. *Poultry Science* **88**: 1666-1675.

DONA, A.C., PAGES, G., GILBERT, R.G. and KUCHEL, P.W. (2010) Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers* **80**: 599-617.

DONKOH, A. and ATTOH-KOTOKU, V. (2009) Nutritive value of feedstuffs for poultry in Ghana: chemical composition, apparent metabolizable energy and ileal amino acid digestibility. *Livestock Research for Rural Development* **21 (3)** http://www.lrrd.org/lrrd21/3/donk21032.htm.

DOUCET, F.J., WHITE, G.A., WULFER, F., HILL, S.E. and WISEMAN, J. (2010) Predicting in vivo starch digestibility coefficients in newly weaned piglets from in vitro assessment of diets using multivariate analysis. *British Journal of Nutrition* **103**: 1309-1318.

ENGLYST, H.N., KINGMAN, S.M. and CUMMINGS, J.H. (1992) Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* **46**: S33-50.

FANG, Z.F., LIU, Z.L., DAI, J.J., QIAN, H.Y., QI, Z.L., MA, L.B. and PENG, J. (2009) Effects of enzyme addition on the nutritive value of broiler diets containing hulled or dehulled chinese double-low rapseed meals. *Journal of Animal Physiology and Animal Nutrition* **93**: 467-476.

FERNANDEZ, F., SHARMA, R., HINTON, M. and BEDFORD, M.R. (2000) Diet influence the colonisation of Campylobacter jejuni and distribution of mucin carbohydrates in the chick intestinal tract. *Cellular and Molecular Life Sciences* **57**: 1793-1801.

FRANCESCH, M. and GERAERT, P.A. (2009) Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralisation of broilers fed reduced nutrient corn-soybean -based diets. *Poultry Science* **88**: 1915-1924.

GABRIEL, I., QUILLIEN, L., CASSECUELLE, F., MARGET, P., JUIN, H., LESSIRE, M., SÈVE, B., DUC, G. and BURSTIN, J. (2008) Variation in seed protein digestion of different pea (Pisum sativum L.) genotypes by cecectomized broiler chickens: 2. Relation between in vivo protein digestibility and pea seed characteristics, and identification of resistant pea polypeptides. *Livestock Science* **113**: 262-273.

GEHRING, C.K., BEDFORD, M.R., COWIESON, A.J. and DOZIER, W.A. (2012) Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. *Poultry Science* **91**: 1908-1914.

HETLAND, H., SVIHUS, B. and KROGDAHL, A. (2003) Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science* **44**: 275-282.

JAMROZ, D., JAKOBSEN, K., BACH KNUDSEN, K.E., WILCZKIEWICZ, A. and ORDA, J. (2002). Digestibility and energy value of the non-starch polysaccharides in young chickens, ducks and geese, fed diets containing high amounts of barley. *Comparative Biochemistry and Physiology* **A 131**: 657-668.

JANAS, S., BOUTRY, S., MALUMBA, P., VANDER ELST, L. and BÉRA, F. (2010) Modelling dehydration and quality degradation of maize during fluidized-bed drying. *Journal of Food Engineering* **100**: 527-534.

JANKOWSKI, J., JUSKIEWICZ, J., GULEWICZ, K., SLOMINSKI, B.A. and ZDUNCZYK, Z. (2009) The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolateof different oligosaccharide content on growth performance and gut function of young turkeys. *Poultry Science* **88**: 2132-2140.

JANSSENS, D. and GAETHOFS, B. (2007) Endo-xylanase, a possible way of supplying prebiotic oligosaccharides? *Proceedings of the 19th Australian Poultry Science Symposium* **19**: 165-168.

JENKINS, D.J., THORNE, M.J. and RAO, A.V. (1987) The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *American Journal of Clinic Nutrition* **45**: 946-951.

JIANG, Z.Y., ZHOU, Y.M., LU, F.Z., HAN, Z.Y. and WANG, T. (2008) Effects of different levels of supplementary alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. *Asia-Australasian Journal of Animal Science* **21**: 97-102.

JIMENEZ-MORENO, E., GONZALEZ-ALVARADO, J.M., GONZALEZ-SERRANO, A., LAZARO, R. and MATEOS, G.G. (2009) Effect of dietary fiber and fat on performance and digestive traits of broilers from one to twenty-one days of age. *Poultry Science* **88**: 2562-2574.

JØRGENSEN, H., ZHAO, X.Q., THEIL, P.K. and JAKOBSEN, K. (2008) Effect of graded levels of rapeseed oil in isonitrogenenous diets on the development of gastrointestinal tract, and utilisation of protein, fat and energy in broiler chickens *Archives of Animal Nutrition* **62**: 331-342.

JØRGENSEN, H., ZHAO, X.Q., KNUDSEN, K.E.B. and EGGUM, B. (1996) The infuence of dietary fibre souce and level on the development of gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *British Journal of Nutrition* **75**: 379-395.

KLASING, K.C. (2007) Nutrition and the immune system. *British Poultry Science* **48**: 525-537.

KOCHER, A., CHOCT, M., PORTER, M. and BROZ, J. (2000) The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. *Poultry Science* **79**: 1767-1774.

LIU, N., RU, Y.J., WANG, J.P. and XU, T.S. (2010) Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. *British Journal of Nutrition* **103**: 862-868.

LOSADA, B., GARCÍA-REBOLLAR, P., ÁLVAREZ, C., CACHALDORA, P., IBÁÑEZ, M.A., MÉNDEZ, J. and DE BLAS, J.C. (2010) The prediction of apparent metabolisable energy content of oil seeds and oil seed by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Animal Feed Science and Technology* **160**: 62-72.

MATEOS, G.G., JIMÉNEZ-MORENO, E., SERRANO, M.P. and LÁZARO, R.P. (2012) Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. *Journal of Applied Poultry Research* **21**: 156-174.

MATEOS, G.G. and SELL, J.L. (1981) Metabolizable energy of supplementalb fat as related to dietary fat level and methods of estimation. *Poultry Science* **60**: 1509-1515.

MENG, X. and SLOMINSKI, B.A. (2005) Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. *Poultry Science* **84**: 1242-1251.

MOOSAVI, M., ESLAMI, M., CHAJI, M. and BOUJARPOUR, M. (2011) Economic value of diets with different levels of energy and protein with constant ratio on broiler chcikens. *Journal of Animal and Veterrinary Advances* **10**: 709-711.

MOORE, S.M., STALDER, K.J., BEITZ, D.C., STAHL, C.H., FITHIAN, W.A. and BREGENDAHL, K. (2008) The Correlation of Chemical and Physical Corn Kernel Traits with Production Performance in Broiler Chickens and Laying Hens. *Poultry Science* **87**: 665-676.

MUSHTAQ, T., SARWAR, M., AHMAD, G., MIRZA, M.A., AHMAD, T., MOREEN, U., MUSHTAQ, M.M.H. and KAMRAN, Z. (2009) Influence of sunflower meal based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility and carcass of broiler chickens. *Animal Feed Science and Technology* **149**: 275-286.

PETTERSSON, D. and AMAN, P. (1989) Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition* **62**: 139-149.

RAVINDRAN, V. and BRYDEN, W.L. (1999) Amino acid availability in poultry:in vitro and in vivo measurements. *Australian Journal of Agricultural Research* **50**: 889-908.

RAVINDRAN, V., HEW, L.I., RAVINDRAN, G. and BRYDEN, W.L. (2005) Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science* **81**: 85-97.

ROSEN, G.D. (2010) Holo-analysis of the efficiency of exogenous enzyme performance in fam animal nutrition, in: BEDFORD, M.R. & PARTRIGE, G.G. (Eds) *Enzymes in Farm Animal Nutrition*, pp. 273-303 (London, CAB International).

RUBIO, L.A. and CLEMENTE, A. (2010) Molecular size distribution affects portal absorption rate of casein amino acids in rats. *Journal of Animal Physiology and Animal Nutrition* **94**: E145-E153.

SAUVANT, D., PEREZ, J.M. and TRAN, G. (2004) in: SAUVANT, D., PEREZ, J.M. & TRAN, G. (Eds) *Tables of composition and nutritional value of feed materials* pp. 13-302 (Paris, Wageningen Academic).

SELLE, P.H. and RAVINDRAN, V. (2007) Microbial phytase in poultry nutrition. *Animal Feed Science and Technology* **135**: 1-41.

SINGH, P.K. (2008) Significance of phytic acid and supplemental phytase in chicken nutrition: a review. *World's Poultry Science Journal* **64**: 557-580**.**

SLOMINSKI, B.A. (2011) Recent advances in research on enzymes for poultry diets. *Poultry Science* **90**: 2013-2023.

SLOMINSKI, B.A., MENG, X., CAMPBELL, L.D., GUENTER, W. and JONES, O. (2006) The use of enzyme technology for improved energy utilisation from full-fat oilseeds. Part II: flaxseed. *Poultry Science* **85**: 1031-1037.

SMINK, W., GERRITS, W.J.J., HOVENIER, R., GEELEN, M.J.H., LOBEE, H.W.J., VERSTEGEN, M.W.A. and BEYNEN, A.C. (2008) Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil. *Poultry Science* **87**: 506-513.

SOPADE, P.A. and GIDLEY, M.J. (2009) A rapid in-vitro digestibility assay based on glucometry for investigating kinetics of starch digestion. *Starch/ Stärke* **61**: 245-255.

SVIHUS, B. (2011) Limitations to wheat starch digestion in growing broiler chickens: a brief review. *Animal Production Science* **51**: 583-589.

SVIHUS, B., UHLEN, A.K. and HARSTAD, O.M. (2005) Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology* **122**: 303-320.

THOMAS, D.V., RAVINDRAN, V. and RAVINDRAN, G. (2008) Nutrient digestibility and energy utilisation of diets based on wheat, sorghum or maize by the newly hatch broiler chickens. *British Poultry Science* **49**: 429-435.

THOMPSON, L.U. and YOON, J.H. (1984) Starch digestibility as affected by polyphenols and phytic acid. *Journal of Food Science* **49**: 1228-1229**.**

THORNE, M.J., THOMPSON, L.U. and JENKINS, D.J.A. (1983) Factors affecting starch digestibility and the glycemic response with special reference to legumes. *American Journal of Clinical Nutrition* **38**: 481-488.

VAHJEN, W., GLÄSER, K., SCHÄFER, K. and SIMON, O. (1998) Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. *Journal of Agricultural Science* **130**: 489-500.

WALL, J.S. and PAULIS, J.W. (1983) Corn and sorghum grain proteins, in: KOLAR, C.W., RICHET, S.H., DECKER, C.D. & STEINKE, F.H. (Eds) *Advance in cereal science and technology* pp. 135-219 (Madison, American Association of Cereal Chemists).

WEURDING, R.E., ENTING, H. and VERSTEGEN, M.W.A. (2003) The effect of site of starch digestion on performance of broiler chickens. *Animal Feed Science and Technology* **110**: 175-184.

WEURDING, R.E., VELDMAN, A., VEEN, W.A.G., VAN DER AAR, P.J. and VERSTEGEN, M.W.A. (2001) In vitro starch digestion correlates well with rate and extent of starch digestion in broiler chickens. *The Journal of Nutrition* **131**: 2336-2342.

WHITE, G.A., DOUCET, F.G., HILL, S.E. and WISEMAN, J. (2008) Physicochemical properties and nutritional quality of raw cereals for newly weaned piglets. *Animal* **2**: 867-878.

WIDYARATNE, G.P. and DREW, M.D. (2011) Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens1. *Poultry Science* **90**: 595-603.

WISEMAN, J. (2006) Variations in starch digestibility in non-ruminants. *Animal Feed Science and Technology* **130**: 66-77.

WISEMAN, J., EDMUNDA, B.K. and SHEPPERSON, N. (1992) The apparent metabolisable energy of sunflower oil and sunflower acid oil for broiler chickens. *Animal Feed Science and Technology* **36**: 41-51.

WISEMAN, J. and SALVADOR, F. (1991) The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fats fed to broilers. *Poultry Science* **70**: 573-582.

WOLF, W.J. (1970) Soybean proteins: their functional, chemical, and physical properties. *Journal of Agricultural Food Chemistry* **18**: 969-976.

WONG, J.H., MARX, D.B., WILSON, J.D., BUCHANAN, B.B., LEMAUX, P.G. and PEDERSEN, J.F. (2010) Principal component analysis and biochemical characterisation of protein and starch reveal primary targets for improving sorghum grain. *Plant Science* **179**: 598-611.

WOOLNOUGH, J.W., MONRO, J.A., BRENNAN, C.S. and BIRD, A.R. (2008) Simulating human carbohydrate digestion in vitro: a review of methods and the need for standardisation. *International Journal of Food Science & Technology* **43**: 2245-2256.

WOYENGO, T.A. and NYACHOTI, C.M. (2011) Review: Supplementation of phytase and carbohydrases to diets for poultry. *Canadian Journal of Animal Science* **91**: 1-16.

YU, S., COWIESON, A., GILBERT, C., PLUMSTEAD, P. and DALSGAARD, S. (2012) Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5 isomers with dietary protein and iron and inhibition of pepsin *Journal of Animal Science* **90**: 1824-1832.

ZANELLA, I., SAKOMURA, N., SILVERSIDES, F., FIQUEIRDO, A. and PACK, M. (1999) Effect of enzyme supplementation of broiler diets based on corn and soybeans. *Poultry Science* **78**: 561-568.

ZELENKA, J. and ČEREŠŇAKOVÁ, Z. (2005) Effect of age on digestibility of starch in chickens with different growth rate. *Czech Journal of Animal Science* **50**: 411-415.