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THE USE OF ENZYMES TO IMPROVE UTILIZATION OF NUTRIENT IN POULTRY DIETS

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Abstract

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Substitution of corn and wheat with barley, rye, triticale and oats has been associated with its variable energy content and detrimental effect on litter quality. The amount of available energy is influenced by fiber content and the amount and composition of cell wall polysaccharides. The predominant Non Starch Polysaccharides (NSP) in barley and oats are the mixed-linked β-glucans, while in rye, wheat and triticale they are the pentosans (arabinixylans). It is now established that the soluble β -1-3, 1-4 D-glucan and soluble arabinoxylan exert antinutritive activity when high concentrations of these cereals are present in broiler diets. Both nonstarch polysaccharides create a viscous environment in the gastrointestinal tract of broiler chickens thereby interfering with the digestion and absorption of nutrients. The increased viscosity of the intestinal contents slow the rate of mixing of digestive enzymes with the substrate and the thickened unstirred water layer at the mucosal surface of the intestine impede the absorption of amino acids and glucose across the microvilous membrane. The ability of dietary fiber to bind bile acids and the ability of microbiota to deconjugate them can cause bile acid insufficiency within the intestine. Whether the formations of β-glucan and arabinoxylan gels in the intestine exert their adverse effect on growth through food intake depression or through impaired efficiency of digestion is unresolved.

Corn alone does not contain soluble NSP in amounts to produce depression of performance Soybean cell walls contain pectic polysaccharides (galacturonans and arabinogalactans), which are half of the NSP.

Dietary supplementation of cereal containing diets for broiler chickens with microbial and fungal β-glucanase and pentosanase activity improved feeding value of barley, oats, rye, wheat and triticale resulting in improved growth, feed conversion and reduction in sticky droppings. The improved performance has been attributed to the viscosity reduction and a breakdown of β-glucans and pentosans. Enzyme supplementation of chicken cerals based diets has resulted in improved starch and nitrogen digestability as well as improved absorption of starch, amino acids and lipids.

The deleterious effects of β-glucans and pentosans, and the response to dietary β-glucanase or pentosanase are also affected by the age of the chickens.

Key words: NSP (nonstarch polysaccharides); ANF (antinutritive factors); carbohydrases; β-glucanase; xylanase; broiler chickens; layers

Introduction

Corn is primary dietary energy source in poultry diets, as it have highest concentration of energy. In some countries it is replaced with wheat, barley, triticale and oats. Cereal grains, however, have a higher fiber level and lower energy content than corn (Herstad, 1987).

The amount of available energy from a cereal will be influenced by fiber content and amount of composition of cell wall polysaccharides (Table 1).

Grain cell walls

Knowledge of the composition of grain cell walls has improved in recent years. Cell walls are primarily composed of carbohydrate fractions with lesser amounts of protein and phenolic acids (Bacic et al., 1988). Complex carbohydrates

Table 1
Dietary fiber and dietary components (% of dry matter)

Components	Spring wheat	Rye	Triticale	Oats	Barley
Dietary fiber	10.6	12.8	10.3	29.6	17.2
Arabinose	2.1	2.6	2.4	1.5	2.2
Xylose	3.3	4.5	3.6	5.4	4.5
Mannose	0.3	0.6	0.5	0.3	0.5
Galactose	0.5	0.6	0.5	0.7	0.4
Glucose	3.1	3.9	2.9	12.2	7.9
Uronic acid	0.4	0.8	0.4	1.1	0.5
Klason lignin	0.9	1.7	1.2	8.4	1.2
β-glucan	0.8	1.3	0.5	3.2	3.8

(From Hesselman, 1989)

are loosely termed nonstarch polysaccharides (NSP). The predominant nonstarch polysaccharides in barley and oats are the mixed-linked β-glucans, while in wheat, rye and triticale they are the arabinoxylans (pentosans) (Henry, 1987; Annison and Choct, 1991). The mixed–linked (1-3) (1-4) β-glucans occurs together with arabinoxylans and mannose containing polysaccharides in cereal cell walls.

• β-glucans

β-glucan is a polymer of glucose with a β-1,4-linked backbone and β-1,3 side linkage – HPLC of lichenase fragments and 13C-NMR of the β-glucans both indicate that there is a small difference between the structures of the mixed-linked β-glucans from barley and oats (Wood et al., 1991). Both soluble and insoluble β-glucan fractions are similar in chemical structure and the solubility relates to the degree of association of glucan with insoluble cell wall fractions.

• Arabinoxylans (pentosans)

Arabinoxylans consist of a backbone of β-1,4-linked xylopyranosyl residues with terminal 1,2 and 1,3 arabinofuranosyl substitutions.

Rye contains a high level of a arabinoxylans. Some wheats have a low apparent metabolisable energy when included in poultry diets. The pentosans of wheat also have antinutritive activities in broiler diets and are probably the cause of the low ME (Choct and Annison, 1990). The pentosans of wheat are similar to those of rye consisting of a (1-4)-β-xylan chain with arabinose substituted at the 02 and 03 positions of the xylose.

Nutritional effects of nonstarch polysaccharides

Common properties of the different nonstarch polysaccharides are their resistance to digestive enzymes and tendency to create a viscous environment in the intestine (Choct and Annison, 1992; Choct et al., 1996). The water-soluble nonstarch polysaccharides are usually the most viscouse.

To date most studies have investigated the anti-nutritive activities of the nonstarch polysaccharides from barley and rye. They depressed growth of broiler chickens, create a viscous environment in the gastrointestinal tract of chickens, resulting in a reduced nutrient utilization and excretion of sticky droppings and cases wet litter conditions (Hesselman and Aman, 1986; Salih et al., 1990; Teitge et al., 1991). Such effects are pronounced in young birds, while older birds are more tolerant to these polysaccharides (Choct and Annison, 1992; Chesson, 2001).

The increase in digesta viscosity is considered to be a major factor influencing the nutritional value of cereal greens (barley, wheat, triticale, rye). The water soluble nonstarch polysaccharides occurring in barley, rye, wheat, oats and triticale are believed responsible for the reduction of growth performance and decreased digestability of starch, protein and lipids in broiler chickens and pigs (Fengler and Marquardt, 1988; Choct and Annison, 1992; Mateo et al., 2008).

The exact effects of viscosity has a not been established but possible mechanisms include reduced rates of diffusion of endogenous enzymes and nutritional substrates. When the viscosity of a solution increased, decreases the rate of nutrient diffusion (Fengler and Marquardt, 1988). In broiler barley diets reduced ileal starch, protein and lipids digestabilities to varying degrees. The digestability of fat was most affected than the others (Martinez et al., 1992; Wang et al., 1992). It was suggested that the low lipid digestability in broiler chickens fed diets with a high content of nonstarch polysaccharides may be due to bacterial overgrowth in the small intestine and subsequent excessive deconjugation of bile acids, which reduce their efficacy in solubilizing lipids (Salih et al., 1991; Smith et al., 1998; Langhout, 1999). In broiler chickens ileal starch and protein digestabilities were lower in those fed barley than in those fed corn. Viscosity seemed to delay the absorption of starch in the small intestine, though starch was almost completely digested at the terminal ileum and cecum (Hesselman and Aman, 1986; Rotter et al., 1990). Feeding barley has been shown to reduce feed passage time in chickens (Salih et al., 1991).

The presence of viscous materials in the stomach and intestine slowed gastric emptying and small intestinal transit (Meyer and Doty, 1988; Smulikowska et al., 2002). The formation of a viscous solution in the intestine impedes the mixing of the gut contents and consequently influences absorption of nutrients (Hesselman and Aman, 1986; Salih et al., 1991; Van der Klis et al., 1993). Nonstarch polysaccharides can increase the depth of the unstirred water layer adjacent to the epithelial lining of the small intestine, which is considered to be a rate limiting step in the absorption (Johnson and Gee, 1981; Chesson, 2001). The unstirred layer at the mucosal surface of intestine reduces the contact between the feed and digestive enzymes and slows the uptake in the gut of released sugars, amino acids and lipids (Bedford, 1995). These polymers slow the absorption of glucose *in vitro* and *in vivo* (Elsenhans et al., 1980; Johnson and Gee, 1981; Flourie et al., 1984). The thickness of unstirred layers is affected by the viscosity of the intestinal contents and it seems reasonable that increase in the viscosity of the digesta will slow down the migration of nutrients and decrease their accumulation in the intestine. The dietary nonstarch polysaccharides inhibit nutrient absorption in broiler chickens not only by raising the viscosity of the digesta, but also by enhancing bacterial fermentation.

It is well known that some dietary fibers have cholesterol lowering effect, guar gum; pectin and barley have repeatedly been shown to reduce plasma cholesterol concentration (Martinez et al., 1992; Wang et al., 1992). Increased viscosity of intestinal contents reduced cholesterol concentration in hamsters fed hydroxypropyl methylcellulose with varying viscosity (Gallaher et al., 1993; Carr et al., 1996).

There are several hypotheses explaining the cholesterol lowering effect of fibers. One of them explained that nonstarch polysaccharides cause a depressed fat emulsification or micelle formation by reduced mucosal uptake of lipids and by increased amount of unabsorbed endogenous fat in the chime (Smits and Annison, 1996). The hypocholesterolemic effect of dietary water-soluble fibers such as guar gum, pectin and psyllium has been well documented (Anderson et al., 1990). Many water-soluble fibers from a viscous matrix within the small intestine could interfere with bile acid and cholesterol absorption (Eastwood and Morris, 1992). Several investigators have reported increased bile acid excretion by dietary fibers and have attributed their findings to either direct binding of bile acids or entrapment of bile acids within the viscous medium (Vahouny et al., 1980; Vahouny and Cassidy, 1985). Consequently, impaired reabsorption of bile acids by water-soluble fibers results in increased hepatic bile acid synthesis (Matheson and Story, 1994).

Corn-soybean diets

Corn has cellular endosperm and a cell wall composition similar to that of wheat. In corn present highly branched arabinoxylans, mixed linked glucan and a small amount of cellulose.

Soybean cell walls contain pectic polysaccharides (galacturonans and arabinogalacans). The energy in leguminosae, however, is stored in the form of oil or oligosaccharides. Their nonstarch polysaccharides composition is quite different, with high viscosity.

Sunflower meal is rich in protein, but also has a high content of crude fiber and could be used at a dose of up to 7% without adverse effect of growing turkeys (Juskiewicz et al., 2010).

Enzyme preparation applied to a diet caused a tendency towards lower ileal viscosity and decrease in caecal total volatile fatty acids concentration.

Enzymes are also used to improve the nutritive value of corn and its byproducts, soybean meal and sunflower meal. There are enzyme products available for corn based diets which contains glucanase, amylase, xylanase, lipase, protease, pectinase, mannanse and galactosidase. It was suggested that about 30% of the undigested fraction can be digested by enzyme supplementation (Cowieson, 2010). Supplementing the corn/soy diet with α-galactosidase and α-mannanase gives significant improvements in growth rate, feed conversion, energy and ileal amino acid digestability in broiler chickens, layers and turkeys (Ghesquiere, 2004). The efficacy of feed enzymes declines as the nutritional value of the control diet improves (Cowieson, 2010).

Enzyme sources and substrate hydrolysis

The β-glucans and arabinoxylans in the cell wall are resistant to digestive enzymes of animal including poultry. They protect starch and protein from digestion in the small intestine (Hesselman and Aman, 1986). Enzymes capable of hydrolyzing grain cell walls in particular β-glucans and arabinoxylans are found in a wide range of microbial sources. Dietary enzymes are primarily derived from bacterial and fungal sources including *Baccilus amyloliquefaciens*, *B. subtilis*, and *Trichoderma viride*.

Enzyme activities necessary for hydrolysis of β-glucan and arabinoxylan are shown in Table 2.

Arabinoxylan hydrolysis is primarily accomplished by endo-1,4-β-xylanase activity which cleaves (1,4) linkages of the xylan backbone.

Table 2

Primary enzyme activity required for hydrolysis of cereal cell wall β-glucan and arabinoxylan

Enzyme
1. β-glucans
Endo-1,3 : 1,4- β-glucanase (EC 3.2.173)
Endo-1,4-β-glucanase (EC 3.2.1.4)
β-glucosidase (EC 3.2.1.21)
β-glucan solubiase
2. Arabinoxylans
Endo-1,4-β-xylanase (EC 3.2.1.8)
β-xylosidase (3. 2. 3.37)
β-L-arabinofuranosidase (EC 3.2.1.55)

β-glucan destruction has primarily been accomplished by the use of enzyme sources containing endo-β-glucanase activity.

Methods of enzyme supplementation

Enzymes are produce in various forms for supplementation in poultry diets. They can be used as powder, granules and liquids. Some enzymes are thermolabile and processing at high temperatures may reduce their activity (Silversides and Bedford, 1999).May used several methods to mix enzymes with dietary ingredients in poultry diets:

- Enzyme powders are added to premix before their mixing with ingredients;
- Enzyme powders and granules are mixed with ingredients;
- Enzymes can be used as liquids after pelleting;
- Enzymes as liquids are mixed with other ingredients or added to the drinking water.

These enzymes cause a partial depolymerization of the nonstarch polysaccharides which reduce their anti-nutritive activity (Bedford et al., 1991; Choct and Annison, 1992; Francesch et al., 1994; Mombaerts and Gaethofs, 2004). Enzyme sources with β-glucanase and pentosanase activity significantly improved feeding value of barley, rye, oats and wheat (Friesen et al., 1991; Choct et al., 1995; Danicke et al., 1997). They increased the nutritional value of cereal ingredients, resulting in improved growth, feed efficiency and reduction of sticky droppings (Hesselman and Aman, 1986; Choct and Annison, 1990; Chotinsky et al., 1994; Anjum and Chaudhry, 2010). Enzyme supplementation had a beneficial effect on weight gain and feed intake but not on eggs production, initial egg weight, average egg weight, egg specific gravity, Haugh unit score and mortality (Brenes et al., 1993). Enzymes hydrolyze the β-glucans and pentosans into smaller polymers in the alimentary tract and reduce the viscosity of the intestinal contents (White et al., 1983; Choct et al., 1995). The effects of enzymes on intestinal viscosity can be seen in laying hens also (Almirall et al., 1995; Chotinsky et al., 1995).

Enzyme supplementation improved overall CP digestability by 2.9%, but this improvement was not equal for all amino acids. The digestabilities of lysine, methionine and arginine were not improved significantly by enzyme supplementation, but that of valine was improved by 2.3% and that of triptophan by 3.0% (Zanella et al., 1999). Olofs et al. (1999) found significantly improvements in starch, fat and protein digestabilities in hen fed on enzyme supplemented wheat and barley based diets. In recent studies it has been shown that the effect of enzyme addition to wheat and rye based diets on performance and digestibility is considerably influenced by the fat sourced used (Langhout et al., 1997; Danicke et al., 1999).

The results of other digestibility test show that the use of enzymes in corn soybean based feed ration significantly increases metabolizing energy 65 kcal/kg for broilers and turkeys (Ziggers, 2006). An experiment with layers fed corn-based diets demonstrated a significant reduction of feed consumption resulting in decrease of feed conversion by 5.8%. Kaczmarek et al. (2009) reported that hominy diets had a lower AME value. Enzyme addition improved the apparent digestibility of all nutrients except crude fat. The natural combination of enzymes on average improved AME 65 kcal/kg for broiler chickens and layers fed on corn and soybean meal diets, 85 kcal/kg for wheat-based diets, 140 kcal/kg for barley-based diet and 65 kcal/kg for pig diets (Liu and Gaert, 2003).

Combination of xylanase and phytase improved nutrient digestibility and retention in broilers fed wheat based diets (Wu et al., 2004). Others also reported the impact of xylanase and phytase on the performance of broiler chickens (Olukosi et al., 2007, 2008). Supplementation of xylanase and phytase in wheat based diets improved growth performance and whole body accretion of minerals and protein and the improvement of protein accretion is an indication of improvement in nutrient utilization resulting from phytase use (Olukosi and Adeola, 2008). Combination of carbohydrases and protease improved bone mineralization of broiler chicks receiving wheat-based diet (Zyla et al., 1999a,b).

Gruzauskas et al. (2007) showed that a combination of enzymes and organic acids improved body weight of broiler chickens and feed conversion. The beneficial effects of organic acids on the performance were noticed by Jozefiak et al. (2007). Modeva et al. (2004) established that the XTRACT supplementation upon the diet in which the corn has been replaced by oats as energetic source and 0.1% β-glucanase increased protein retention and decreased fat retention in the chicken carcasses after XTRACT supplementation.

The supplementation of enzymes in the diets of broiler chickens enhances meat production. Sarvestani et al. (2006) observed increases in the weight of breast, thigh, heart, liver and abdominal fat in broiler chickens fed 0.75 kg/ton Biozyme in a pellet diet. Breast meat yield also increased when Versazyme supplement in the diets of broiler chickens (Wang et al., 2006).

Enzyme supplementation of broiler chickens barley, wheat, rye and corn based diets has resulted in improved starch and nitrogen digestibility, as well as improved absorption of starch, fat and amino acids (Oloffs et al., 1999; Bedford, 2000; Zigers, 2006; Serena et al., 2009).

Conclusion

Enzyme supplementation of poultry feed has evolved in recent years from a trail to more precisely targeted science based on the increase in knowledge of the antinutritional components of feedstuffs employed. The most common application and the most thoroughly documented is the use of β-glucanase preparation in barley containing poultry diets. More recently pentosanases for wheat, rye, and triticale–based diets have also found some application. Enzyme preparations for corn/soybean diets contain a variety of NSP enzymes usually blended with protease and other activities. Further advances in the understanding of the structure of endosperm cell walls and of the rate of release and composition of cell wall components in the digestive tract will allow identification of the remaining obstacles to nutrient utilization.

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