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Enzymes in broiler diets with special reference to protease

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Enzyme supplementation of poultry diets is nutritionally, economically and environmentally justified. Enzymes are used to increase the energy value of feed ingredients and enhance the utilisation of protein, fats, carbohydrates and phytin phosphorus from plant materials, leading to a lower excretion rate of undigested nutrients into the environment and, hence, reduced environmental pollution. This is especially important regarding proteases, as the correct digestion of nitrogenous compounds in feed materials is essential for reducing N excretion – a major pollutant worldwide. Numerous studies have shown no adverse effects of enzyme supplementation in broiler diets on body weight, mortality, health, feed intake, FCR, nutrient digestibility, meat quality and production costs. However, there is still a large amount of uncertainty regarding the use of enzymes.

Keywords: broiler; diet; enzymes; protease

Introduction

Modern poultry production involves the use of breeds that give high quality meat and feed formulations that ensure optimum meat quality, along with high yields. Market requirements have substantially changed in the last years, and in contrast to the great demand in the previous decades for maximum possible yields of broiler meat, increasing attention is focused on improving the nutritional quality of poultry meat (Bogosavljević-Bošković *et al.*, 2012), whilst maintaining weight gain. Moreover, increasing importance in broiler production has been attributed to environmental standards to help prevent environmental pollution and ensure improved rearing conditions for birds.

Feed costs account for about 60-75% of broiler production costs. This fact suggests that an increase in profit is most easily attainable through feed cost reduction. These cost

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savings require knowledge of digestive tract characteristics of poultry, nutrient requirements, digestion and nutrient utilisation at certain growth and development stages *i.e.* fattening stages. In recent years, the goal of broiler industry has been to obtain faster broiler growth in minimal time (Longo *et al.*, 2007). In order to achieve this improvement, broilers should be fed diets that can meet all their nutrient requirements.

Recent research has shown that physiological changes occur in the digestive tract during the first week of life in broilers. This period is followed by an increase in the secretion and activity of digestive enzymes, leading to greater nutrient breakdown and availability. In the starter period, rapid feed passage rate and deficiencies in innate enzyme availability can result in a lower digestibility of proteins (Uni *et al.*, 1999), which are required in high amounts in young growing birds. In order to eliminate these problems, some authors (Garcia *et al.*, 2006) have recommended providing a good-quality pre-starter diet to compensate for the initially immature digestive system, and give the young birds access to more digestible ingredients and a higher concentration of nutrients.

Normal growth and development of broilers necessitates the optimisation of a range of factors. One of the most important nutritional prerequisites for optimum performance is to ensure adequate dietary protein levels (Bregendahl *et al.*, 2002), in light of the fact that proteins have numerous functions in the living organism. However, some studies have evaluated practices that allow a reduction in dietary protein levels, since this would not only reduce nitrogen excretion into the environment (Aletor *et al.*, 2000; Bregendahl *et al.*, 2002) but would also reduce diet costs, since protein, and its constituent amino acids, are relatively expensive.

In order to attain optimum animal performance, diets should be supplemented with proteins in order to ensure both an adequate level of total proteins and an optimum ratio of amino acids that ensure the ideal amino acid profile for the development stages of the birds. In general, most protein ingredients supplied to animals are obtained from vegetable proteins, with oil crops and legumes being their major sources. Vegetable proteins are inadequate for poultry, not only in terms of quantity but also in terms of quality, *e.g.* amino acid profile. Moreover, their nutrient composition shows frequent variations in both crude protein level and amino acid composition (Thorpe and Beal, 2001).

Young broilers require relatively high dietary protein for optimum growth. Soybean meal is a highly digestible protein feed with a relatively high protein content and a well balanced amino acid profile. However, its nutritional value is often decreased by the presence of anti-nutritional factors (ANFs) such as trypsin inhibitors, lectins, and non-starch polysaccharides, which can mean that soybean meal is incompletely digested by poultry (Marsman *et al.*, 1997; Pack and Bedford, 1997). Huo *et al.* (2001) and Palacios *et al.* (2004) reported that soybean meal (SBM) contains a high level of a few heat-stable anti-nutritional factors, such as antigenic proteins, flatulence factors and lectin, which can decrease the fattening performance and health status of broilers. Many efforts have been made to increase the nutritional value of soybean meal through protease and carbohydrase supplementation before or after processing (Ghazi *et al.*, 1997; Rostango *et al.*, 2000). Corn as a commonly used, high energy feed material, on the other hand, has a relatively low protein levels (8.5 to 8.8%), but due to its high percentage in poultry diets, it can provides a significant protein in the diet. Although considered highly digestible, conventional corn-soybean meal diets for broilers can contain diverse protein complexes that are not easily digested by young chicks due to the rapid passage of feed through the digestive tract and the deficiency of innate enzymes in early stages of life (Uni *et al.*, 1999).

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Given the above, the objective of this review is to stress the importance of enzymes, primarily protease, in broiler diet.

Enzymes

Enzymes are defined as catalysts of biochemical processes that initiate and control the rate of biological reactions by which substrates are transformed into products. They are highly specific in terms of the type of substrate and the range of temperature and pH over which they function.

Since the first enzyme (urease) was isolated and purified by Sumner in 1926, enzymes have been extensively used in various industries. Enzymes have been used as feed supplements for more than 50 years, but the rapid growth in their use has only been within the last 10 years (Tuoying, 2005). Almost all exogenous enzymes used in animal diets are either from fungal or from bacterial sources. When enzymes are added to diets, they must be active under physiological conditions prevailing in the animal's digestive tract in order to realise their functions, and they must also compliment the characteristics of dietary ingredients and additives included in the feed. Furthermore, enzyme activity in poultry diets must be sufficiently high to compensate for the relatively short transit time of digesta through the tract. Enzyme activity is significantly affected by the pH of the digesta (Tuoying, 2005), and Baas and Thacker (1996) and Ademark *et al.* (2001) reported that both fungal and bacterial enzyme preparations typically exhibit optimum activity at pH 4.0-5.0.

Exogenous enzymes, in combination with endogenous secretions, degrade complex compounds to the size of particles that can be easily used by animals. Therefore, exogenous enzymes selected for in-feed use should be complementary to the natural enzymes produced by animals. Secondly, enzymes must prevent an increase in digesta viscosity that would reduce absorption. Young animals, poultry in particular, are highly sensitive to changing digesta viscosity.

The possibility of using exogenous enzymes in non-ruminant diets has provided nutritionists with a very important tool to improve feed digestibility, reduce environmental pollution and lower feed costs, thus allowing more flexibility in diet formulation (Zakaria *et al.*, 2010).

In the past, research has mostly focused on improving the energy value of corn/soybean-based diets and enhancing the utilisation of phytin phosphorus from plant-based diets, with a much less attention being paid to proteins and amino acids.

Although the efficacy of carbohydrases, proteases, amylases, xylanases, lipases, cellulases and phytases in poultry diets has been well established, there is still a large amount of uncertainty regarding the modes of action of exogenous enzymes. Furthermore, the countless interactions between enzymes and the host animal, its microflora, and the digestion of ingredients are not yet fully understood (Bedford, 2002).

Thorpe and Beal (2001) reported that differences in the anatomy and physiology of the digestive tract most likely affect exogenous enzyme activity. Partridge (1993) and Dierick and Decuypere (1994) observed the following differences between pigs and poultry:

1. Anatomical differences - in poultry, feed passes into the crop, where any added enzyme can act for several hours at a pH of about 6.0 before passing into the acid environment of the gizzard, whereas in pigs, feed passes directly into the acid environment of the stomach immediately after ingestion.
2. Digestive capacity - poultry have a shorter small intestines and thus reduced time for enzyme inactivation by the microflora, a shorter retention time in the small intestine

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(1-2 h in poultry *vs.* 4-5 h in pigs) and a lower water content in the upper part of the gastrointestinal tract.
3. Bacterial activity - the importance of the microflora in the gut of poultry is much lower than in pigs.
4. Fibre digestion is lower in poultry than in pigs, due to their widely different gut capacities.

The nutritional value of several major feed ingredients can be improved by exogenous enzyme supplementation. Major benefits of using dietary enzymes for animals include:
- Higher feed utilisation (better FCR), faster animal growth, improved health, and reduced pollution via litter and faecal material (Bedford, 2000);
- Improved nutrient digestibility (Sun, 2007); however, the mechanism used by enzymes to ensure these improvements is insufficiently elucidated, but changes may occur in gastrointestinal activity, absorption, secretion and immune response or through an increase in the production of total digestive enzymes;
- Decreased viscosity of intestinal digesta, reduced incidence of sticky excreta, and improved laying performance (Broz and *Ward*, 2007);
- Reduced moisture content of the faeces, to keep the litter dry (Alam *et al.*, 2003);
- Reduced loss of non-digestible nutrients to the environment – reduced pollution, particularly in aquaculture (Officer, 2000);
- Improved flock uniformity (Olukosi *et al.*, 2007);
- An 'environmentally responsible' alternative to some growth promotion hormones and antibiotics (Officer, 2000);
- Simple to use and safe for human health (Bedford and Apajalahti, 2001; Alam *et al.*, 2003);
- Higher profits and a quality of poultry products (Cowieson, 2005).

Enzyme effects

Enzymes produce different effects on different feedstuffs. Knowledge of the nutritional value of feed ingredients with the addition of exogenous enzymes is essential for establishing their nutritional matrix. In a corn/soybean meal-based diet, although it is difficult to determine whether the response was due to the effect of enzymes on the corn or on the soybean meal, the positive effect was most likely the result of the beneficial effects on both ingredients.

The responsiveness of corn-based diets to exogenous enzymes has received considerable attention in recent years due to rising ingredient prices. Biofuel production will likely induce a rapid continuation of the consumption of nutritionally important starch sources, such as corn and wheat. With increasing prices of starch and fat sources, the cost-effectiveness of these nutrient sources in animal fattening programs has become increasingly difficult (Bedford, 2000; Choct, 2006).

The price of traditional sources of starch and fat, such as corn, wheat and vegetable oils, has increased to the point where the profitability of poultry production is seriously at risk. Therefore, economic potential has been bestowed upon supplemental ingredients such as enzymes that offer a higher energy value to the diet via sparing mechanisms or digestibility enhancements. These are included in least cost formulation programs through the use of matrix values for energy, amino acids, calcium and phosphorus. The effect of increasing the dose rate of an enzyme product will be dependent on the relative sensitivity of the system, this being influenced by a multitude of interacting

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factors, including the susceptibility and concentration of substrate, the age of the host animal and the balance of nutrients in the diet (Cowieson and Ravindran, 2008).

Enzymes as feed supplements are used most commonly in broiler production, which has been attributed to the following (Annison, 1997):

1. Broilers are sensitive to digestive upsets, which can be overcome by specific feed enzymes.
2. Enzyme activities have enabled nutritionists to use feedstuffs that contain components that may reduce animal performance or those that lack nutritional value without enzymes.
3. Research into the effect of enzymes in broilers is inexpensive compared to larger animals.

Favourable costs of research in broilers suggests that observations can be based on scientific principles in terms of statistics, chemistry and physiology. Moreover, experiments with broilers yield rapid results, which in turn increase the rate of new product development. Many experiments with broilers have provided results regarding the effect of enzymes on chickens from one to 21 days of age, or over the entire fattening period of 35 to 49 days.

Enzyme supplementation methods

Nutritionists use two methods of enzyme supplementation (Pack and Bedford, 1997; Barbosa *et al.*, 2008): enzyme addition to reduced nutrient specification diets or enzyme supplementation in standard rations, if economically justified. A further alternative is to change the diet formulation by reducing nutrient levels and supplementing exogenous enzymes to provide identical nutritional values ('matrix values') to the diet without adversely affecting bird performance.

Exogenous enzymes used in the diets of young chicks can be very beneficial in improving nutrient digestibility in at least two ways (Olukosi *et al.*, 2007):

By supplying enzymes that the chick cannot produce in sufficient quantity by itself, which results in enhanced catalytic and hydrolytic activities and an increased rate of digestion, absorption and metabolism of complex organic feed nutrients (Douglas *et al.*, 2000; Engberg *et al.*, 2002),

Although the chick can produce sufficient amounts of an enzyme by itself, exogenous enzymes may reduce the requirement for endogenous enzymes, thus making more nutrients and energy available for growth.

Modes of enzyme action

Different authors have reported that different enzyme combinations have different effects, including antagonistic (Naveed *et al.*, 1999; Saleh *et al.*, 2004), sub-additive (Wu *et al.*, 2004b; Leslie *et al.*, 2005), additive (Zyla *et al.*, 1996; Mulyantini *et al.*, 2005) and synergistic effects (Ravindran *et al.*, 1999). Cowieson and Adeola (2005) highlighted the key importance of obtaining information and drawing conclusions regarding the effect of each enzyme combination on performance parameters, and Aćamović (2001) observed an obvious dependence of the positive effects of these additives on the amount and quality of feed ingredients, energy level, type of enzyme, and fattening conditions.

Numerous researchers have not detected any positive effects of dietary enzyme supplementation for broilers (Iji *et al.*, 2003; Günal *et al.*, 2004; Sayyazadeh *et al.*,

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2006), whereas other investigators, such as Brake *et al.* (2003) and Wu *et al.* (2004a), apart from reporting the positive effects of enzyme supplementation on profitability, documented that there were possible negative maternal effects on the progeny when enzyme cocktails were fed to the parent flock. In this case, the inclusion of a mixture containing phytase, protease and carbohydrase reduced broiler progeny body weight, as a consequence of a reduction in feed consumption.

Most studies have shown a certain effect of enzymes on digestible energy (Douglas *et al.*, 2000; Kocher *et al.*, 2001; Olukosi *et al.*, 2007), whereas Zhou *et al.* (2009) reported that enzyme supplementation in broiler chicken diets can improve the efficiency of energy utilisation, particularly in diets with lower levels of metabolisable energy.

The supplementation of corn/soybean meal-based broiler diets with commercial enzymes typically results in a significant increase in body weight gain and FCR (Zanella *et al.*, 1999; Brufau *et al.*, 2002). The increase is due to the ileal digestibility of crude proteins (Simbaya *et al.*, 1996), starch and fat (Zanella *et al.*, 1999), as well as to an improvement in ileal non-starch polysaccharide (NSP) digestibility (Cowieson and Adeola, 2005), with magnitude of response depending on enzyme activity and dosage (Kocher *et al.*, 2001).

Use of enzymes in broiler starter and finisher diets

Cmiljanić *et al.* (2007) observed positive results of enzyme supplementation in young chicks in particular, as opposed to less pronounced positive effects in old chicks.

Zhao and Wang (2008) reported that an adequately formulated corn/soybean meal-based diet supplemented with an enzyme complex improved performances and nutrient utilisation in broilers to a certain degree. Exogenous enzymes, such as protease and amylase, primarily affect growth and nutrient utilisation through digestion in young broilers in the starter phase, but this is much reduced in the grower phase. This may be due to the fact that the underdeveloped intestines of young broilers cannot produce sufficient amounts of digestive secretions, making an exogenous supplement necessary to overcome the deficiency. In the grower phase, however, the mature digestive tract of broiler can produce enough endogenous enzymes, and an exogenous supply (through feed) is no longer needed. A diet containing more low-nutrition ingredients (such as wheat bran and rapeseed meal) may increase the necessity for multiple enzymes containing non-structured polysaccharide enzymes; but an overdose of these enzymes should be avoided since the content of these polysaccharides in the corn-soybean-meat by-product diet is relatively low compared with that found in wheat and barley-based diets.

Assuming that digestive enzyme production and secretion are limited after hatching, Mahagna *et al.* (1995) used amylase and protease of bacterial origin as a supplement in a starter diet fed to broilers aged up to 14 days and studied their effect on performance, development of the gastrointestinal tract, digestive enzyme activities and FCR. In order to verify these effects, the enzymes were eliminated from the feed at the age of 14 days, and the same variables were determined at 42 days of age. The authors suggested that enzyme supplementation had no significant effect on growth performance and feed intake over the trial period (1-14 days), but differences were observed in FCR from 1 to 7 days of age. The difference was significant for enzyme levels of 250 g/t as compared to the control group, but not for supplementary level of 1,000 g/t. After day seven, no differences in these parameters were observed. At 42 days of age, the broilers in all experimental groups had a similar body weight, feed intake and, accordingly, FCR.

Conversely, Hübner *et al.* (2002); Alam *et al.* (2003) and Józefiak *et al.* (2004)

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achieved better body weight gain in broilers receiving enzyme-supplemented diets than in broilers fed the control diet in the finisher phase.

PROTEASE

Protease supplementation was reported in the early 1950s and 1960s. Burnett (1966) reported beneficial effects of amylase and protease supplementation on animal performance, whereas Jensen *et al.* (1957) observed no improvement in poultry performance after protease supplementation of cereal crop-based diets.

Numerous potential modes of action have been proposed to explain favourable effects of proteases in poultry diets. The addition of protease along with endogenous peptidase secretions reduces the need for amino acids and energy to be supplied in the diet. Moreover, protease can improve protein hydrolysis in the presence of anti-nutritional factors such as lectins or trypsin inhibitors (Huo *et al.*, 1993; Ghazi *et al.*, 2002), by increasing the efficiency of amino acid utilisation in poultry. Douglas *et al.* (2000) showed that the supplementation of a corn/soybean diet with commercial carbohydrase and protease improved the energy value of feed.

The inclusion of protease in a multi-enzyme complex proved beneficial for broilers (Ranade and Rajmane, 1992; Morgan and Bedford, 1995; Simbaya *et al.*, 1996) and had a positive effect on egg production in laying hens (Adams, 1989). However, not all experiments showed improved performance in animals fed protease-supplemented diets; for example, Officer (1995) reported no improvement in pig performance after supplementation with a multi-enzyme complex containing two proteases.

The addition of protease to laying hen diets has had a number of beneficial effects, including: increased nutrient digestibility and feed conversion efficiency, improved eggshell thickness, and lower production costs. Yadav and Sah (2006) used these results to suggest that dietary crude protein content could be reduced by 1%, provided protease was added to the diet, with an optimum level in laying hens being 0.1% supplementation.

Effect of protease used in combination with other enzymes on the productive performance of broilers

Ebert *et al.* (2000), García *et al.* (2000) and Fischer *et al.* (2002) found that the multi-enzyme complex Vegpro (protease, amylase, cellulase, α-galactosidase and xylanase) did not improve broiler performance and nutrient digestibility. These studies involved broilers fed corn/soybean-based diets, kept under different rearing conditions. However, different conditions (*e.g.* housing) are known to affect broiler response to enzymes (notably, the contact with microorganisms from the litter and the stress status under farm conditions). Madrid *et al.* (2010) observed no significant improvement in the performance of broilers fed a wheat/soybean-based diet supplemented with the Vegpro multi-enzyme complex under ideal rearing conditions. However, ileal nutrient digestibility was improved by multi-enzyme complex supplementation in 42 day-old broilers reared on used litter or on commercial farms. Therefore, the effect of the multi-enzyme complex on nutrient digestibility was found to be better under poorer rearing conditions.

Perić *et al.* (2008) studied the effect of an enzyme complex (protease, amylase, β-glucanase, xylanase, pectinase, cellulase and phytase) in diets with different nutritional value on broiler performance. Enzyme supplementation had a positive effect on body weight gain and FCR, regardless of whether the enzyme was added to the nutritionally adequate diet or to a diet having a reduced nutrient value (*i.e.* 0.3 MJ energy, 0.1%

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available phosphorus and 0.1% calcium less than in the control diet). The enzyme supplement had no effect on broiler mortality in the experiment. The performance index showed the highest performance in the full specification, positive control group, whereas those receiving diets with reduced energy, calcium and phosphorus plus an enzyme supplement gave results comparable to those in the positive control. In economic terms, the feed price expressed per kg of live broiler weight was lower in broilers fed enzyme-supplemented diets than in either the positive or negative control groups.

Ghazi *et al.* (2002), Gracia *et al.* (2003) and Onderci *et al.* (2006) reported an increase in feed intake with protease and amylase supplementation, as opposed to Cowieson and Ravindran (2008) who found improved digestibility of corn/soybean diet in broilers at a similar feed intake rate compared to a control group. Nevertheless, the increased feed intake seems to be justified, with improved feed conversion and a higher body weight gain obtained with the highest enzyme doses, suggesting that the enzyme did not induce any dietary imbalances in terms of nutrient restriction. These results have been confirmed by other authors (Zanella *et al.*, 1999; Douglas *et al.*, 2000), who reported improvements in the ileal digestibility of nitrogen, amino acids and energy as the result of the use of a xylanase/amylase/protease cocktail.

The supplementation of broiler diets with protease and amylase in combination induced significant improvements in fattening performance (Café *et al.*, 2002; Odetallah *et al.*, 2005). In their study on broiler sensitivity to two different doses of a xylanase/amylase/protease cocktail, Cowieson and Ravindran (2008) found that the xylanase/amylase/protease combination improved the nutrient value of corn/soybean meal-based diet in a dose-dependent manner. Dose sensitivity may be associated with the level and quality of ingredients, as well as with the enzyme combination in the cocktail. Higher cocktail doses gave the highest improvement in weight gain, FCR, ileal nitrogen digestibility and dry matter retention, but this is not necessarily the best economic choice. The decision on an enzyme dose should be based on the economic optimisation of factors that take into account ingredient prices and enzyme effect on energy utilisation and nutrient digestibility.

The results by Yadav and Sah (2005) showed that crude protein reduction increased the feed intake of broilers aged 28 and 42 days. Generally, in both periods, feed consumption was reduced in broilers fed a reduced crude protein plus protease diet. The increased feed intake in broilers fed reduced crude protein diets can be due to their tendency to consume more feed in order to ingest a required level from a low-protein diet. Conversely, the reduced intake of protease-supplemented low-protein feed may be due to exogenous protease which most likely induced a significant increase in protein digestibility. The results show that feed conversion efficiency was depressed when decreasing protein levels were fed, and slightly improved with protease supplementation. Depression in feed conversion in broilers fed diets with reduced protein levels can be attributed to decreased weight gain and increased feed intake. The low improvement in the conversion of the reduced crude protein feed supplemented with protease can be the result of a higher weight gain and a low feed intake.

Broilers fed diets supplemented with amylase, lipase and protease mostly consumed lower amounts of feed as compared to broilers receiving control diets (Espino *et al.*, 2000). Supplementing diets with an enzyme complex (cellulase, amylase and protease) in Hubbard broilers (Rahman *et al.*, 2009) showed similar mean values for body weight gain ($P > 0.05$) across all four groups of broilers that consumed either the control diet or experimental enzyme-supplemented diets, but no changes ($P > 0.05$) were found in dry matter consumption and feed conversion in experimental broilers. These authors suggested that the enzyme complex had no beneficial effect when included in the

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finisher diet on the productive performance of broilers. Broilers receiving a soybean meal diet supplemented with protease had higher body weights, due to nitrogen retention, and higher metabolic energy, with no changes in feed conversion being observed (Ghazi *et al.*, 1997). In another experiment involving Ross broilers fed corn/soybean meal-based diets, Ouhida *et al.* (2002) studied the effect of mannase and protease supplementation on productive performance and nutrient digestibility. Results showed no effect of these enzymes on body weight, feed intake, feed conversion, and crude protein, fat and organic matter digestibility.

Effects of protease used in combination with other enzymes

Rostagno *et al.* (2000) reported a 3% improvement in the actual digestibility of crude protein from a corn/soybean meal-based diet supplemented with protease, amylase and cellulase, whereas Rodrigues (2000) achieved a 1.9% improvement in the protein, starch and energy digestibility coefficient for corn/soybean-based diets supplemented with a combination of protease, xylanase and amylase enzymes. In broilers, protease supplementation of a diet formulated with lupins (Rubio *et al.*, 2003) or soybean (Ghazi *et al.*, 2002; 2003; Marsman *et al.*, 1997) led to improved protein digestibility.

Yadav and Sah (2005) showed a 1% reduction in dietary crude protein levels significantly decreased the protein digestibility in broiler starter and finisher diets, whereas protease supplementation of the same reduced specification diet consistently and significantly improved protein digestibility in both starter and finisher diets. Crude fibre digestibility was significantly improved at 0.1% and 0.075% protease inclusion in broiler starter diets and 0.1% protease addition in broiler finisher diets.

Ghazi *et al.* (2002, 2003) reported that the nutritional value of SBM was improved by protease supplementation in broiler diets. Some authors, such as O'Doherty and Forde (1999), observed that protease and α-galactosidase had no effect on protein and energy digestibility, but led to feed conversion improvements. Others, like Szczurek *et al.* (2001) found no effect of protease on protein and energy digestibility.

Barbosa *et al.* (2008) studied the effect of combined enzymes (phytase, amylase, protease and xylanase) in a corn/soybean meal-based broiler diet having reduced nutrient levels on the performance and ileal digestibility of nutrients (proteins, calcium and phosphorus) and energy, and found improvements in protein, calcium and phosphorus utilisation, and suggested that broiler performance was maintained due to better nutrient utilisation. However, the performance of these broilers differed from birds fed the recommended diet without enzyme supplementation. It has been suggested that protease can increase the solubilisation of soybean meal proteins, by reducing the effect of trypsin inhibitors (Caine *et al.*, 1998).

In order to define a nutritional matrix value for corn and soybean meal when exogenous enzymes are used, Dourado *et al.* (2009) observed that the addition of phytase, xylanase, amylase and protease was efficient in increasing corn metabolisable energy, however no increase in metabolisable energy of soybean meal was evidenced with the addition of the test enzymes.

Ghazi *et al.* (2002) found that the use of different protease sources can increase true metabolisable energy (TME) and true nitrogen digestibility in broilers, although there were large differences in bird responses between protease sources.

Broilers receiving a low energy diet, supplemented with an enzyme cocktail containing amylase, protease and xylanase, achieved the same growth performance as those fed a basal corn-soybean diet, and showed a 2.9% improvement in protein digestibility. This

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showed that protease supplementation can increase protein utilisation efficiency and, accordingly, reduce dietary protein requirements (Zanela *et al.*, 1999).

Yu *et al.* (2007) performed experiments with low-protein low-amino acid diets as a control/basal diet or supplemented with an enzyme cocktail or a single protease enzyme, which had previously shown in vitro protein hydrolysis. Enzyme supplementation increased feed intake and reduced feed intake for the low-protein diet compared to the negative control. Enzyme supplementation resulted in increased body weight; but the addition of protease alone improved the FCR of broilers as compared to both diets with or without the enzyme cocktail. Logically, the inclusion of protease showed better hydrolysis of proteins from soybean meal under *in vivo* conditions, since broiler performance also improved. Therefore, supplementing a broiler diet with specific enzymes that demonstrate in vitro soybean meal hydrolysis can improve productive performance in broilers and enable formulation of low-protein diets, resulting in reduced nitrogen excretion.

Yadav and Sah (2005) investigated the effect of protease supplementation of corn/soybean-based diets on broiler performance (weight gain, feed consumption, nutrient digestibility and production economics). The results showed that crude protein reduction decreased the weight gain of broilers at 28 days of age. Protease supplementation at 0.075 and 0.1 % in a reduced crude protein diet increased body weight gain in broilers at 28 and 42 days of age. The results showed that protease inclusion improved body weight gain only at 0.075% levels, whereas the higher protein level had no effect. The improvement in body weight gain of broilers with protease supplementation of diets can be attributed to increased nutrient digestibility.

Effect of protease used in combination with other enzymes on dressing percentage

Espino *et al.* (2000) observed a slight increase (non significant) in the dressing percentage of broilers fed diets containing protease, amylase and lipase. An improvement (non significant) in the dressing percentage of broilers fed enzyme-supplemented diets has been reported by Richter *et al.* (1991) and Osei and Oduro (2000), as opposed to Hartman (1996) who observed a significant increase in the dressing percentage of broilers fed wheat-based diets supplemented with commercial enzymes. This difference in dressing percentage may be due to different diets, and different types and levels of enzymes used, but from the published papers to date, it seems there is no significant enzyme effect on this parameter.

Effect of protease on the productive performance of broilers, nutrient digestibility, meat quality and feed costs, with special reference to Ronzyme ProAct

Recently, an increasing number of researchers have focused on analysing the effect of protease alone on broiler productive performance and nutrient digestibility. Two experiments (Frietas *et al.*, 2011) were conducted to determine the effect of adding exogenous protease to broiler diets. In the first experiment, the dietary treatments included a positive control and a negative control (formulated with a 4.4% reduction in ME and protein) compared to diets containing a mono-component protease (75,000 protease/g), which was added to the negative control diets at 0, 100, 200, 400, 800 and 1,600 ppm of feed. Protease supplementation had no effect on body weight gain, but feed

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conversion was improved. No effect was observed for feed consumption, mortality or carcass traits. In the second experiment, the effect of two protein levels and energy levels in diets with two protease levels (0 and 200ppm) was examined. No interactions between the three factors were observed for life performance measures. Protease supplementation improved feed conversion and the digestibility of fat and crude protein ($p \leq 0.01$), regardless of dietary protein and energy levels.

Angel *et al.* (2011) used six different diets: a control diet and five experimental groups (reduced protein level with protease supplementation at 0, 100, 200, 400 and 800 mg/kg of feed). At 22 days of age, broilers fed the control diet had the highest weight gain as compared to the other experimental groups, and a similar feed intake, which resulted in the highest FCR. Birds receiving protease supplementation showed similar body weight gain to the control birds. FCR was highest with diets supplemented with protease at 400 mg, followed by 800 and 200 mg, and lowest in broilers fed low-protein diets without protease or with 100 mg protease. It was calculated that protease addition increased crude protein digestibility by 6.1%, and improved amino acid digestibility.

Fidelis *et al.* (2010) evaluated the effect of protease (Ronozyme ProAct) in broilers *via two* experiments. In the first experiment, control-group broilers received no enzyme supplement, and positive control group were fed the same diet + 200 ppm/kg of protease in feed. In the first 21 days, enzyme addition had a significant effect on body weight gain and FCR, particularly in male broilers. In the grower phase, the effects of enzyme supplementation on body weight gain and FCR were not statistically significant. Protease induced a significant increase in protein digestibility, total fat digestibility and digestible energy. The second experiment involved two protein levels, with or without protease supplementation. In the starter phase, enzyme supplementation had no significant effect on body weight gain regardless of the dietary protein level, although broilers fed enzyme-supplemented diets consumed less feed than those receiving diets without enzyme supplementation. In the grower phase, the enzyme had no significant effect on body weight gain.

In a trial investigating different levels of protein and ME in broiler diets supplemented with protease (Ronozyme ProAct, 0 and 200ppm/kg feed), Favero *et al.* (2009) found that ileal digestibility of crude protein, fat and gross digestibility energy increased by approximately 5% when the treatment with the highest crude protein and energy level was compared to diets with and without protease supplementation. The study suggested that protease supplementation significantly improved the performance of broilers reared up to 42 days of age, regardless of dietary protein and energy levels, with significant ($p \leq 0.01$) improvements in FCR as compared to broilers fed diets without protease.

Rosa *et al.* (2009) reported that body weight, weight gain and feed intake in broilers fed reduced protein and amino acid (-6%) diets supplemented with protease (Ronozyme ProAct, 200ppm/kg feed) were similar to those of broilers fed a normal crude protein level (21.50%).

Maiorka *et al.* (2009) observed no significant differences in body weight between control broilers (*without* protease) and broilers fed enzyme (200 ppm/kg feed-Ronozyme ProAct) supplemented diets. Differences in feed intake were observed during the grower phase. FCR was significantly ($P<0.05$) better in broilers fed the enzyme-supplemented diet than in the control group, as well as over the whole fattening period. Enzyme addition improved ($P<0.05$) the digestibility of fat, energy, crude protein and some amino acids (Met, Cys, Val and Ala) by 8% or more.

In a feeding trial on broilers using diet supplemented with 0, 100, 200, 400, 800 and 1,600 g protease/ton (Ronozyme ®ProAct), Vieira *et al.* (2009) observed no significant effect on body weight gain, but detected a linear improvement in feed conversion ($p \leq 0.01$) with increasing enzyme dose. The ileal digestibility of methionine and

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histidine showed a trend for improvement ($p \leq 0.10$), with enzyme supplementation, regardless of the enzyme level. Under in vitro conditions, Fischer *et al.* (2009) determined a strong ability of protease (Ronozyme ® ProAct) to increase protein solubilisation and protein hydrolysis for different ingredients, including meat and bone meal, corn, sorghum, feather meal, full-fat soybean meal and defatted soybean meal.

Carvalho *et al.* (2009a) reported that 200 ppm of protease (Ronozyme®ProAct) effectively increased the average utilisation of amino acids in a commercial meat-and-bone meal by 6.3%.

Supplementation of protease significantly ($P<0.05$) improved for certain amino acids, resulting in an increase for the digestibility of total amino acids overall. The use of this protease resulted in an average 3.2% improvement of the corn amino acid metabolism during the broiler starter period (Carvalho *et al.*, 2009b).

Bertechini *et al.* (2009) found significant ($P<0.05$) increases in the digestibility of most amino acids from a soybean meal when feeding 200 ppm protease (Ronozyme® ProAct). The authors concluded that the use of this particular protease resulted in an average 5.3% improvement in the utilisation of total amino acids of soybean meal.

Dietary protease inclusion at 0.075% significantly improved average weight gain in broilers at 28 days of age. The cumulative feed consumption was significantly lower with 0.05% protease supplementation in broiler finisher diets. The increase in protease levels in the reduced protein diet had no significant effect on the dressing percentage of broilers (Yadav and Sah, 2005). In this study, these authors also analysed the effect of protease supplementation on production costs. The highest increase in income over feed costs was derived from broilers fed diets supplemented with 0.075% protease. Protease supplementation of reduced crude protein diets increased profitability as compared to the basal diet.

Conclusions

Enzyme supplementation of poultry diets is nutritionally, economically and environmentally justified. Numerous research projects carried out so far have shown no adverse effects of enzyme supplementation of broiler diets on body weight, feed intake, FCR, nutrient digestibility, meat quality and production costs. However, there is still a large amount of uncertainty regarding the use of enzymes. Future research should be conducted to establish efficient cocktail-enzyme complex formulations, improve existing nutritional matrices for precise measurement of the activity of individual enzymes and enzyme combinations, and identify enzyme combinations that can reduce or even eliminate the negative effects of certain anti-nutritional factors occurring in animal feed. Moreover, focus should be placed on improving the use of enzymes in poultry production in an environmentally friendly way in order to help reduce pollution.

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