

ANIMAL SCIENCE

A review on role of exogenous enzyme supplementation in poultry production

Khusheeba Munir^{1*} and Sajid Maqsood²

¹*Division of Veterinary Medicine, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Science and Technology-Kashmir, J&K, 190012, India*

²*Department of Food Science, Faculty of Food and Agriculture, United Arab Emirates University, Al-Ain, 17551, United Arab Emirates*

Abstract

Monogastric animals like poultry, pigs, etc. lack the alloenzymes from rumen microflora and thus it become necessary to incorporate the enzymes in their diets in order to derive optimal nutrient utilization from complex feed matrix. Feed enzymes are added to animal feed to increase the availability of nutrient by digesting the feed components during storage or after consumption within the gastrointestinal tract. Some of the enzymes that have been used over the past several years and have potential for use in the feed industry include cellulase (β -glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases. Most of the enzymes used in the feed industry have been applied for poultry to neutralize the effects of the viscous, non-starch polysaccharides in cereals such as barley, wheat, rye, and triticale. Recently, considerable interest has been shown in the use of phytase as a feed additive. The addition of phytase to feed improves phosphorus utilization in both ruminant and monogastric animals, reducing the need for supplemental inorganic phosphate and helps in reducing the environmental problems that arise from organic phosphate excretion. Thus, enzyme supplementation in the feed play an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components. This review aims to elucidate the different enzymes being used in the poultry industry and their efficiency in increasing the poultry production.

Key words: Enzyme, Poultry nutrition, Growth, Phytase, Phosphate excretion

Introduction

The application of feed enzymes to poultry diets for the enhancement of nutrient availability had been reported since 1926. Previously, the research conducted on feed enzymes in poultry nutrition focused on non-starch polysaccharide (NSP) degrading enzymes, especially xylanase and β -glucanase, in diets containing wheat, rye and barley (Choct, 2006). The use of unconventional feedstuff for poultry production is however limited due to their fibrousness and inability of birds to possess the cellulase enzyme that can digest the fibre (Adebisi et al., 2010). Exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains,

including wheat and barley (Bedford and Schulze, 1998), initiated this practice. Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed-linked β -glucans and arabinoxylans (Parsippany, 2008). Depolymerisation of these NSPs requires specific enzymes, these enzymes are specific to the main and side chain structure of the NSP. The first development and use of NSP-degrading enzymes was applied in barley and then in wheat based broiler diets. Researchers found that the use of NSP-degrading enzymes in barley and wheat based broiler diets improved litter quality and performance. The use of NSP-degrading enzymes in wheat and barley based diets for broilers are therefore well established and accepted (Bedford, 2009).

The animal rearing practice depends on the application of feeds that are uniform in quality and have high nutritive value. The complex plant materials that are commonly used as feed ingredients, such as coarsely processed grains and high-fiber feedstuffs (e.g., cereal grains, forages,

Received 02 December 2011; Revised 09 July 2012; Accepted 21 August 2012; Published Online 24 November 2012

*Corresponding Author

Khusheeba Munir
Division of Veterinary Medicine, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Science and Technology-Kashmir, J&K, 190012, India

Email: khusheeba@gmail.com

and crop residues) have nutritive components that are resistant to endogenously-produced digestive enzymes. Some feed components also have anti-nutritive effects, for example phytate, which reduces bioavailability of certain minerals, and oligosaccharides and other soluble carbohydrates that increase viscosity and reduce nutrient absorption. Hence, obtaining the maximal nutritive value from such complex feedstuffs typically requires supplementation of autoenzymatic activity with alloenzymatic activity (i.e., exogenously produced digestive enzymes from non-host sources) (Klasing, 1998). Feed enzymes are typically added to animal feed to increase availability of nutrient by acting on feed components prior to or after consumption, i.e., within the gastrointestinal tract (Pariza and Cook, 2010). The effect of dietary enzyme on the animal is influenced by the type and concentration of the undesirable carbohydrate present in the feedstuff and the class and age of the livestock and poultry that consume it. Young chicks are affected to a greater degree by anti-nutritional compounds than older birds (Marquardt et al., 1996). Enzymes that appear to be beneficial for non-ruminant animals are the xylanases, or more specifically the endoxylanases for the feed which contain wheat, triticale and rye and the β -glucanases or cellulases for those which contain barley and oats (Marquardt et al., 1996). Most commercial enzymes contain a spectrum of different enzymes including xylanases and β -glucanases and therefore can be used effectively with the above cereals. It is, nevertheless, essential to ensure that the enzyme preparation has the appropriate activities of the specific enzymes that are required. Phytase, in addition to the above mentioned enzymes, is an enzyme, which increases the availability of phosphorus from phytate, a bound form of phosphate found in cereals and other plant material (Marquardt et al., 1996). It has become available for use in the feed industry and may assist in reducing phosphorus requirements in non-ruminant animals and therefore it can solve problems associated with environmental pollution. During the past decade, the inclusion of microbial phytase in poultry diets has increased drastically with proved success, mainly in response to heightened concerns over phosphorus pollution of the environment.

While supplementing the poultry feed with enzymes, it is important to consider several factors which are as follows (Marquardt et al., 1996):

1. The enzyme supplement must contain the proper spectrum of enzymes, so that anti-

nutritive effects of target substrate will be neutralized.

2. Different cereals contain different amounts of the enzyme-sensitive anti-nutritional factor. Therefore, the response to enzyme treatment may vary within a given cereal (i.e. barley and probably wheat).
3. Outcomes of the enzyme supplementation are affected by grade and age of poultry. The responses in swine are usually less dramatic than those of poultry and have not been clearly established.
4. The activity of the enzymes must not be affected by processing or by the low pH (<4) or digestive enzymes in the gastrointestinal tract.

Ruminant animals (e.g., cattle and sheep) have the advantage of alloenzymatic digestion provided by rumen microflora, which enables ruminants to obtain nutrients from complex feed matrices that are not made available through autoenzymatic digestion (Pariza and Cook, 2010). Pigs, poultry and other monogastric animals lack the alloenzymes from rumen microflora, so for these species to derive optimal nutrient benefit from complex feed matrices, it is necessary to provide added enzyme supplementation. Enzymes may act during feed processing, while feed is present in storage and feeder bins, and also following the ingestion by acting within the digestive system itself (Pariza and Cook, 2010). Moreover, the addition of enzyme like phytase has also been known to improve phosphorus utilization in both ruminant (Kincaid et al., 2005; Knowlton et al., 2007) and monogastric animals (Nnenna et al., 2006), thus, reducing the need for supplemental inorganic phosphate and the environmental problems that arise from organic phosphate excretion. Therefore, the main objective of exogenous enzyme supplementations is to improve feed efficiency for monogastric animals like poultry.

Various enzymes currently used in poultry feed

The exploitation of microbial enzymes at industrial level started 100 years ago with the patenting of a process for the production of α -amylase from the fungus *Aspergillus oryzae* (Pariza and Cook, 2010). Most of the enzymes currently used in the food and beverage industry are from *Aspergillus*, however, hemicellulases and cellulases are derived from *Trichoderma*. Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase (β -glucanases), xylanases and associated enzymes, phytases, proteases, lipases,

and galactosidases (Table 1). For instance the use of combination of xylanase and β -glucanase has been proved to be beneficial in terms of growth increment in the poultry. Pettersson and Aman (2007) tested the addition of an enzyme cocktail, consisting of xylanase and β -glucanase to an

unpelleted poultry diet containing rye and wheat. The enzyme cocktail was added at various inclusion levels. The addition of the enzyme cocktail in Pettersson and Aman's (2007) trail resulted in a significant increase in body-weight and feed intake.

Table 1. Enzymes currently used in poultry feed.

Enzyme Name	Classification	Production organism	Function
α -Amylase	Carbohydrase	<i>Aspergillus spp.</i> , <i>Bacillus spp.</i> , <i>Rhizopus spp.</i>	Hydrolyzes starch
Maltogenic α -amylase	Carbohydrase	<i>Bacillus subtilis</i> , <i>d-Bacillus stearothermophilus</i> .	Hydrolyzes starch with production of maltose
β -Amylase	Carbohydrase	Barley malt	Hydrolyzes starch with production of maltose
Cellulase	Carbohydrase	<i>Aspergillus niger</i> .	Breaks down cellulose
α -Galactosidase	Carbohydrase	<i>Aspergillus niger</i> , <i>Mortierella vinaceae</i> var <i>Saccharomyces sp.</i>	Hydrolyzes oligosaccharides
β -Glucanase	Carbohydrases	<i>Aspergillus spp.</i> , <i>Bacillus spp.</i>	Hydrolyzes B-glucans
β -Glucosidase	Carbohydrase	<i>Aspergillus niger</i>	Hydrolyzes cellulose degradation products to glucose
Glucoamylase (amylolytic)	Carbohydrase	<i>Aspergillus niger</i> , <i>Aspergillus oryzae</i> , <i>Rhizopus niveus</i> , <i>Rhizopus oryzae</i> .	Hydrolyzes starch with production of glucose
Hemicellulase	Carbohydrase	<i>Aspergillus spp.</i> , <i>Bacillus spp.</i> , <i>Humicola sp.</i> , <i>Trichoderma sp.</i>	Breaks down hemicellulose
Invertase	Carbohydrase	<i>Aspergillus niger</i> , <i>Saccharomyces sp.</i>	Hydrolyzes sucrose to glucose and fructose
Lactase	Carbohydrase	<i>Aspergillus niger</i> , <i>Aspergillus oryzae</i> , <i>Candida pseudotropicalis</i> , <i>Kluyveromyces marxianus</i> .	Hydrolyzes lactose to glucose and galactose
β -Mannanase	Carbohydrase	<i>Aspergillus niger</i> , <i>Bacillus lentus</i> , <i>Trichoderma reesei</i>	Hydrolyzes beta-mannans
Pectinase	Carbohydrase	<i>Aspergillus aculeatus</i> , <i>Aspergillus niger</i> , <i>Rhizopus oryzae</i>	Breaks down pectin
Pullulanase	Carbohydrase	<i>Bacillus acidopullulyticus</i>	Hydrolyzes starch
Xylanase	Carbohydrase	<i>Aspergillus spp.</i> , <i>Bacillus spp.</i> , <i>Humicola sp.</i> , <i>Penicillium sp.</i> , <i>Trichoderma sp.</i>	Hydrolyzes xylans
Lipase	Lipase	<i>Aspergillus niger</i> , <i>Candida sp.</i> , <i>Rhizomucor sp.</i> , <i>Rhizopus sp.</i>	Hydrolyze triglycerides, diglycerides, and glycerol monoesters
Bromelain	Protease	Pineapple (<i>Ananas comosus</i>) stem and fruit	Hydrolyzes proteins
Ficin	Protease	<i>Ficus glabrata</i>	Hydrolyzes proteins
Keratinase	Protease	<i>Bacillus licheniformis</i>	Hydrolyzes proteins
Papain	Protease	Papaya (<i>Carica papaya</i>)	Hydrolyzes proteins
Pepsin	Protease	Animal stomachs	Hydrolyzes proteins
Protease	Protease	<i>Aspergillus niger</i> , <i>Aspergillus sp.</i> , <i>Bacillus spp.</i>	Hydrolyzes proteins
Trypsin	Protease	Animal pancreas	Hydrolyzes proteins
Catalase	Oxidoreductase	<i>Aspergillus niger</i> var., <i>Micrococcus lysodeikticus</i>	Produces water and oxygen from hydrogen peroxide
Glucose Oxidase	Oxidoreductase	<i>Aspergillus niger</i>	Degrades glucose to hydrogen peroxide and gluconic acid
Phytase	Phosphatase	<i>Aspergillus spp.</i> , <i>Penicillium sp.</i> , <i>Phytase canola</i> , <i>Pichia pastoris</i> , <i>d-Aspergillus niger</i> , <i>d-Escherichia coli</i> .	Hydrolyzes phytate

Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in feed but also reduces environmental pollution (Pariza and Cook, 2010). Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, β -galactosidases to neutralize certain anti-nutritive factors in non-cereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals (Marquardt, 1997).

Phytate and phytate-bound phosphorus (P) is present in all poultry diets and the partial availability of phytate-P has long been recognized. Possibly, Warden and Schaible (1962) were the first to show that exogenous phytase enhances phytate-P utilisation and bone mineralisation in broiler chicks. Nevertheless, three decades elapsed before an *Aspergillus niger*-derived phytase feed enzyme, with the capacity to liberate phytate-bound P and reduce P excretion, was commercially introduced in 1991. It was then considered that the use of microbial phytases would be confined to areas where financial penalties are imposed on excessive P levels in effluent from intensive pig and poultry units (Chesson, 1993). Contrary to this forecast, the inclusion of phytase feed enzymes in monogastric diets has been far more widely accepted and now exceeds that of NSP degrading enzymes (Bedford, 2003). Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment.

The principle rationale for the use of enzyme technology is to improve the nutritive value of feed stuffs. All animals use enzymes in the digestion of food, those produced either by the animal itself or by the microbes present in the digestive tract. However, the digestive process is nowhere near 100% efficient. For example, swine are unable to digest 15-25% of the food they eat. Therefore, the supplementation of the animal feed with suitable enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestion process (Pariza and Cook, 2010). In many animal production systems, feed is the biggest single cost and profitability depends on the relative cost and nutritive value of the feeds available. Often, the limiting factor when formulating rations is the animal's ability to digest different constituent parts of the feed raw material differently, particularly the fibre. Despite recent advances, the

potential nutritional value of feedstuffs is not achieved at the animal level. This inefficiency in the utilization of nutrients can result in an extra cost to the farmer, the food company and the environment (Sheppy, 2003). Thus, the feeds need to be supplemented with the exogenous enzymes and these are the four main reasons for using enzymes in animal feed:

i) To breakdown anti-nutritional factors that are present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous digestive enzymes, can interfere with the normal digestion, causing poor performance and digestive upsets (Sheppy, 2003).

ii) To increase the availability of the starches, proteins, and minerals that are either enclosed within the fibre-rich cell wall and, therefore, not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (e.g, phosphorus as phytic acid).

iii) To break down specific chemical bonds in raw materials that are not usually broken down by animal's own enzymes, thus releasing more nutrients.

iv) To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate (Sheppy, 2003).

In addition to improving diet utilization, enzyme addition can reduce the variability in nutritive value between feedstuffs and improving the accuracy of feed formulations. Experimental trials have shown that ensuring feed consistency in this way can increase the uniformity of groups of animals, thus aiding management and improving profitability. The general health status of animals can also be indirectly influenced, resulting in fewer of non-specific digestive upsets that are frequently provoked by the fibre components in the feed (Sheppy, 2003).

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades. The role of exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley has been elucidated by Bedford and Schulze (1988). Thus, enzyme supplementation in the feed plays an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components.

Improvement obtained in the poultry with application of different enzyme preparations

The improvements obtained by adding enzymes to the diet of poultry depends on many factors, including the type and amount of cereal in the diet; the level of anti-nutritive factor in the cereal, which can vary within a given cereal; the spectrum and concentration of enzymes used; the type of bird and their age (young bird tend to respond better to enzymes than older birds). Enzyme supplementation to field bean diets has been shown to be effective in improving chick performance (Castanon and Marquardt, 1989). Addition of a cell-wall degrading multi-enzymes complex and α -galactosidase improved the feed-to-gain ratio of raw high-tannin pea diets fed to chickens, whereas no improvement of chick performance was observed for animals fed with a tannin-free pea variety (Brenes et al., 1993b). Pectinase supplementation of pea-based diets for broiler chickens improved weight gain and feed consumption, whereas feed conversion ratios were unaffected (Igbasan and Guenter, 1996). Pectinase or α -galactosidase supplementation in the diets of chicken increased the performance of the birds. Supplementation with α -galactosidase to the milled diets gave the highest cumulative feed intake, whereas no effect was seen for the crushed diets with larger particle size (Daveby et al., 1998). Pectinase supplementation significantly increased cumulative feed intake on the 10th day for both the milled and the crushed diets, compared to the unsupplemented diets (Daveby et al., 1998). Marquardt et al. (1994) reported an improvement obtained in growing chicks when five different cereals were supplemented with an enzyme preparation high in xylanase and β -glucanase activities. In general, they found a close relationship between the amount of soluble arabinoxylans or β -glucans in the different cereals, their corresponding viscosities, and the performance of the chicks. Brenes et al. (1993a) found that naked oats resulted in the greatest degree of response to enzyme treatment. Enzyme treatment improved weight gain by 46%, apparent metabolizable energy (AME) by 33% and fat digestibility by 193%. On the other hand, adding enzymes to a corn-based diet had no effect on chick performance (Brenes et al., 1993a). Enzyme supplementation also reduces water intake and water content of the excreta, which is probably related to a reduced ability of the partially hydrolyzed water-soluble non-starch polysaccharide (WSNPS) to absorb water (Marquardt et al., 1994). The excreta with reduced water content is of immense importance, as high moisture excreta will

lead to vent pasting; soiled birds and eggs; enhanced ammonia production due to continued fermentation by microorganisms and an increased growth of fungal spores, including those that may be responsible for damage to farmer's lungs (aspergillosis). A number of research studies with layers have reported positive responses to the addition of Avizyme with respect to reduced diet cost (Cook et al., 2000); improved FCR; liveability (Cook et al., 2000); and egg weight. In broilers and ducks, improvements in FCR, body weight, uniformity and nutrient digestibility were reported. Greenwood et al. (2002) reported that addition of Avizyme 1502 into reduced ME diet (2, 3 and 4% in starter, grower and finisher, respectively) improved performance in terms of 42-day body weight and FCR. Additionally, Kidd et al. (2001) found that Avizyme 1500 improved significantly 49-day mortality corrected feed conversion when applied into a diet mimicking industry standard (IS) and a diet with increased lysine and TSAA levels compared to the ones found in IS. These observations indicate that enzyme supplementation of cereal-based diets can have a dramatic effect on the performance and probably also the physiological responses of chickens.

Increased nutrient digestibility and decreased excreta output by supplementing enzymes in the diets of the poultry

In recent years, enzymes have been widely used in monogastric diets to increase nutrient digestibilities and to decrease nutrient waste in the excreta. In densely populated parts of the world, such as Asia and Europe, excretion of large amounts of organic matter, especially those containing high levels of nitrogen and phosphorus, presents serious environmental threats. The large amount of excreta output in the poultry industry is a serious concern for the environment as well as for it results in slower growth performances. Dry-matter digestibility (DMD) in animals ranges from 50 to 80%; the remainder of the dry matter (DM) is lost via the excreta. In the poultry industry, the amount lost via excreta is about 9 000–22 000 t of high-N manure per million birds annually. The effect of enzyme supplementation on DMD in poultry depends on the type of diet and the type of animal. An increase in DMD ranges from 0.9 to 17% in poultry has been reported by enzyme supplementation (Annison and Choct, 1993; Schutte et al., 1995). The enzyme supplementation improved DMD by 17%, apparent metabolizable energy (AME) by 24%, and feed-conversion rate (FCR) by 31% (Annison and Choct, 1993).

The enzymes currently used in monogastric diets are predominantly glycanases, which are known to cleave non-starch polysaccharides (NSPs) into simpler form, thereby eliminating their ability to form viscous digesta and improving nutrient digestibilities. The effects of glycanases are generally non-specific, except for their effect on fat, which is known to have a greater effect on saturated fat than on unsaturated fat. Another enzyme which is being used in feed on a large scale is phytase. Phytase is known to increase the utilization of phytate phosphorus. The ability of phytase to enhance the digestion of phytate phosphorus and subsequently to reduce the output of organic phosphorus to the environment has attracted a great deal of interest. In poultry, use of phytase was reported to reduce phosphorus excretion by as much as 40% for broilers (Simons and Versteegh, 1991). When phytase was added to layer diets, increased egg production and positive effects on egg weight and tibia ash were also noted (Simons and Versteegh, 1991).

Cereal by-products, such as rice bran, are important feed ingredients used in Asia, but their efficient use in monogastric diets is hindered by the presence of high levels of NSPs and phytase. Martin (1995) demonstrated that supplementing duck diets with microbial phytase which allowed rice bran to be used at high levels (up to 60%) without detrimental effects. Phosphorus excretion was reduced by 9.6%, and significant decreases in excretion of manganese, copper, and zinc were also noted. Thus, enzyme supplementation in the diets of the poultry increased the nutrient digestibility and decreased the excreta output.

Impact of exogenous enzyme supplementation on growth performance of poultry

The role of exogenous enzyme capable of degrading NSP in broiler diets based on 'viscous' grains, including wheat and barley has been studied by Bedford and Schulze (1998). Supplementation of the maize/soy-based diets with both glucanase and xylanase displayed improvement in feed conversion ratio (FCR) and ileal nutrient digestibility (Cowieson et al., 2010). When both enzymes were added simultaneously, a synergistic effect, resulted in greater benefits than either enzyme independently but less than the sum of the individual effects was observed. The best performance was achieved with the combination of xylanase (16 000 BXU/kg) and glucanase (30 000 BU/kg) (Cowieson et al., 2010). The enzyme (Yemzim B) containing 300 IU of xylanase/g, 20 IU of β -glucanase/g, 20 IU of hemicellulase/g and 260 IU of amylase/g with or

without essential oil was incorporated in wheat—soybean meal based, in mash form diet for broilers. Birds fed on diets containing enzyme, essential oil (250 mg/kg) and enzymes plus essential oil (250 mg/kg) had significantly higher weight gain than those given control diet from d 0 to 7 (Basmaciog et al., 2010). Supplementation with enzyme significantly decreased viscosity and increased dry matter of digesta, but did not alter pH of digesta. Enzymes and essential oil had different modes of actions. The supplementation of enzyme with essential oil in diets is more likely to be effective in view of performance, nutrient digestibility, enzyme activities and immune system (Basmaciog et al., 2010).

In another study, Maize-soyabean based diet supplemented with mixture of xylanase, amylase, and protease (XAP) at 650, 1650 and 4000U, respectively, or *Escherichia coli*-derived phytase (1000 phytase units/kg) individually or in combination was fed to the chicks for 21 days (Tiwari et al., 2010). It was found that a cocktail of XAP alone did not improve performance, but phytase supplementation improved weight gain (Tiwari et al., 2010). The enzymes were additive in their effects on growth performance of the chicks. The enzymes had no effect on ileal digestible energy. Ileal N digestibility was higher in diets with XAP or phytase individually compared with negative control diet. Both phytase and XAP individually and in combination improved ileal P digestibility compared with negative control (Tiwari et al., 2010). This study shows that a combination of XAP and phytase improved performance, but the enhancement in performance appears to be due mainly to phytase. Both XAP and phytase were effective in improving P digestibility and retention of chicks receiving nutritionally marginal maize—soybean meal (Tiwari et al., 2010). In the study of Simon et al. (2009), phytase addition (1500 FTU kg⁻¹) to diets containing 4.5 g kg⁻¹ total P increased weight gain (733 g versus 338 g) and feed efficiency (1.50 versus 1.85) of broilers from 0 to 24 days of age. Subsequently, Cabahug et al. (1999) reported that phytase addition (400 and 800 FTU kg⁻¹) to 2.3 g kg⁻¹ non-phytate-P diets increased weight gain (18.8%), feed intake (9.0%) and feed efficiency (7.9%) of broiler chicks from 7 to 25 days of age. However, responses to phytase by broilers offered at 4.5 g kg⁻¹ non-phytate-P diets were more modest (respective increases of 5.0, 5.0 and 0%), with a significant interaction between non-phytate-P level and phytase addition for weight gain. In general, the responses to phytase in feed intake and weight gain are more robust and consistent than feed efficiency responses. Rosen (2003) reported that feed efficiency

responses to phytase have been declining with time which was attributes to concurrent improvements in broiler strains, feeds and management techniques. Phytase supplementation of P-adequate broiler diets has been shown to generate misleading responses regarding the growth performance, which may be mediated by dietary nutrient specifications (Selle et al., 1999). The magnitude of responses to phytase will be more pronounced with increasing inclusion rates of the feed enzymes and, presumably, greater degradation of phytate. Shirley and Edwards (2003) investigated phytase supplementation of maize-soy broiler diets (4.60 g total P kg⁻¹; 2.72 g phytate-P kg⁻¹); responses in selected parameters to graded phytase inclusion levels to a maximum of 12,000 FTU kg⁻¹. Increasing the phytase inclusion is associated with substantial increases in total tract phytate degradation ranging from 0.403 to 0.948. Moreover, highest phytase inclusion rate of 12,000 FTU kg⁻¹ helped in phytate degradation which was correlated with large increases in P retention, tibia ash, weight gain, feed intake, nitrogen (N) retention, feed efficiency, AME and Ca retention (ranked in descending order of significance). At these extreme inclusion rates, there is a possibility that any minor enzymic side-activities that may be present in the phytase preparation may become significant, and thus having an independent impact on nutrient utilization. Increasing phytase from 750 to 1000 FTU kg⁻¹ slightly benefited amino acid digestibility; in contrast, weight gain, feed efficiency and AME responses to phytase reached a plateau at 750 FTU kg⁻¹, which is quite different to the observations reported by Shirley and Edwards (2003). Different studies have reported effect of enzymes on nutrient utilization and growth performance of poultry differently. Thus, the inclusion of different feed enzymes in monogastric diets has been far more widely accepted and have been found to be effective (Bedford, 2003).

Effect of exogenous enzyme supplementation on the overall performance of the poultry

Many enzyme supplementations are known to have a positive impact on the overall performance of the poultry. Different feed ingredients are known to shown different level of response to different enzyme supplementation. For example, cereal grains which are not well digested by the poultry are more greatly influenced by enzyme addition than those which are well digested (Lobo, 2000). Some enzyme are known to destroys anti-nutritional factors or increases the digestibility of indigestible nutrients which can help in saving energy and

improves amino acid digestibility (Lobo, 2000). In addition, enzymes can be useful to reduce the digesta viscosity which is important for improving fat digestion and for increasing the availability of fat-soluble vitamins. Some enzymes plays a role in improving the efficiency of absorption of natural dietary pigments – chlorophylls and xanthophylls – that are essential for good yolk color in eggs and broiler beaks as well as feet (Nahm, 2007).

The addition of a complex enzyme to broiler diets (Allzyme Vegro, Alltech, Inc., UK) increased true digestibility of all amino acids except valine (Charlton and Pugh, 1995), where substantial increases in the bioavailability of many amino acids for broilers including most limiting amino acids (lysine, methionine, cystine and threonine) were reported. It is well established that the young chick responds to high amino acid density (AAD) diets optimizing growth performance and meat yield (Corzo et al., 2005; Dozier III et al., 2006), but high AAD diets may not be appropriate as broilers approach market weight. Therefore, decreasing dietary AAD late in development should translate to decreased N excretion and ammonia production of the excreta without negatively influencing production efficiency.

Table 2 shows the effect of enzyme supplementation on performance of different type of poultry. Enzyme supplementation is most commonly done by direct addition of an enzyme or enzyme combination to the feed. The most effective enzyme addition to feed has been when the enzyme action was directed at a specific feed component (Table 2). Data from this table indicates that different species require different levels of dietary enzyme supplementation and different basal diets also require different levels of enzyme supplementation, in order to achieve better performance of poultry. Jo' Zefiak et al. (2010) reported that the cereal type as well as the exogenous enzyme supplementation influence the microbiota in broiler chicken caeca, and may have the effect of reducing potentially pathogenic Enterobacteriaceae populations. Influence of supplementation of xylanase to rye-based diets and β -glucanase to barley-based diets is not only limited to the ileal phase. β -glucanase supplementation could have an important impact on gut health in chickens, in particular with respect to the caecal lumen reflux and possible migration of some of the bacterial species to upper parts of the gastro-intestine (Jo' Zefiak et al., 2010).

Table 2. Enzyme Supplementation and their effects on different types of poultry performance.

Source	Type of poultry	Type of enzyme	Amount of enzyme	Increased performance	Based diets
(Mandal et al., 2005)	Pullets, Guinea fowl, Quail	Commercial enzyme preparations	Two levels added (0 or 0.57g/kg feed)	The apparent metabolizable energy (AMEN) value of rapeseed meal did not improve, while sunflower seed meal increased	Solvent-extracted rapeseed and sunflower seed meal
(Wu et al., 2005)	Layers	β -Mannanase	Recommended levels	1. No significant differences in overall average egg production and egg mass among treatments 2. Increased average egg production and egg mass during 5–8 weeks 3. No significant differences in feed intake, production performance and body weight	Corn–soybean meal
(Lazaro et al., 2003)	Layers	Fungal β -glucanase/xylanase	Four enzyme concentrations (0, 250, 1250, 2500mg/kg feed)	No effect on egg production of feed efficiency	Wheat, barley and rye-based diets
(Mathlouthi et al., 2003a)	Turkeys	Xylanase β -glucanase	560 and 2800IU/kg feed of xylanase and β -glucanase, respectively	Improved body weight gain and feed efficiency	Wheat or wheat and barley-based diets
Mathlouthi et al., 2003b)	Layers	Xylanase and β -glucanase, used as two enzyme preparations	1. Wheat/barley based diet with 20mg 2. Corn/soybean meal with 20mg	1. Decreased viscosity of wheat, barley, corn and soybean meal. 2. Other beneficial effects were not significant.	1. Wheat/barley based diets 2. Corn/soybean meal
(Odetallah et al., 2002a)	Turkeys	Enzyme mix supplementing β -glucanase units (BGU) and endo-xylanase units	BGU: 300g/ton feed, EXU: 300g/ton feed, EXU: 100g/ton feed, BGU 150g/ton feed	Marginal improvement in growth performance depending upon enzyme formulation	Wheat-based diets
(Odetallah et al., 2002b)	Turkeys	Mannan-endo-1,4- α -mannosidase	With or without 100 million units (MU) Hemicell/ton (1MUD106 enzyme activity U)	Some of the adverse effects of anti-nutritional factors of SBM on turkey growth performance can be alleviated by enzyme supplementation	SBM-44 or SBM-48 was found in the basal diet
(Zhang et al., 2000)	Pullets	Five preparations using: <i>T. viride</i> , <i>T. reesei</i> , <i>A. niger</i> (Finizym), <i>A. niger</i> (SP 249), <i>Bacillus subtilis</i>	1. 0.003125% 2. 0.00625% 3. 0.0125% were added respectively	1. Can accurately evaluate profitability 2. Selects the most profitable cereal	Barley-based diets
(Zhang et al., 2001)	1-d-old single combed white leghorn cockerels	Xylanase	Six amounts of two enzymes: 1. 0, 0.25, 0.75, 2.25, 6.75, and 20.25g/kg feed 2. 0, 0.1, 0.3, 0.9, 0.27, 8.1g/kg feed	1. Evaluate the efficacy of enzyme preparations 2. Predict chick performance	Rye-based diets

Effect of microbial phytase supplementation on ileal digestibility of amino acids and in reducing the phosphorus excretion into the environment

Many research studies have reported improvements to varying extents in the coefficient of apparent ileal digestibility (CAID) of amino acids following phytase addition to practical broiler diets. Several research studies which have reported the effect of phytase on digestibility of amino acids has been determined. In these studies, the inert markers selected include chromic oxide (Kornegay et al., 1999; Zhang et al., 1999; Camden et al., 2001; Dilger et al., 2004; Onyango et al., 2005), acid insoluble ash (Selle et al., 1999) and titanium oxide (Rutherford et al., 2002; Ravindran et al., 2006). It was reported that effect is greater when acid insoluble ash or titanium oxide were used as dietary markers in comparison to chromic oxide. It can be seen that, added phytase increased the mean digestibility coefficient of essential amino acids by 4.4% in the acid insoluble ash and titanium oxide assays, whereas in the chromic oxide assays, the mean digestibility coefficient was increased by 1.4%.

The incorporation of microbial phytases in pig and poultry diets has achieved a good momentum irrespective of the cereal grain base. As reviewed by Selle and Ravindran (2007), the implications of phytate in poultry diets are numerous but essentially, phytases liberate phytate-bound phosphorus (P) and helps in reducing P excretion, which is of ecological importance. Consequently, the combined addition of xylanase and phytase in wheat-based broiler diets is gaining importance. Previous studies have already proved the efficacy of using a combination of enzymes (Ravindran et al., 1999a; Zyla et al., 1999a,b). Xylanase pre-treatment was found to reduce the dietary concentration of arabinoxylan by 15–30% (Denstadli et al., 2010). Xylanase top-dressing tended to improve ileal protein digestibility but, in general, xylanase treatment had no major effect on overall performance in male broilers (Denstadli et al., 2010). Inclusions of xylanase and phytase in broiler diets showed that xylanase increased mean apparent ileal digestibility (AID) coefficients of 17 amino acids by 8.6%, which exceeded the increases of 4.8% and 5.5% obtained by xylanase and phytase, respectively (Selle et al., 2009). Increases in AID were significant ($P < 0.05$) for the majority of amino acids in response to enzyme inclusions with indications of synergistic responses for five amino acids. Xylanase plus phytase increased

($P < 0.05$) nitrogen-corrected AME by 0.63 MJ kg^{-1} , ileal energy digestibility by 0.89 MJ kg^{-1} and energy AID coefficient by 6.7% relative to the negative control diet (diet supplemented with xylanase: 2000XU kg^{-1} , or phytase: 500 FTU kg^{-1} , or xylanase plus phytase: 2000XU kg^{-1} and 500 FTU kg^{-1}). The enzyme combination increased ($P < 0.05$) coefficients of ileal nitrogen digestibility by 9.1% and 8.0% relative to positive and negative control diets, respectively (Selle et al., 2009). Phytase increased ($P < 0.05$) ileal calcium digestibility by 32.2% and ileal P digestibility by 28.0% relative to the negative control. Ravindran et al. (1999a) reported that xylanase plus phytase increased AID coefficients of 14 amino acids by an average of 8.7% (0.868 versus 0.800) in wheat-casein diets. Individually, xylanase increased AID coefficients by an average of 5.0% and phytase by an average of 5.1%. Subsequently, Selle et al. (2003) found that the combination increased AID coefficients of 16 amino acids by an average of 4.6% (0.816 versus 0.781) in practical diets based on a wheat-sorghum blend. Phytase increased amino acid AID coefficients by an average of 3.6% but xylanase had a negligible impact (0.4%). The possible mechanisms whereby phytate and phytase influence ileal amino acid digestibility have been considered (Selle et al., 2000, 2006; Cowieson et al., 2006; Selle and Ravindran, 2007). It has also been established that phytate improves the endogenous amino acid flows in broilers which is counteracted by phytase (Cowieson and Ravindran, 2007; Cowieson et al., 2008). These workers reported that sodium phytate addition to an enzyme-hydrolysed casein-based diet markedly increased endogenous amino acid flows with the largest increases being recorded for serine, threonine, glutamic acid, glycine and aspartic acid. The simultaneous inclusion of xylanase and phytase in wheat-based broiler diets generates greater responses than either individual component of the enzyme combination. Inclusion of both xylanase and phytase increase digestibility of amino acids by ameliorating the negative influences on protein digestibility of their respective substrates. The simultaneous inclusion of xylanase plus phytase in wheat-based broiler diets was beneficial in terms of nutrient utilization and growth performance.

Phytase has been shown to increase the CAID (Ravindran et al., 1999b) and true ileal digestibility (CTID) (Rutherford et al., 2002) of amino acids of individual feed ingredients in broilers to quite marked extents. Phytase increased the CAID of the

diets given to broilers (Olukosi et al., 2010). Phytase improved the coefficient of the apparent total tract DM retention independently of maize Distillers' Dried Grain with Solubles (mDDGS) and tended to improve the coefficient of apparent phosphorus retention in the diets without mDDGS.

Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment. The incorporation of microbial phytases in poultry diets was induced by the need to reduce P excretion and its loss into the environment, where P pollution is known to be hazardous to water quality. Excessive P drain off into the rivers, lakes and reservoirs are the main reason for their eutrophication (Correll, 1999). Surface runoff from soils with accumulated P accelerates eutrophication, which may result in toxic algal blooms and fish kills (Sharpley, 1999). Consequently any reduction in P excreted by poultry is of benefit to both the environment and sustainable production. Simons et al. (1990) demonstrated the efficient use of microbial phytase to reduce P excretion. They found that 1500 FTU kg^{-1} phytase activity, coupled with reductions in dietary P (7.5–4.5 g kg^{-1}) and Ca (9.0–6.0 g kg^{-1}), reduced P excretion by an average of 61% in two broiler experiments. Zyla et al. (2001) totally eliminated dicalcium phosphate from wheat–soy broiler diets, which reduced non-phytate-P (4.1–1.7 g kg^{-1}) and Ca (9.8–5.9 g kg^{-1}) levels. A combination of phytase and acid phosphatase was included in this modified diet. In a 43-day feeding study, this regime generated a 45% reduction of P in litter (14.8 g/bird versus 26.8 g/bird). Additionally, exogenous enzymes significantly enhanced toe ash (164 g kg^{-1} versus 150 g kg^{-1}), carcass yield (71.3% versus 69.1%) and feed efficiency (1.86 versus 1.97), although there was a numerical reduction in weight gain (2124 g/bird versus 2215 g/bird). In another study, Paik (2003) reduced non-phytate-P levels in starter (4.5–3.5 g kg^{-1}) and finisher broiler diets (3.5–2.5 g kg^{-1}) without, and with, the addition of 500 FTU kg^{-1} phytase. Decreasing the level of non-phytate-P was proven to reduce P excretion by 14.8%, but the weight gain was decreased. Phytase inclusion further increased the reduction in P excretion by 29.6% but, in contrast, growth performance was not compromised. The impact of phytase on P excretion was based on total P assessments in the above studies. From a limited number of investigations, the phytate-P proportion of total P excreted by poultry offered non-supplemented diets

is probably less than anticipated. This proportion ranged from 0.28 to 0.38 in excreta from maize, wheat, triticale and barley-based broiler diets (Pintar et al., 2005) and, in turkeys, a range from 0.16 to 0.32 has been reported (Toor et al., 2005). McGrath et al. (2005) reported that phytate-P represented 0.57 of total P in the excreta of broilers fed non-supplemented diets and 0.50 in those fed phytase-supplemented diets. These relative levels of phytate-P excretion may be indicative of excessive dietary P levels, undigested inorganic P, endogenous P losses and inherent phytase activity in the gut. There is an ecological concern that phytase supplementation may increase P solubility in excreta and litter as soluble P in run-off is more likely to exacerbate eutrophication. For example, Miles et al. (2003) reported that while phytase supplementation of maize–soy diets reduced total P in broiler litter, levels of soluble P were increased (2.85 g kg^{-1} versus 2.17 g kg^{-1}). In contrast, however, Applegate et al. (2003) compared standard maize–soy broiler diet with three different phytase-supplemented diets. Overall, phytase reduced total P in fresh litter by 32.2% (7.55 g kg^{-1} DM versus 11.14 g kg^{-1} DM) and soluble P by 43.1% (1.23 g kg^{-1} versus 2.16 g kg^{-1}). It is noteworthy that dietary total P concentrations were lower in the study of Applegate et al. (2003). The ecological benefits of phytase supplemented broiler diets, formulated to reduced non-phytate-P specifications, coupled with minimal increases in litter moisture during storage were emphasised by McGrath et al. (2005).

Conclusion and future directions

Enzyme supplementation in the feed play an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components. This is the area of research that requires the most attention in the future. For new feed enzyme, products derived from a safe strain lineage should be used, it is important to ensure a sufficiently high safety margin for their intended use, and that the product complies with appropriate specifications for chemical and microbial contamination.

References

- Adebisi, O. A., A. D. Ologhobo, O. A. Adu and T. O. Olasehinde. 2010. Evaluation of the nutritional potentials of physically treated cowpea seed hulls in poultry feed. *Emir. J. Food Agric.* 22:232-239.
- Annisson, G. and M. Choct. 1993. Enzymes in poultry diets. In: C. Wenk and M. Boessinger

- (Eds.). pp.61–68. Enzymes in animal nutrition, Kartause Ittingen, Thurgau, Switzerland.
- Applegate, T. J., B. C. Joern, D. L. Nussbaum-Wagler and R. Angel. 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. *Poult. Sci.* 82:1024-1029.
- Basmaciog, L. M. L., H., S. Baysal, Z. Misirliog-Lu, M. Polat, H. Yilmaz and N. Turan. 2010. Effects of oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat—soybean meal diets. *Brit. Poult. Sci.* 51:67-80.
- Bedford, M. R. 2003. New enzyme technologies for poultry feeds. *Brit. Poult. Sci.* 44:S14-S16.
- Bedford, M. R. and H. Schulze. 1998. Exogenous enzymes of pigs and poultry. *Nut. Res. Rev.* 11:91-114.
- Bedford, M. R. 2009. The use of NSPases for improving efficiency of nutrient extraction from corn for poultry. *Poultry Bulletin* April: 193.
- Brenes, A., M. Smith, W. Guenter and R. R. Marquardt. 1993a. Effect of enzyme supplementation on the performance and digestive tract size of broiler chicks fed wheat- and barley-based diets. *Poult. Sci.* 72:1731-173.
- Brenes, A., B.A. Rotter, R.R. Marquardt and W. Guenter. 1993b. The nutritional value of raw, autoclaved and dehulled peas (*Pisum sativum* L.) in chicken diets as affected by enzyme supplementation. *Can. J. Ani. Sci.* 73: 605–614.
- Cabahug, S., V. Ravindran, P. H. Selle and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase as influenced by dietary phytic acid and non-phytate phosphorus levels I. Effects on bird performance and toe ash content. *Brit. Poult. Sci.* 40:660-666.
- Camden, B. J., P. C. H. Morel, D. V. Thomas, V. Ravindran and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize–soya-bean meal diets for broilers. *Ani. Sci.* 73:289-297.
- Castanon, J. I. R. and R. R. Marquardt. 1989. Effect of enzyme addition, autoclave treatment and fermenting on the nutritive value of field beans (*Vicia faba* L.). *Ani. Feed Sci. Technol.* 26:71–79.
- Charlton, P. and R. Pugh. 1995. Expanding enzyme applications: Higher amino acid and energy values for vegetable proteins. In: Official Proceedings 13th Annual Pacific Northeast Animal Nutrition Conference, October 10–12, Marriott Hotel, Portland, OR, USA, 1–8.
- Chesson, A. 1993. Feed enzymes. *Ani. Feed Sci. Technol.* 45:65–79.
- Choct, M. 2006. Enzymes for the feed industry: Past, present and future. *Worlds Poult. Sci. J.* 62(01):5-16.
- Cook, M. E., B. M. Drake, and E. Ernest Pierson. 2000. Effects of Avizyme 1500 on laying hen performance. *Poult. Sci.* 79(7): Abstract.
- Correll, D. L. 1999. Phosphorus: a rate limiting nutrient in surface waters. *Poult. Sci.* 78:674–682.
- Corzo, A., M. T. Kidd, D. J. Burnham, S. L. Branton and R. Gonzalez-Esquerra. 2005. Dietary amino acid density effects on growth and carcass of broilers differing in strain cross and sex. *J. App. Poult. Res.* 14:1–9.
- Cowieson, A. J., T. Acamovic and M. R. Bedford. 2006. Phytic acid and phytase: implications for protein utilization by poultry. *Poult. Sci.* 85:878–885.
- Cowieson, A. J. and V. Ravindran. 2007. Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. *Brit. J. Nutr.* 98:745–752.
- Cowieson, A. J., V. Ravindran and P. H. Selle. 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. *Poult. Sci.* 87:2287–2299.
- Cowieson, A. J., M. R. Bedford and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *Brit. Poult. Sci.* 51:246-257.
- Daveby, D. Y., A. Razdan and P. Aman. 1998. Effect of particle size and enzyme supplementation of diets based on dehulled

- peas on the nutritive value for broiler chickens. *Anim. Feed Sci. Technol.* 74:229-239.
- Denstadli, V. B. Westereng, H. G. Biniyam, S. Ballance, S. H. Knutsen and B. Svihus. 2010. Effects of structure and xylanase treatment of brewers' spent grain on performance and nutrient availability in broiler chickens. *Brit. Poult. Sci.* 51:419-426.
- Dilger, R. N., E. M. Onyango, J. S. Sands and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. *Poult. Sci.* 83:962-970.
- Dozier III, W. A., R. W. Gordon, J. Anderson, M. T. Kidd, A. Corzo and S. L. Branton. 2006. Growth, meat yield, and economic responses of broilers provided three- and four-phase regimens formulated to moderate and high nutrient density during a 56 day production period. *J. Appl. Poult. Res.* 15:312-325.
- Friesen, O. D., W. Guenter, R. R. Marquardt and B. A. Rotter. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. *Poult. Sci.* 71:1710-1721.
- Greenwood, M. W., C. A. Fritts, and P. W. Waldroup. 2002. Utilization of Avizyme 1502 in corn-soy meal broiler diets with and without antibiotics. *Proceeding of SPSS. Abstract number 25.*
- Igbasan, F. A. and W. Guenter. 1996. The evaluation and enhancement of the nutritive value of yellow-, green- and brown-seeded pea cultivars for unpelleted diets given to broiler chickens. *Anim. Feed Sci. Technol.* 63:9-24.
- Jo' Zefiak, D., A. Rutkowski, S. Kaczmarek, B.B. Jensen. R. M. Engberg and O. Højberg. 2010. Effect of b-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. *Brit. Poult. Sci.* 51:546-557.
- Kidd, M. T., G. W. Morgan, Jr., S. R. Rogers, E. E. Pierson, and C. Wyatt. 2001. Commercial broiler responses to enzyme and diet density. *Poultry Sci.* 80(7): Abstract 114.
- Kincaid, R. L., D. K. Garikipati, T. D. Nennichand and J. H. Harrison. 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. *J. Dairy Sci.* 88:2893-2902.
- Klasing, K.C. 1998. *Comparative Avian Nutrition.* CAB International, Wallingford, UK.
- Knowlton, K. F., M. S. Taylor, S. R. Hill, C. Cobb and K. F. Wilso. 2007. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. *J. Dairy Sci.* 90:4356-4360.
- Kornegay, E. T., Z. Zhang and D. M. Denbow. 1999. Influence of microbial phytase supplementation of a low protein/amino acid diet on performance, ileal digestibility of protein and amino acids, and carcass measurements of finishing broilers, *Phytase in Animal Nutrition and Waste Management* (second revised ed.), BASF Corporation, Mount Olive, NJ: 557-572.
- Lazaro, R., M., Garcia, M. J. Aranibar and G. G. Mateos. 2003. Effect of enzyme addition to wheat, barley- and rye-based diets on nutrient digestibility and performance of laying hen. *Brit. Poult. Sci.* 44:256-265.
- Lobo, P. 2000. Enzyme to cut animal waste. *Feed Manag.* 51:15-16.
- Mandal, A. B., A. V. Elangovan, P. K. Tyagi, A. K. Johni and S. Kaur, 2005. Effect of enzyme supplementation on the metabolizable energy content of solvent-extracted rapeseed and sunflower seed meals for chicken, guinea fowl and quail. *Brit. Poult. Sci.* 46:75-79.
- Marquardt, R. R. 1997. Enzyme enhancement of the nutritional value of cereals: role of viscous, water-soluble, non-starch polysaccharides in chick performance. In: *Enzymes in Poultry and Swine Nutrition*, edited by *Marquardt, R. R. and Han, Z.* The International Development Research Center: 5-18.
- Marquardt, R. R., D. Boros, W. Guenter and G. Crow. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reesei* enzyme preparation. *Anim. Feed Sci. Technol.* 45:363-378.
- Marquardt, R. R., A. Brenes, Z. Zhang and D. Boros. 1996. Use of enzymes to improve nutrient availability in poultry feedstuffs. *Anim. Feed Sci. Technol.* 60:321-330.

- Martin, E. A. 1995. Improving the utilisation of rice bran in diets for broiler chickens and growing ducks. The University of New England, Armidale, Australia. Ph.D. Thesis.
- Mathlouthi, N., H. Juin and M. Larbier. 2003a. Effect of xylanase and β -glucanase supplementation of wheat- or wheat- and barley-based diets on the performance of male turkeys. *Brit. Poult. Sci.* 44:291–298.
- Mathlouthi, N., M. A. Mohamed and Larbier, M. 2003b. Effect of enzyme preparation containing xylanase and β -glucanase on performance of laying hens fed wheat/barley- or maize/soybean meal-based diets. *Brit. Poult. Sci.* 44:60–66.
- McGrath, J. M., J. T. Sims, R. O. Maguire, W. W. Saylor, C. R. Angel and Turner, B. L. 2005. Broiler diet modification and litter storage: impacts on phosphorus in litters, soils and runoff. *J. Environ. Qual.* 34:1896–1909.
- Miles, D. M., P. A. Moore, D. R. Smith, D. W. Rice, H. L. Stilborn, D. R. Rowe, B. D. Lott, S. L. Branton and J. D. Simmons. 2003. Total and water-soluble phosphorus in broiler litter over three flocks with alum litter treatment and dietary inclusion of high available phosphorus corn and phytase supplementation. *Poult. Sci.* 82:1544–1549.
- Nahm, K. H. 2007. Feed formulations to reduce N excretion and ammonia emission from poultry manure. *Biores. Technol.* 98:2282–2300.
- Nnenna, O. P., Emeka, N. P. and C. L. Okpoko. 2006. Performance of broiler chicks (*Gallus domesticus*) fed maize offal-based diets supplemented with roxazyme G enzyme. *Internat. J. Poult. Sci.* 5(7):607–610.
- Odetallah, N. H., P. R., Ferket, J. L. Grimes and J. L. McNaughton. 2002a. Effect of mannan-endo-1,4- β -mannosidase on the growth performance of turkeys fed diets containing 44% and 48% crude protein soybean meal. *Poult. Sci.* 81:1322–1331.
- Odetallah, N. H., C. W. Parks and P. R. Ferket. 2002b. Effect of wheat enzyme preparation on the performance characteristics of tom turkeys fed wheat-based rations. *Poult. Sci.* 81:987–994.
- Olukosi, O. A., A. J. Cowieson and O. Adeola. 2010. Broiler responses to supplementation of phytase and admixture of carbohydrases and protease in maize—soyabean meal diets with or without maize Distillers' Dried Grain with Solubles. *Brit. Poult. Sci.* 51:434–443.
- Onyango, E. M., M. R. Bedford and O. Adeola, 2005. Efficacy of an evolved *Escherichia coli* phytase in diets for broiler chicks. *Poult. Sci.* 84:248–255.
- Paik, I. 2003. Application of phytase, microbial or plant origin, to reduce phosphorus excretion in poultry production. *Asian-Aus. J. Anim. Sci.* 16:124–135.
- Pariza, M. W. and M. Cook. 2010. Determining the safety of enzymes used in animal feed. *Reg. Toxicol. Pharmacol.* 56:332–342.
- Parsippany, N. J. 2008. Non-starch polysaccharide enzymes for poultry. Proceedings of the 6th MIDatlantic nutrition conference, University of Maryland.
- Pettersson, D. and P. Aman, 2007. Enzyme supplementation of a poultry diet containing rye and wheat. *Br. J. Nutr.* 62:139–149.
- Pintar, J., B. Homen, K. Gazic, Z. Janjecic, M. Sikiric and T. Cerny. 2005. Effects of supplemental phytase on nutrient excretion and retention in broilers fed different cereal based diets. *Czech. J. Anim. Sci.* 50:40–46.
- Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby and J. S. Sands. 2006. Influence of an *E. coli*-derived phytase on nutrient utilization in broiler starter fed diets containing varying concentrations of phytic acid. *Poult. Sci.* 85:82–89.
- Ravindran, V., P. H. Selle, W. L. Bryden. 1999a. Effects of phytase supplementation, individually and in combination, with glycanase on the nutritive value of wheat and barley. *Poult. Sci.* 78:1588–1595.
- Ravindran, V., S. Cabahug, G. Ravindran and W. L. Bryden. 1999b. Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. *Poult. Sci.* 78:699–706.
- Rosen, G. 2003. Microbial phytase in broiler nutrition. In: P.C. Garnsworthy and J. Wiseman, Editors, *Recent Advances in Animal Nutrition*, Nottingham University Press, Nottingham, UK: 105–117.
- Rutherford, S. M., T. K., Chung, P. C. H. Morel and P. J. Moughan. 2004. Effect of microbial

- phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. *Poult. Sci.* 83:61–68.
- Rutherford, S. M., T. K. Chung and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in broiler chickens. *British Poult. Sci.* 44:598–606.
- Schutte, J. B., J. De Jong and D. J. Langhout, 1995. Effect of a xylanase enzyme supplementation to wheat-based diets in broiler chicks in relation to dietary factors. In: W. Van Hartingsveldt, M. Hessing, J. P. Van der Lugt, and W. A. C. Somers (Eds.), *Proceedings of the Second European Symposium on Feed Enzymes*, 25–27 Oct, Noordwijkerhout, Netherlands. TNO Nutrition and Food Research Institute, Zeist, Netherlands; 95–101.
- Selle, P. H., V. Ravindran, P. H. Pittolo and W. L. Bryden. 1999. An evaluation of microbial phytase in sorghum-based broiler diets. *The Proceedings of the Australian Poultry Science Symposium*, 11:97–100.
- Selle, P. H., V. Ravindran, R. A. Caldwell and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. *Nutr. Res. Rev.* 13:255–278.
- Selle, P. H. and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed. Sci. Technol.* 135:1–41.
- Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo and W. L. Bryden. 2003. Influence of phytase and xylanase supplementation on growth performance and nutrient utilisation of broilers offered wheat based diets. *Asian-Aus. J. Anim. Sci.* 16:394–402.
- Selle, P. H., V. Ravindran, W. L. Bryden and T. Scott. 2006. Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: a review. *J. Poult. Sci.* 43: 89–103.
- Selle, P. H., V. Ravindran and G. G. Partridge. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. *Anim. Feed. Sci. Technol.* 153:303–313.
- Sharpley, A. 1999. Agricultural phosphorus, water quality and poultry production: are they compatible? *Poult. Sci.* 78:660–673.
- Sheppy, C. 2003. The current feed enzyme market and likely trends. In: M. R. Bedford and G. G. Partridge (Eds.), *Enzymes in Farm Animal Nutrition* CABI Publishing Wallingford, Oxon, UK.
- Shirley, R. B. and H. M. Edwards. 2003. Graded levels of phytase past industry standards improves broiler performance. *Poult. Sci.* 82:671–680.
- Simons, P. C. M. and H. A. J. Versteegh. 1991. Application of microbial phytase in poultry nutrition. *Poult. Sci.* 70:110.
- Simons, P. C. M., H. A. J. Versteegh, A.W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Brit. J. Nutr.* 64:525–540.
- Tiwari, S. P., M. K. Gendley, A. K. Pathak and R. Gupta. 2010. Influence of an enzyme cocktail and phytase individually or in combination in Ven Cobb broiler chickens. *Brit. Poult. Sci.* 51:92–100.
- Toor, G. S., J. D. Peak and J. T. Sims. 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. *J. Env. Qual.* 34:687–697.
- Warden, W. K. and P. J. Schaible. 1962. Preliminary investigations concerning utilization of phytin phosphorus by the chick. *Poult. Sci.* 4:1692.
- Wu, G., M. M. Bryant, R. A. Voitle and D. A. Roland Sr. 2005. Effect of β -mannanase in corn-soy diets on commercial Leghorn in second-cycle hens. *Poult. Sci.* 84:894–897.
- Zhang, X., D. A. Roland, G. R. McDaniel and S. K. Rao. 1999. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids in broilers. *Poult. Sci.* 78:1567–1572.
- Zhang, Z., R. R. Marquardt and W. Guenter. 2000. Evaluating the efficacy of enzyme preparations and predicting the performance of Leghorn chicks fed rye-based diets with a dietary viscosity assay. *Poult. Sci.* 79:1158–1167.

- Zhang, Z., R. R. Marquardt, W. Guenter and G. H. Crow. 2001. Development of a multipurpose feed enzyme analyzer to estimate and evaluate the profitability of using feed enzyme preparations for poultry. *Poult. Sci.* 80:1562–1572.
- Zyla, K., J., Koreleski, S., Swiatkiewicz, J., Piironen, D. R., Ledoux. 2001. Influence of supplemental enzymes on the performance and phosphorus excretion of broilers fed wheat-based diets to 6 weeks of age. *Anim. Feed Sci. Technol.* 89:13–118.
- Zyla, K., D. Gogol, J. Koreleski, S. Swiatkiewicz and D. R. Ledoux. 1999a. Simultaneous application of phytase and xylanase to broiler feeds based on wheat: *in vitro* measurements of phosphorus and pentose release from wheats and wheat-based feeds. *J. Sci. Food Agric.* 79:1832–1840.
- Zyla, K., D. Gogol, J. Koreleski, S. Swiatkiewicz and D. R. Ledoux. 1999b. Simultaneous application of phytase and xylanase to broiler feeds based on wheat: feeding experiment with growing broilers. *J. Sci. Food Agric.* 79:1841–1848.