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Enzyme supplementation in poultry diet formulation: A review

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Abstract

In recent years there have been concerted efforts to improve the nutritive worth of feedstuffs by using exogenous enzymes. On the basis of many reports it may be concluded that the nutritional and, therefore, economic value of corn, soybean meal (SBM) and other ingredients commonly used in poultry diets can be improved by the addition of appropriate preparations of phytase, carbohydrase and other enzyme activities. It is our understanding, that an increase in the productive value with enzyme supplementation can be achieved by: (1) release of available phosphorus from phytate hydrolysis (2) elimination of the nutrient encapsulating effect of the cell walls and therefore improved energy and amino acid availability (3) solubilization of cell wall, non-starch polysaccharides (NSP) for more effective hindgut fermentation and improved overall energy utilization (4) hydrolysis of certain types of carbohydrate-protein linkages and therefore improved availability of amino acids and (5) elimination of the anti-nutritive properties of certain dietary components including NSP, by their enzymatic hydrolysis to the prebiotic type components which in turn may facilitate gut development and health in young chickens.

Keywords: Enzyme; phytase; poultry; nutrition

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Introduction

Enzyme supplementation to improve the productive value of high fibre diets

Studies conducted on the effect of *in vitro* digestion on the chemical composition of broiler feed incorporated with enzymes suggest an increase in available carbohydrate (A-CHO) and acid soluble nitrogen fraction (ANSF) and reduction in acid detergent fibre (ADF) and neutral detergent fibre (NDF) of low fibre (LF) and high fibre diets (Rutherfurd et al., 2007). One gram of EFS contained amylase, 7,500 units; cellulase, 400 units; protease, 200 units and lipase, 300 units. LF and HF diets contained un-autoclaved and autoclaved rice bran (RB), wheat bran (WB) and sunflower cake (SC). The observations have also been supported by *in vivo* studies. These studies involved two types of diets namely LF (fibre, 3.2%) and HF (fibre, 6.4%) containing 22.18% crude protein; 2900 kcal ME and 22.68% crude protein; 2600 kcal/kg ME, respectively. Third diet (HF_1) had 1.5-g/kg enzyme feed supplement. LF diet contained autoclaved rice bran (RB) as higher fibre feed ingredient whereas HF diet contained autoclaved RB, wheat bran and sunflower cake as high fibre feed ingredients. Body weight gain, feed efficiency and performance index were significantly higher in chicks fed diet with EFs (Tamim et al., 2004). Cost of production of 1 kg live weight of broiler fed LF diet was 0.34 USD, whereas for producing the same live weight gain of broiler fed HF diet with EFS, the cost involved was 0.36 USD. Therefore, in the production of 1 kg live weight in broiler fed HF diet with EFs, there was saving of 12 paisa approximately (Applegate et al., 2003). It was also suggested that for large-scale broiler production, use of autoclaved high fibre ingredients along with EFs was economical.

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Effect of non-starch polysaccharide hydrolyzing enzymes (cellulase, xylanase, pectinase and α-galactosidase etc.) on performance of broiler fed maize-soya diets

Experiments on utilization of non-starch polysaccharides (NSP) indicated that the performance of broilers in terms of weight gain, efficiency of feed utilization, livability and carcass attributes are not affected by supplementation of different NSP degrading enzymes.

The insignificant effect of enzyme supplementation on performance indicates that the enzymes at the concentration used did not elicit any beneficial response on the utilization of NSP in broilers. The calculated total NSP content in starter and finisher diets is 9.79 and 9.58% respectively, out of which the major component is glucose, which might be a monomer of cellulose. The observed significant increase in weight gain in xylanase and pectinase fed birds at 28 d of age and subsequent disappearance of this effect in these experiments indicate that the chicken at the younger age are not able to utilize xylans and pectins and thus required additional supplementation of enzymes to hydrolyze these compounds. This observation also indicates that as the age of the bird advances the anti-nutritive effect of these NSP declines.

The growth depression observed in broilers fed xylanase + pectinase + α-galactosidase might be due to incomplete hydrolysis of the NSP and consequent increased viscosity of intestinal digesta. The α-galactosidase concentration employed was very low. The increased viscosity of intestinal digesta is known to inhibit the digestion and subsequent absorption of nutrients from the feed. The growth depressing effect of above enzymes on broilers was alleviated by the addition of cellulase to the above enzyme complex. Since, cellulose is the major component of the NSP in the experimental diets, Cellulase supplementation might have increased the hydrolysis of ß-1-4 linkage of glucose monomers utilization in broilers fed a combination of xylanase + pectinase + α-galactosidase + cellulose (Rutherfurd et al., 2007). It is also possible that on breakdown of cellulose, the major component of the cell wall the release of encapsulated intracellular nutrients might have been released.

Several workers also did not observe any significant effect on broiler performance due to supplementation to corn soya diet of either individual or multi-enzymes hydrolyzing NSPs. Contrary to this; a significant improvement in broiler performance has starting and finishing diets. However, on repeated trials on maize-soya-deoiled rice bran diets for three times in broiler chickens, twice in Japanese quails and twice in native chickens, enzyme supplementation did not prove beneficial in terms of growth or feed conversion efficiency (Tamim et al., 2004).

been reported in broilers when fed either corn soya diet or soyabean meal supplemented with either individual or combination of NSP hydrolyzing enzymes. The reported beneficial effects of NSP enzymes on broiler performance may be due to increase in digestibility and retention of nitrogen, increase in dry matter and energy utilization. The reported wide variation in the performance of broilers due to NSP enzymes supplementation to corn soya diet might be due to variations in the concentration and composition of NSP in the diet and source and/or activity of the enzymes supplemented. The activity of a specific enzyme produced even from the same microorganism varies significantly. Further, the variation could also be due to the variation in dose and composition of enzyme cocktail. The improvement in broiler performance observed by some workers due to supplementation of enzyme could be due to incorporation of higher dose of cellulase (1000 to 3000 IU/kg diet) compared to the levels used in some of other studies (420 and 560 IU/kg diet) and also be earlier workers (15 to 97 IU/kg diet). The lack of response due to supplementation of only xylanase in corn-soya is expected because, the monomers released from by the action of the enzyme on arabinoxylan i.e., arabinose and xylose are poorly metabolized by poultry and therefore of little value to animal performance. The addition of a mixture of enzymes considering the composition of NSP in a given diet may yield better response compared to supplementation of individual exogenous enzyme. A series of experiments have also been conducted in CARI Experimental Station employing various maize-soya, pearl millet, sorghum, finger millet, mustard cake and un-decorticated sunflower seed meal based diets in broiler chickens, native chickens and Japanese quails. The commercial preparation was analyzed and found to contain sufficient enzyme activities (Rutherfurd et al., 2007). The enzyme preparation was incorporated in diet at level of 50g/100 kg. The activities of various enzymes in a gram of commercial enzyme preparation were: ß glucanase, 35732; ß-D xylanopyrosidase, 98466; xylanase, 2202; FT Pase, 397; amylase, 5773 and CM cellulase, 1906 MIU/kg. There was marginal improvement in growth of broiler chicks in pearl millet based diets during starting phase while significant improvement was observed in finger millet based broiler starting and finishing diets. Enzyme supplemented was also found beneficial to improve utilization of sunflower seed meal supplemented in maize-finger millet or maize-sorghum based broiler

In vitro evaluation of non-starch polysaccharide digestibility of feed ingredients by enzymes

Some of the commonly used feed ingredients for poultry (corn, sorghum, finger millet, deoiled rice bran, soya bean meal, peanut meal, sunflower meal and rapeseed meal) were screened for pentosans, cellulose, pectin and total non-starch polysaccharides. The ingredient *in vitro* digestibilities by enzymes were evaluated (Lumpkins et al., 2004). Cereal samples screened contained mainly pentosans. Pectin content was rich in oilseed meals. Sunflower meal, soybean meal, deoiled rice bran and a broiler starter diet were subjected to a 2 stage in vitro digestion assay with 3 different enzyme mixtures *viz*. Enzyme-I (xylanase + cellulase from Trichoderma viridae), Enzyme-II (xylanase + Cellulase + beta-glucanase from Huminicola insolens) and Enzyme-III (xylanase + cellulase + pectinase + beta-glucanase from *Aspergillus aculeatus*) by incubating 0.1 g of the sample with 3 ml of a pepsin-HCl mixture (2000 U pepsin/ml of 0.1 N HCl) for 45 min to simulate the peptic phase of bird digestion. A panereatin-$NaHCO_3$ mixture (2 mg panereatin/ml of 1 M $NaHCO_3$) was used for 2 h at 40°C to simulate the panereatic phase. Digestibility was assessed by measuring the relative viscosity of the digesta supernatant and the total sugars released. Enzyme-I produced the least relative viscosity and highest total sugars in sunflower meal, deoiled rice bran and broiler starter diet, and whereas, Enzyme-III was very effective in soyabean meal subjected to *in vitro* digestion (Jia et al., 2008).

Prospects of microbial phytase in phosphorus utilization by poultry

Upto about 85% of the total phosphorus found in feedstuffs of vegetable origin, particularly the cereals, cereal by-products and oil cakes is present in form of phytic acid (inositol hexa-hosphate). Under most

Factors affecting phosphorus bioavailability

The practical poultry rations are largely composed of vegetable oil cakes, meals, grains, and their byproducts. Phosphorus bioavailability from such feedstuffs is only about 30%. The hydrolysis and absorption of phytate phosphorus by monogastric animals are complex processes that are affected by certain factors namely- dietary calcium and inorganic phosphorus (or available phosphorus) levels, vitamin D3, age and type (genotype) of birds, types of dietary ingredients, sources of fibre in diet and feed processing etc. The values reported in the literature on the availability in most of the single and many feed mixtures are variable. In feedstuffs of plant origin, the P occurs in association with phytates and the variation in its availability may party be due to the presence of the endogenous phytase which hydrolyses phytate and affects P bioavailability.

High dietary levels of calcium have been reported to have a slightly negative effect on phytate P utilization in pullets and laying hens. It has been reported that a chick's ability to retain phytate P increases when the diet

dietary conditions the phytate phosphorus is poorly utilized by monogastrics including poultry and, consequently, excreted via the feces. The bird's need for phosphorus, therefore, has to be met through supplementing diet with inorganic phosphorus sources such as defluorinated phosphate or the dicalcium phosphate (Tamim et al., 2004). The traditional practice of adding inorganic phosphorus in poultry ration adds to the cost of feeding besides constraints in finding the right kind of stuff for regular use.

Phytate problem

Presently, about 12 million tones of compounded poultry feed are being manufactured annually. Considering the minimum level of l% addition in diet, about 0.12 tones of dicalcium phosphate of worth USD400, 000 is required.

Besides being an indigestible constituent the phytate phosphorus tends to act as an anti-nutritional factor in diet in that it possesses strong chelating properties thereby markedly reducing the bioavailability of several multivalent cations mainly the Ca^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} and Fe^{3+} by forming insoluble, phytate-metal, complexes. Moreover, the phytate also decreased protein digestibility through formation of indigestible protein-phytate complex and strongly inhibit the activity of amylases (Nitsan et al., 1991).

Most feed ingredients of plant origin contain from 55-85% of the total phosphorus in phytate form. The cereal by- products like wheat or rice brans and oilseed meals usually possess higher levels of total phosphorus than that in whole grains and with a larger proportion bound as phytate (Rutherfurd et al., 2007).

contained inadequate levels of non-phytin phosphorus (NPP) compared to normal phosphorus levels. Phytate P is more efficiently retained when chickens are fed suboptimal levels of NPP. It is generally conceded that older birds hydrolyze phytate phosphorus to a greater extent than chicks, the basis of this being that there is more of the phytase activity present in the gastrointestinal tract of older birds.

Limited reports indicate that there may be breed and strain differences in the utilization of phytate phosphorus. In view of such undesirable effects of phytic acid, it is preferred to either remove it altogether or reduce its amount in poultry feed or ingredients. Efforts have been made in different laboratories to either eliminate or reduce phytic acid content in plant feedstuffs through chemical methods, solid state fermentation technology, autolysis or by the use of phytase enzyme in diet. Of these, supplementation of diet with microbial phytase appears to be more promising (Angel et al, 2006). In early seventies, Dr.T.J.Nelson and his group at International Minerals and Chemical Corporation, Libertyville, Illinois were among the first to report that inclusion of fungal phytase from *Aspergillus ficuum* in diet of broilers resulted in a marked improvement in the utilization of phytate phosphorus and also in bone mineralization. However, because of the high-anticipated production cost of enzyme and a relatively lower cost of di-calcium phosphate or the de-fluorinated rock phosphate, results of their work could not be commercialized (Camden et al., 2001).

Making enzyme to work

Considering an increased awareness amongst the masses and the environmentalists against high phosphorus in animal wastes in many of the developed countries and a rapid advancement in fermentation technology, interest in application of phytase enzyme in improving phosphorus utilization by poultry has further been renewed. In this direction, a great number of phytase, hydrolyzing microorganisms have been isolated. The strains of *Aspergillus* capable of producing phytase enzyme can greatly meet expectations of the nutritionists. Moreover, steps have been taken towards economic production of phytase through conveying phytase gene in the original wild *Aspergillus* strain on to the production strain thereby resulting in a considerable increase in production efficiency (Hughes et al., 2008).

Effect of microbial phytase on the performance of meat type birds

Considerable work has been done in recent years on the effectiveness of supplemental microbial phytase in improving the P bioavailibity in poultry. Microbial phytase supplementation @ 250-500 units/kg in diet increased body weight gain and feed intake in broiler chicken. However, as a result of simultaneous increase in body weight gain and feed intake, several reports have indicated that microbial supplementation had no significant effect on feed to gain ratio in broiler chicken. Improvement in chick performance, however, has been related to an improved utilization of dietary phosphorus. The phytase supplementation of diet was found associated with a significant increase in plasma inorganic phosphorus concentrations, tibial ash percentages, as well as improved absorption and reduced excretion of dietary phosphorus. However, contrary to these reports, the addition of dietary phytase to corn-soya diet containing less phosphorus than the NRC (1994) recommendation did not improve either body weight gain or feed intake, but it did increase toe and tibia ash and plasma inorganic phosphorus in broiler chickens, growing quails and guinea fowl. The apparent cause of dissimilar results may partly be attributed to the complex nature of factors influencing the bio-efficacy of phytase and utilization of phytate phosphorus.

Nonetheless, the phytase supplementation to a standard broiler diet can allow the reduction of the usual addition of inorganic phosphate in the diet and also reduce the amount of P excretion into the environment. Enhanced level of vitamin D_3 can also be used as an alternative to phytase to improve phytate availability in low Ca and NPP diets (Meng et al., 2004).

Effect of microbial phytase on the performance of laying birds

As layer feed differs from broiler feed in respect of among other things, a very high calcium content, the supplemental phytase may just not be as effective in presence of high dietary calcium (3.5%) as in case of broilers (1.0%). Experiments carried out in this direction indicated that phytase supplemented layer diets @ 250-300 units/kg improved laying performance, tibial bone ash and phosphorus absorption. The phosphorus excretion also decreased significantly in birds fed the phytase-supplemented diet. Quite contrary to these findings. Some workers did not find any improvement in the production performance of laying birds. However, it has been demonstrated that phytase supplementation @ 250 units/kg in layer eliminated inorganic P supplementation without affecting laying performance.

Effects of microbial phytase on mineral utilization

Phytic acid has strong chelating potential and forms a variety of complexes with cations and proteins, rendering, these nutrient biologically unavailable. Theoretically, when phytic acid is hydrolysed by microbial phytase, all minerals bound to it should be released.

Relatively, very little work has been done on the effect of phytase supplementation on the availability of minerals. Reports on this aspect suggested that contents of ash and minerals in tibia such as Ca, P, Mg, Zn and Fe were significantly higher in phytase supplemented layer diets (Nitsan et al., 1991).

Effects of phytase on nutrients digestibility

Phytase not only improves mineral and trace element utilization, but may also enhance the protein digestibility and energy utilization. Some of the recent reports conducted abroad have clearly shown that the supplemental phytase improved apparent ileal digestibility of nitrogen and amino acids (lysine, methionine,cysteine, threonine) and dietary energy (Tamim et al., 2004).

At the present time, the benefit from adding microbial phytase to poultry diets on the utilization of minerals and trace elements is undisputed, because many studies have shown consistent effects with differing diets. Thus, it is widely accepted that microbial phytase is an effective means of improving the economics of broiler/layer production. The use of phytase has become accepted all over the world, especially in countries with intensive animal production on limited acreage, where reduction in phosphorus output from animal manure is of ecological concern. Phytase contributes to alleviating excessive P output.

Conclusion

Phytase Efficacy

Given the scale of resources that have been applied to phytase research over the last several years, the degree of success in terms of liberation of P from PA and improving P retention is disappointing. From the studies reviewed it appears that a number of causes can be identified that are leading to this reduced efficacy including insufficient enzyme distribution within the feed matrix, feed processing practices such as excessive pellet temperatures that reduce enzyme activity, over conservative ingredient and dietary P specifications and inaccessibility of PA to phytase within the intestine. In light of these findings and new data showing additive effects of phytase and various carbohydrases, it seems pertinent that we rethink how phytase is incorporated into commercial feeding programs if optimal economic returns are to be realized.

NSP Enzymes

In parts of the world where hard cereal ingredients predominate poultry diets, NSP enzymes have been successfully applied into poultry programs. However, when these same enzyme activities are applied to corn/SBM based diets, performance responses have been less successful. When the actual constituent NSP in corn and SBM are analyzed it becomes clear that an extensive blend of carbohydrases must be supplemented if any performance response is to be achieved. This is likely why minimal performance improvements have been reported in corn/SBM based poultry diets supplemented solely with xylanase and β-glucanase or a combination of xylanase, amylase and protease. Rather a complex blend of multiple carbohydrases is required to depolymerize the NSP present in the diet, which will lead to predictable improvements in BWG, FCR and nutrient digestibilities. Thus, in order to achieve viable and consistent economic returns in commercial poultry feeding programs, the correct blend of multiple carbohydrases, including cellulases, pectinases, xylanases, glucanases, mannanases, and galactanases must be applied.

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