Role of microbial phytase in broiler nutrition- A review

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Abstract

During the past decade, the inclusion of microbial phytase in poultry diets has increased remarkably, mainly in response to heightened concerns over phosphorus (P) pollution of the environment. The capacity of this feed enzyme to release phytate-bound P and reduce P excretion is now well documented. Effectively, phytase is an alternative, economical P source and, as global phosphate reserves are not renewable, this is beneficial to their preservation. Alternatively, dietary phytate concentrations may be reduced by the inclusion of selected, low-phytate feedstuffs or dephytinised feed ingredients. There is a distinct possibility that phytate negatively influences protein and energy utilisation in poultry and, as these influences would be ameliorated by phytase. Responses in amino acid digestibilities following phytase supplementation are variable and the underlying mechanisms have not been completely understood. The impact of phytase on protein and energy utilisation may be more positive than generally realized, but this should become increasingly evident if greater phytate degradation rates can be achieved. The practical acceptance of microbial phytase in poultry diets will re-define nutrient requirements, particularly in relation to P and calcium, and increasingly contribute to ecologically sustainable poultry production in the future. This would be facilitated by a more fundamental research focus, which, arguably, has been lacking in the past.

Keywords: microbial phytase; phytate; broiler; poultry.

Introduction

The inclusion of feed enzymes in poultry diets to enhance nutrient utilisation and performance by counteracting the negative influence of targeted substrates has become commonplace within the last two decades. Most of the poultry diets in our country are formulated with maize-soybean as a basic raw material. Due to increase in price and non-availability of these ingredients, the nutritionists have to formulate the diets alternatively with the agro-industrial by products and non-conventional feed resources. Despite the availability of the agro-industrial by products in abundance of relatively cheaper prices, their inclusion in poultry diets is limited because of presence of incriminating factors. Among these, Phytate and Non-Starch Polysaccharides (NSP) present in the endosperm cell walls adversely affected the performance by lowering the nutritive value of the feedstuffs. Because of lack of endogenous enzymes to hydrolyze Phytate, phosphorus is biologically less available to poultry. In order to counteract the adverse effect of such substances feed enzymes are added to the diet not only to improve the nutritive value but also to economize poultry production and reduce environmental pollution. A number of studies have shown that inclusion of microbial phytase in broiler diet release the phytate bound P and to improve the utilisation of P in plant derived ingredients including energy and amino acids (Ravindran et al., 1995; Coelho and Kornegay, 1999; Selle et al., 2000).

Phytate

Phytic acid (*Myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate*) is the major storage form of P in plants. Phytate P consists of 60-80% of the total P in grains and their by-products (Ravindran et al., 1995).

Phytate bears six P groups on one 6-carbon molecules. At neutral pH the phosphate groups in phytic acid have either one or two negatively charged oxygen atoms (Reddy et al., 1982). Therefore, various cations can chelate strongly between two phytate groups or weakly with a single phosphate group. As a result, phytic acid can bind various mineral elements and amino acids, and reduce their bio-availability.

Phytate phosphorus concentration in various feed ingredientS

Ingredients	Phytate P (g/100g dry matter)	Phytate P (as % of total P)
Cereals		
Barley	0.27	64
Corn	0.24	72
Rice(unpolished)	0.27	77
Sorghum	0.24	66
Wheat	0.27	69
Cereal by products		
Rice bran	1.03	80
Rice polishing	2.04	89
Wheat bran	0.92	71
Grain legumes		
Field peas	0.24	50
Oilseed meals		
Rapeseed meal	0.70	59
Sesame meal	1.02	81
Soyabean meal	0.39	60
Sunflower meal	0.89	77

(Ravindran et al., 1995)

The level of phytate P in a feedstuff generally depends on the part of the plant from which it is derived. In general, oilseed meals and cereal by-products contain large amount of phytate P, where as cereals and grain legumes contains only moderate amount (Ravindran et al., 1995). The proportion of phytate P varies from 60-80% of the total P in the seed of cereals, grain legumes and oil bearing plants. In most cereals, phytic acid is not uniformly distributed in kernel, but associated with specific morphological components of the seed (Oberleas et al., 1990). Phytate concentration in plant materials depend on several factors including the stage of maturity, degree of processing, cultivar, climate, water availability, soil, geographical location and year (Ravindran, 1999b).

What is phytase?

The phytate can be hydrolysed by phytases. There are three sources of phytases namely plant phytase, intestinal phytase and microbial phytase.

(i) Plant phytase

Endogenous phytase activity in feedstuffs is variable. The highest activities are reported in rye, wheat and wheat bran (Ravindran et al., 1995). In contrast corn, sorghum, oilseeds have very little endogenous phytase activity. Published data on the effects of plant phytase activity on animal performance is limited.

(ii) Intestinal phytase activity

The presence of intestinal phytase activity in poultry is controversial. Liebert et al. (1993) reported that the phytase activity in the contents of the crop, stomach and small intestine of chicken is negligible. Kornegay (1999) stated that the significance of phytase produced by microorganism residing in the intestinal tract is negligible. Maenz and Classen (1998) however reported that intestinal brush border alkaline phosphatase could contribute to degradation of phytate P. The specific and total activities of alkaline phosphatase in intestinal brush border were highest in the duodenum and declined in the jejunum and ilium.

(iii) Microbial phytase:

Microbial phytase can be found in numerous bacteria, yeast and fungi. *Aspergillus* is the most widely used fungi in the commercial production of microbial phytase.

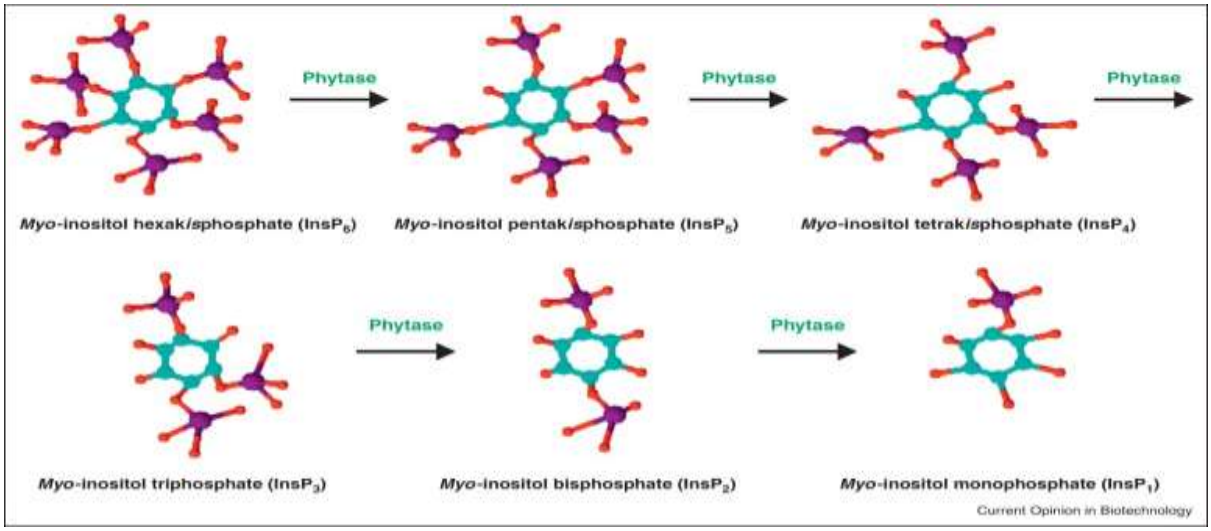
Two types of commercial phytase products are available:

1. One is derived from **submerged liquid fermentation** that uses genetically manipulated organisms to achieve maximum enzyme production.
2. The other type of product is based on **Solid-state fermentation** that uses normal organisms for enzyme production.

Phytase feed enzymes fall into two categories depending on the site where the hydrolysis of the phytate molecule is initiated. 3-Phytase (EC 3.1.3.8) preferentially liberates the P moiety at position C3, whereas 6-phytase (EC 3.1.3.26) commences at position C6 of the myo-inositol hexaphosphate ring.

Several distinct microbial phytase products are now commercially available. The three commonly used phytase feed enzymes are derived from *A. niger*, which is a 3-phytase and *Peniophora lycii* and *Escherichia coli*, which are 6-phytases. Phytase feed enzymes may be included in poultry rations as granulates or as liquids, via post-pelleting application systems, to avoid thermostability problems at high pelleting temperatures (>80 ◦C).

Mechanism of action



(Kebreab et al., 2012)

In theory, enzymic hydrolysis of phytate generates a series of lower myo-inositol phosphates esters (IP6⇒IP5⇒IP4⇒IP3⇒IP2⇒IP1), via a progression of step-wise dephosphorylation reactions, to yield inositol and six inorganic P moieties. Consequently, hydrolysis of phytate by phytase is more likely to yield myo-inositol monophosphate (IP1) and five inorganic P moieties.

At last due to presence of phytase with water at pH 5.0 myo-inositol hexaphosphate is converted into free myo-inositol and onorganic orthophosphate is liberated from the phytate structure.

Units of measurements

i) One unit of phytase (U): It is defined as amount of enzyme that liberates 1 µmol inorganic P per minute from 0.0015 mol/L sodium phytate at pH 5.5 at 37^{o}C. (Camden et al., 2001)

(ii) One unit of phytase activity (FTU): It is defined as amount of enzyme required to liberate 1 µmol of inorganic orthophosphate from 0.0015 mol/L sodium phytate per min. at 37oC and pH 5.5. (Engelen et al., 1994).

Effect on growth performance and feed efficiency

Since the report of Simons et al. (1990), several hundred investigations into the effects of various microbial phytases on growth performance of poultry have been completed, which precludes their individual

consideration. Predictably, the addition of phytase to P-inadequate diets has been consistently shown to enhance growth performance. In the study of Simons et al. (1990), 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 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. Generally, responses to phytase in feed intake and weight gain are more robust and consistent than feed efficiency responses which were suggested by Onyango et al. (2005) and Bahadoran et al. (2011). Rosen (2003), from multi-factorial analyses of phytase feeding trials, argues that feed efficiency responses to phytase have been declining with time, which he attributes to concurrent improvements in broiler strains, feeds and management techniques.

Phytase supplementation of P-adequate broiler diets has been shown to generate equivocal growth performance responses, which may be mediated by dietary nutrient specifications. For example, in a study reported by Selle et al. (1999), standard and modified sorghum-based diets were offered to broilers from 7 to 25 days of age, without and with 600 FTU kg−1 phytase. The modified diets contained reduced specifications in P, Ca, protein/amino acids and energy density. Phytase did not influence growth performance of broilers on standard diets but significantly increased weight gain (7.6%) and feed efficiency (4.7%) in modified diets. Moreover, there was a significant interaction between diet type and phytase addition for feed efficiency.

Logically, the magnitude of responses to phytase will be more pronounced with increasing inclusion rates of the feed enzyme 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 phytase inclusions are associated with substantial increases in total tract phytate degradation ranging from 0.403 to 0.948. At such extreme inclusion rates, however, the possibility arise that any minor enzymic side-activities that may be present in the phytase preparation may become significant, impacting independently on nutrient utilisation.

In diets with higher P levels, increasing phytase inclusion rates do not necessarily generate more pronounced responses in broilers. For example, there were remarkably little differences in responses to 400 or 800 FTU kg−1 phytase over a wide range of parameters in broilers as reported by Cabahug et al. (1999) and Ravindran et al. (2000). Moreover, the addition of seven levels of phytase activity (0–1000 FTU kg−1) to broiler diets containing 7.5 and 3.0 g total P kg−1 were investigated by Ravindran et al. (2001). While 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).

Effect on Amino acid digestibility

The magnitude of amino acid responses with supplemental phytase appears to be dependent on the ingredient used. This may be related to the concentration, structure and storage site of phytate in a particular ingredient and the examples of soybean meal, canola meal, cottonseed meal, sunflower meal, sorghum, wheat, maize, rice polishing and wheat middling are discussed below by Ravindran et al., (1999a) that there was significant increase in indispensible and dispensible amino acid (%) in treatment group as compare to control group and highest response were seen in soybean meal, wheat and rice polishing as followed by other ingredients. In study of Dilger et al., (2004), both type of amino acid indispensible and dispensible are significantly increase in treatment group supplementing with two levels (500 and 1000 FTU/kg) of phytase as compare to negative control group.

Effects on apparent metabolizable energy (AME)

The possibility that supplementary phytase has a positive effect on energy utilisation in poultry has considerable practical implications. Early studies involving dephytinised feed ingredients suggested that phytate negatively influences energy utilisation in broilers (Rojas and Scott, 1969; Miles and Nelson, 1974). Very few studies have been completed in layers; however, Scott et al. (2001) found that phytase increased AME in both maize (13.84 MJ kg−1 DM *versus* 13.36 MJ kg−1 DM) and wheat (14.57 MJ kg−1 DM *versus* 14.04 MJ kg−1 DM) based layer diets. Alternatively, Liebert et al. (2005) reported that phytase supplementation of maize–soy diets did not enhance N-corrected AME in layers. Thus, this discussion is confined to broilers and exogenous phytase has quite consistently increased AME of broiler diets based on Park et al. (2000) wheat and/or sorghum in studies completed at the University of Sydney (Ravindran et al., 1999b, 2000, 2001; Selle et al., 1999, 2001, 2003, 2005).

In phytase experiments, wheat may be pre-pelleted separately to eliminate intrinsic phytase activity as it might compromise responses to microbial phytase. This approach was adopted in one study (Selle et al., 2001), in which phytase did not enhance energy utilisation. Interestingly, there are indications that extrusion of wheat reduces solubility of protein and phytate (Ummadi et al., 1995a,b), which may render phytate less susceptible to hydrolysis. Interestingly, have shown that heat-treatment of rapeseed meal reduces phytate degradation by ruminal fermentation in sheep. It seems possible, therefore, that the prior steam-pelleting of wheat *per se* may have contributed to the lack of a response to phytase. However, Edwards et al. (1999) found that extruding maize–soy

broiler diets reduced phytate-P retention by 13.1%, whereas steam-pelleting diets did not influence phytate-P retention.

Instructively, Camden et al. (2001) evaluated two phytase feed enzymes (*Bacillus subtilis* at 250, 500, 1000; *A. niger* at 500 FTU kg−1) in broilers offered maize–soy diets. Overall, phytase increased ileal digestibility coefficients of fat by 3.5% (0.954 *versus* 0.921), protein by 2.6% (0.866 *versus* 0.844) and starch by 1.4% (0.978 *versus* 0.964). This was associated with phytase-induced increases in AME of 0.17 MJ (15.29 MJ kg−1 DM *versus* 15.12 MJ kg−1 DM) and apparent ileal digestibility of energy of 0.26 MJ (16.34 MJ kg−1 DM *versus* 16.08 MJ kg−1). Thus, this study indicates, as suggested earlier by Baker (1998), that the positive impact of phytase on energy utilisation stems from an accumulation of increases in fat, protein and starch digestibilities.

Effect on toe and tibia ash

Kornegay et. al. (1996) and Cabahug et al. (1999) reported that the positive response of different inclusive rate of phytase was observed at all levels of nP, although magnitude of the response seemed to decrease as level of nP increased and among them better result was shown at 400 FTU/kg phytase. Both tibia and toe ash increased with supplementation at different inclusion levels of microbial phytase (500 to 1000 FTU/kg) to the negative control diet, ranging from 42 to 47% for tibia and 10 to 12% for toe sample (Camden et al., 2001, Dilger et al., 2004, Onyango et al., 2005). Mondal et al., 2007 observed that the improvement of length and width of tibia by supplementing phytase is indicative of deposition of minerals in bone.

Future directions and implications

The present usage of phytase feed enzymes by poultry producers are substantially greater than anticipated when they were first introduced. Increasing ecological concerns in relation to pollution, a better appreciation of the application of microbial phytases, and their decreasing inclusion costs, has contributed to this increasing acceptance. During the past 15 years, research on the evaluation of microbial phytases in diets for simple-stomached species has rapidly expanded, but much of the focus of this research has been on the evaluation of various phytases from different sources rather than the investigation of the underlying factors causing variability in phytase responses. Fundamental information in respect of phytate and phytase is lacking in many aspects, which needs to be generated and integrated for a more complete understanding of this subject.

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