

## Broiler Performance in Response to Phytate and Supplemented Phytase

### Review Article

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### ABSTRACT

Phosphorus (P) is a macro mineral in broiler nutrition. In growing broilers, besides its requirement for proper bone development, it is also involved in almost all metabolic processes. Poor P availability results in decreased productivity and poor health status. Phosphorus availability from plant derived feeds is affected by an anti-nutritional factor “phytate”, which forms a variety of insoluble salts with most of the minerals including P, calcium (Ca), magnesium, zinc (Zn) and copper (Cu) due to its reactive anion capability. So, phytate is responsible for considerable nutrient losses as vegetable sources form a major portion in broiler diet formulations. Phytate has also been reported to form complexes with protein and proteolytic enzymes (pepsin and trypsin). Mono-gastric animals lack endogenous phytase (an enzyme capable of hydrolyzing phytate bound P, Ca, protein and other nutrients), so phytate decreases the nutrient availability at the intestinal level in poultry. Application of phytase in poultry rations may liberate cations and other nutrients bound by phytate-P complexes resulting in improved production parameters and body structure characteristics in broilers. However, efficacy of supplemental phytase rests on its rate of application, Ca: P in ration, composition of diet, genotype and age of birds. Phytase could ensure the economical poultry production by the exploitation of inherent nutritional potential of feedstuffs. Some studies, however, showed that phytase does not degrade dietary phytate efficiently and thus the negative influence of phytate on protein digestibility is not completely removed by phytase supplementation. More focused research on currently available phytase feed enzymes and their potential improved action by the simultaneous use of other exogenous enzymes, which complement their activity is recommended.

**KEY WORDS** digestibility, phosphorus, phytase, phytate, pollution, poultry, protein.

### INTRODUCTION

Feed has a major economic impact on broiler production, as it contributes 60-70% of total production cost (Coon, 2002). Broiler production may become a more profitable enterprise by obtaining efficient growth and improved feed conversion ratio (FCR). Efficiency of feed utilization may be improved by the application of different feed additives e.g. antibiotics, antioxidants, anticoccidials, pellet binders,

herbal preparations, enzymes (e.g. phytase) and probiotics to broiler rations.

Phosphorus (P) is an essential mineral for growth and skeletal development in chickens and if deficient results in deleterious effects such as skeletal deformities and impaired metabolic processes and ultimately poor nutrient utilization and decreased performance (Scott *et al.* 1982). As P plays an important role in the metabolism of primary nutrients, so its deficiency results in poor health and performance of

chickens (Waldroup, 1999; Hatten *et al.* 2001). Conventional vegetable feed sources have low (up to 30-40%) availability of P (Nelson, *et al.* 1968; NRC, 1994), as P is in bound form phytate. Non-ruminants lack the endogenous enzyme for the hydrolysis of phytate, so there is a need for supplementation of inorganic P (di-calcium phosphate, DCP) to overcome the P deficiency (Sebastian *et al.* 1998). Phytate binds amino acids by the formation of binary protein-phytate complexes in the gastrointestinal tract (as shown in figure 2). These complexes are resistant to pepsin activity. Phytate has also been reported to promote the flow of endogenous amino acids (Thompson, 1988).

### Phytate

In literature, three names are used for the substrate of the enzyme phytase; these are phytate, phytin and phytic acid, although "phytate" is most frequently used, referring to the mixed salt of phytic acid (*myo* inositol hexaphosphate; IP6). Phytin, in particular is the deposited complex of IP6 with calcium (Ca), potassium (K) and magnesium (Mg) that is present in plants. Phytate was discovered more than a century ago (Hartig, 1855). Limited availability of the phytate-P (282 g kg<sup>-1</sup>) in mono-gastric animals is important because of non-renewability of rock phosphate reserves, which, in future, could create a problem fulfilling the high demand for P (Abelson, 1999; Mullaney *et al.* 2000). Worldwide, crop seed and fruit yields contain probably 14.4 million tonnes of phytate bound P, which is about 65% of the annual fertilizer P sales (Lott *et al.* 2000). On average, a commercial poultry ration may contain 2.5 to 4.0 g/kg of phytate-P (Ravindran, 1995). Averill and King (1926) estimated phytate concentrations in human foods but at present many records regarding phytate contents of feed ingredients used in poultry feed are reported by many researchers Table 1 (Nelson *et al.* 1968; Kirby and Nelson, 1988; Eeckhout and de Paepe, 1994; Ravindran *et al.* 1994; Viveros *et al.* 2000; Selle *et al.* 2003; Godoy *et al.* 2005).

Derived from studies by (Nelson *et al.* 1968; Kirby and Nelson, 1988; Eeckhout and de Paepe, 1994; Ravindran *et al.* 1994; Viveros *et al.* 2000; Selle *et al.* 2003; Godoy *et al.* 2005).

Phytate is a poly-anionic molecule and has twelve sites (Figure 1) for chelating positively charged nutrients in the gastrointestinal tract (GIT). Phytate mostly bounds Mg to make complexes with a molar ratio 4.4:1 and a pH of 6 (Evans and Pierce, 1982).

Several researchers have proposed that phytate may be one of the aetiological agents of osteomalacia, or rickets, as it limits the availability of Ca and P in the GIT by binding them (Bruce and Callow, 1934; Krieger *et al.* 1940; Mellanby, 1949; Bhaskaram and Reddy, 1979; Abugassa and Svensson, 1990). At low pH the tendency of phytate to

bind protein increases (Wise, 1983). Cosgrove (1966) concluded that at lower pH phytate reacts with essential amino acids (lysine, histidine, arginine) and sodium phytate also precipitates glycine (Okubo *et al.* 1976). Similarly, Rajendran and Prakash (1993) reported maximum interaction between sesame seed  $\alpha$ -globulin and sodium phytate at pH 2.3; this interaction was also dependent upon the protein to phytate ratio.

Phytate may however also act as an anticarcinogen, probably by acting as antioxidant by the means of metal binding properties (Harland and Morris, 1995).

### Phytate interactions

#### Phytate / protein interactions

Phytate is most commonly associated with the protein of the plant (O'Dell *et al.* 1979). The possibility that phytate has a negative influence on protein utilization in poultry, was first suggested by Rojas and Scott (1969). During ripening, physical phytate-protein association starts in the seed when phytate accumulates in a protein-rich aleurone layer and the protein bodies of monocotyledonous and dicotyledonous seed, respectively (Prattley *et al.* 1982; Ravindran *et al.* 1995a). Feed ingredient processing (e.g. oil extraction and heat treatment) further strengthens the phytate-protein complex. Boland *et al.* (1975) found that phytate from soybean flakes was soluble, whereas phytate from isolated soybean was completely insoluble. Figure 3 demonstrated possible associations of phytate with protein.

Resistance to proteolysis is due to the complex formations between phytate and some proteins. Carmovale *et al.* (1988) conducted an *in vitro* digestibility study of protein using faba beans, peas, whole flour, protein concentrate, protein isolate, lactalbumin, casein, serum albumin and zein. In all cases there was a negative effect of Phytate addition (up to 10 mg) to any protein containing 10 mg of N after incubation for 1 hr at room temperature. This presents strong evidence that phytate-protein interactions negatively affect protein digestibility *in vitro*.

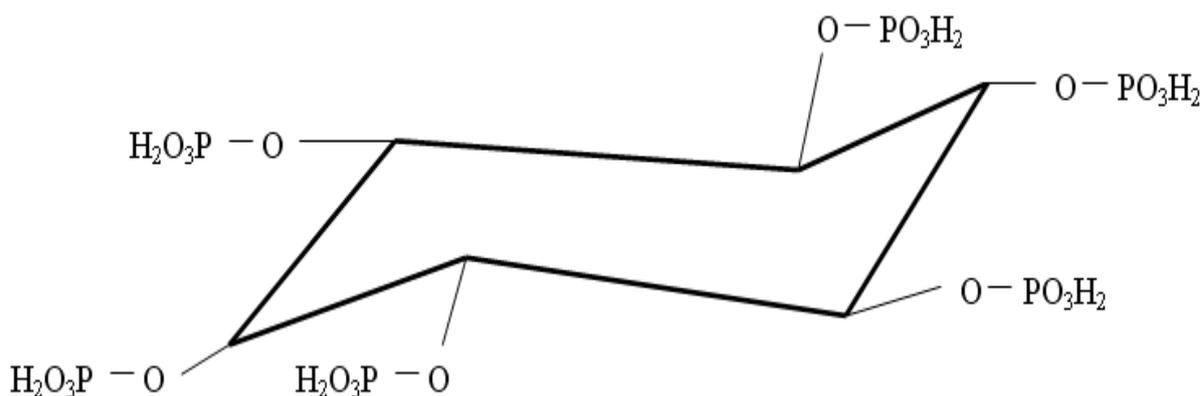
#### Phytate / phosphorus and calcium interactions

In the presence of phytate and added Ca, interference with mineral absorption occurred as a result of the formation of insoluble complexes (Sandberg *et al.* 1993). Normally, when phosphate groups are removed from the inositol hexaphosphate, the mineral binding strength becomes progressively lower and solubility increases.

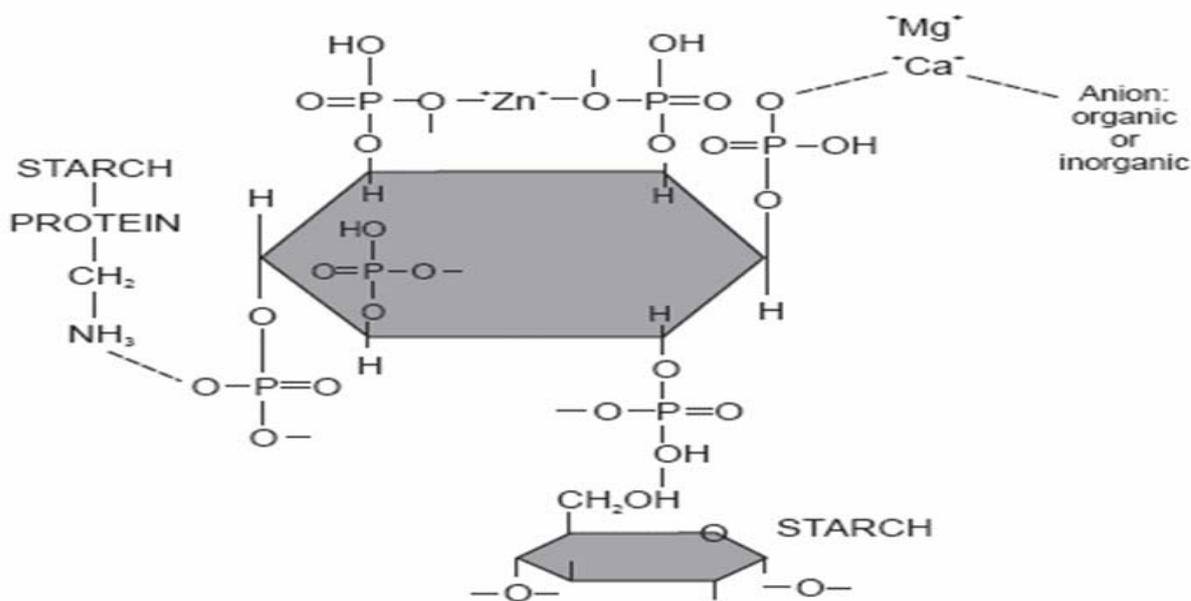
The highest retention of phytate P in chicks (79.4%) was obtained when phytase and 1, 25(OH)<sub>2</sub> D<sub>3</sub> were present in the diet (Edwards, 1993). In animal feeds of plant origin the main source of P is phytate but absorption of P cannot take place until the phosphate groups are removed from the inositol molecule.

**Table 1** Weighed mean (and range) of total P and Phytate-P concentrations, and proportion of phytate-P in key poultry feed ingredients

Feed ingredients	Number of data sets / samples	Total P (g kg <sup>-1</sup> )	Phytate-P (g kg <sup>-1</sup> )	Proportion of phytate P from total P (%)
<b>Cereals</b>				
Barley	4 / 41	3.21±0.48	1.96±0.17	61.0 (59-68)
Maize	7 / 45	2.62±0.30	1.88±0.25	71.6 (66-85)
Sorghum	5 / 41	3.01±0.5	2.18±0.40	72.6 (65-83)
Wheat	6 / 97	3.07±0.17	2.19±0.55	71.6 (55-79)
<b>Oilseed meals</b>				
Canola meal	4 / 28	9.72±1.5	6.45±1.89	66.4 (36-76)
Cottonseed meal	3 / 21	10.02±2.1	7.72±2.1	77.1 (70-80)
Soybean meal	6 / 89	6.49±0.62	3.88±0.5	59.9 (53-68)
<b>By-products</b>				
Rice bran	6 / 37	17.82±6.7	14.17±8.15	79.5 (42-90)
Wheat bran	6 / 25	10.96±3.02	8.36±1.3	76.3 (50-87)

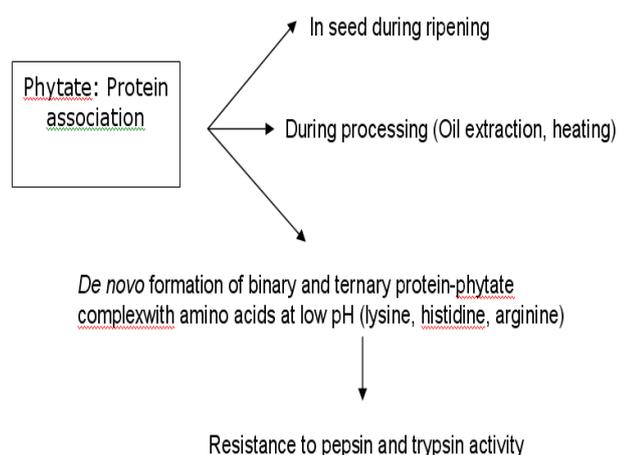


**Figure 1** Structure of fully protonated Phytate (*myo*-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate). Adapted from Graf (1986)



**Figure 2** Phytate-protein-starch complex molecule: a potential structure Adapted from Kies, 2001)

Phytate P in plants is present in the form of phytate complexes. In most of the foods of plant origin, about 50-80% of the total P is bounded in phytate complexes. Biological preparations containing phytase, when incubated with the feed at 1000 units of phytase/kg of feed, have been reported to increase P availability in chickens. Supplementation of phytase and 1, 25 (OH)<sub>2</sub> D<sub>3</sub> has also been shown to improve P utilization as determined by growth and bone ash of broilers (Ketola *et al.* 1993).



**Figure 3** Phytate / protein association (Cosgrove 1966 and Selle *et al.* 2000)

### Phytate / other mineral interactions

Mineral absorption takes place in the upper part of the intestine, where the pH is about 6.0. Zinc (Zn) forms an extremely insoluble complex with phytate at this pH, thus leading to Zn deficiency in broilers fed a diet high in phytates (Maddaiah *et al.* 1964). Deficiency of Zn due to zinc-phytate complexes may be overcome by the supplementation of Zn in diet (O'Dell, 1962; Edwards, 1966; Lease, 1966). Davies *et al.* (1961) demonstrated that phytate-rich diets also decrease the availability of magnesium and copper. McWard (1969) observed poor growth performance of broilers fed a 4% Phytate-soy protein complex in a diet supplemented even with 75 mg/kg of magnesium.

### Phytate / vitamin D<sub>3</sub> interaction

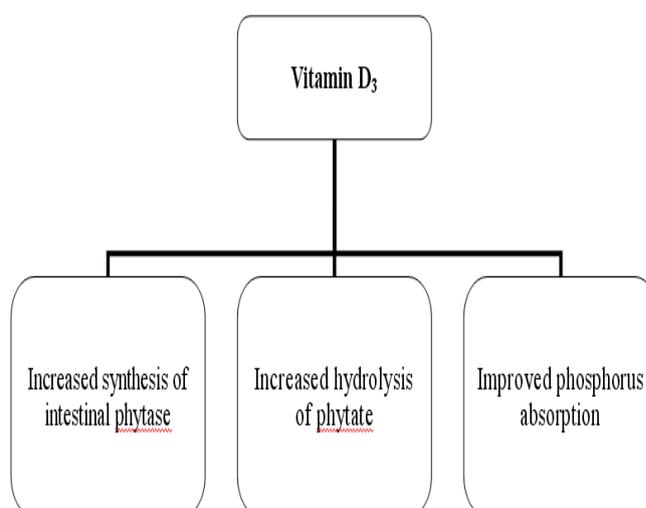
Derivatives of vitamin D<sub>3</sub> such as 1-hydroxycholecalciferol (1-OHD<sub>3</sub>) and 1, 25 (OH)<sub>2</sub> D<sub>3</sub> have been reported to increase phytate P utilization up to 68% (Edwards, 1993).

The effect of these derivatives was more intense when diets low in P were supplemented with a combination of phytase and 1, 25 (OH)<sub>2</sub> D<sub>3</sub> (Yan and Waldroup, 2006). A proposed mechanism through which vitamin D<sub>3</sub> improves phytate P utilization is presented in figure 4.

### Phytate / carbohydrate and fat interaction

Phytate interferes with fat and carbohydrate metabolism. Thompson and Yoon (1984) reported decreased (60%) digestibility values for wheat starch in chickens fed phytate supplemented diets possibly due to inhibition of amylase activity by phytate.

Phytate forms complexes with Ca ions, which are necessary for the activity of  $\alpha$ -amylase (Cawley and Mitchell, 1968), this problem may be alleviated by Ca supplementation (Yoon *et al.* 1983; Thompson *et al.* 1984). Phytate also reduces the digestibility and absorption of fat in the GIT by making insoluble Ca-phytate-fatty acid complexes, as shown in figure 5 (Leeson, 1993). Many researchers reported deleterious effects of phytate on endogenous secretion and energy utilization in chickens (Cowieson *et al.* 2004, 2005; Ravindran *et al.* 2006; Cowieson and Ravindran, 2007; Liu *et al.* 2008).



**Figure 4** Vitamin D<sub>3</sub> and phytate interaction

### Phytase

Suzuki *et al.* (1907) discovered an enzyme capable of degrading phytate to inositol and orthophosphoric acid, while pursuing hydrolyzing studies for rice bran.

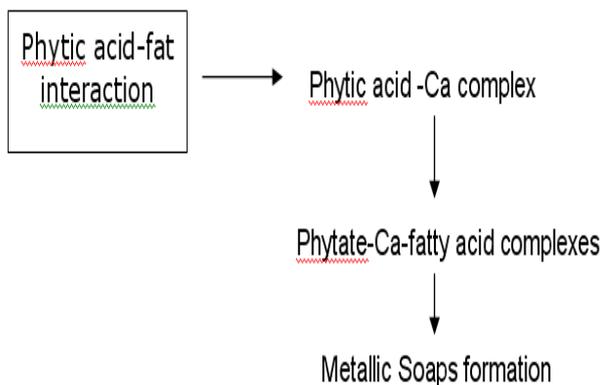
That enzyme which was present in rice bran was termed as "phytase" or "myo inositol hexakisphosphate phosphohydrolase" (EC 3.1.3.8). However, commercial production for phytase started in 1991 in Netherlands, in response to legislation regarding increased P pollution. Phytase is now commercially available and incorporated in poultry diets to achieve better feed utilization efficiency.

### Source, action and activity of phytases

Phytase may be of plant, animal or microbial origin. Sebastian *et al.* (1998) reported four possible sources for phytase:

1. Intestinal phytase found in digestive secretions (animal origin)
2. Phytase originating from microbes in the digestive tract (e.g. as in ruminants)
3. Endogenous phytase from plant feed-stuffs
4. Phytase produced by exogenous micro-organisms

Microbial sources are considered to be more widely used for commercial phytase production. *Aspergillus sp.* is commonly used, particularly the strains *A. niger* and *A. ficuum*, by commercial producers. Shieh and Ware (1968) reported possible microbial sources for phytase (Bacterial and fungal sources).



**Figure 5** Phytate and fat interaction (Selle *et al.* 2006)

Phosphorus in plants normally exists in the form of a P-phytate complex. Phytate consists of a sugar (similar to glucose) called *myo* inositol, to which phosphate (PO<sub>4</sub>) groups are covalently linked. Phytase releases these phosphates from the inositol ring as shown in figure 6. Phytase activity depends upon the intestinal pH (Baruah *et al.* 2004).

Phytases from different origins may have different optimal pH (Onyango *et al.* 2005). Most of the microbial phytases have their optimum activity around pH 5.0 (acid phytase) although phytases from *Bacillus* spp. work optimally around pH 8.0 (alkaline phytase; Baruah *et al.* 2007b; Selle *et al.* 2007). However, almost all plant phytases work optimally around pH 5.0 (Wodzinski and Ullah, 1996). Phytase activity is measured as Phytase Units (FYT or PU), or Units (U). One FYT is the amount of enzyme required to liberate 1μMol inorganic phosphate / minute from 5.1 mM of sodium phytate at pH 5.5 and 37 °C (Engelen *et al.* 1994). Phytase activity varies between sources (Steiner *et al.* 2007). Activity of phytase for different ingredients is shown in Table 2.

### Classification of phytase

There are two types of phytase, EC 3.1.3.8 or 3-phytase and EC 3.1.3.26 or 6-phytase (The International Union of Biochemistry and Molecular Biology (IUBMB) in consultation

with the International Union of Pure and Applied Chemistry-International Union of Biochemistry (IUPAC-IUB), Joint Commission on Biochemical Nomenclature (JCBN).

Although 3-phytase and 6-phytase are regarded to be of microbial and plant origins, respectively (Reddy *et al.* 1982) and Phillippy *et al.* (1985) reported 3-phytase activity in soybeans, and 6-phytase activity has been observed in *E. coli* (Greiner *et al.* 1993). Optimum pH for microbial phytase is 2 to 6 and for plant phytases is 5 (Wodzinski and Ullah, 1996).

#### 1. EC 3.1.3.8

3-phytase (systematic name: *myo* inositol hexakis phosphate-3-phosphohydrolase) hydrolyzes the ester bond at the third position of *myo* inositol hexakis phosphate to produce d-*myo*-Ins-1, 2, 4, 5, 6-pentakisphosphate and orthophosphate.

#### 2. EC 3.1.3.26

6-phytase (*myo* inositol hexakis phosphate-6-phosphohydrolase) hydrolyzes the ester bond at the 6<sup>th</sup> position of *myo* inositol hexakisphosphate to produce d-*myo*-Ins-1, 2, 3, 4, 5-pentakisphosphate and orthophosphate. While, Mullaney and Ullah (2003) proposed three classes of phytases based on their structural formula and differences in catalytic properties.

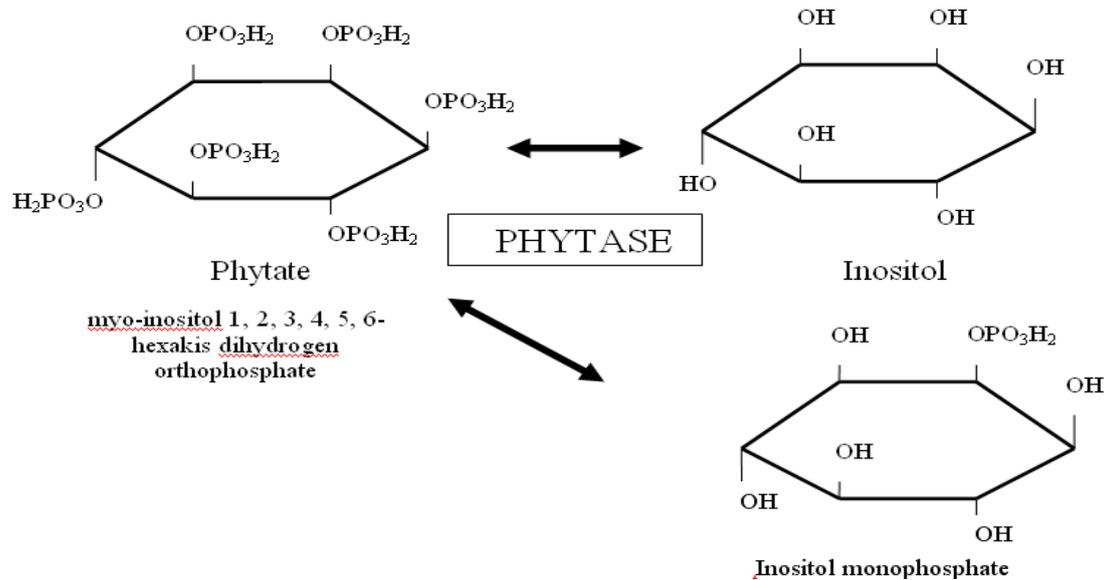
These include members of histidine acid phosphatases (HAPs), betapropeller phytase (BPP) and purple acid phosphatases (PAP).

### Interactions between Phytase and phosphorus or calcium

Phytase supplementation can be an important tool to release P from plant derived feed ingredients.

Nelson *et al.* (1968) found that the utilization of phytate P supplemented with crude phytase was similar to that of inorganic P. In Nelson *et al.* (1971) found better utilization of phytate P in a phytase supplemented corn-soy diet. Ravindran *et al.* (2000) found increased P availability in wheat-sorghum-soy based diets containing low (0.29%), medium (0.37%) and high (0.44%) phytate P supplemented with phytase (800 FYT kg<sup>-1</sup>).

Simons *et al.* (1990) observed increased bio-availability of P (up to 60%) and decreased dropping P (up to 50%) in diets supplemented with phytase. Release of P from phytate depends upon the sources of phytate and phytase and Ca and vitamin D3 contents (Simons *et al.* 1990; Edwards, 1993; Schoner *et al.* 1993; Yi *et al.* 1994; Ravindran *et al.* 1995b; Qian *et al.* 1995; Kornegay *et al.* 1996). Phytase supplementation results in increased blood P (Perney *et al.* 1993; Broz *et al.* 1994). Sebastian *et al.* (1994) also found increased plasma P in corn-soy diets supplemented with phytase (500 FYT/kg diet).



**Figure 6** Schematic mode of action of microbial phytase on dietary phytates (Baruah *et al.* 2004)

**Table 2** Phytase activity, total phosphorus and phytate phosphorus content in legume seeds, cereals and cereal by-products<sup>a</sup>

	n <sup>b</sup>	Phytase activity (FYT/kg <sup>-1</sup> )	Total P (g/kg <sup>-1</sup> )	Phytate P (g/kg <sup>-1</sup> )	Proportion of total P
<b>Legume seeds</b>					
Field beans	11	290±78	5.7±0.92	3.9±0.75	0.70±0.052
Peas	18	262±73	4.1±0.46	2.4±0.45	0.58±0.060
Lupins	14	324±59	5.7±1.48	3.5±0.92	0.63±0.037
<b>Cereals</b>					
Oats	6	496±35	3.7±0.14	2.5±0.17	0.67±0.054
Wheat	18	2886±645	4.0±0.40	2.9±0.37	0.73±0.081
Barley	15	2323±648	4.2±0.42	2.6±0.31	0.63±0.035
Triticale	12	2799±501	4.0±0.34	2.8±0.30	0.70±0.054
Rye	13	6016±1578	3.6±0.19	2.4±0.23	0.67±0.050
<b>Cereal by products</b>					
Wheat bran	3	9945±0427	8.8±0.71	7.9±0.05	0.90±0.073
Rye bran	3	9241±1452	5.8±0.25	4.9±0.23	0.85±0.029

<sup>a</sup> mean±standard deviation (dry matter basis).

<sup>b</sup> number of observations (Steiner *et al.* 2007).

Mitchell and Edwards (1996) reported that supplementation of phytase with 1, 25-dihydroxy cholecalciferol (5 µg/kg diet) resulted in increased plasma P. Sebastian *et al.* (1994) observed increased P retention (up to 12.4%) in male broiler chicks by the supplementation of phytase (500 FYT/kg diet). Likewise, Francesch and Geraert (2009) found decreased P excretion in corn-soy diets supplemented with a multi-enzyme complex containing non-starch polysaccharide enzymes and phytase.

Many studies have indicated that the supplementation of phytase results in improved Ca availability in broiler chicks (Schoner *et al.* 1993; Yi *et al.* 1994; Sebastian *et al.* 1996a; Singh *et al.* 2003a, b). Qian *et al.* (1996) observed a linear increase in Ca retention with increasing phytase supplementation. Sebastian *et al.* (1994) found increased Ca retention (up to 12.2%) in male broilers supplemented with phytase (500 FYT/kg diet).

Ahmad *et al.* (2000) also reported that phytase increased Ca availability in low P diet. Bone ash contents are a more susceptible and reliable index for the determination of phytate P usage and are rendered more precise than the body weight gain.

Nelson *et al.* (1971) supplemented a corn-soy diet with phytase and found increased tibial bone ash in broiler birds, which might be credited to improved bio-availability of phytate P. Increased tibial and toe ash was also observed by Perney *et al.* (1993) in phytase supplemented broilers. They further stated that phytase supplementation resulted in better mineralization of bone by improving the availability of Ca and P.

Broz *et al.* (1994) also reported higher tibial ash in phytase supplemented broilers as compared to the control. Denbow *et al.* (1995) found a linear increase in tibial and toe ash in broilers fed diets containing 0.20, 0.27 and 0.34%

phytate P supplemented with increasing phytase level (200, 400, 600, 800, 1000 and 1200 FY/kg<sup>-1</sup>). Cabahug *et al.* (1999) reported improved ash contents in wheat-sorghum-soy based diets with different phytate P concentrations (2.9, 3.7 and 4.4 g kg<sup>-1</sup>diet) supplemented with phytase. Zanini and Sazzad (1999) also observed improved tibial ash contents in broilers fed high energy diets (11.72 and 12.25 MJ kg<sup>-1</sup> diet) supplemented with phytase (1000 FYT/kg<sup>1</sup>).

#### Interactions between Phytase and other minerals

Phytate has strong chelating potential to form a variety of complexes with cations (Ca, Mg, Zn, Mn and Cu), rendering those cations biologically unavailable (Maddaiah *et al.* 1964; Davies and Reid, 1979). Zinc has been reported to have the strongest affinity to form complexes with phytate (Reddy *et al.* 1992; Gifford and Clydesdale, 1990). Zn deficiency is commonly believed to result from feeding a high phytate containing diet (Oberleas and Harland, 1996). Sebastian *et al.* (1996b) reported that supplementing the diet with microbial phytase resulted in increased Zn retention from 27.6% to 34.7%. Yi *et al.* (1996b) also found microbial phytase to be effective in improving Zn utilization (as judged by Zn retention and concentration in tibial ash) in broilers fed corn-soy diets with a lower Zn concentration.

They also reported that 100 units of phytase released about 0.9 mg of Zn. Brenes *et al.* (2003) concluded that the addition of phytase to maize and soybean meal based diets improved the performance and Zn utilization in chicks. Little work has been done to evaluate the effect of phytase on the utilization of other phytate-bound minerals. Sebastian *et al.* (1996b) reported increased copper retention (from 24.6% to -5.4%) in broilers fed phytase supplemented corn-soy diet having lower P contents. Improvements in copper (Aoyagi and Baker, 1995) and manganese (Mohanna and Nys, 1999) utilization in response to phytase supplementation in broilers have also been reported.

#### Interactions between Phytase and protein and amino acids

Theoretically, phytase should be capable of releasing protein that is bound by phytate. Many researchers have reported increased availability of protein in diets that are supplemented with phytase. Farrell *et al.* (1993) found increased nitrogen retention in fed a sorghum-soy diet supplemented with phytase (750 FTU kg<sup>-1</sup> diet). Kornegay *et al.* (1999) also reported that supplementation of protein deficient diets with phytase results in improved protein utilization. Zanini and Sazzad (1999) observed a significant increase in nitrogen utilization in fed a corn-soy diet supplemented with phytase (500 FYT/kg diet). Ravindran *et al.* (2000) reported improved digestibility of nitrogen in diets

with varying levels of phytate (0.29, 0.37 and 0.44%), supplemented with phytase. Significantly increased nitrogen retention in broilers fed low phytate diet supplemented with phytase has also been reported by other researchers (Pourreza and Ebadi, 2006; Panda *et al.* 2007; Centeno *et al.* 2007). Phytase supplementation also results in increased amino acid digestibility especially histidine, arginine, leucine, threonine and valine. Biehl *et al.* (1995) reported a significant increase in lysine, methionine and / or valine in response to phytase supplementation (1220 FTU/kg diet). Yi *et al.* (1996a) observed increased ileal digestibility of amino acids other than methionine and cystine from a diet having 22.5% crude protein (CP) and 0.45% phytate. Whereas, Sebastian *et al.* (1997) found increased ileal digestibility of all amino acids other than methionine and phenylalanine in male broilers fed diets supplemented with phytase. While, Kornegay (1996a) found improved amino acid digestibility except for the methionine in diets having CP (17, 20 and 23%) supplemented with phytase. Zhang *et al.* (1999) reported significant increase in amino acids digestibility especially arginine, cystine, threonine and serine in broilers supplemented with phytase. Namkung and Leeson (1999) observed increased in amino acid digestibility especially valine and isoleucine in phytase supplemented diets. Ravindran *et al.* (2001) reported improved amino acid digestibility in diets deficient in lysine and supplemented with phytase. Decreased flow and increased ileal amino acid digestibility in broilers fed phytase-supplemented diets has also been reported by Cowieson and Ravindran (2007) and Cowieson *et al.* (2008). While, Newkirk and Classen (1995) found unaltered amino acid digestibility in broilers fed diets supplemented with or without phytase or crude phytase.

#### Interactions between Phytase and carbohydrate and fat

Roland (2006), found increased carbohydrate digestibility in diets supplemented with phytase. Liu *et al.* (2008) found that phytase has a positive impact on the endogenous carbohydrate degrading enzymes as their activity is impeded by phytate. Cowieson *et al.* (2008) reported that phytase supplementation resulted in reduced amino acid flow and increased energy digestibility. Mroz (1998) observed that in broilers fed diets having high phytate, digestibility of energy is improved (up to 32-46 kJ kg<sup>-1</sup> of diet) by phytase supplementation. However, improvements in energy digestibility depend upon (1) the acidity / buffering capacity of individual ingredients, feeds, and intragastric / intraluminal contents; (2) sources and levels of dietary phytate, phytase, protein, and energy; (3) feeding regimen (restricted/ad libitum); (4) specific configurations and stability of phytate complexes; and (5) the degree of synchrony of energy and nitrogen release in the small intestine with body

protein / fat accretion patterns. Francesch and Geraert (2009) concluded that supplementation of diet with a multi-enzyme complex containing non-starch polysaccharide enzymes and phytase was efficient in reducing the energy specifications of corn-soybean meal diets. In a study on broiler chicks, Akyurek *et al.* (2005) observed that supplemental phytase improved ileal digestibility of crude fat.

### Phytase and broiler performance

Adverse effects of phytate can be overcome by the supplementation of phytase in the diet (Ravindran *et al.* 2000). Guo *et al.* (2009) found better performance and bone characteristics in broilers fed diets supplemented with sodium gluconate and phytase. Many researchers reported that phytase supplementation improved growth performance, feed intake and feed efficiency in broiler chickens (Singh and Khatta, 2002; Singh *et al.* 2003a; Karim 2006; Pillai *et al.* 2006; Singh and Sikka 2006; Selle *et al.* 2007). Efficacy of phytase depends upon the enzyme application level and the amount of phytate present in the diet (Cabahug *et al.* 1999). Broz *et al.* (1994) observed that supplementation of phytase at rates of 125, 250 and 500 FYT kg<sup>-1</sup> diet, resulted in 4.6, 6.4 and 8.5 per cent increased weight gain, respectively. However, Johnston and Southern (2000) observed that increasing the level of phytase had little effect on growth performance. Denbow *et al.* (1995) investigated the effect of varying non-phytate P level supplemented with phytase on weight gain and feed intake in broilers; they found that the response was maximal at the lowest non-phytate P level. Lim *et al.* (2000) also found lower activity of phytase with same addition rate, at higher levels of phytate in corn-soy diet. Likewise, Cabahug *et al.* (1999) observed the growth performance of broilers fed wheat-soy diets containing different concentrations of phytate supplemented with the same levels of phytase and found that growth was greater in broilers fed diets containing lower concentrations of phytate. Other researchers also reported similar findings (Singh *et al.* 2003a, b; Singh and Khatta, 2003a, b). So overall what do you think this means.

### Economic impacts

Phytase supplementation, by improving overall production performance of broilers, may lead to economic benefits. Vinil *et al.* (2000) found a reduction in feed cost in soy-wheat bran diets supplemented with phytase (25 g 100 kg<sup>-1</sup>) of about 1.00 Indian rupee (INR). Net income increased up to 9.47% in response to 300 g/ton of phytase supplementation (Kundu *et al.* 2000). Singh and Khatta (2004) reported that phytase supplementation resulted in 10% and 6% reductions in cost per unit gain in broilers fed corn and wheat based diets, respectively. Plumstead *et al.* (2008) observed that less expensive broiler diets low in P and other nutrients

supplemented with phytase resulted in optimum production. Supplementation of phytase leads to safe, economic and almost complete replacement of dietary P (dicalcium phosphate), that ultimately causes reduction in feed cost kg<sup>-1</sup> of weight gain (Singh *et al.* 2003a, b; Singh and Khatta, 2003a, b).

### CONCLUSION

Phytate affects broiler chickens by reducing amino acid digestibility, making association with proteins, interfering with minerals (Ca, P, Zn, Mg, Mn, Cu), carbohydrates and fat absorption. Supplementation of phytase reduces the effects of phytate by increasing amino acid digestibility, absorption of minerals, carbohydrate digestibility, digestible energy of diet and ultimately the growth of broiler chickens. However, more work is required to evaluate the efficacy of current phytase feed enzymes that can be further enhanced by the simultaneous use of other exogenous enzymes, which complement their activity by increasing substrate access and/or absorption of liberated nutrients.

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