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Review Article

Biochemistry of phytate and phytases: Applications in monogastric nutrition

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ABSTRACT: **Phytic acid is important for plant germination as the primary store of phosphorus but has become very important in animal nutrition due to the sheer volume of plant feedstuffs that are used in feeding non-ruminant animals. Phytases on the other hand enable the utilisation of the phosphorus that is bound in phytic acid. Animals do not produce phytase in any appreciable amount and hence the phytase primarily used in animal feed are of microbial origin. Biochemical studies have provided insights into the role of this vital compound, and have enabled development of a spectrum of enzymes that are capable of tolerating the heat treatment of some animal feed, escape the denaturing action of the gastric HCl and the digestive action of both gastric and intestinal proteases. In spite of the progress in understanding of phytic acid and phytase in monogastric animals, much still need to be learnt. A better understanding of the action of phytic acid in the digestive tract of animals is still needed and newer generation of phytases that allowed greater reduction in the use of inorganic phosphorus are continually being discovered and developed. The future of animal feeding will continue to require a better understanding of the biochemical principles underpinning nutrient utilisation by animals.**

PHOSPHORUS AND ENVIRONMENT

The objective of this article is not to present a treatise of the effects of phytic acid in non-ruminant (monogastric) nutrition or to extensively characterise and discuss the modes of action of phytase but to briefly show how understanding the underlying biochemistry of the substrate (phytic acid) and the enzyme (phytase) has helped non-ruminant nutritionist harness a vital nutrient that will otherwise be largely unavailable to non-ruminant animals.

Phosphorus is a limiting nutrient for growth for both plants and animals and this makes the elemental nutrient a double-edged sword. Plants ensure a steady supply of the vital element during germination by storing it in the form of phytin (the form of phytic acid in plants) which is hydrolysed by the plants' endogenous phytase during germination. When these cereal grains or oilseeds (e.g. soybean) are fed to non-ruminant animals or pre-ruminant calves, utilisation of the phytic-P by the animals trumps the necessity for the plant to use it for germination. However because of insufficient endogenous phytase in these animals the phytic P is almost wholly unavailable to them. In view of this, inorganic P (mainly from rock phosphate) supplementation is an integral part of animal diets and this raises several issues (Sutton *et al.*, 1996; Olukosi and Adeola, 2007) relating to the release of unused phytic acid P to the environment causing environmental concerns (e.g. algae bloom and its negative effect on aquatic life) and the threat of possible depletion of rock phosphate among others.

Although animal sources of P can also be used in animal feeds they are either expensive (e.g. fish meal) or their use is banned or severely curtailed (e.g. bone meal and meat and bone meal) in some countries. In view of the importance of using plant feedstuffs in animal diets therefore, a good understanding of the chemistry of phytic acid and its effect inside the animal as well as the action of phytase in ameliorating the negative effects of phytic acid is important for animal nutrition and the environment. Furthermore this helps demonstrate how the challenge of meeting the meat demands in the coming decades depends not only on research activities of applied and basic animal nutrition but also on a good understanding of the chemistry of these important substances.

OCCURRENCE AND NUTRITIONAL SIGNIFICANCE OF PHYTIC ACID

Phytates, the salts of *myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or phytic acid found in plant-based feedstuffs, are the main storage form of P in plants and may hold approximately 70% of the total P in plants (Eeckhout and DePaepe, 1994). Steiner *et al.* (2007) reported that about 67% of the total P in legume seeds, cereals and cereal by-product are bound to phytic acid mainly located in the outer layers of cereal grains. At pH 1 to 6, the normal pH range in the stomach of pigs or the proximal gastrointestinal

tract of poultry, phytates have 3 to 6 negative charges (Bebot-Brigand *et al.*, 1999) and thus are able to form a complex with divalent cations such as K, Ca, Mg, and Zn. At this pH range, phytic acid can also form complexes with protein either via direct phytate-protein interaction at low pH or via ternary phytate-divalent ion-protein interaction at higher pH (Cheryan, 1980).

The unavailability of phytate-bound P is the first obvious antinutritive effect of phytic acid. This is abundantly demonstrated when P bioavailability in conventional soybean meal (SBM) is compared to that of low-phytate SBM. Sands et al (2003) using slope-ratio assay with tibia mineralization and tibia ash weight as response criteria, showed that the bioavailability of P was higher in low-phytate SBM compared to conventional SBM with higher phytate-P content. Cowieson *et al.* (2004) reported that up to 82% of phytate P fed to broilers by direct delivery of feed into the crop was recovered in the excreta. Cowieson *et al.* (2006) demonstrated using purified diet that inositol-6-phosphate reduced the digestibility of amino acids and nitrogen in casein and increased the excretion of endogenous minerals. Baxter *et al.* (2003) reported that feeding low-phytate corn led to significantly reduced faecal contents of total and dissolved reactive P in swine.

Apart from reduced availability of P in phytic acid, complexation of the compound with other cations or protein is of interest in non-ruminant nutrition. Tervilä-Wilo *et al.* (1999) and Bohn *et al.* (2007) demonstrated that wheat phytate globoids consist of phytic acid and protein in greatest relative proportion but also other minerals especially K, Mg, Ca and Fe in decreasing order of concentration. The presence of these minerals in the globoids strongly supports the notion that phytic acid makes other minerals unavailable to the animal. Similar observation was made by Woyengo *et al.* (2010) in which addition of phytic acid decreased Ca and Mg concentration in the jejunum digesta (Figure. 1). Martin and Evans (1989) showed that although phytate does not by itself reduce the activity of carboxypeptidase-A, the formation of phytate-Cu^{2+} complex caused a decrease in the enzyme activity. This is thought to be mediated via a cation exchange mechanism and demonstrates how phytate may have negative nutritional impacts. Martin and Evans (1991) also demonstrated the inactivation of alkaline phosphatase in the presence of phytic acid complexed with Cu^{2+}, the authors suggested a metal ion exchange mechanism similar to that observed for carboxypeptidase inactivation as being responsible. Champagne and Fisher (1990) noted that the complexation of Cu^{2+} to phytate seems to be stronger than that of Zn^{2+} and that in some systems the two ions potentiate the binding of each other but competes for the binding sites on phytate in other systems. The data of Sanderg and Svanberg (1991) suggested that, in vitro, only the hexa- and penta-inositol phosphates are the ones that have the strongest inhibition on Fe availability.

Lastly, Cowieson *et al.* (2004) demonstrated that phytic acid increases loss of endogenous nutrients. In the study, addition of phytic acid to a glucose diet resulted in the loss of amino acids, Ca, Na, P and sialic acid whereas Onyango *et al.* (2008) demonstrated that increasing levels of phytate caused a decrease in the uptake of free amino acids. Taken together, these studies have demonstrated how an understanding of the chemical mechanisms surrounding phytic acid and its salts helps answer the question of why non-ruminant animals are unable to effectively use the P that is present in all plant-based feedstuffs. However, phytase which began to be available in commercial quantities in the 1990s can be used to hydrolyse phytic acid and thus help reduce the negative influence of this compound.

OPTIMIZING NUTRITION UTILIZATION WITH THE AID OF PHYTASE

Phytases are myo-inositol hexaphosphate phosphohydrolase that are able to hydrolyse phytate. Phytases catalyse the stepwise removal of inorganic orthophosphates from phytic acid via inositols-pentaphosphate to -monophosphates as well as intermediate products. Frølich (1990) observed that hydrolysis of phytate proceeded in a step wise manner in an isolated system with lower inositols being prevalent at the various stages and only the tri-, di- and mono-phosphates being the inositols that could be detected after 5 hours. Zyla (1993) proposed that acid phosphatase with optimum pH at 2.5 acts independently of phytase. Whereas phytase hydrolyses the ester linkage, the phosphatase acts on inositol phosphate intermediates. Phytase-producing microorganisms include bacteria such as *Bacillus subtilis*, *Pseudomonas* sp., *Escherichia coli*, yeasts such as *Schwanniomyces castelli* and *Saccharomyces cerevisae* and fungi such as *Aspergillus ficuum* and *Aspergillus terreus* (Nagashima *et al.*, 1999).

Although several plants (wheat and rye for the most part) have some significant intrinsic phytase activity (Schwartz and Nevins ,1989) this article will focus on microbial phytase because the latter are more widely used in the livestock industry. Important qualities of desirable phytases in animal nutrition include thermostability and resistance to protease and HCl inactivation in the stomach and small intestine and it stands to reason that phytases from different origins will differ in these properties. Yin *et al.* (2007) compared fungi- and transformed fungi-phytases and observed that fungal phytase with phytase gene from *Aspergillus niger* had higher thermostability than bacterial phytase from *Escherichia coli* principally because of additional glycosylation of the fungal phytase. Bacterial phytases also had lower acidic pH for optimum activity in comparison to fungal phytases and so bacterial phytases had greater residual activity at pH 2 than fungal-phytases and this makes them more relevant in feed formulation because of being able to maintain potency in the acidic environment of the stomach (Bohn *et al.*, 2007). Rodgriguez *et al.* (1999) compared the 3-phytase (phytase that initiates dephsophorylation of phytic acid from C3) *A. niger* to a 6-phytase (begins dephosphorylation from C6) *E. coli* and noted that *A. niger* phytase is more resistant to trypsin whereas *E. coli* phytase is more resistant to pepsin. The nutritional significance of this is that *E. coli* phytase has the potential of being more resistant to proteolytic activity of pepsin in the stomach and thus more likely to survive longer in the digestive tract than *A. niger* phytase. Pillai *et al.* (2006) in comparing *E. coli* phytase with 2 fungal phytases reported that *E. coli* phytase had greater efficacy in improving growth performance or bone mineralization. Pillai *et al.*

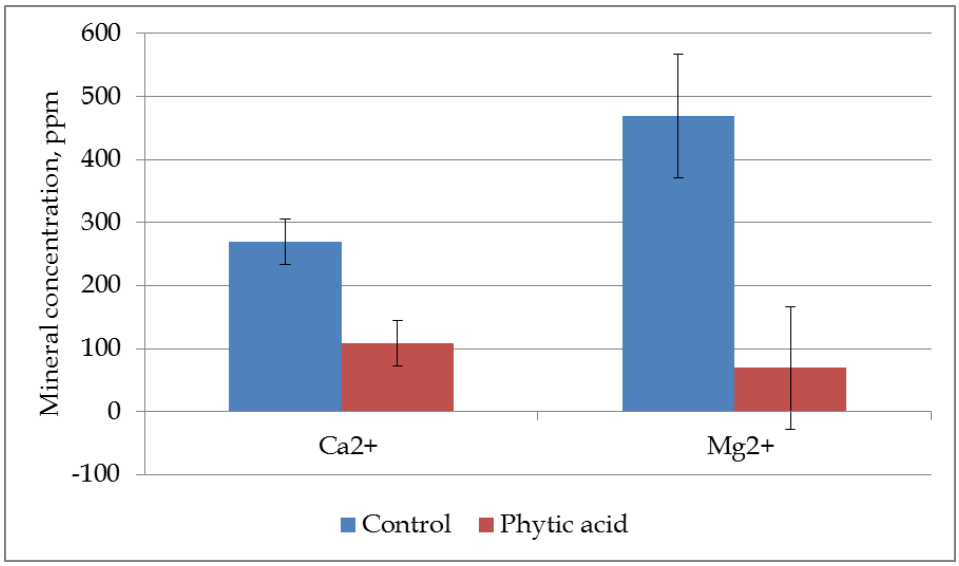

FIGURE 1 **Nutrition model to demonstrate the effect of phytic acid on mineral concentration in the digesta from jejunum of broiler chickens, addition of phytic acid to the diet decreased the digesta concentration of free Ca and Mg. In addition, phytic acid increased jejunal concentration of Na (data not shown) indicating how phytic acid may negatively influence dietary electrolyte balance. Adapted from Woyengo *et al.* (2010).**

(2006) found that *E. coli* phytase released 0.119 to 0.239% P from phytic acid compared to 0.03 to 0.18% released by fungal phytases.

Most phytases are inherently susceptible to heat treatment and thus enhancing the heat tolerance of phytase is of importance in animal feed industry where pelleted feed are subjected to temperature in excess of 60°C. Lei *et al.* (1993) suggested that the lower effectiveness of yeast phytase compared to *A. niger* phytase might be due to inactivation of the enzyme in the stomach or the yeast phytase being less thermo-tolerant than the microbial phytase. Although plant phytase are heat labile, genetic engineering of phytase in transgenic wheat that expresses *Aspergillus fumigatus* phytase activity enabled it to withstand temperature up to 89°C (Brinch-Pedersen *et al.*, 2006). The data suggests that the heat resistance ability of these phytase is due to their ability to fold into active form when cooled following unfolding of their tertiary structure during heat processing. Han *et al.* (1999) showed that heavy glycosylation of *A. niger* phytase expressed in *S. cerevisae* improved the thermostability of the enzyme and deglycosylation minimally reduced its activity but substantially decreased its thermostability. Rodriguez *et al.* (2000) also showed that mutagenesis of *E. coli* phytase to increase its glycosylation decreased its K_m but that additional glycosylation did not necessarily improve the enzyme's thermostability. It appears that mutation to remove the disulphide bond in the G helix and GH loop was more responsible for improved thermostability. The foregoing help demonstrate how the understanding of higher structures of an enzyme helped in enhancing its practical relevance in animal feed.

Wyss *et al.* (1999) observed that different expression systems of phytase may alter the enzyme's activity by modification of its optima pH, thermostability, specific activity or resistance to proteolysis. Onyango *et al.* (2004) supplemented phytase produced by *Escherichia coli* phytase but expressed in *Pichia pastoris*, *Schizosaccharomyces pombe* or *Schizosaccharomyces cerevisae* which thus confer slight differences in their glycosylation pattern to low-P broiler diets. The authors reported no difference in the effect of the different phytases on growth performance but that only *Pichia pastoris* phytase outperformed the low-P diet. All the phytase improved bone strength above the low-P diet, the result indicated that the differences in post-translational modification of the enzymes did not affect their ability to release phytic acid P from the feedstuffs for the broilers.

The concluding paragraphs will document the reported effects of phytase supplementation in non-ruminant animal nutrition. Orban *et al.* (1999) reported that phytase supplementation to P-deficient finisher duck diet promoted growth performance similar to what was observed for P-supplemented diets. Dilger *et al.* (2004) reported improvement in growth performance, total tract retention of nutrients and some amino acids and bone mineralization of broilers up to 42 days of age when fed corn-SBM diet supplemented with *E. coli* phytase expressed in *Schizosaccaromyces pombe*. Jendza *et al.* (2005) noted that *E. coli* phytase improved growth performance in starter, grower and finisher pigs receiving corn-soybean meal diet and there was also an increased in Ca and P digestibility and absorption. Adeola *et al.* (2006) indicated that 500 units of phytase activity (usually added to 1 kg of diet) from *E. coli* and *P. lycii* can release 770 and 572 mg of phytic acid P, respectively. This is a considerable saving of P that could have come from rock phosphate. Olukosi *et al.* (2007a,b; 2010) showed that phytase supplementation by itself or in combination with an admixture of carbohydrases and protease promoted growth performance and nutrient utilisation in monogastric species. In addition, Olukosi *et al.* (2008a,b) showed

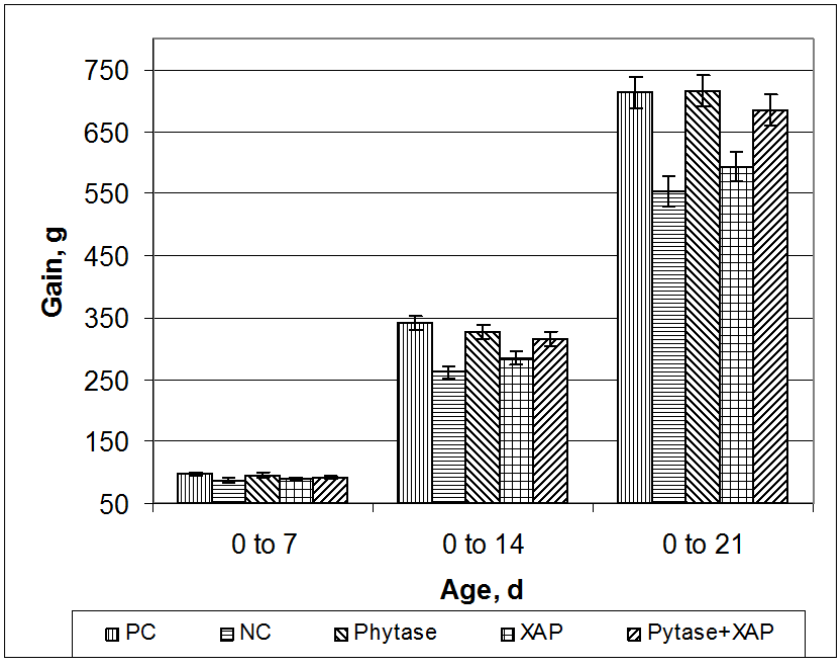

FIGURE 2 **Weight gain response of broilers to supplementation of phytase into a P-deficient diet; The positive control (PC) diet had adequate P whereas the negative control (NC) had 60% of required P. Note that the phytase-supplemented diet restored performance to the level obtained with PC. Supplementation of a mixture of xylanase, amylase and protease (XAP) did not interfere with efficacy of phytase.**

that phytase supplementation increased the whole-body retained energy and preferentially favoured partitioning of nutrients for protein rather than fat accretion in poultry. The positive effects of phytase supplementation on growth performance of broiler chickens are depicted in Figure 2.

Mroz *et al.* (1994) reported improved ileal digestibility of protein and most amino acids in 45-kg pigs receiving corn-tapioca-soybean meal diet supplemented with *A. niger* phytase but Traylor *et al.* (2001) reported in growing pigs that *A. niger* phytase supplementation to soybean meal did not significantly improved apparent and true ileal protein and amino acid digestibility demonstrating that the response of animals to phytase varies depending on feed type and age of animals among other factors. Similarly, Liao *et al.* (2005) reported no improvement in ileal digestibility of protein and amino acids in weanling pigs fed *A. niger* supplemented diets. The effect of phytase on amino acid utilisation is inconclusive however Cowieson *et al.* (2004) reported that phytase supplementation to glucose-phytic acid diet (directly delivered to the crop of chickens) resulted in reduction in excretion of endogenous amino acids and sialic acid and the authors subsequently suggested that phytase may mediate its positive effects on amino acid availability through a reduction in endogenous amino acid loss.

Preponderance of evidence shows the benefit of using phytase in non-ruminant animal feeding. The benefits of phytase are most manifest when feeding diets that are lower in P than requirement thus making it possible to use less P in diets and at the same time meeting P needs of the animals. Increased understanding of the genetic make-up of the different microorganisms producing phytase has made it possible to produce newer-generation phytases that are better able to release more P per unit of enzyme supplementation as well as cope with high temperature associated with some types of animal feed processing. The advancements in the understanding of phytase and phytate were only possible as a result of close relationship between applied and basic nutritional sciences as well as improvement in the understanding of the biochemistry of the enzyme. It is clear that future development will require more cooperation among these important fields of investigation.

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