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THE USES OF MICROBIAL PHYTASE AS A FEED ADDITIVE IN POULTRY NUTRITION – A REVIEW

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Abstract

Most of the phosphorus (P) in feed ingredients is present as phytate, which is poorly available for absorption in the gastrointestinal tract of different poultry species due to the lack of endogenous phytase. The supplementation of phytase increases the utilization of P by hydrolyzing phytate, which consequently may reduce the excretion of P in the environment. In addition, it has been suggested that phytase may improve the feed utilization, weight gain, egg production and egg traits, nutrient digestibility, energy availability, retention of important minerals in blood and bones. Thus, the effectiveness of phytase on performance and Ca and P absorption in layer chickens fed corn-soybean based diets has been well recognized. The current review briefly discusses the supplementation of phytase in the diet of poultry on performance and egg production and characteristics as well as amino acids and minerals availability.

Key words: phytase, poultry, performance, egg quality, digestibility, blood

Phosphorus (P) remains complexed as phytate in plant feed ingredients. Phytate P generally accounts for approximately two-thirds of the total P in plant seeds (Maenz, 2001; Vieira et al., 2016). Phytate is required to be hydrolyzed by phytase enzyme to release P. Although phytase activity is present in the brush border membrane of the digestive tracts of poultry (Maenz and Classen, 1998), phytate P is feebly utilized by poultry. Consequently, inorganic P is needed to include in the diet of poultry to attain optimal growth and production. This eventually leads to nonutilization of a high proportion of dietary P by the animals and excretion of P in feces. Exogenous phytase can be included in diets to hydrolyze phytate within the gastrointestinal (GI) tract, which may make more phytate P available for absorption by poultry and allow the reduction of dietary inorganic P supplementation without compromising performance and decreased excretion of P from poultry feces under intensive production thus lowering the harmful environmental impact of P (Mohammed et al., 2010).

The supplementation of phytase in the diets of laying hens has been shown to improve the availability of phytate P and other minerals including Ca, Mn and Zn (Jalal and Scheideler, 2001; Ghosh et al., 2016). Moreover, phytase supplementation may increase the availability of other nutrients such as protein, amino acids (Rutherfurd et al., 2004) and energy (Newkirk and Classen, 2001) in poultry. Microbial phytases supplementation improved the phosphate utilization from phytate phosphorus, reduced P excretion, and subsequently decreased phosphate pollution (Jondreville et al., 2007; Lalpanmawia et al., 2014). The ability of phytase to improve the availability of dietary nutrients in poultry depends upon the diet composition, dietary mineral content, endogenous conditions as GIT pH range, sources of phytase, bird species and age. The present review aims to highlight the nature of phytate and the beneficial uses of in-feed phytase in poultry.

Phytate metabolism

Phytate is a natural organically bound compound present in most cereal grains (Maenz, 2001). The P linked with phytate is defined as phytate P, which is mostly unavailable to the monogastric animals including poultry (Oatway et al., 2001; Olukosi et al., 2010). Because phytate P is poorly utilized by monogastric animals, their dietary P requirement is not fulfilled from dietary phytate P alone. Inorganic P is needed to supplement with their feeds to meet dietary P requirement and to achieve optimal growth and production performance. Phytate has also been identified as an anti-nutritional factor. It can bind with other minerals, protein and starch, preventing their absorption in the digestive tract and make them unavailable to the animals (Urbano et al., 2000; Mazzuco and Bertechini, 2014).

Phosphorus in plant based feedstuffs principally remains bound as phytate form that is weakly digested and absorbed by monogastric animals (Woyengo and Nyachoti, 2013). Distiller co-products such as dried distillers grains with solubles (DDGS) and brewers grains contain high concentrations of total P with higher proportion of available P and low proportion of phytate P compared with cereal grains due to yeast fermentation (Deniz et al., 2013). Many strategies like phytase addition to diets and the use of low-phytate ingredients have been developed in an attempt to improve the P availability in monogastric animals. Several studies have demonstrated that low

phytate P variety vegetable ingredients like maize, soybean meal, pea and barley is more available and better utilized by birds compared with the conventional varieties (Thacker et al., 2013; Kahindi et al., 2015).

For the availability of phytate P to the chickens, phytate must be hydrolyzed to inositol and inorganic P within the digestive tract (Sandberg, 2002). The degree of phytate P utilization by poultry is quite variable ranging from 37 to 50% in poultry (Robertson and Edwards, 1994). Although birds are capable of degrading phytate to inorganic P, the endogenous phytase activity in the brush border membrane of intestinal tracts and the intrinsic phytase activity in feeds of poultry are very low to effectively hydrolyze the phytate molecule (Maenz et al., 1999). This necessitates the supplementation of exogenous phytase to the diets to improve the utilization of phytate P. Onyango et al. (2004) stated that dietary phytate prompted an increase in the endogenous amino acids losses in chickens and ducks, but phytase addition to the diets did not decrease the endogenous losses. In a nutshell, the nature of phytate and its importance for poultry necessitates the inclusion of phytase in the diet.

Phytase

Phytase (myo-inositol hexaphosphate phosphohydrolase) is an enzyme that hydrolyzes phytate in the digestive tract to inositol phosphates and inorganic P (Wyss et al., 1999). There are two primary classes of phytases according to phosphate group position on the ring of myo-inositol, the first is 3-phytase that hydrolyzes the phosphate group from position 3. The second is 6-phytase which acts first at position C6 (Zyła et al., 2004). In the background of animal nutrition, as feed additives, phytases may be created from the intestine of animal, from intestinal bacteria, from feed ingredients containing phytase or be added, as exogenous enzyme, as feed additives in the feed (Nys et al., 1996). Extensive research has been performed with the addition of phytase in diets of monogastric animals to improve phytate digestibility (Augspurger et al., 2007). The phytase activity is very low in the brush border membrane of the digestive tracts of monogastric animals (Maenz and Classen, 1998). Therefore, the phytase enzyme is included in diets to maximize the hydrolysis of the phytate molecule. Supplementation of phytase to wheat-based diets may alleviate the anti-nutritional effects that are associated with phytate due to its ability in phytate hydrolyse to release the digestive enzymes and bound nutrients (Bedford, 2000). Microbial phytase usefulness for its ability to release phytate-bound phosphorus and improve bioavailability of phosphorus in poultry feed is well recognized (Cowieson et al., 2006; Selle and Ravindran, 2007).

Factors affecting the efficacy of phytase

Various factors can have an influence on phytase efficacy, including dietary-related factors, animal-related factors and phytase-related factors as the following:

Diet composition

Due to the variation in composition, level and location of phytate, as well as the contribution of intrinsic phytase in some oilseeds and cereals, the rate of phytate hydrolysis by microbial phytase can differ to a large extent in these plant-based

ingredients (Akter et al., 2017). The release of P due to added phytase varies in different feed ingredients. For example, addition of phytase increased the available P in corn, soybean meal, wheat, wheat midds, barley, defatted rice bran, and canola from 30.8 to 59.0%, 34.9 to 72.4%, 30.7 to 46.8%, 29.1 to 52.2%, 32.2 to 71.3%, 33.2 to 48.0%, and 36.7 to 55.8%, respectively, with higher percentage of phyate degradation in canola (19%) compared to SBM (37.5%) (Leske and Coon, 1999). This necessitates calculation of phytase equivalency for individual feeds for precise diet formulation.

Dietary mineral content

Phytase is added in poultry diets to relieve the effects of anti-nutritional for phytate and consequently develop the performance. Microbial phytase supplementation to poultry diet is recognized to be the most effective means to utilize and release phytate-linked minerals (Selle and Ravindran, 2007). The ability of phytase to improve the availability of dietary nutrients in poultry depends upon the mineral content of the diets, specifically Ca and P, which can influence the effectiveness of phytase to hydrolyze phytate in the digestive tract (Sandberg et al., 1993). The Ca-phytate complexes are not easily hydrolyzed by the phytase, resulting in unavailability of phytate P and bound Ca to poultry. Earlier research has demonstrated that high dietary Ca concentrations in diets decrease the availability of phytate P in laying hens (Scheideler and Sell, 1987; Mazzuco and Bertechini, 2014; Kahindi et al., 2017). Poultry feed containing calcium can have a great impact on phytase efficacy and phytate P utilization. Van der Klis et al. (1997) reported that increasing dietary Ca (from 30 to 40 g/kg) in laying hens, decreased degradation of phytate P from about 33 to 9% in the diet without phytase supplementation. Also, it has been detected that limestone particle size can have an effect on the phytase efficacy due to the Ca high solubility in fine limestone (Manangi and Coon, 2007). Plumstead et al. (2008) stated that increasing Ca (from 4.7 to 11.6 g/kg) in broiler diets linearly decreased digestibility of ileal phytate P by 71%. While in the diet supplemented with phytase (500 FTU kg^{-1}), the reduction in phytate P degradation was from 76 to 65%. With regard to the ratio between Ca and P, increasing the ratio of Ca: P may have a bad impact on activities of phytase: reducing the Ca: P ratio in the diet from 2:1 to 1.2:1 augmented phytase efficiency by around 16% and enhanced digestibility and performance in turkey (Qian et al., 1996). Plumstead et al. (2008) illustrated that the optimum ratio between Ca and non-phytate P that caused the lowest P excretion and highest P retention was 2.34:1, 2.53:1 and 2.40:1 for diets with 0.10%, 0.28% and 0.24% phytate P. Increasing levels of dietary Ca decreased the P digestibility and phytate P hydrolysis with regard to Fe impact on phytase efficacy. Abudabos (2012) declared that the increasing dietary iron significantly decreased the activity of intestinal phosphatase in chickens. Also, Akter et al. (2017) point out that high dietary Fe (100 mg/kg) inhibited efficacy of phytase and subsequently lowered the nutrient utilisation and overall performance of broilers.

Sources of phytase

There are several sources of phytase that are available in the markets as fungal-derived phytase, for example from *Aspergillus niger* and *Aspergillus ficum*. Other

sources for commercial phytase result from the bacteria *E. coli* or from the fungi *Peniphora lycii* (Selle and Ravindran, 2007). Furthermore, phytase from several sources may have various characteristics, such as resistance to degradation in GIT, thermal stability and the suitable pH of the optimal enzymatic activity (Onyango et al., 2004). These characteristics can influence the P release, so it is important to indicate its effects in the enzyme efficacy. Phytase equivalency for Ca, P, Mn, energy and CP and amino acids varies due to sources of phytase and diet composition. Ribeiro et al. (2016) reported that phytase of *Citrobacter braakii* (Ronozyme® HiPhos) at 500, 1000, and 2000 U/kg had inorganic P equivalency of 0.63, 1.09, and 2.02 g/kg diet from dicalcium phosphate, respectively while phytase of *Escherichia coli* (Quantum® Blue) at 250, 500, and 1000 U/kg had P equivalency of 0.76, 1.31, and 2.40 g of inorganic phosphorus from dicalcium phosphate, respectively. In general, nutrient equivalency for SunPhase® 5000 is suggested to be 88 kJ/kg metabolisable energy (ME), 1.68 g/kg CP, 1 g/kg Ca, 1.15 g/kg available P, 0.07 g/kg lysine, 0.02 g/kg methionine, 0.04 g/kg methionine+cysteine, 0.01 g/kg tryptophan and 0.07 g/kg threonine (Deniz et al., 2013).

Endogenous conditions as GIT pH range and other enzymes

The efficiency of various commercial phytases differs over a pH range of 2.5–4.5 (*in vivo*). *E. coli* phytases are more active at pH 2.5–4.5 range than fungal phytases. Also, the phytase activity curve at different pH values can differ for various *E. coli* phytases as well, due to the bacterial expression and production technology (Kumar et al., 2003; Morales et al., 2011; Dersjant-Li et al., 2015). *E. coli* phytase (*Schizosaccharomyces pombe*) showed the highest activity even when compared with *E. coli* phytase (*Pichia pastoris*) in the pH range 2–5.5. *Aspergillus niger* phytase revealed low activity in the same pH, while the *P. lycii* phytase showed optimal activity at pH 4–5 (Tran et al., 2011). Phytase activity was significantly higher with *E. coli* phytase than *P. lycii* phytase in total digestive tract sections of broilers (Onyango et al., 2005). Phytase activities depend upon the pH of the GIT, all phytases have pH optima which are source dependent, and this pH optimum will affect the functioning ability of the phytase within the GI tracts of the animals (Oatway et al., 2001). Natuphos® phytase (BASF Animal Nutrition, Ludwigshafen, Germany) has two pH optima at 2.5 and 5.5, while Quantum™ phytase (Syngenta Animal Nutrition, Inc.) has a relatively broad pH optimum ranging from 2.5 to 6.0. The broad pH range may provide an advantage in its ability to function more efficiently for an extensive period of time within the GI tract. Phytase may also make available phytate-bound protein by releasing protein for utilization by poultry. Phytase (protein molecule) can be hydrolyzed by endogenous enzymes as protease in the GIT of animals. Also, the high resistance of *E. coli* phytase to protease was detected by Morales et al. (2011). There is a huge variation in the resistance of several commercial phytases against protease in the stomach. The higher efficacy in the resistance endogenous enzymes was described with *E. coli* phytase compared with *P. lycii* phytase in poultry (Augspurger et al., 2003). Phytase which can hydrolyze phytate, works under wide range of gut pH but the efficacy may be influenced by several factors.

Poultry species

In poultry, the major activity site for supplementary microbial phytase is firstly in the crop then in the upper part of the digestive tract (Akter et al., 2017). While, Truong et al. (2016) reported that the primary site from the avian digestive tract for degradation of phytate is the gizzard by bacterial phytases. The bound P release by phytase (100 FTU) in broilers is clearly lower than in laying hens. Hydrolysis of phyate of SBM without supplementation of phytase was greater in laying hens than broilers. The different response to phytase between laying hens and broilers may be related to the difference in digesta retention time and intestinal microbial balance (maturity) between layers and broilers (Leske and Coon, 1999). We think that laying hens had extra-phosphoric effect due to the importance of P for both bone strength and eggshell strength compared with broiler chicks. But this point needs more attention in future studies to compare the phosphoric effect of poultry species. Where, research on the inclusion of phytase in broiler diets has been widespread, but that on layer nutrition has been limited.

Effect of phytase on nutrients digestibility

Phytate may exert inhibitory effect on the activity of endogenous digestive enzyme secreted in the GI tract of animals by chelating the co-factors needed for the optimum endogenous enzyme activity, forming phytate-protein complexes at pH below the isoelectric point of proteins and binding the digestion products (Katayama, 1997). Phytase supplementation may improve utilization of other nutrients. For example, dry matter (DM) digestibility of broiler chicks increased when phytase was increasingly added from 250 to 2500 FTU/kg diet compared with basal diet containing 0.19% NPP without phytase (Zhang et al., 2000). Attia et al. (2002) reported that phytase supplementation at 1000 FTU/kg in broiler diets increased the digestibility of crude fiber (CF), which may provide additional advantageous effect from hydrolyses of cell walls. Thus, an increase in apparent metabolizable energy (AME) value of feedstuffs may be noted owing to the slightly higher apparent digestibility of nitrogen free extract (NFE) and ether extract (EE). Abd-Elsamee (2002 b) reported that the digestibility of DM, crude protein (CP), EE and NFE as well as nitrogen retention were improved with the addition of microbial phytase (750 FTU/kg of diet) in broiler diets. Abd-Elsamee (2002 a) also observed that the use of optimum concentration of available P (0.45%) in laying hen diet significantly improved the digestibility of organic matter (OM), CP, EE and NFE, while CF digestibility was not affected. Similarly, supplementation of microbial phytase to the diets fed to laying hens substantially improved the digestibility of nutrients particularly CP and EE. Phytase supplementation increased the digestibility of amino acids in layer diets (Liu et al., 2007). Increased protein utilization and amino acid digestibility by phytase may partly be mediated through reduced endogenous loss of amino acids (Cowieson and Ravindran, 2007). Fayza et al. (2003) reported that the amounts of DM digested by broiler chickens were greater in groups fed high P concentrations (0.45 and 0.40%) than the low P concentrations (0.35 and 0.30%). In this study, the supplementation of phytase at 600 FTU/kg to low P diets increased digestibility of DM. Phytase augmented ($P<0.05$) digestibility of ileal calcium by 32.2% and digest-

ibility of ileal P by 28.0% compared to the negative control (Selle et al., 2009). In conclusion, phytase is very effective in improving the digestibility of different feed ingredients in broiler and layers.

Phytase impact on P utilization

A large number of bioassay procedures have been proposed to evaluate the efficacy of phytase inclusion in diets on bioavailability of phytate P, which includes growth and laying performance, bone traits, P utilization, blood P level, and alkaline phosphatase activity (Kornegay and Qian, 1996; Yi and Kornegay, 1996). Early studies have described the details of the replaced or released amount of nonphytate P by levels of phytase addition in poultry diets. The decision of non phytate P replacement by an amount of phytase in poultry diets is difficult due to the fact that the released amount of phytate P depends on different concentrations of the several phytases available commercially. Each phytase has special chemical features that are unlike in part depending on the derived source. These characteristics can affect the main position of activity as well as the phytate P release efficacy in GIT and the relative importance of Ca and Ca:P (Maenz et al., 1998). Phytase supplementation of the diets released phytate-bound P at different percentages depending upon the used phytase level. Denbow et al. (1995) stated that P released by phytase reached from 31 to 58% with phytase at levels 250 and 1,000 U/kg in broilers diet, respectively. Yi et al. (1996) assessed that up to 37% of the nPP in SBM was free by phytase (1,000 U/kg) broilers diet. Also, Waldroup et al. (2000) illustrated that adding phytase released about 50% from the nPP in the corn–SBM diet of broiler. Rapeseed meal contains high concentration of total P (1.12%), but availability of P is low (25–37%) in poultry (Rutkowski et al., 1997; Leske and Coon, 1999) and efficacy of phytase addition to rapeseed containing diet is low which needs an addition of higher amount of phytase in diets (Leeske and Coon, 1999). In a bioassay study, phytase supplementation increased the available P content from 36.7% to 55.8%; whereas for soybean and corn these values were increased from 30.8% to 59.0% and 34.9% to 72.4% (Leske and Coon, 1999). A number of studies have shown that phytase supplementation is effective in improving the utilization of phytate P, total P and P retention in the body, which lead to a reduction in P excretion in the environment (Ahmad et al., 2000; Onyango et al., 2005; Augspurger et al., 2007). Excretion of P was reduced by 42–51%, 32–36% and 37.5% due to supplementation of phytase to low P diets as compared to NRC (1994) dietary P recommendations in these studies (Augspurger et al., 2007; Ahmad et al., 2000; Onyango et al., 2005). The reduction of phytate P excretion in manure will decrease P pollution in the environment when this manure is applied to the land. Thus, the use of supplemental phytase in combination with decreased dietary P concentrations could be effective in improving phytate P utilization as well as decreasing P excretion in the manure (Waldroup et al., 2000; Panda et al., 2005). Zyla et al. (2011) found that phytase significantly increased the amounts and proportion of phytate P and Ca retained by hens. A non-significant increase in ileal digestibility of Ca and P was noted due to phytase supplementation to the diets (Englmaierová et al., 2017). A meta-analysis study suggests that P retention may be increased by 5.02% in laying hens receiving an average exogenous phytase at

371 FTU/kg (Bougouin et al., 2014). Recently, Vieira et al. (2015) stated that adding phytase at level of 1,000 FYT/kg provided bioequivalence of NPP that averaged 40% higher compared with adding phytase at level of 500 FYT/kg. Moreover, some researchers reported that phytase plays a key role in modulating the gut microflora (Englmaierová et al., 2015; Ptak et al., 2015). As a final point, the variations in reported values for P release are probably associated with type and dose of phytase and the levels of dietary Ca and phytate. With slight variations in the results, most of the reported literature suggests that phytase increases the availability of P for the animal to be used for biochemical functions in the body.

Phytase impact on dietary energy and amino acids availability

The effect of phytase addition in the diets of poultry on energy utilization is quite variable in different studies. Newkirk and Classen (2001) reported that phytase supplementation improved AME in diets, while Onyango et al. (2005) noted that phytase supplementation did not influence AME concentration in diets. The variations in energy retention among the studies may be attributed to the differences in phytase source, production stage of the birds and dietary ingredients that are used in the studies (Onyango et al., 2005). Previous research demonstrated that the influences of phytase on amino acid availability are much variable, but generally they tend to suggest improvements of protein and amino acid digestibility and availability in chickens (Rutherfurd et al., 2004). Few amino acids are more available than others when phytase is added to the diets. For example, Rutherfurd et al. (2002) noted an increase in isoleucine digestibility in soybean meal with exogenous phytase supplementation. Microbial phytase addition may improve amino acid nutrition by reducing their endogenous losses in the chicken's intestine (Liu and Ru, 2010). Phytase supplementation improves availability of amino acids and energy utilization in the poultry (Selle and Ravindran, 2007). Phytase impacts liberation of Ca from calcium phytate, because the last component may induce the metallic soaps formation in the gut lumen, resulting in lower utilization of energy from fats (Ravindran et al., 2000), subsequently, phytase improves lipid utilization from the diets. In addition, energy utilization improved by addition of phytase into diets may be due to that liberation of Ca ions is essential for activity of α-amylase which is involved in digestion of starch (Kies and Van Hemert, 2001).

Effect of phytase on performance of broilers

Phytase enzyme is beneficial for weight gain in broilers. It is a general concept that phytase hydrolyses the phytate and reduces its anti-nutritional effects, therefore improving the birds performance. Rations supplemented with phytase increase weight gain, making feed to be efficiently utilized. This has been proved by a number of researchers. Shirley et al. (2003) presented feed to the broilers, containing different levels (0, 93.75, 187.5, 350, 750, 1500, 3000, 6000 and 12000 U/kg of diet) of phytase to 0- to 16-day-old mixed-sex chicks. They found that supplementing phytase from 0 to 12000 U/kg significantly increased body weight gain from 287 to 515 g/chick. Similarly Sohail et al. (1999) came up with results that phytase treatment significantly increased body weight gain at 21 day in both male and female

chickens by 13.2 and 5.8%, respectively. De Souza et al. (2015) carried out a research study to determine the effect of dietary phytase on performance and digestive, bone and blood characteristics. They found that diet which was supplemented with phytase, presented best performance. Broilers which were fed with phytase supplemented diet showed 4.40, 11.04 and 7.14% improvement in feed intake, weight gain and feed conversion ratio, respectively. Lim et al. (2001) in a research study with three control groups having 0, –0.1 and –0.2% reduced dietary levels of non-phytate phosphorus while two levels of phytase (0 and 500 U/kg) were added to the low level non-phytase phosphorus diet, found that supplementing reduced dietary levels of non-phytate phosphorus with phytase enzyme increased weight gain and reduced mortality. Sebastian et al. (1996) conducted a three-week trial on 240-day-old broiler chicks to determine the efficacy of microbial phytase at different levels of dietary Ca on performance and utilization of minerals in broiler chickens. The study design was 3 × 2 factorial arrangement for treatment; Ca at 0.6, 1.0 and 1.25% and phytase at 0 and 600 U/kg diet. The results of the experimental study revealed that phytase supplementation regardless of Ca levels, increased body weight gain, feed intake and feed efficiency.

Effect of phytase on laying hens

Body weight

The impacts of dietary microbial phytase supplementation to layer diets are shown in Table 1. Sukumar and Jalaudeen (2003) reported that the supplementation of fungal phytase (200, 300 and 400 FTU/kg diet) in P deficient layer diets numerically improved body weight. Metwally (2005) studied the effect of dietary P concentration with and without supplementation of phytase or dried yeast on the performance of Dandarawi laying hens and found that birds fed low available P (0.25%) diet supplemented with phytase enzyme (1000 FTU/kg diet) or dried yeast had higher final body weight. Silversides et al. (2006) observed that the supplementation of phytase (34 to 49 wks of age) had significant effects on body weight when diets had reduced P and unsupplemented with xylanase, but phytase had no effects on body weight when these diets were supplemented with xylanase. Hughes et al. (2008) reported that hens fed 0.15% available P diet with phytase supplementation (200, 400 and 600 FTU/kg diets) had lower body weight at 61 wk of age compared with the control treatment containing 0.35% non-phytate P (NPP). El-Deek et al. (2009) evaluated the utilization of corn gluten feed (CGF) as a feed ingredient in laying hen diets containing 0, 4, 8, 12, 16 and 20% of CGF with phytase addition (300 FTU/kg diet). They reported that inclusion of CGF in diets supplemented with phytase had no significant effects on body weight. In conclusion, the supplementation of phytase improved the weight gain of layers; however, it is difficult to pinpoint the exact level of dose, age and duration of the experiment.

Table 1. Impacts of microbial phytase supplementation to layer diets

References	Results	NPP in the diet	Total P in the diet	Age	Diet	Phytase levels in the diet	Type and source of phytase
Jalal and Scheideler (2001)	improved egg mass, feed conversion and feed intake; elicited a response in egg components and shell quality at the low NPP	0.35, 0.25, 0.15, or 0.10%	0.56, 0.46, 0.36 or 0.31	40 to 60 wk hens	corn-soybean meal diets	250 to 300 FTU/kg	natuphos (phytase from *Aspergillus niger*)
Lim et al. (2003)	increased egg shell quality	2 levels of NPP (0.15 and 0.25%)	2 levels of TP (0.41 and 0.51%)	from 21 to 41 wks	corn and SBM-based laying diets	0 and 1000 U/kg	phytase from Aspergillus oryzae
Sukumar and Jalaudeen (2003)	improved hen-day egg production; increased body weight	0.30%		White Leghorn pullets, 18 wks old	corn and SBM-based diets	(200, 300 and 400 FTU/ kg	fungal phytase
Cabuk et al. (2004)	increased daily feed consumption	0.30–0.45 g/kg	5.0–6.3 g/kg	54-wk-old Nick-Brown hens	diets containing corn, SBM and SFM	300 FTU/kg	natuphos (phytase from *Aspergillus niger*)
Musapuor et al. (2005)	no significant effects on egg production and egg shell quality; increased plasma P concentration; increased total ash weight, ash % and P content in tibia	0.175 and 0.25%		30- to 42-wk-old White Leghorn	corn and SBM-based laying diets	0, 500 and 1000 FTU/kg	natuphos (phytase from *Aspergillus niger*)
Casartelli et al. (2005)	improved egg production	0.12 and 0.36%	0.31–0.55%	32–64 wks of age Brown laying hens	corn and SBM-based diets	0 and 100 FTU/kg	phytase (unknown source)
Silversides et al. (2006)	increased serum P level from 5.17 mg/dl (with no phytase addition to diets) to 6.3 mg/dl with addition of 700 PPU/kg of phytase	0.25–0.3% and 0.15–0.20%		from 33 to 64 wks of age	wheat-based laying diets	(0, 300, 500, and 700 PPU/kg;	phyzyme (phytase from *E. coli* species)

Ahmadi et al. (2007)	improved egg production				diets containing wheat bran	0, 150 and 300 FTU/kg	phytase (unknown source)
Hughes et al. (2008)	lowered body weight at 61 wk of age compared with control; hens fed 0.15% NPP diet with phytase addition decreased egg production; significantly increased feed consumption and feed conversion	0.15, 0.25 and 0.30%	0.37, 0.47, 0.57%	21–61wk Leghorn laying hen	corn and SBM-based diets	200, 400 and 600 FTU/kg	quantum (*E. coli* 6-phytase)
Kannan et al. (2008)	phosphorus excretion was significantly reduced	0.20, 0.25, 0.30 and 0.5 %	0.52, 0.58, 0.63 and 0.83%	21 to 52 wks of age, layer hen	diets containing corn, SBM, SFM and rice bran	300, 600, 900 and 1200 IU/kg	phytase (unknown source)
El-Deek et al. (2009)	no significant effects on body weight; decreased values of egg production, weights, and egg mass	0.50%	0.72%	32 weeks of age, Hy-Line laying hens	corn gluten -based diets	300 FTU/kg	natuphos (phytase from *Aspergillus niger*)
Mohammed et al. (2010)	improved performance and egg shell percentage	0.67%		22-wk-old, Hy-line hens	diets containing corn, SBM and rice bran	1, 1.5, 2 and 2.5 kg/ton	phytase from *Aspergillus niger*
Englmaierová et al. (2015)	increased the minerals digestibility and changed the microflora of GIT	1.8 and 2.1 g/kg	4.38 and 4.71 g/kg	38-wk-old Lohmann Brown hens	wheat-maize-SBM-based diet.	0,150, 250, and 350 FTU/kg	natuphos (phytase from *Aspergillus niger*)
Ponnuvel et al. (2015)	improved feed efficiency without affecting feed intake; increased egg production	0.30 and 0.50%		21 to 40 weeks	corn and SBM-based laying diets	0, 500 and 1000 units/kg	phytase (unknown source)
Kim et al. (2017)	positively impacted on egg production. did not affect egg quality traits	0.26 and 0.38%		42-wk-old Hy-Line Brown laying hens	corn and SBM-based laying diets	10,000, 20,000 or 30,000 FTU/kg	phyzyme (phytase from *E. coli* species)
Englmaierová et al. (2017)	improved egg production and maintained eggshell quality and egg content in older hens	1.80 g/kg	4.36 g/kg	older hens aged 60–71 wks	wheat-maize-SBM-based diet	350 FTU/kg	natuphos (phytase from *Aspergillus niger*)

Egg production

Supplementation of phytase in layer diets has been shown to improve production performance and feeding efficiency, especially with diets containing low P. Sukumar and Jalaudeen (2003) showed that supplementation of fungal phytase (200, 300 and 400 FTU/ kg diet) in P deficient layer diets numerically improved hen-day egg production. Snow et al. (2004) studied the minimum P requirement of one-cycle and two-cycle (molted) hens. They noted that egg production and egg mass were significantly reduced by all lower available P levels (0.10, 0.11 and 0.13) except 0.15% available P when compared to the 0.45% available P diet. Casartelli et al. (2005) evaluated the effects of phytase (0 and 100 FTU/kg) in diets formulated with different P sources (Ca and sodium phosphate, micro-granulated di-Ca phosphate and triple super phosphate). They showed that phytase supplementation significantly affected the egg production traits. In a study with low-protein and energy diets, phytase (500 and 1000 U/kg) increased egg production (Ponnuvel et al., 2015). However, Musapuor et al. (2005) noted that phytase supplementation (1000 FTU/ kg diet) did not influence egg production. Metwally (2005) reported that diets supplemented with phytase (1000 FTU/ kg diet) or dried yeast had significant effects on egg number, egg mass and egg production (%) compared to the unsupplemented diets during the whole experimental period (32–48 wks).

Ahmadi et al. (2007) investigated the effect of different doses of phytase supplementation (0, 150 and 300 FTU/kg diet) on the performance and egg quality of laying hens. They noted that egg production was considerably higher in phytase supplemented group than unsupplemented groups. Hughes et al. (2008) studied the efficacy of phytase in a 40-wk production trial in White Leghorn laying hens fed a corn-soybean meal based diet and measured production performance from 21 to 61 wks of age. Hens fed 0.15% NPP diet with phytase supplementation decreased total housed egg production at 61 wk of age compared with the positive control diet (0.35% NPP). El-Deek et al. (2009) noted that increasing corn gluten feed inclusion up to 20% with phytase addition (300 FTU/kg) insignificantly decreased values of egg production, weights, and egg mass. In a similar way, supplementing phytase at level of 20,000 FTU/kg diets had a positive impact on egg production in laying hens (Kim et al., 2017). Addition of phytase at 350 FTU/kg to a wheat-maize-soybean meal-based laying hen diet improved egg production while maintaining eggshell quality and egg content in older hens (Englmaierová et al., 2017). This information may be due to our knowledge that phytase mobilizes calcium, phytate and other nutrients. Then at the end of the laying period, a higher concentration of calcium can increase egg production and improve eggshell quality. Gao et al. (2013) observed that the source of phytase in laying hen diets profoundly impacts on egg production.

Although by-products such as DDGS contain high concentration of available P and low phytate P, phytase supplementation with diets containing DDGS may improve laying performance. Supplementation with xylanase (200 U/kg) and phytase (2000 U/kg) in a diet containing 200 g/kg of DDGS increased total egg and egg mass production compared with the diet containing 200 g/kg of DDGS without any enzyme (Świątkiewicz et al., 2013). Egg quality parameters were not affected in this study. Similarly, a diet containing 100 g/kg of DDGS and phytase (300 U/kg) formu-

lated using Ca and P equivalency of phytase resulted in similar laying performance (Deniz et al., 2013). Most of the studies have documented beneficial effect of phytase on egg production; however, the dose level is not consistent.

Feed utilization

Phytase may increase feed efficiency and or/feed intake in layer diets. Sukumar and Jalaudeen (2003) indicated an increase in feed intake by the addition of phytase. In other study, supplementation of phytase (500 and 1000 U/kg) improved feed efficiency without affecting feed intake (Ponnuvel et al., 2015). Addition of 200 FTU of phytase/kg diet may help in reducing available P level to 0.3% in layer diet. Cabuk et al. (2004) reported that phytase supplementation (300 FTU/kg diet) significantly increased daily feed consumption. Snow et al. (2004) noted that the minimum available P requirements of first cycle hens and molted hens in their second cycle are 0.16 and 0.20%, respectively. Hens had significantly lower feed efficiency in low available P diets than those fed 0.45% available P. Metwally (2005) showed insignificant effect of dietary supplemental phytase, P levels and yeast on feed intake but hens fed 0.45% NPP diets performed better than those fed 0.25% NPP diets. Feed conversion was better for birds fed diets supplemented with 1000 U of microbial phytase or dried yeast than those fed the control. Nezhad et al. (2007) studied the effects of citric acid supplementation on phytate P utilization and efficiency of microbial phytase in laying hens. They stated that hens fed to negative control (0.2% available P) and supplemented diets with only citric acid addition had lower feed consumption than the positive control (0.3% available P) and microbial phytase added diets. Also microbial phytase (300 FTU/kg diet) supplemented diets were used as efficiently as the positive control diet (0.3% available P). Hughes et al. (2008) observed that addition of exogenous phytase as 200, 400 and 600 U/kg of diets with 0.15% NPP significantly increased feed consumption and feed conversion. Similar to weight gain and egg production in laying hens, feed consumption was also improved under the supplementation of phytase enzyme.

Egg quality traits

Inclusion of phytase in the diets of layer chickens may improve egg quality traits depending upon the Ca and NPP concentrations. Phytase was associated with increased egg weight (Ponnuvel et al., 2015). Lim et al. (2003) noted that Ca, NPP levels and phytase interactions were significant on Haugh units during 31–41 weeks of age. Metwally (2005) found that hens fed 0.45% NPP diet supplemented with 1000 U of phytase/kg diet resulted in greater albumen weight and albumen percentage than those fed control diets or fed the same diet supplemented with dried yeast. Nezhad and Kandi (2008) studied the combination effect of ethylenediamine tetraacetic acid (EDTA) and microbial phytase on the egg quality in commercial laying hens (Hyline strain w-36) at 53–64 weeks of age. They reported that the interaction effect of EDTA and phytase on Haugh unit was found to be significant.

Lim et al. (2003) noted that low NPP improved egg shell thickness in the period of 31–41 weeks of age; whereas high NNP diet lowered the percentage of broken and soft-shell eggs in the second 10 weeks of age. Low Ca diet decreased egg shell strength and egg shell thickness in both periods. Phytase supplementation (1000 U/kg diets) significantly increased eggshell quality. Liu et al. (2007) observed that

the lowering Ca and P concentrations from the positive control diet (Ca 3.30%, total P 0.50% and NPP 0.28%) significantly reduced egg shell hardness, but supplementing phytase (300 FTU/kg diet) in the negative control diet (Ca decreased by 0.12%, total P decreased by 0.14% and NPP decreased by 0.13%) resulted in an improvement in egg shell quality comparable to the positive control diet. Nezhad and Kandi (2008) showed that the interaction effect of EDTA and phytase on egg shell weight was significant, and phytase addition in low available P diets, significantly increased egg shell weight and egg shell thickness. Also, Englmaierová et al. (2015) observed that supplementation of phytase at 350 FTU/kg to a layer diet with 1.8 g/kg of NPP improved shell quality in comparison with eggs from hens fed diets with only 2.1 g/kg of NPP. Gao et al. (2013) found that different sources of phytase significantly improved egg quality in hens at 50 to 66 weeks of age. On the contrary, Musapuor et al. (2005) studied the effects of different doses of phytase (0, 500 and 1000 FTU/kg diet), Ca (2.28 and 3.25%) and available P (0.175 and 0.25%) on phytate P utilization in laying hens. They observed that phytase had no beneficial effects on egg shell quality. It was difficult to find the clear reason for this observation (no beneficial effects on egg shell quality) because there are no data describing this point. Also, supplementing phytase at levels of 10,000, 20,000, or 30,000 FTU/kg diets did not affect egg quality of laying hens (Kim et al., 2017), which might be due to adequate concentrations of P and Ca present in the diets. In a nutshell, the supplementation of phytase improved the egg quality traits at different doses and age of birds.

Blood mineral concentrations and tibia mineral

Metwally (2005) reported that hens fed diets containing 0.45% NPP had higher Ca and lower P concentrations in plasma than those fed the diets containing 0.25% NPP. Supplementation of phytase or yeast in diets particularly increased both concentrations of Ca and P in plasma. Also, the latest author indicated that supplementation of phytase increased the concentration of tibia ash in hens fed 0.45% NPP diet. Musapuor et al. (2005) noted in laying hens that dietary phytase increased plasma P concentration. Dietary phytase significantly decreased plasma alkaline phosphatase activity. Significant interactions among phytase, Ca, available P levels and plasma Ca were noted. Also, with the same authors, dietary phytase increased total ash weight, ash percentage and P content in tibia. Available P concentration in diets had substantial effect on tibia ash weight percentage and dietary Ca concentration had pronounced effect on tibia ash weight. Interaction between phytase and Ca on tibia P was significant in this study (Musapuor et al., 2005). In another study, Musapuor et al. (2006) indicated that dietary phytase (500 and 1000 FTU/kg diet) increased plasma P concentration in laying hens. In addition, dietary phytase (both levels) improved tibia ash weight and percentage. The effect of dietary Ca (2.28 and 3.25%) was significant on tibia ash weight. Also, an interaction between phytase and available P on tibia P was found to be significant. Whereas, phytate binds some of the important minerals and decreases its availability for the consumption, phytase separates these minerals and makes them available for utilization. Silversides et al. (2006) reported that phytase supplementation in laying hens diet increased serum P level from 5.17 mg/dl (with no phytase addition to diets) to 6.3 mg/dl with addition of 700 U/kg of phytase. However, Kannan et al. (2008) showed that various levels

of phytase enzyme in layer diets had no significant differences in serum Ca, P and alkaline phosphatase among treatment groups at 52 weeks of age. Similarly, Shehab et al. (2012) reported that serum Ca and P were not affected by supplementation of phytase. Phytate can form complexes with some minerals such as Ca, Mg, Cu, Zn, Fe and K, consequently reducing their solubility (Oberleas and Harland, 1996). This confirms the beneficial effect of supplementation of phytase on tibia bone. Phytase enzyme can also release iron from inositol and thus improve iron reserve in hens (Abbasi et al., 2015).

Conclusions

In summary, most of the published literature supports that dietary supplementation of phytase can improve the poultry performance, feed efficiency and minerals contents. Poultry feed containing amounts of calcium can have a great impact on phytase efficacy and phytate P utilization. Phytase supplementation to poultry diet is recognized to be the most effective means to utilize and release phytate-linked minerals. Also, the decreased excretion of P will also reduce the environmental concerns. However, the results are largely conflicting and the exact dose, duration, species of birds, microbial nature of the phytase and some other important factors are still needed to be elucidated. In addition, large commercial experiments are also needed to ascertain the beneficial impact of phytase on poultry production.

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Conflict of interest

The authors do not have conflict of interest in this work.

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