Review Paper:

Effect of phytase supplementation in swine and poultry nutrition - A Review

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Abstract

Phytase, an enzyme which initiates the removal of phosphate from phytate has been extensively used in animal feed. The phytases are generally dispersed in microorganisms, plants and animals and the use of phytase in animal feed will grab the anti-nutritional effects of phytate, will diminish the environmental pollution, will increase availability of starch, protein, amino acids, calcium and P and will eliminate the surplus addition of inorganic phosphate in animal feed. Besides it is a protein molecule and it can be hydrolyzed by endogenous protease in the digestive tract of animals.

The addition of phytase to poultry feed can significantly enhance the immune system, increase poultry weight and also improve egg quality, eggshell quality and egg quantity. Hence, in-depth research in the past few years aimed to establish methods to increase the utilization of phosphorus (P) in livestock production. However, the common method of adding phytase releases the inherent phosphorus component in pigs and poultry is quite inadequate. Thus, in this review, we aimed to focus the effects of different sources of phytase, the mode of action on phytase and the factors that affect the phytase activity in the gastrointestinal tract of pigs and poultry.

Keywords: Phytase, Growth performance, Poultry, Swine.

Introduction

Global meat consumption per capita is expected to increase. Consequently, the ratio of total cropland worldwide currently being used to produce livestock feed to meet the demand cannot be increased. One solution to reduce cropland use for animal feed is to increase the production of alternative feed additive. Optimization programs for formulating commercial feeds over the past few decades have to meet minimum nutrient constraints to minimize feed costs. Phytase, an enzyme which initiates the removal of phosphate from phytate has been extensively used in animal feed, especially in swine and poultry nutrition. The phytases are generally dispersed in microorganisms, plants and animals and the use of phytase in animal feed will grab the anti-nutritional effects of phytate, will diminish the environmental pollution, will increase availability of starch, protein, amino acids, calcium and P and will eliminate the surplus addition of inorganic phosphate in animal feed[14].

The first marketable phytase product was launched in 1991[39]. The main purpose of adding this phytase additive in monogastric animal diets is to increase the digestibility of phytate-related phosphorus[58].

Subsequently, a new generation of phytase was developed and sold on the market. However, the efficacy of this phytase varies from generation to generation[46,57]. In addition, considerable efforts have been made to improve the nutritional value of animal feed by supplementing exogenous enzymes. The phytase can be divided into plant phytase, microbial phytase (fungal and bacterial phytase), intestinal macrofloral phytase, exogenous microbial phytase and endogenous mucosal phytase[26]. Compared with its counterparts in plants and microorganisms, the phytase activity of animals is negligible, because some plants such as wheat and barley are rich in intrinsic phytase and have a narrower pH spectrum activity and low thermal stability than the activity of microbial phytase[77].

However, most of the scientific work has been done on microbial phytases, especially phytases from mucor piriformis and Cladosporium species. For instance, Cao et al[10], Baruah et al[7] and Sardar et al[64] reported that the addition of phytase significantly increased the digestibility of protein, calcium, zinc and utilization of phosphorus. In addition, Greiner and Farouk[18] demonstrated that using phytase as a feed additive can effectively release phytate phosphate from the digestive tract, stably resist heat inactivation during feed processing and storage. Apart from this, the production cost is also very less.

In pigs, phytase supplement is mainly activated in the stomach and upper part of small intestine and the added phytase activity is not restored in the ileum. Despite, in poultry, the supplemental activity of phytase mainly exists in the upper part of the digestive tract including crops, proventriculus and gizzard. In addition, adding phytase to poultry feed can significantly enhance the immune system, increase poultry weight and also improve egg quality, eggshell quality and egg quantity[14].

Hence, in-depth research in the past few years aimed to establish methods to increase the utilization of phosphorus (P) in livestock production. However, the common method of adding exogenous phytase to dephosphorylate phytase and release the inherent phosphorus component in pigs and poultry is quite inadequate[68]. Therefore, in this review, we aimed to focus the effects of different sources of phytase, the mode of action on phytase and the factors that affect the

phytase activity in the gastrointestinal tract of pigs and poultry.

Phytase: A general description

The enzyme phytase (myo-inositol hexaphosphate phosphohydrolase) promotes hydrolysis of the mineral phosphate of InsP6 from myo-inositol to InsP1 via InsP5. Phytases are present in plant, microorganism and animal tissues[19]. According to the initial stage of hydrolysis and the preferred pH value, phytase is divided into two sites[37]. The two international classifications of phytases are: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26) named after the site where the phytic acid molecule begins to hydrolyze[68]. EC 3.1.3.8- is mainly derived from microorganisms while EC 3.1.3.26- is mainly isolated from plant sources[12].

However, some exceptions were found for 3-phytase and 6-phytase because soybean phytase is 3-phytase and *E. coli* phytase is 6-phytase respectively[63]. In addition, these phytases can usually be classified according to their optimal alkaline phytase (optimum pH value: 7.0-8.0) and acid phytase (optimum pH value: 3.0-5.5)[74,80]. Alkaline phytase that has been identified in Bacillus species does not accept myoinositol phosphates with three or less phosphate groups as a substrate while acid phytase releases five of the six phosphate groups of phytase and their common final degradation product seems to be InsP1[21].

In 2010, Jacela et al[28] stated that in order to maintain the efficacy of phytase, we should consider thermal stability, proper storage and handling procedures because phytate can be complexed with minerals, starch and protein. They are very sensitive to high temperatures and can cause denaturation when overheated. In addition, phytase is very sensitive to humidity. The best solution to keep feed pellets active is to sprinkle liquid phytase on the cooled pellets. In addition to the above problems, there are several factors that affect the efficiency of phytase: for example, the acidic pH stability of the small intestine (stomach and neutral), proteolytic stability and temperature stability[27,34]. The optimum temperature for most phytases is about 50 to 60 °C[27]. The animal body temperature exceeding this optimum temperature is another reason why the phosphorus digestibility is as low as 65%, despite the addition of phytase[77]. There are four possible sources of phytate degrading enzyme activity in pig and poultry nutrition including plant phytase (some feeds), microbial phytase (fungal and bacterial phytase), intestinal flora phytase, exogenous microbial phytase (small intestine mucosa) and endogenous phytase, mucosal phytase.

In addition, a new generation of phytase has been developed and sold in the market. Some examples of 3- and 6-phytases currently on the market and their properties are listed in table 1. These commercial phytases differ in their optimal pH, resistance to endogenous proteases and affinity to phytic acid substrates, which may be the main factors affecting their *in vivo* efficacy.

Different source of phytases and their roles

Plant phytase: For more than 60 years, it has been well known that plant phytases can produce hydrolyzed phytic acid[24,55]. Phytase activity varies greatly among plant species. Among these grains, rye, triticale, wheat and barley have the highest phytase activity[16,77] while high-protein feeds such as oats, corn and sorghum have the lower phytase activity[70,75]. Most of the phytase in whole grains is located in the scutellum and alluron layers[51]. Therefore, by-products such as wheat bran or rye bran usually have the highest activity in-plant feeds[70].

Consequently, dietary ingredients with high phytase activity, such as wheat, wheat bran, triticale and rye, can promote higher absorption of phytic acid P. The activity of this enzyme mainly depends on moisture content, temperature and pH [11, 22]. Due to lack of water activation, phytase is inactive in dry grains. The maximum activity of phytase was observed at weakly acidic pH (approximately 5.0) [51] and optimum temperature (approximately 50 °C) [17,20]. As mentioned earlier, when the raw materials are subjected to high temperatures, the phytase activity of plants will be significantly reduced [16, 35].

Table 1
Commercial 3 and 6 phytase and their characteristics

Type*	Protein origin	Expression	Optimal pH	Optimal Temperature (∘C)	Trade name
3	*A. niger*#	*A. niger*, non-recombinant	6.0	-	Allzyme® SSF
3	*A. niger*#	*Trichoderma reesei*	2.5	-	Finase® P/L
3	*A. niger*#	*A. niger*	2; 5–5.5	65	Natuphos®
6	*Escherichia coli*#	*Pichia pastoris*	4.5	-	Quantum®
6	*Escherichia coli*#	*Trichoderma reesei*	-	-	Quantum Blue®
6	*Peniophora lycii*#	*Aspergillus oryzae*	4–4.5	58	Ronozyme®
6	*Citrobacter braakii*	*Aspergillus oryzae*	-	50–55	Ronozyme Hiphos®
6	*Buttiauxella* spp.	*Trichoderma reesei*	3.5–4.5@	60@	Axtra® PHY

#Adapted from Lei *et al.* with modifications; *3- or 6-phytase; —, no information available;
@ personal communication (C Evans).

In addition, Blaabjerg et al[9] pointed out that heat treatment resulted in a 74% reduction in phytase activity. In addition, Jongbloed and Kemme[30] pointed out that phytase activity will be eliminated when the diet is steam pelleted at a high temperature exceeding 85 °C and cold pelleting will not have a negative effect on phytase activity.

Microbial phytase: Exogenous microbial phytases are usually isolated from bacteria, fungi and yeasts [23]. Nelson et al[49] explored the effect of exogenous microbial phytase on the hydrolysis of phytic acid and observed that chickens fed with corn-soybean meal diets containing *Aspergillus* had increased phosphorus utilization. Since the 1980s, the environmental pressure to reduce P excretion has always existed and the cost of adding exogenous enzymes is very high. Advanced biotechnology gave birth to fungal genetic modification technology. However, advanced fermentation technology has led to the development of commercial phytase, which can be economically used in pork and poultry feed[35].

Although the original phytase feed enzyme was mainly produced by fungi, recent advances in enzyme production by other forms of microorganisms (such as bacteria and yeast) have produced new external phytases. Compared with fungal-derived phytase, this bacterial phytase appears to be more effective for broilers[6].

In addition, Adeola et al[2] reported that 500 FTU/kg of *P.lycii* and *E. coli* phytase are equivalent to 0.572 and 0.770 g/kg P respectively. The increased release of phytate bound P in diets supplemented with bacterial phytases may be explained by a greater resistance to the pepsin activity of *E. coli* phytases compared to fungal phytases[27,62]. In addition, the effect of phytase in releasing phosphorus may be different in pigs and chickens. Although the fungal phytase showed higher phosphorus release in pigs compared with broilers, the phosphorus release value of E. coli phytase was found to be similar across the species[6].

In addition, the different research effects of adding phytase on P digestion can sometimes be further explained by changes in the basal diet, especially the concentration of Ca and total P, as well as other factors, such as the source and concentration of phytate P, the source of inorganic enzymes and inclusion levels of P and phytase[66]. Especially mineral-phytate complex and calcium-phytate can reduce phytase activity. It has been suggested that Ca may inhibit the activity of phytase [5].

Some studies have shown that the calcium to phosphorus ratio (Ca/P) of feed affects the response to phytase utilization[42,59]. However, there is little conclusive evidence that Ca directly inhibits external phytase activity, but the data on this function is contradictory[43]. Nevertheless, in order to increase phytase activity, it is generally recommended to maintain low calcium levels when adding phytase to pig and poultry diets without affecting bone integrity or growth performance[67]. Furthermore, Liu et al[42] recommended that 1.0:1 Ca/P ratio could be optimal for pigs. This result was supported by Selle and Ravindran[66] who concisely believed that the Ca/P ratio should be "narrow" around 1.1: 1. On the other hand, Qian et al[60] stated that broilers and turkeys[59] have calcium/phosphorus levels between 1.1 and 1.4:1. Although previous studies have shown that feed ingredients exhibit significant phytase activity, they can increase the utilization of phosphorus in *in vivo* studies[52] but Eeckhout and de Paepe[15] reported that the activity of acid enzyme is lower than that of microbial phytase and this result was confirmed by Zimmermann et al[84] who pointed out the comparable efficiency of wheat and rye phytase with *Aspergillus niger* phytase in growing pigs.

This is because the acidic pH of the stomach is more conducive to the growth of microorganisms than the activity of plant phytase and plant phytase is easily decomposed by pepsin [56]. In recent years, people have paid more and more attention to the effect of unconventional high-dose phytase. This "super" dose of phytase (i.e. >2500 FTU/kg from *Aspergillus niger* or *E. coli*) attempts to remove phytic acid from the diet[13]. Although the exact mechanism needs to be elucidated, the effects of overdose phytase have shown beneficial effects on animal performance in pigs and poultry[13,76]. Genetically modified industrial strains of fungi and bacteria are used as hosts to overproduce phytase. Traditionally, the genes encoding these enzymes are randomly integrated into the host's genes.

However, the new generation of strains is produced by targeting genes into predetermined regions in the mutant genome. These "new generation" phytases have the ability to dephosphorylate large amounts of phytic acid. Therefore, the more nutritional benefits of phytase feed enzymes can be consideration.

Gut microflora phytase: It is generally believed that animal feces mainly contain undigested phytic acid P[54]. In 2004, Leytem et al[40] did an experiment and asked pigs to eat barley diets with different concentrations of phytic acid. They expected to find the positive effects of phytic acid in pig manure based on the concentration in the diet. However, the amount of phytic acid in feces is so small that it cannot be detected which prevents accurate quantification. Finally, they revealed that phytate is hydrolyzed by hindgut fermentation. A study by Baxter et al[8] found that in four treatments, phytic acid-bound phosphorus in pig manure accounted for <16% of total phosphorus including corn-soybean diet based on normal and high-available phosphorus corn, with or without addition of phytase and observation to phytic acid P by a method based on iron precipitation.

Therefore, due to the overestimation of InsP6 content in feces, it is impossible to distinguish InsP6 from low-substituted inositol phosphates[78]. Similarly, Seynaeve et al[69] pointed out that the phytate in chyme contains a lot of phytate. The bacteria in the hindgut are hydrolyzed and the

poultry and crop microorganisms seem to be involved in the hydrolysis of phytate[33]. In addition, the absorption of phosphorus by pigs mainly occurs in the upper part of the small intestine. The absorption of phytic acid from the colon is physiologically irrelevant because the absorption of phosphorus in the lower part of the intestine is limited[65].

Endogenous phytases: Contents of the stomach and intestine in pigs have negligible phytase activity[31,65,79]. There is controversy that mucosal phytase activity is an indicator of unspecified acid activity of alkaline phosphatase[3]. Due to this very low phytase activity, environmental pollution occurs as a result of excessive release of P in the feces[61]. Hu et al[25] stated that the hydrolytic enzyme activity in the pig intestinal mucosa increased with Insp3-Insp6 and decreased with the number of phosphate groups. Therefore, the low inositol phosphates formed in the diet are further hydrolyzed in the intestine.

Although the activity of mucosal phytase in poultry is often dismissed as of minor importance, Tamim et al[72] found 69.2% of ileal disappearance of in broilers fed a phytase un supplemented maize–soybean meal diet. Furthermore, the ability of chicks to reduce phytate is reported to be inheritable[83]. The dietary Ca levels may have a negative effect on endogenous phytase and phosphatase among others[67,71]. However, when P does not provide adequate nutrition, broilers themselves have the adaptive ability to increase intestinal phytase and phosphatase function[45].

Factors that affects phytase activity

Many factors affect phytase activity in the body such as the optimal pH range, phytase and protease resistance and the relationship between specific phytase activity and substrates. Animal-related factors including species, age and residence time of the animal may also be included.

Optimal pH range: The function of phytase is measured in units of phytase. In the official standardized phytase function measurement, 1 unit is the phytic acid that releases 0,0051 mol L^{-1} sodium biotite per minute per 1 mmol of mineral phosphate at pH 5.5 and 37 ^0C[4]. As mentioned above, the best way to reduce the anti-nutritional effects of phytate is to hydrolyze the phytate in the upper digestive tract as soon as possible [82]. However, the pH in the stomach is significantly lower than pH 5.5, which is the pH used in the measurement of the standardized phytase function.

Therefore, the "real" function of phytases may be different from commercial phytases, especially in the body, due to their different optimal properties. The optimal pH range will indicate the effectiveness of phytase in the stomach and upper small intestine. Table 2 compares the performance of these different commercial phytase products in the pH range of 2.5-5.5. These values are from Tran et al[73] where we could find a clear illustration with the huge difference in the relative functions of these phytases.

Phytase resistance to protease: Phytase is a protein molecule that can be hydrolyzed by endogenous protease in the digestive tract of animals. Kumar et al[36] evaluated the response of phytase derived from *P. lycii*, *A. niger* and *E. coli* to endogenous protease and these three phytases were incubated in a buffer containing protease for 2 h and the remaining phytase was measured at pH 5. Finally, phytase derived from *E. coli* had greater protease resistance than *P. lycii* and *A. niger* phytase (Table 3). The researchers recommended that this may moderately differ in bio-efficiency between commercial phytases in monogastric animals.

Table 2
Activity of different phytase at different pH valuers measured on IP6 -lysozyme substrate complex

	E. coli	***A. niger***	***P. lycii***
Pepsin	76.7a	31.4b	5.42c
Trypsin	23.0 a	0.45 b	1.25 c
Chymotrypsin	65.8 a	2.95 b	5.77 c
Means within a row without the same letter are significantly different (P <0.05).			

Table 3
Percentage residual activities of different types of commercial phytases when treated with endogenous proteases for 2h[28]

pH	***E. coli* 1**	***E. coli* 2**	***A. niger***	***P. lycii***
2.5	130	84	38	13
3.5	122	52	32	26
4.5	124	59	54	54
5.5	88	52	90	48
Reactions were carried out in 50mmolL−1 glycine–HCl (pH 2.5–3.5) and 50 mmol L−1 sodium acetate (pH 3.5–5.5) containing 0.3 mmol L−1 IP6 and 0.23 mmol L−1 lysozyme in a total volume of 120 μL at 37 ∘C. The enzyme dose for each reaction was 0.1 FTU mL−1 based on inorganic P release fromIP6 in conventional phytase activity assay. Values are derived from figures from the original study.[73]				

Similarly, Morales et al[47] observed the high resistance of *E. coli* phytase to protease. In addition, *E. coli* and *Peniophora lycii* phytases were incubated with pepsin or a gastric crude extract from rainbow trout[1]. These in vitro results are consistent with *in vivo* studies, where higher phytase activity was observed in broilers digesta with *E. coli* phytase compared to *P. lycii* phytase[53]. Furthermore, Newkirk et al[50] reported that *E. coli* phytase compared to *P. lycii* phytase in both poultry and swine higher efficacy.

Phytase specific activity against targeted substrates: The target substrate may have some influence on phytase activity[73]. The study was evaluated at pH 3.0 and 37^{0}C to determine the relative activity of four commercial phytases to hydrolyze IP6 from IP6-soybean protein or IP6-lysozyme complex compared with sodium phytase. All phytases are added to the equivalent FTU basis (measured according to standards). As shown in table 4, pH 3 phytase activity is significantly different between commercial phytases and is related to the target substrate. Compared with the standard substrate IP6-Na used to measure phytase activity, when soy protein and IP6-lysozyme are used as target substrates, a greater difference in activity between different phytase sources is observed.

A. niger phytase reduces the relative activity on these substrates, while compared with IP6-Na. *E. coli* phytase has an increased activity on the complex of soy protein and IP6-lysozyme. For *E. coli* phytase, the activity of IP6-soy protein or IP6-lysozyme complex is higher than that of IP6 sodium. At pH 3, *E. coli* phytase hydrolyzes IP6 several times faster than fungal phytase and the efficacy varies with the type of substrate[73]. However, due to bacterial exposure, there is also a huge difference in the relative functions between the two *E. coli* phytases. Therefore, the interaction of phytase with different types of substrates may be one of the factors affecting the effectiveness of phytase.

Factors that affect the animal species, age and retention
Swine: In pigs, research data indicates that if microbial phytase is not added to the feed, the main phytase activity is observed in the colon. After supplementing with microbial phytase, the main active sites are the stomach and upper small intestine. Yi and Kornegay[79] study showed the sites of phytase activity in the gastrointestinal tract of piglets fed diets containing *Aspergillus niger* fungal phytase and found that the phytase activity in the gastric chyme was higher than that in the upper small intestine. In pigs fed with a diet containing 1050 FTU kg^{-1} of microbial phytase, the phytase activities in the stomach, upper and lower chyme of the small intestine were 579, 348 and 53 FTU kg-1 dry matter (DM) respectively.

Phytase activity in the digesta of pigs fed with a basal diet was around 30 FTU kg^{-1} in the stomach and small intestine. These data indicate that the stomach is the site with the highest activity of microbial phytase due to a more favorable pH. Kemme et al[32], Lantzsch et al[38] and Mroz et al[48] also report a similar conclusion. The stomach is the main part where we found phytase in pigs. Jongbloed et al[31] reported that exogenous phytase activity was found in the digesta. Pagano et al[54] noted that in piglets receiving diets supplemented with *E. coli* phytase, the highest phytase activity was observed in the stomach and upper jejunum while no activity was detected in the chyme of the lower jejunum or ileum.

The high endogenous phytase activity in the colon may not increase the utilization of phosphorus, but it can increase the excretion of soluble phosphorus. This is consistent with the observation of Seynaeve et al[69]. The endogenous phytase activity in the large intestine does not increase the utilization efficiency of phosphorus, but converts the organic phosphorus in the feces into inorganic phosphorus. In the basal diet without added phytase, the degradation rate of IP6 in the terminal ileum was only 16.2%, while in pigs fed with a diet supplemented with 500 FTU kg^{-1} phytase (*Aspergillus niger* phytase), the decomposition rate of IP6 has increased by these two factors.

Compared with the non-supplemented group, phytase supplementation reduced the total P ($P = 0.09$) and IP6-P ($P < 0.05$) of the ileal digesta. In the ileal digesta, the phytate phosphorus content of the phytase treatment group was 42% lower than that of the control group on average.

Table 4
Relative activity of different phytase measured at pH3 using IP6 sodium, IP6-soy protein and IP6- lysozyme complex as substrates[29]

	IP6–Na+	IP6–soy protein	IP6–lysozyme complex
E. coli 1 (*S. pombe*)	100	164	229
E. coli 2 (*P. pastoris*)	103	138	152
A. niger	37	32	23
P. lycii	10	25	13
The assay was carried out in a total volume of 120 μL in 50 mmol L^{-1} glycine–HCl (pH 3.0) at 37 °C for the different phytases added at a dose of 0.1 FTUmL^{-1}. The reaction rate was measured as inorganic P release (μmol inorganic P mL^{-1} min^{-1}) by stopping the reaction at different time intervals and analyzing inorganic P on Konelab. Activity of *E. coli* 1 phytase (0.096 μmol inorganic PmL−1 min−1) on IP6–Na+was set at 100%. Activities of the phytase on the other substrates were reported relative to the activity of *E. coli* 1 phytase on IP6–Na+			

However, the fecal IP6-P of the phytase-treated group and the untreated group was lower, indicating that the control group had higher endogenous phytase activity in the large intestine, resulting in higher inorganic phosphorus in the stool. Phytase treatment reduced total phosphorus and inorganic phosphorus in feces (36% lower on average than the control group). In the pig's stomach, when it is relatively empty, the pH is usually 2-2.5 and it increases after feeding. Newly weaned piglets have a lower ability to secrete hydrochloric acid (HCl); therefore, the pH in the stomach may be higher than that in growing pigs. In the first part of the small intestine, the pH ranges from 3.5 to 5.5. Jongbloed and co-authors [31] observed that the pH of the duodenal (approximately 25 cm behind the pylorus) chyme was about 6 at the time of feeding, dropped to 5 1 hour after feeding and remained at about 4 2 to 5 hours after feeding. Therefore, microbial phytase with high activity at low pH is more effective for pigs.

Poultry: In poultry, the main active sites for adding microbial phytase are crops and the upper digestive tract which is similar to pigs. In laying hens, wheat, corn and SBM-based diets were used to study phytase activity in chyme, but no microbial phytase was added[44]. The diet contained 2 and 4.37 g kg^{-1} of phytate and non-phytate P respectively. The phytase activity in a 1 kg basal diet hydrolyzes 160.2 µmol of phytic acid (sodium phytate) into phosphate, inositol and lower inositol phosphate per minute. The highest specific activity of phytase (per gram of chyme) was observed in the cecum. There were no significant differences in phytase activity between crops, stomach, small intestine contents, or mucosa (Table 5).

Generally speaking, when the microbial phytase is not supplemented, the cecal phytase activity level is high, the small intestine is medium and the phytase activity level in crops and stomach is low. The results of this study indicate that in the absence of microbial phytase supplements, phytase activity is mainly observed in the cecum (similar to pigs). In this study, phytic acid digestibility and phosphorus retention were measured at the ileum and total intestinal levels. It has been observed that the digestibility of ileal phytic acid is lower than the total digestibility of the digestive tract (20% and 18% and 33% and 35% in layers and broilers respectively), which may be due to the relative amount of phytase activity in the cecum. However, the total intestinal retention of P is lower than the intestinal retention (22% and 19% and 52% and 42% in layers and broilers respectively), indicating that the degradation of phytic acid in the cecum does not contribute to the total P reserved.

This study shows that adding microbial phytase to the diet is essential to increase the hydrolysis of phytate in the upper digestive tract, to reduce the negative impact of phytate on nutrient digestion and to increase the utilization of phosphorus by poultry. Since the pH of the crop is 5.2 to 5.8 and the pH of the proventriculus is 2.8, these GI fragments are expected to be the main sites of exogenous phytase activity in poultry [81]. The activity of exogenous phytase (*P.lycii*) was observed to gradually decrease along the small intestine and no activity was detected in the ileum of broilers. The low activity of the lower part of the small intestine may be due to the activity of endogenous digestive proteases capable of decomposing exogenous phytase. However, as mentioned above, different phytases have different resistances to endogenous proteases. For example, when expressed in FTU kg^{-1} DM intake, in all parts of the digestive tract of broilers, the phytase activity of *E. coli* phytase was significantly higher than that of *Pseudomonas* litchi[53] (Table 6).

In this study, the phytase activity of 22-day-old broilers fed mash diets with or without microbial phytase was measured. Broilers were fed a low-P negative control diet or a control diet supplemented with 1000 FTU kg^{-1} of *E. coli* phytase or Lycium barbarum phytase at 8 to 22 days of age. It was expressed in FTU kg^{-1} DM intake, the exogenous phytase activity was the highest in crops followed by the stomach and stomach and the activity in the ileum was very low (Table 6). The phytase activity of broiler digesta fed with a basal diet without adding phytase was very low throughout the digestive tract.

Table 5
Phytase activity in the intestinal tract of laying hens fed wheat- corn- soybean meal-based diet without microbial phytase supplement.

Segment	Specific, g−1 digesta	Total, per segment
Crop	10.2^{a}	98^{a}
Stomach	9.2^{a}	97^{a}
Small intestine	14.6^{a}	359^{b}
Small intestinal mucosa	11.5^{a}	227^{ab}
Sum pre-caecal		781
Caeca	135.4^{b}	663
Sum total		1444
Means within a column not sharing a common letter differ significantly (P <0.05). Source: Marounek et al[44]		

Table 6
Phytase activity in digesta of broiler chicks fed diet with or without the inclusion of microbial phytase from 8 to 22 days of age (at day 22)

	NC: Low-P diet	**NC +1000 FTU *E. coli* phytase kg−1**	**NC +1000 FTU *P. lycii* phytase kg−1**
Feed, FTU kg^{-1}	14	825	1152
Digesta, FTU kg^{-1} (DM intake)	-	-	-
Crop	67	649a	404b
Proventriculus and gizzard	28	406a	63b
Jejunum	29	554a	25b
Ileum	16	91a	6b
Means within a row not sharing a common letter differ significantly (P <0.05). Source: Onyango *et al.*[30]			

Supplementing *E. coli* phytase significantly increased the phytase activity in crops, stomach and stomach, jejunum and ileum, while litchi phytase only increased the phytase activity in crops. Libert et al[41] measured the phytase activity in the digestive tract of chickens (3-5 weeks old) fed with a control diet and supplemented with 500 or 1000 FTU kg^{-1} phytase (Niger). Basically, no phytase activity (<50 FTU kg^{-1}) was found in the feed and intestinal contents of chickens fed with the control diet. In diets supplemented with phytase, the main phytase activity is found in crops (250-575 FTU kg^{-1} lyophilized samples) and stomach (100-225 FTU kg^{-1} lyophilized samples). No activity was detected in the small intestine (<50 FTU kg^{-1}).

These data indicate that different types of phytase may have different activities in the digestive tract. When phytate is not completely hydrolyzed in the stomach, it may cause re-precipitation of phytate in the small intestine. Libert et al[41] measured the concentration of phytate phosphorus in the digestive tract of chickens and observed that the addition of 1000 FTU kg^{-1} phytase to the diet reduced the content of phytate phosphorus in the crop, stomach and small intestine compared with the control diet.

In the end of the small intestine, the disappearance rate of phytate phosphorus is as high as 65%, while in a diet without added phytase, this proportion is only 15-23%. In different digested slices, all processed crops and stomach had lower phytic acid P content and small intestine phytic acid P content increased, but the degree of phytase treatment group was smaller, indicating that due to the increase in pH and phytic acid P in the small intestine the salt re-precipitates Onyango et al [53] also reported similar results in 2005.

Conclusion

In short, it is generally believed that the use of phytase can reduce feed costs and increase the utilization efficiency of phosphate and other nutrients in plant-based feed materials, thereby generating economic and environmental benefits. In addition, phytase works in a wide pH range and is active in the stomach and upper intestine and will be an ideal phytase for animal feed.

Scientists must be more active in isolating new and best phytate hydrolase microorganisms and optimizing their catalytic properties, heat resistance and specific activity through genetic engineering to produce an idyllic phytase for feed applications.

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