

## Manipulation of factors affecting phytate hydrolysis in enhancing phytase efficacy in poultry: A review

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### Abstract

Phosphorus in phytate is largely unavailable to chickens unless they are provided with dietary phytase. Phytase was shown to increase phytate degradation in the crop and proventriculus-gizzard and very little phytate degradation occurred in the duodenum-jejunum or ileum. These previous investigations were conducted on chickens fed corn based diet but not with wheat based diet. Increase in digesta passage or mean retention time (MRT) along the gastrointestinal tract could enhance phytase efficacy as the prolonged reaction time between substrates and phytase may further facilitate phytate dephosphorylation. Dietary fat and fibre supplementation have been shown to influence intestinal MRT in chickens therefore it is expected that inclusion of both dietary fat and fibre could be manipulated to further improve phytase efficacy in broiler chickens. This paper provides a brief review of *in vitro* phytate hydrolysis, phytate hydrolysis in the gastrointestinal tract of broilers and factors that affect phytate hydrolysis that can be manipulated to enhance the efficacy of phytase in poultry diets.

**Keywords:** Phytate, phytate dephosphorylation, phytases, poultry

### Introduction

Phosphorus has a critical role in cell metabolism, bone development and bone mineralization in animals and P deficiency could hinder the animals from attaining their optimum genetic potential in growth and feed efficiency as well as skeletal development. In the case of monogastric animals, particularly poultry, P is present in plant-based feed ingredients and approximately 70% of it is in the form of phytate-P. Phytate is able to reduce the bioavailability of other nutrients, particularly calcium, proteins and starch in poultry. Due to the low availability of phytate-P to poultry, dietary P (inorganic P) is added to poultry diets in order to meet the P needs of the bird. It is a common practice

in the commercial environment to overfeed dietary P exceeding the published requirement (Applegate and Angle, 2008). Calcium phosphate, a phosphate supplement in poultry diet, is produced from rock phosphate which is non-renewable and was predicted to decline in its production in near future (Ulrich and Schnug, 2013). Besides having higher cost of feeding due to inclusion of expensive inorganic P in poultry diet, the excess of soluble P from overfeeding of dietary P and undigested phytate-P may increase the total and soluble P content of excreta and litter. This will lead to a higher risk of environmental pollution (Angle *et al.*, 2002). Thus, the efficient approach to reduce feed cost and ecological hazards posed by P is by reducing or

avoiding the use of inorganic P supplementation and increasing the bioavailability of phytate-P and other nutrients in the feed. This can be done via degradation of phytate using a phytase enzyme (inositol hexaphosphate phosphohydrolase) and solubilization of phytate at pH values below 4.5 (Graham *et al.*, 2009).

Several excellent reviews have appeared covering the use of microbial phytase in poultry nutrition in relation to P utilisation, the extra phosphoric effects of phytase and factors affecting phytase efficacy in phytate hydrolysis (Maenz, 2001; Kornegay, 2001; Selle and Ravindran, 2007; Selle *et al.*, 2010; Greiner and Konietzny, 2011). This paper will provide a brief review of *in vitro* phytate hydrolysis, phytate hydrolysis in the gastrointestinal tract (GIT) of chicken and factors that affect phytate hydrolysis which could be manipulated to enhance phytase efficacy in poultry.

A number of factors have been identified to influence the efficacy of phytases and

these factors could be manipulated in order to enhance the positive responses in chickens, particularly broilers. However, one has to understand the avian digestive system, the condition of the GIT of broilers and nature of phytate and phytases in order to formulate strategies in improving phytase efficacy.

**Gastrointestinal tract of chickens**

Avian digestive system consists of an elementary canal from the beak/mouth to the cloaca/vent, liver and pancreas. Feed enters the beak into the mouth and passes through the esophagus into the crop, proventriculus and ventriculus/gizzard (Figure 1). Digesta in the gizzard is discharged through the pylorus into the duodenum, passes into the lower small intestine (jejunum and ileum) and finally faecal materials are discharged at the cloaca via the large intestine or colon. The retention time (RT) of feed/digesta is the time taken for feed to retain in each segment of the gut before passing through the GIT.

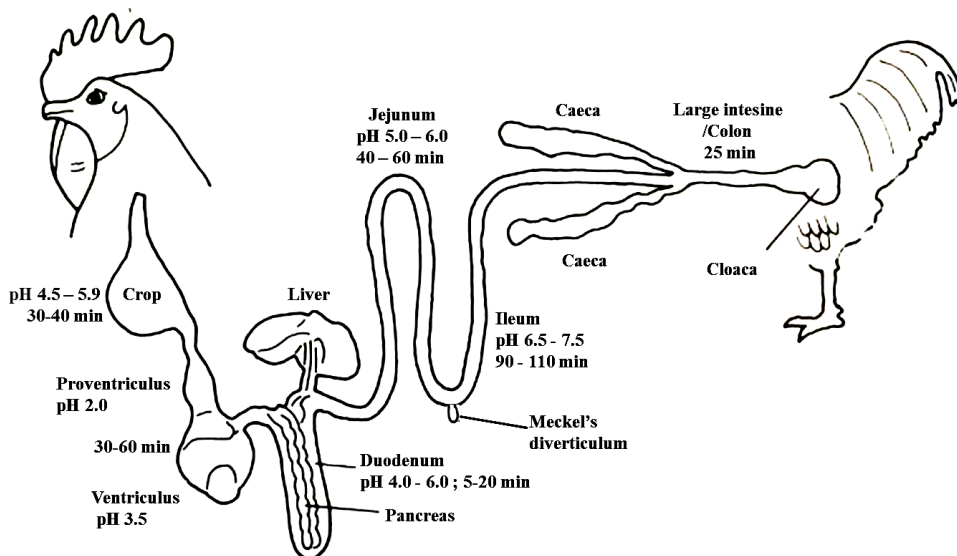


Figure 1. Retention time and pH of the digestive content along gastrointestinal tract of chicken.

Usually the pH of crop content is similar or close to the pH of the feed which is between 4.5 to 5.9 and digesta remains in the crop for 30 to 40 min (Svihus 2011). As the feed enters the proventriculus or true stomach, the pH of the digesta is drastically reduced to as low as pH 2.0 due to secretion of hydrochloric acid by submucosal glands in the proventriculus. pH of gizzard content was reported to be highly variable, ranging from 1.9 and 4.5, with an average value of 3.5 (Svihus, 2014). After 30 to 60 min in the proventriculus and gizzard, the digesta is peristaltically moved into the small intestine.

Small intestine starts from the exit from gizzard through duodenum, jejunum and ileum to the end of small intestine at the junction of ileum, caeca and colon. Digesta passes through duodenum within a short time (<5 min, Chee *et al.*, 2010) but the pH of duodenal content is increased from 2.0 to more than 6.0 as duodenum receives pancreatic juice that contains sodium bicarbonate for hydrochloric acid neutralization.

The digesta then enters the jejunum, the site for digestion and absorption of fat, starch and protein with mean RT of 40 to 60 min (Weurding *et al.*, 2001, Chee *et al.*, 2010). Although the length of the ileum is about the same as that of the jejunum, digesta passage through the ileum is slower ranging between 90 and 110 min (Weurding *et al.*, 2001, Chee *et al.*, 2010). In addition to some major nutrient digestion and absorption, minerals and water are thought to be mainly absorbed in the ileum. In the lower small intestine, digesta pH is less variable in comparison to those in the crop and gizzard with an average pH of 6.5 to 7.5 (Svihus, 2011). About 18% of ileal digesta dry matter enters caeca, 2 blind pouches that are located between the end of ileum and before large intestine, and the rest passes into the large intestine. According to Svihus *et al.* (2013), only finely-ground particles and/or soluble, low

molecular weight and non-viscous molecules enter the caeca.

### ***Feed components, particle size and form***

Carbohydrate, protein, fat, mineral, vitamin and water are the main components of the feed that provide energy and nutrients to the chickens. Corn, wheat and barley are some of the major carbohydrate sources which are used in chicken feed as the source of energy. Most carbohydrates in the form of starch are readily digestible in young chicken. However, other types of carbohydrate known as non-starch polysaccharide or fibre are less digestible and some of them are resistant to digestive enzyme such as cellulase. Fibres such as  $\beta$ -glucan and arabinoxylan become antinutrients that interfere with other nutrient utilization by creating viscous environment that reduces the nutrient absorption in the small intestine and consequently detrimentally affects the performance of the chicken.

Fat on the other hand provides higher calories per gram of carbohydrate compared to cereal grains. Usually fat is added into feed to increase the overall energy concentration of the feed. Besides reducing grain dust during feed processing, fat also improve the palatability of the feed. Supplementation of fat also reduces food passage along the digestive tract that warrants better digestion and nutrient absorption (Mateos and Sell, 1980). Meanwhile, soybean meal, corn gluten meal and fishmeal are among the common protein sources used in chicken feed to provide amino acids required for body protein synthesis and construction of body tissues including muscles, nerves and cartilage. Limestone and oyster shells are the sources of calcium and dicalcium phosphate is the source for both calcium and phosphorus. Calcium and phosphorus are essential for the

formation of bones. Deficiency of either Ca or P in young chicks results in abnormal bone development.

In the effort to provide a highly digestible feed to post hatched chicks, ingredients with high energy and high protein content are used and consequently the feed contains very low crude fibre (CF). On the other hand, chickens fed on diets with very low CF were found to have poor GIT development (Gonzales-Alvarado *et al.*, 2008). According to Mateos *et al.* (2012), about 2-3% of insoluble dietary fibre (DF) with particle size of more than 1 mm is required to stimulate a proper development of GIT. The examples of insoluble DF are oat hulls, sugar beet pulp, soybean hulls and sunflower hulls. Although microcrystalline cellulose is also insoluble DF, it does not affect the development of GIT and growth performance. It was thought to be due to its lack of physical structure (Jimenez-Moreno *et al.*, 2009). On the other hand, fine DF may accumulate in gizzard and reduce the passage of digesta through the GIT. The passage rate of digesta containing fine DF may be further reduced with the presence of coarse fibre (Mateos *et al.*, 2012).

Use of whole wheat in chicken diet also contributes in development of GIT, particularly gizzard and improves ileal nutrient absorption (Hetland *et al.*, 2002). Increase in pancreas and liver secretions may also contribute in more efficient digestion and absorption of diet with whole wheat compared to diet ground wheat which indicates the role of wheat form (whole or ground) in development of digestive functions (Svihus *et al.*, 2004). According to Amerah *et al.* (2015), the effect of feed particle size is more critical on growth performance and development of GIT when chickens fed on mash feed compared to pelleted feed. Chickens fed on mash feed with coarse particle size have higher body weight gain and large size of gizzard than

those fed on fine particle size. Meanwhile, pelletization of the feed reduces the performance gap between different particle sizes. On the other hand, less developed gizzard was observed in chickens fed on crumble-pellet feed although the growth performance was better than those fed on mash feed. During pelletizing, feed ingredients are finely ground, mixed and mechanically pressed to form 'artificial grain'. Besides having well balanced nutrients, pelleting improves palatability, reduces selective feeding and feed wastage and consequently improves feed intake, body weight gain and feed conversion ratio. However, the form of pellet feed is more readily disintegrated as feed enters the mouth and further breaks up due to grinding activity by gizzard muscles. The retention time of small particle feed in the gizzard is shorter than coarse feed and less mechanical stimulation leads to size reduction of digestive organs.

Although early development of digestive system in chickens is critical in ensuring adequate nutrient intake that is necessary for growth, the process of feed digestion is not 100% efficient. This is due to the lack of or very low activity of specific enzymes in the GIT to break down certain components of the feed. The presence of indigestible anti-nutritive factors in most of feed ingredients such as NSPs and phytate also interfere the digestion process. Feed enzymes are used to reduce the adverse effects of anti-nutritive factors by breaking down fibre or phytate and improving the availability of nutrients including starch, amino acids, calcium and phosphorus from the feed.

Broiler chicken is one of the fastest growing farmed animals and presently the chicken can reach a weight of approximately 2 kg in 35 d while consuming only 3.2kg of feed. Broiler growth rates have increased about 300% over the last 50 y of production intensification and genetic selection. Leg

disorders are considered as welfare issues and have been a considerable problem to the broiler industry. Leg-bone abnormalities can lead to severe walking problems and lameness and even death due to starvation and dehydration. There is evidence that indicates the importance of early nutrition on chick development to prevent initiation of bone defect in a very young chick (Fleming, 2008). Rickets is commonly observed in young broilers, which indicates deficiency or imbalance in dietary calcium, phosphorus, or vitamin D<sub>3</sub>. Tibial dyschondroplasia, characterized by abnormal cartilage mass in the proximal head of the tibiotarsus, is another common disease related to imbalanced calcium: phosphorus ratio. The diet phosphorus level is relatively higher than calcium. The deformation of bone can be prevented or alleviated with the balance supply of Ca and P at 2:1 ratio in the starter diet.

### **Phytate**

Phytate is a salt form of phytic acid or myo-inositol-6-phosphates (InsP<sub>6</sub>), which is a major storage form of P and myo-inositol in mature plant seed. It was reported that the biosynthesis of phytate begins soon after flowering and during development of seed (Bohn *et al.*, 2007; Woyengo and Nyachoti, 2011). Almost all P that is taken up by the root of a crop is translocated to the seed and usually they are more than required for cellular function. Phytate is synthesized via 2 possible pathways: lipid independent pathway (Raboy, 2009) and lipid dependent pathway to yield phytic acid (Loewus, 2001).

Phytate is present in major plant feedstuffs in the form of phytate-mineral complexes, mainly Mg-K-phytate (Shears and Turner, 2007; Lott *et al.*, 2000). Due to its chemical structure, InsP<sub>6</sub> is capable of binding to positively charged molecules and nutrients to form a very stable insoluble

complex, which is a main anti-nutritive character of InsP<sub>6</sub>. Besides Mg and K, phytate can also form complexes with other minerals including Cu, Zn, Ni, Co, Mn, Fe and Ca but Cu and Zn have the strongest binding affinity (Cheryan, 1980). Besides the type of cations being involved during the formation of phytate-mineral complexes, phytate solubility is also dependent on pH. Phytate is more soluble at lower pH values than at higher pH values. Na-phytate and K-phytate are soluble at all encountered pH values. Zn-phytate, Ca-phytate and Mg-phytate are insoluble above pH values of 4.3, 5.5 and 7.2, respectively. Meanwhile, Fe-phytate is insoluble at pH below 3 but slowly becomes soluble as pH value increases above 4 (Selle and Ravindran, 2007; Kumar *et al.*, 2010).

With reference to other monogastric animals, a study on phytate solubility in pigs conducted by Schlemmer *et al.* (2001) has shown that InsP<sub>6</sub> and less phosphorylated inositol phosphates (lower InsPs) have different levels of solubility, where the lowest InsPs has the highest solubility. It was also observed that the complexes formed between lower InsPs with minerals were proportionately weaker, suggesting that the hydrolysis of phytate to at least InsP<sub>3</sub> is necessary in order to achieve higher solubility of both minerals and InsPs at higher pH (Cowieson *et al.*, 2011). Although most of the phytate-P present in feed ingredients is in the form of Mg- and K-phytate, Ca-phytate was shown to play a crucial role in phytate-P bioavailability in poultry. The formation of Ca-phytate along the GIT of chicken was assumed to be important (Selle *et al.*, 2009). The formation of Ca-phytate complexes occurs over a broad pH range, between pH 2 to pH 12, and the affinity of phytate for Ca ion increases with pH (Marini *et al.*, 1985). The Ca-phytate complexes were soluble below pH 4 and became insoluble at pH above 5 (Grynsan

and Cheryan, 1983). However, pH 5 and pH 5.4 were found critical for the formation of Ca-phytate complexes from phytate/InsP6 and InsPs (InsP1 to InsP5), respectively (Selle *et al.*, 2009).

Due to its strong negative charge, phytate is also capable of interacting with proteins to form binary phytate-protein complexes or ternary mineral-phytate-protein complexes (Figure 2), which was thought to be mediated by pH values in the gut (Singh, 2008). Binary phytate-protein complex forms at pH values below 5 through strong electrostatic interaction between negatively charged phytate and positively charged protein, which only redissolves at pH values below 3 (Dersjant- Li *et al.*, 2014). At pH above 7,

the formation of completely insoluble ternary mineral-phytate-protein complexes occurs via involvement of multivalent cations in the interaction (Dersjant- Li *et al.*, 2014). Phytate can also directly bind with starch via hydrogen bonds or indirectly via proteins associated with starch or with starch (Truong *et al.*, 2015) and fat in the presence of Ca to form faecal soap fat (Atteh and Leeson, 1984). The presence of these complexes along the GIT will lead to a reduction in amino acids, energy and nutrient digestibility and reduction of protein functionality, especially with regards to a number of digestive enzymes in chickens (Selle and Ravindran, 2007).

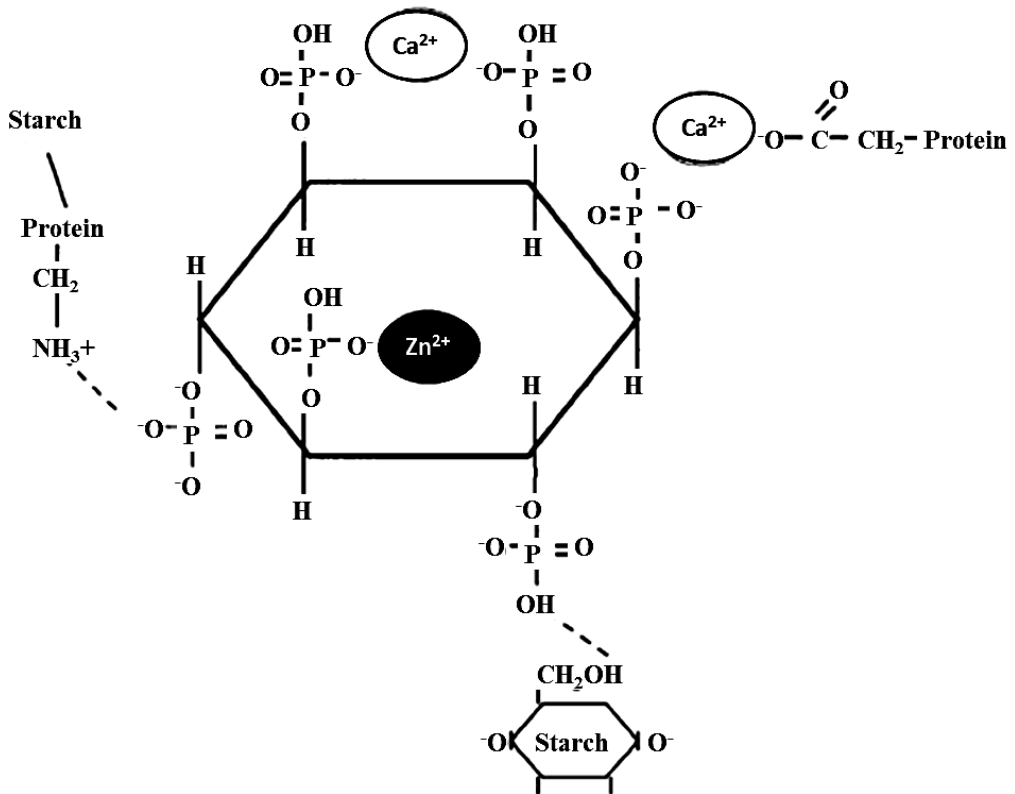


Figure 2. Structure of phytate and possible bonds (after Thompson, 1988).  
Adopted from Kornegay (2001)

### *Phytase*

Phytate, an anti-nutrient that is present in most plant based feed ingredients but yet it is a useful source of nutrients. Degradation of phytate could lead to the release of P and other bound nutrients and makes them available for absorption in poultry. The most practical and effective approach to breakdown phytate in poultry feed is via supplementation of exogenous microbial phytases. Phytases (inositol hexaphosphate phosphohydrolase), a sub-group of phosphatases, are important enzymes that are capable of hydrolyzing of phytate and release phytate bound phosphorus (phytate-P) in stepwise manner. Phytases have been identified in plants, microorganisms and in some animal tissues. In most feed ingredients, the detected phytase activity is considerably low and due to a narrow pH spectrum of activity, plant phytases become less effective at a low pH, more susceptible to proteolytic digestion and thermal destruction during feed processing. Animal phytases are produced by intestinal mucosa and are largely found in the duodenum. Their production seems to be

regulated by the presence of phytate and products of its hydrolysis (Woyengo and Nyachoti, 2011). Meanwhile, microfloral phytases, produced by the hindgut microbial population, are considered to have some influences in further hydrolyzing the undigested phytate-P as concluded by Kerr *et al.* (2000). In comparison to the intestinal mucosa phytases, microfloral phytases are more capable of hydrolyzing phytate-P.

Another type of phytases is microbial phytase, currently being used in animal feeds,, is produced using fungi (e.g, *Aspergillus niger*), bacteria (e.g., *Escherichia coli*) and yeast. Most of them are derived by over expressing phytase genes in a suitable host. Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK), Ronozyme P (DSM Nutritional Products, Basel, Switzerland), Quantum (AB Vista, Marlborough, UK), Natuphos (BASF, Germany) and OptiPhos (Phytex LLC, Sheridan, IN) are some of the commercially available phytases for animal feed. The biochemical properties and relative catalytic performance of selected commercialized phytases were reported by Menezes-Blackburn *et al.* (2015) (Table 1).

Table 1. Enzymatic properties of selected commercially available phytases (Adopted from Menezes-Blackburn *et al.*, 2015)

Trademark	Quantum	Quantum Blue	PhyzymeXP	AxtraPHY	Ronozyme Hiphos	Ronozyme NP	Natuphos
Supplier	AB Vista	AB Vista	Danisco	Danisco	Novozyme/DSM	Novozyme/DSM	BASF
Recommended dosage for broiler	500 FTU/kg	500 FTU/kg	250 FTU/kg	250 FTU/kg	500 FTU/kg	1500 FTU/kg	500 FTU/kg
Donor organism	<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Escherichia coli</i>	<i>Buttiauxella sp.</i>	<i>Cytobacter braakii</i>	<i>Peniphora lycii</i>	<i>Aspergillus niger</i>
Production organism	<i>Trichoderma reesei</i>	<i>Trichoderma reesei</i>	<i>Schizosaccharomyces pombe</i>	<i>Trichoderma reesei</i>	<i>Aspergillus oryzae</i>	<i>Aspergillus oryzae</i>	<i>Aspergillus niger</i>
pH range (80% of optimal activity)	4.0 – 5.0	3.5 – 5.0	3.0 – 5.0	3.0	3.0 – 4.5	4.5 – 5.5	4.5 – 5.5
Phytase activity at pH 3.0 <sup>a</sup> (%)	92.5	101.3	82.8	235.1	145.7	12.5	64.2
Phytase activity at pH 7.0 <sup>a</sup> (%)	0.8	2.2	1.7	.5	.6	7.8	7.0
K <sub>M</sub> (μM) for phytate at pH 5.0 and 37°C	228	142	285	272	364	75	35
K <sub>cat</sub> (s <sup>-1</sup> ) for phytate at pH 5.0 and 37°C	1545	1821	1327	1054	1478	1532	318
K <sub>M</sub> (μM) for phytate at pH 3.0 and 37°C	257	178	302	311	427	98	142
K <sub>cat</sub> (s <sup>-1</sup> ) for phytate at pH 3.0 and 37°C	1012	1274	984	768	1061	824	170
Residual activity (%) (pH 3.0, 37°C, 45 min)							
Without pepsin	95	98	92	87	93	58	81
With 3000 U pepsin	93	98	92	85	92	34	47
Optimal ionic strength (mMNaCl)	50-100	50-200	100-200	50-200	50-200	50-200	50-600
Phytase activity needed to achieve % of maximum reachable values							
50% reduction of InsP <sub>6</sub>	326 (0.95)	319 (0.86)	395 (0.92)	323 (0.92)	445 (0.87)	418 (0.80)	586 (0.89)
50% reduction of InsP <sub>6</sub>	2194 (0.84)	955 (0.94)	1159 (0.93)	952 (0.94)	2200 (0.97)	2606 (0.89)	2398 (0.93)

<sup>a</sup> Phytase activity at pH 5.5 was taken as 100%; <sup>b</sup> Values (U/kg) obtained by non-linear fit of the observed data; coefficient of determination in parentheses.

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There is a series of quality criteria that should be fulfilled before an enzyme product can be considered as “ideal” for animal feed applications. Those criteria include the effective release of phytate phosphate in digestive tract, product stability during feed processing and storage and the economical production of the enzyme product. Many studies have demonstrated the differences between commercially available phytases and the understanding of those differences is necessary to secure the optimal animal performance. However, the properties of each phytase product could be used as guidelines on potential functionality in animal feeds and digestive systems.

The classes of phytases are based on the initiation sites for dephosphorylation of phytate. The phytases that preferentially

initiate phytate dephosphorylation in position 3 are called 3-phytase, whereas, 6-phytases initially remove phosphate residue from position 6 (Figure 3). Currently, these two classes of phytases are extensively studied in poultry nutrition. Other classes of phytases were also reported (2-phytases, 4-phytases and 5-phytase) with initiation site of phytate dephosphorylation at position 2, 4 and 5 respectively. Most 3-phytases were predominantly observed in microorganisms such as *Aspergillus* sp., *Bacillus* sp., whereas 6-phytases were found in *E.coli* and *P. lycii*. Most of plant phytases are 4-phytases except lupin (3-phytase) and lily pollen (5-phytase) while 2-phytases (intracellular phytases) were observed within animal cells. There is no report on the existence of 1-phytases as yet.

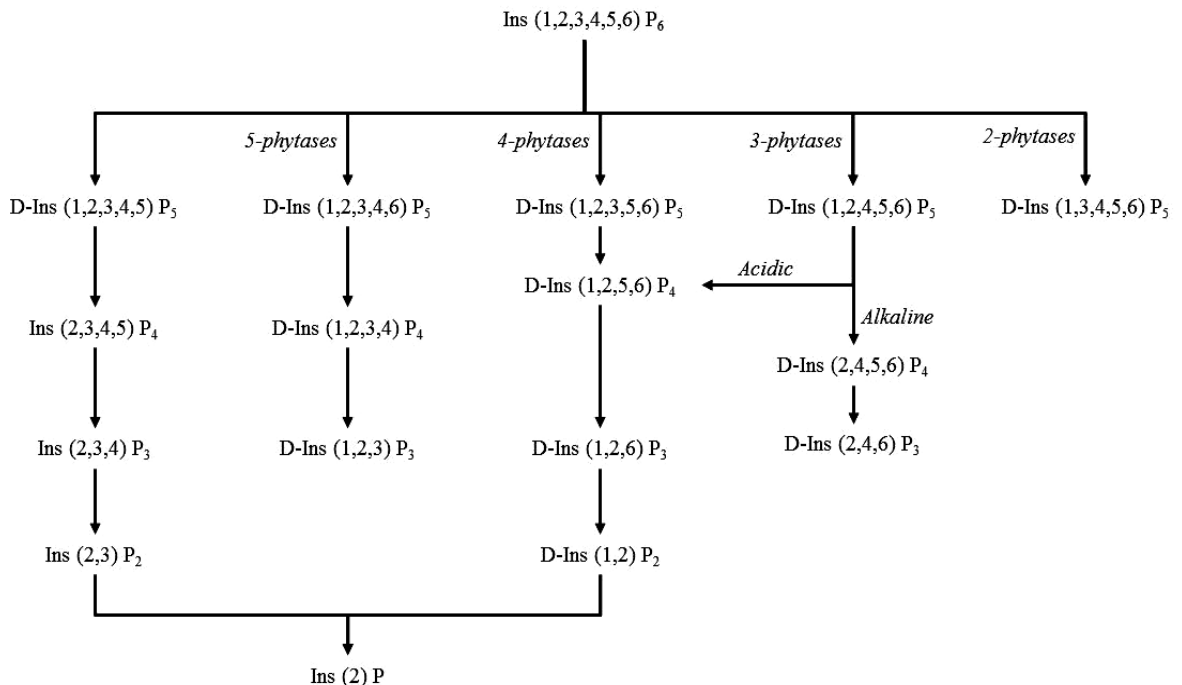


Figure 3. Major phytate degradation pathways for the classes of phytases (Adapted from Greiner and Konietzny, 2011)

Digesta retention time (RT) and pH are recognized as important physiological factors that contribute in determining the efficacy of phytase mainly on phytate degradation and P utilization in the digestive tract of poultry (Selle and Ravindran, 2007). The mechanism of how phytate interacts with nutrients along the GIT of chickens that leads to the reduction of nutrient utilization has been described by Adeola and Cowieson (2011). Degradation of phytate has to happen as quickly as possible in the crop, proventriculus and gizzard in order to minimize the anti-nutritional effects of phytate. Exogenous phytases, mainly microbial origin, should be able to degrade  $\text{InsP}_6$  up to  $\text{InsP}_3$  rather than to inositol and free phosphate in order to limit the passage of phytate esters ( $\text{InsP}_5$  and  $\text{InsP}_4$ ) from gizzard into the duodenum.

The main sites for phytate degradation by microbial phytases are in the crop and gizzard of the chicken. Microbial phytase activity was found relatively higher in the crop, gizzard and followed by duodenum, jejunum and ileum (Yu *et al.*, 2004; Onyango *et al.*, 2005). Beside favourable pH conditions and low protease activity, phytase from *P.lycii* with pH optimum between 4.0–4.5 (Augsburger *et al.*, 2003) and that from *E.coli* with pH optimum of 4.5 (Onyango *et al.*, 2005) would be more active in the crop than in the proventriculus and gizzard of the chicken. In addition, with high solubility of phytate at pH 4 and below, more phytate would be degraded by the time digesta reached the proventriculus and gizzard (Zeller *et al.*, 2015a). Phytases with high stability toward proteolytic activity of the gastric region and broader range of optimal pH (2.5 to 6.0) may be able to continue degrading more phytate in the proventriculus and gizzard (Walk *et al.*, 2014), and perhaps beyond the upper part of the GIT. Higher phytase activity was also detected in duodenum compared to in the ileum

(Onyango *et al.*, 2005), therefore higher degradation of residual phytate from gizzard in duodenum would be expected than in ileum.

Despite very low phytase activity was detected in the diet and in each section of GIT of broilers, P digestibility was considerably high in broilers fed on low P and Ca diet without phytase supplementation (Onyango *et al.*, 2005, Tamim *et al.*, 2004). This is due to the presence of intestinal phytase, phosphatases and intrinsic plant phytase, which contribute to the utilization of phytate-P (Morgan *et al.*, 2015). Higher capacity of P utilization via intestinal phytase was further induced by low dietary P (Abudabos, 2012). Degradation of undigested phytate was also observed beyond ileo-caecal junction indicating the contribution of gut microbiota in phytate degradation. In addition, Zyla *et al.* (2004) demonstrated that further phytate hydrolysis on the myo-inositol rings is achievable by adding nonspecific phosphatases to diet containing exogenous phytases at 500 FTU/kg or higher. Nevertheless, due to short RT and small range of optimum pH in each part of GIT, further improvement of exogenous phytase efficacy on phytate degradation and P utilization is considered challenging.

### ***In vitro* phytate hydrolysis**

Although addition of phytase into diets is able to increase phytate hydrolysis, the *in vitro* phytate hydrolysis is affected by type of phytase, pH, phytate matrix, phytate origin and level of added Ca (Tamim *et al.*, 2004, Brejnholt *et al.*, 2011). Fungal phytases (mainly 3-phytase) and bacterial phytases (particularly 6-phytase) were shown to have different pH for optimal phytate-P hydrolysis. Although 3-phytases have 2 peaks of activity, i.e at pH 3 and pH 5.5, concentration of P released by 6-phytase at

pH around 4.5 was significantly higher than the concentration of P released by 3-phytase. Increasing concentrations of added Ca increased phytate-P hydrolysis by both 3-phytase and 6-phytase at low pH (pH 2.5), while at higher pH (pH 6.5) as low as 0.1 % added Ca negatively influenced phytate-P hydrolysis by both types of phytase (Tamim *et al.*, 2004). The degree of InsP<sub>6</sub> and InsP<sub>5</sub> hydrolysis by endogenous and recombinant wheat phytases was the highest at pH 4 but reduced as pH increased beyond or lower than pH 4. Microbial phytase, otherwise, has a broader pH range (pH 3 to 5) in hydrolysing InsP<sub>6</sub> and InsP<sub>5</sub> (Brejnholt *et al.*, 2011). Microbial phytases reduced more than 75% of the contents of InsP<sub>6</sub> and InsP<sub>5</sub> in wheat, corn, barley and rapeseed meal but recombinant wheat phytases showed a variable degree of InsP<sub>6</sub> and InsP<sub>5</sub> hydrolysis in these feed materials.

The efficacy of phytase in feed materials under feed processing conditions was shown to depend on moisture level of the feed mixture (Denstadli *et al.*, 2006). Concentration of InsP<sub>6</sub> was reduced by between 76 and 86% when a phytase supplemented feed mixture was moistened to 45% (ml/g DM). The moisture level of more than 45% is not recommended due to possible complication in extrusion process. Phytase supplementation at 2500 FTU/kg was not sufficient to completely hydrolyse InsP<sub>6</sub> in feed materials.

Complete phytate hydrolysis or dephosphorylation is achieved when each phytate molecule of feed ingredients is degraded and digested and residual concentration of phytate measured after digestion is negligible. In *in vitro* hydrolysis of phytate, the extent of phytate hydrolysis is measured as the percentage of total P removed from the feed and this criterion of measurement is called dephosphorylation level (DL). Another criterion for *in vitro* phytate hydrolysis, suggested by Zyla *et al.*

(2004), is the measure of dialysed free P (%) due to phytate degradation over the total P removed from the feed, which is also called the conversion degree (CD). Measure of CD indicates the extent of phytate hydrolysis on the myo-inositol rings, which also relates to changes in free myo-inositol concentration. In their study, Zyla *et al.* (2004) demonstrated that addition of a nonspecific phosphatase to a diet containing 3-phytase or 6-phytase at 500 FTU/kg or higher did not increase DL but increased CD. Without addition of nonspecific phosphatases, the concentration of free myo-inositol was not further enhanced by 3-phytase or 6-phytase.

The efficacy of phytase produced via solid state fermentation (SFF) in *in vitro* digestion in comparison to phytases produced via submerged liquid fermentation (SLF) was reported by Wu *et al.* (2004b). The release of dialysed P in wheat-soy and corn-soy diets was higher with SSF phytase compared to SLF phytases, suggesting the presence of unknown factors in SSF phytase that enhanced phytate-P hydrolysis. Besides phytase, other enzymes such as cellulase, amylase, xylanase, glucanase and other side activities were detected in SSF phytase while in SLF phytase, usually side enzyme activities were undetectable (Sabu *et al.*, 2003).

Another approach in determining the *in vitro* efficacy of phytase is by measuring the solubility of P and Ca. At gastric pH (pH 2.75 to 3.5), phytate, P and Ca are soluble and have high solubility value. While at the small intestine pH (6.5), phytate, P and Ca are likely to precipitate and low solubility values of P and Ca are expected (Selle *et al.*, 2009). The effect of phytase doses and any factors affecting phytase efficacy, therefore, would change the value of solubility of P and Ca. An increasing phytase level increased P and Ca solubility in soybean meal, rapeseed meal and diets containing high level of soybean meal or rapeseed meal in both

gastric and small intestine phase (Morgan *et al.*, 2014). Phytase increases P solubility of corn based and soybean meal based diets. When the diets contain either adequate or lower P and Ca, the solubility P and Ca in the diets are further increased by phytase supplementation. The P solubility in the diets is further increased with addition of phytase together with dicalcium phosphate but reduces when phytase is added together with limestone (Walk *et al.*, 2012a, Walk *et al.* 2012b). This is largely related to the Ca:P ratio in the diets which reflects the ability of phytate to form insoluble phytate-Ca-P complexes and make it inaccessible for phytase-phytate reaction. Solubility of P and Ca could be further increased by phytase with smaller particle size diets (Manangi and Coon, 2007).

The *in vitro* performance of commercial 6-phytases and 3-phytases using ground wheat that contained inactivated intrinsic phytase as the source of phytate was found to be different suggesting that the *in vitro* degradation system cannot be used to rank phytases based on their bioefficacy (Menezes-Blackburn *et al.*, 2015). The generated results did not precisely reflect their performance in animals but these systems can be useful in evaluating the potential benefits of phytases as feed supplement. Nevertheless, Morgan *et al.* (2014) demonstrated a strong relationship between *in vitro* and *in situ* evaluation of phytase efficacy on P and Ca solubility. In order to fully compare and determine the efficacy of potential feed phytases, dose response feeding trials in animal species of interest would need to be performed in spite of the successful prediction of phytase efficacy via *in vitro* assays (Brejnholt *et al.*, 2011, Morgan *et al.* 2014)

### ***Phytate hydrolysis in the chicken's gastrointestinal tract***

In contrast to *in vitro* degradation, phytate hydrolysis in the GIT of chickens could be affected not only by supplemental phytase and the intrinsic phytase in feed materials but also by intestinal mucosa phytase and phytase produced by the intestinal microflora. Zeller *et al.* (2015a) reported the efficacy of phytases in different sections of the digestive tract of broilers. Supplementation of either 3- or 6- phytase at 500 FTU/kg increased phytate hydrolysis in the crop but did not significantly affect phytate hydrolysis in the duodenum/ jejunum or ileum. However, the presence of InsP<sub>5</sub> and InsP<sub>4</sub> isomers at high concentration in the duodenum/jejunum and ileum in the 6-phytase supplemented group was assumed to be due to further activities of the enzymes in lower gut sections. Hydrolysis of phytate by 3-phytase was also reported to be higher compared to 6-phytase in the crop (Zeller *et al.*, 2015a). In the gizzard, phytate hydrolysis was almost complete in chickens fed with low P and Ca diets when phytase supplementation level was increased up to 1500 FTU/kg (Walk *et al.*, 2014). The gizzard concentrations of InsP<sub>3</sub>, InsP<sub>4</sub> and InsP<sub>5</sub> were also reduced resulting in a higher concentration of inositol.

Considerably high phytate hydrolysis was detected in the duodenum/jejunum and ileum, ranging from 55% to 59% and 67% to 74%, respectively, when chickens were fed low Ca and P diets without dietary phytase (Zeller *et al.*, 2015a; 2015b). About 91% of caecal phytate hydrolysis was also reported (Zeller *et al.*, 2015a). These studies demonstrated the occurrence of phytate hydrolysis in small intestine and caeca was either due to endogenous mucosa phytase, the activity of the intestinal microbiota or combination of both endogenous phytase and intestinal microbiota activities. When the

level of Ca and P in the diet was increased, the level of phytate hydrolysis in the small intestine was reduced, which may be due precipitation of insoluble and undegradable Ca-phytate at the pH of the small intestine (Zeller *et al.*, 2015b). However, the possibility of a decrease in the level of endogenous or microbiota-related phytase due to higher Ca and P level is undeniable.

At a very high level of supplemental phytase, although it did not result in a complete ileal hydrolysis of InsP<sub>6</sub>, more complete ileal hydrolysis of InsP<sub>5</sub> was observed. In addition, ileal hydrolysis of InsP<sub>6</sub> and InsP<sub>5</sub> due to phytase supplementation at 12,500 FTU/kg was not affected by increasing level of Ca and P in the diet (Zeller *et al.*, 2015b). Therefore, intestinal mucosa phytase and phytase produced by the intestinal microflora did contribute in ileal phytate hydrolysis but it is negatively affected by level of Ca and P in the diet. Very high level of phytase supplementation, however, could be used to further improve ileal phytate hydrolysis.

### ***Ileal P digestibility***

Supplementation of phytase has been shown to improve P digestibility and selected

studies on the effect of phytase on ileal P digestibility are listed in Table 2. Most of the studies were conducted with corn-soybean meal diets, however, Leytem *et al.* (2008) and Rutherford *et al.* (2004) evaluated phytase efficacy on ileal P digestibility in other feed materials beside corn. Leytem *et al.* (2008) reported that ileal P digestibility of corn, wheat, oat and barley based diets containing low P were 56%, 57%, 65% and 64%, respectively. With addition of *A. niger* 3-phytase at 1000 FTU/kg, the percentage increase in ileal P digestibility was small, ranging between 1 and 5%. Rutherford *et al.* (2002), on the other hand, reported higher ileal P digestibility in feed materials including corn, soybean meal, wheat, rice bran and rapeseed meal increasing by from 6 to 17% when supplemented with 750 FTU/kg 6-phytase of *P. lycii* expressed in *A. oryzae*. Wu *et al.* (2004) found 22.7% increase in ileal P digestibility after supplementing wheat-soybean-canola diets with 1000 FTU/kg phytase produced via solid state fermentation (SSF). Besides demonstrating the difference in ileal P digestibility in different feed materials in response to phytase supplementation, these studies also showed that the type of phytase and level of P and Ca influenced P digestibility.

Table 2 Results on the effect of supplemental phytase on ileal P digestibility by several authors

Phytase type	Phytase inclusion (FTU/kg)	PP, P, Ca (g/kg)	Ileal P digestibility		%* above control	Diet	Gender /Age	References
			Without phytase	With phytase				
6-phytase ( <i>E. coli</i> )	500	-, 5.2, 7.5	57	64	7.0	Corn-SBM	Mixed /25 d	Zeller <i>et al.</i> (2015a)
6-phytase ( <i>E. coli</i> )	12,500	-, 4.4, 6.0	52	71	19.0	Corn-SBM	Mixed /24 d	Zeller <i>et al.</i> (2015b)

Continue...

Table 2 Results on the effect of supplemental phytase on ileal P digestibility by several authors

Phytase type	Phytase inclusion (FTU/kg)	PP, P, Ca (g/kg)	Ileal P digestibility		%* above control	Diet	Gender /Age	References
			Without phytase	With phytase				
...continue								
6-phytase ( <i>Buttiauxella</i> in <i>T. reesei</i> )	500	-. 4.8, 7.9	39.5	64.9	25.4	Corn-SBM	Male /22 d	Kiarie <i>et al.</i> (2015)
	2,000	-. 4.8, 7.9	39.5	68.2	28.7			
6-phytase ( <i>Buttiauxella</i> in <i>T. reesei</i> )	1,000	3.2, 5.1, 5.1	55.1	71.9	16.8	Corn SBM	Male /21 d	Amerah <i>et al.</i> (2014)
6-phytase ( <i>T. reesei</i> )	500	-, 4.2, 6.0	40.6	51.7	11.1	Corn SBM	Male /34 d	Kuhn <i>et al.</i> (2012)
6-phytase ( <i>T. reesei</i> )	500	-, 6.0, 4.5	40.8	54.6	13.8	Corn SBM	Male /22 d	Walk <i>et al.</i> (2012c)
	2,500	-, 6.0, 4.5	40.8	67.5	26.7			
3-phytase ( <i>A. niger</i> )	1,000	-, 10.6, 9.5	35.81	45.87	10.06	Corn-SBM	Male /35 d	Nourmoham-madi <i>et al.</i> (2012)
6-phytase ( <i>Peniiphora lycii</i> )	1,000	3.2, 5.6, 8.9	53.3	59.9	6.6	Corn-SBM	Male /22 d	Rutherford <i>et al.</i> (2012)
	2,000	3.2, 5.6, 8.9	53.3	61.9	8.6			
3-phytase ( <i>A. niger</i> )	1,000	2.5, 5.7, 8.4	56	59	3.0	Corn-SBM	Male /21 d	Leytem <i>et al.</i> (2008a)
	1,000	3.0, 5.9, 8.1	57	62	5.0			
Continue...								

Table 2 Results on the effect of supplemental phytase on ileal P digestibility by several authors

Phytase type	Phytase inclusion (FTU/kg)	PP, P, Ca (g/kg)	Ileal P digestibility		%* above control	Diet	Gender /Age	References
			Without phytase	With phytase				
...continue								
3-phytase ( <i>A. niger</i> )	500	3.1, 4.0, 1.7	67.9	75.1	7.2	Corn-SBM	Male /24 d	Tamim <i>et al.</i> , (2004)
	500	3.1, 4.0, 6.5	29.4	50.4	21.0			
6-phytase ( <i>E. coli</i> )	1,000	2.4, 4.3, 7.7	69.3	73.9	4.6	Corn-SBM	Male /22 d	Onyango <i>et al.</i> (2005)
6-phytase ( <i>P. lycii</i> )	500	-, 6.5, 7.8	53.14	63.04	9.9	Corn-SBM	Male /28 d	Rutherford <i>et al.</i> (2004)
phytase ( <i>A. niger</i> ) (solid state fermentation)	500	2.9, 5.7, 8.3	43.3 (35.4)	60.9 (58.8)	17.6 (23.1)	Wheat SBM, Canola	Male (Female) /42 d	Wu <i>et al.</i> (2004)
	1,000	2.9, 5.7, 8.3	43.3 (35.4)	66 (60)	22.7 (24.6)			
	1,500	2.9, 5.7, 8.3	43.3 (35.4)	67.7 (68.8)	24.4 (33.4)			
	2,000	2.9, 5.7, 8.3	43.3 (35.4)	73.5 (67.4)	30.2 (32.0)			
6-phytase ( <i>P. lycii</i> in <i>A. oryzae</i> )	750	15.6, 15, 0.7	29	46	17.0	Rice bran	Male, /35 d	Rutherford <i>et al.</i> (2002)
3-phytase ( <i>Bacillus</i> )	500	3.1, 5.9, 8.0	54.7	61.5	6.8	Corn-SBM	Male /21 d	Camden <i>et al.</i> (2001)
6-phytase ( <i>A. ficuum</i> in <i>A. niger</i> )	400	3.7, 6.3, 10.4-15.7	42.2	58.2	16.0	Wheat	Male, /25 d	Ravindran <i>et al.</i> (2000)
	800	7, 6.3, 10.4-15.7	42.2	58.4	16.2			

\*% above control – significant at P&lt;0.05

The efficacies of 3-phytase and 6-phytase on ileal P digestibility were reported by Tamim *et al.* (2004) and Camden *et al.* (2001). These studies demonstrate Ca is another contributing factor in determining the extent of ileal P digestibility due to phytase supplementation. At 500 FTU/kg supplementation level, lower ileal P digestibility with 6-phytase compared to 3-phytase (Tamim *et al.*, 2004) whereas, Camden *et al.* (2001) reported the opposite finding, which is ileal P digestibility with 6-phytase was higher than those with 3-phytase when supplemented at the same phytase and phytate level in a similar corn-soybean meal diet. The obvious difference between the two studies was the level of Ca that is 1.7 g/kg and 8.0g/kg, respectively.

Extremely low Ca content in diets may promote the hydrolysis of phytate by intestinal mucosa phytase resulting in high ileal P digestibility and then with phytase supplementation, the ileal P digestibility is further improved. High Ca content in the diet may promote precipitation of phytate which hinders the activity of intestinal phytase but dietary phytase enhanced phytate hydrolysis and ileal P digestibility although it was not as high as that achieved with the low Ca diet (Walk *et al.*, 2012c, Amerah *et al.*, 2014, Walk *et al.*, 2012d, Tamim *et al.*, 2004).

The content of P in the diet was also shown to influence ileal P digestibility. By increasing the non-phytate-P from 2.3 to 4.5g/kg in wheat based diets, addition of phytase increased ileal P digestibility from 39.9% to 70.2% and 46.8%, respectively. The increase in ileal P digestibility was greater in the diet with lower P content compared to those with adequate non phytate-P. On the other hand, the increasing concentration of dietary phytic acid in a phytase supplemented diet did not affect ileal P digestibility (Ravindran *et al.*, 2000).

The supplementation level and type of phytase and Ca:P ratio also affected ileal P digestibility. The graded level of phytase from 0 to 1000 FTU/kg added to corn-soybean meal diets increased ileal P digestibility from 54.7 to 66.2% (Camden *et al.*, 2001) and from 39.5 to 69.1% (Kiarie *et al.*, 2015). On the other hand, Rutherford *et al.* (2004) showed the increase of ileal P digestibility was limited as the phytase level was increased from 500 FTU/kg to 750 FTU/kg. Differences in the effects of phytase on ileal P digestibility in these studies were due to different Ca: total P, i.e. 2.3:1 (Kiarie *et al.*, 2015) versus 1.2 (Rutherford *et al.*, 2004) and different source of phytase, i.e fungal origin (Camden *et al.*, 2001) and bacterial origin (Rutherford *et al.*, 2004). The effect of fungal phytase was greater in increasing ileal total P absorption at supplemental level higher than 1000 FTU/kg when compared to bacterial phytases (Chung *et al.*, 2013).

Based on the concept and the kinetics of complete dephosphorylation of phytate proposed by Zyla *et al.* (2004), more effective phytate degradation may occur within the GIT of the chicken when adding phytase at a dose higher than the dose recommended by the manufacturers, which is normally at 500 FTU/kg for broilers (Cowieson *et al.*, 2006). At similar ratio of Ca: total P, an increase of between 8.6 to 12.5 % in ileal P digestibility was observed when 2000 to 2500 FTU/kg phytase was added in corn-soybean meal diet compared to non-phytase supplemented diet (Rutherford *et al.*, 2012; Walk *et al.*, 2012c). A high increase (30%) in ileal P digestibility was observed with the addition of 2000 FTU/kg SSF phytase into a wheat-soybean meal-canola based diet (Wu *et al.*, 2004b). Kiarie *et al.* (2015) also observed a high increase (28.7%) in ileal P digestibility when 2000 FTU/kg phytase was added to a



corn-soybean diet with higher Ca: total P ratio (2.3:1). Zeller *et al.* (2015b) also reported a higher percentage of ileal P net absorption at extremely high phytase dosage (12,500 FTU/kg) compared to manufacturer's recommended dose (500 FTU/kg).

### ***Growth performance and bone mineralization***

Besides ileal P digestibility, growth performance and bone ash are the most commonly used evaluations of phytase efficacy in chickens. According to Selle and Ravindran (2007), hundreds of investigations on the microbial phytase evaluation on growth performance have been reported. Phytase supplementation of diets with inadequate P have been shown to improve growth performance (Selle and Ravindran, 2007) and sometimes bone ash particularly tibia ash was reported together with the growth parameters due to its greatest sensitivity to changes in mineralization (Angle *et al.*, 2006). A comprehensive review of phytase supplementation effects on growth performance, bone characteristics and bone mineralization has summarized that addition of microbial phytases enhanced the performance of growing broilers and at an increased level of supplementation further improved feed efficiency, nutrient utilization, bone growth and mineral retention (Khan *et al.*, 2013).

Supplementation of phytases beyond 500 FTU/kg in broiler diets has been gaining interest, particularly from the commercial sector, due to two main reasons. Firstly, the use of unconventionally high phytase doses apparently improved growth performance due to elimination of anti-nutritional effects of phytate up to below  $\text{InsP}_3$  in the diets and the generation of myo-inositol as a potential growth promotant in broilers (Cowieson *et*

*al.*, 2011). By taking advantages of nutrient release by phytase particularly amino acid and energy, more effective feed could be formulated using nutrient matrix values for phytase which leads to further reduction of feed costs.

Shirley and Edward (2003) demonstrated the benefits of supplementing superdoses of phytase, as high as 12,000 FTU/kg, on growth performance of broilers and numerous similar investigations have been reported since. According to Cowieson *et al.* (2011), phytase supplementation higher than 2500 FTU/kg was considered as superdosing level. Later, the term of 'superdosing phytases' was redefined as supplementation of phytase at 1500 FTU/kg or more either with or without the application of the phytase nutrient matrix (Cowieson *et al.*, 2013). Supplemental phytase from 1500 FTU/kg to as high as 40,000 FTU/kg benefited growth performance particularly weight gain and feed efficiency in growing broilers as early as 14 d old. Walk *et al.* (2012c; 2012d) reported a non-significant effect of phytase supplementation at 2500 and 5000 FTU/kg on growth performance but tibia ash was significantly increased indicating phytase benefited broilers via bone mineral retention.

The impacts of phytase on protein/amino acid availability and energy utilization have been extensively reviewed by Selle and Ravindran (2007) and Selle *et al.* (2010). In general, phytase supplementation improved both total and individual ileal amino acid digestibility in broilers and the effect of phytase on amino acid digestibility was more pronounced in wheat based compared to corn based diets. Selle and Ravindran (2007) also deduced that increases in fat, protein and starch digestibility accumulatively contributed to the positive impact of phytase supplementation on energy utilization in

broilers.

### ***Approaches in enhancing phytase efficacy***

The amount of phytate in the diets, amount of phytases added, the type of phytases used and the gut pH are several factors that influence the efficacy of phytase in poultry. The phytate content varies among commonly used feed ingredients (Table 3) and the level of phytate degradation also varies in different feed ingredients as demonstrated by Leske and Coon (1999). Whereas, Graham *et al.* (2009) reported that at different supplemental levels of Quantum phytase, ranging from 250 FTU/kg to 1000FTU/kg, the release of P has significantly increased by 30%. It was also reported that the release of P by 500 FTU/kg using different types of phytases, i.e. derived from *P. lycii*, *A. niger* and *E. coli*, are significantly different, with 0.67

and 1.30 kg/t, respectively. The efficacy of phytases in hydrolyzing phytate-P could also be affected by dietary levels of inorganic P and calcium, dietary endogenous phytase activity and non-starch polysaccharides (NSPs) (Woyengo and Nyachoti, 2011). NSPs are poorly digested by poultry particularly young ones and the utilization of other nutrients is also low due to encapsulation of nutrients including phytate in the plant cell walls. The soluble NSPs, on the other hand, is capable of increasing the viscosity of the digesta, consequently reducing nutrient digestibility and absorption (Bedford 2000, Bedford and Schulze, 1998). When the insoluble NSPs are hydrolyzed, phytate and other nutrients are released from the cell wall and become accessible to and digested by phytase and other digestive enzymes. Breakdown of soluble NSPs partially reduces digesta viscosity and consequently increases the absorption of nutrients liberated by phytase

Table 3. Typical concentration of calcium, total P, phytate-P, proportion of phytate-P to total P, and phytate in key feed ingredients (Adapted from Selle *et al.*, 2009)

Feed ingredient	Ca (g/kg) <sup>a</sup>	Total P (g/kg) <sup>b</sup>	Phytate-P (g/kg) <sup>b</sup>	Phytate-P/total P (%) <sup>b</sup>	Phytate (g/kg) <sup>c</sup>
Barley	0.30	3.21	1.96	60	7.0
Corn	0.20	2.62	1.88	72	6.7
Sorghum	0.40	3.01	2.18	73	7.7
Wheat	0.50	3.07	2.19	73	7.8
Canola meal	6.80	9.72	6.45	66	22.9
Cottonseed meal	1.50	10.02	7.72	77	27.4
Soybean meal	2.70	6.49	3.88	60	13.8
Rice bran	0.50	17.82	14.17	80	50.3
Wheat bran	1.40	10.96	8.36	76	29.6

<sup>a</sup> NRC (1994);

<sup>b</sup> Selle and Revindran (2007);

<sup>c</sup> Calculated on the basis that phytate contains 282 g/kg

In order to improve the efficacy of phytase in broiler chickens, the above mentioned factors were considered and further studied by several authors (Deepa *et al.*, 2011; Jozefiak *et al.*, 2010; Manangi and

Coon, 2008) and reviewed by Selle *et al.* (2010). However, based on two broiler studies (Camden *et al.*, 2001; Tamim *et al.*, 2004) and one layer study (van der Klis *et al.*, 1997), Selle *et al.* (2010) suggested that

the efficacy of phytase in broiler chickens could be further improved by increasing the digesta retention time in the crop. By delaying the intestinal retention rate of food/digesta, this may facilitate phytate dephosphorylation through extending the time of exposure of substrates to phytases and phytate-P absorptive sites thus improving the nutrient utilization efficiency in chickens (Mateos and Sell, 1980).

Intestinal RT is affected by numerous dietary and husbandry factors. Dietary fat is one of the factors that affects intestinal RT, beside particle sizes and types of fibre and carbohydrate. Mateos *et al.* (1982) showed that the intestinal RT increased as inclusion level of yellow grease increased from 193 min (0% fat) to 270 min (30% fat) in chickens fed with corn-based diet. For rye-based diet, the intestinal RT was higher with addition of 100g/kg soya oil (499 min) compared to beef tallow (414 min) at the same level of inclusion (Danicke *et al.*, 1999). However, they did not report on the effect of fat inclusion level on intestinal RT. Conversely, Golian and Maurice (1992) found the intestinal RT was not affected by addition of poultry fat but it increased as the age of chickens increased from 170 min (1 wk old) to 211 min (6 wk old). Although there was only one report in the literature evaluating the phytase effect on intestinal RT (Watson *et al.*, 2006), the effect of fat addition, type and inclusion level of fat in the presence of phytate on intestinal RT was not reported.

In addition to ambient temperature, lighting schedule was reported to affect the intestinal RT. Chickens reared under a 14L:10D lighting schedule had significantly longer intestinal RT during the scotoperiod (dark) than during the photoperiod (Buyse *et al.*, 1993). The effect of shorter scotoperiod

on growth performance and intestinal RT was described by Duve *et al.* (2011). With continuous 8 h scotoperiod (16L:8D) in a day, the mean intestinal RT in chickens fed on a wheat-based diet was 475 min which was longer than the RT for intermittent 8 h scotoperiod with two equally spaced 4-h darks (351 min). Nevertheless, the RT for these light schedules were much higher than those under continuous lighting program as reported by Hughes (2004) and Watson *et al.* (2006) with mean RT of 206 min (wheat-based diet) and 112 min (corn-based diet), respectively. Amerah *et al.* (2008) also found shorter intestinal RT with the average of 139 min for young chickens fed on both corn-based and wheat-based diets under continuous lighting program. In the case of phytase supplementation, Bedford *et al.* (2007) found that longer lighting time reduced weight gain of chickens fed on phytase supplemented diet but the intestinal RT was not reported. Meanwhile, Watson *et al.* (2006) found that by phytase supplementation, the intestinal RT of chickens fed on corn-based diets was reduced. Since the lighting program was not mentioned, it is assumed the chickens were subjected to continuous lighting program.

Supplementation of other enzymes such as xylanase and glucanase is expected to reduce intestinal RT, shorter than those in non-enzyme supplemented diet. The enzymes hydrolyse non-starch polysaccharides in the diet into oligo and monosaccharides, reduce viscosity of intestinal content (Choct, 1997) and reduce time for passage of digesta through the gastrointestinal tract (Danicke *et al.*, 1997; 1999; 2000). Lázaro *et al.* (2003) and Almirall and Esteve-Gracia (1994) concluded that supplementation of xylanase and glucanase to rye-based and barley-based diets reduced intestinal viscosity and RT.

## Conclusion

Phytate is the primary storage form of both phosphate and inositol in plant-based feed ingredients. It forms insoluble complexes with dietary minerals, especially calcium, magnesium, iron and zinc, and reduces the bioavailability of P, Ca, Zn and other metabolically important minerals in the gut. Phytate also interferes with the digestibility of energy, protein and fat, impediments that have significant nutritional and health consequences. It is worth noting that limitations of intestinal RT and pH within the GIT of chickens do not allow complete degradation of phytate to myo-inositol and inorganic P. Clearly, there are huge possibilities in developing strategies to increase the degradation of phytate-P and P utilization in poultry, particularly broilers. Phytase reactivity under different environmental conditions, the interaction between phytate, protein, carbohydrate and minerals in the intestine, and also the appropriate use of phytate mediation strategies such as phytase, pH manipulation, gut motility manipulation and divalent cation intake need further investigations.

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