

## Current and Future Applications of Phytases in Poultry Industry: A Critical Review

### Abstract

Phytases are enzymes that initiate the removal of phosphate from phytate. This enzyme has been widely utilized in animal feeding especially in the poultry industry to enhance phosphorus intake and minimize environmental pollution. Phytases are widely distributed in microbial, plants and animals. Supplementations of phytase into the diets of poultry have great impact to the improvement of poultry immune systems and increase bird weight. In addition to that, phytase are able to improve both quantity and quality of eggs, egg mass and eggshell quality. This review covers the classifications and distribution of phytases in different biofactors. In addition, it shed more light on the recent trends of application and beneficial impact in poultry farming.

**Key Words:** Phytase, Food and Feed supplements, Poultry feed, Functional Feed, Enzyme Supplement

### Introduction

Animal feed, similar with the humans, is comprised of protein, carbohydrates, and fats. Protein typically comes from soybeans, carbohydrates from corn, and fats from vegetable oils or animal fat. However, animal feed contains many ingredients such as phytate phosphorous and non-starch polysaccharides that are poorly digested by livestock. Therefore, enzymes in animal feed are typically added with the intent to increase nutrient bioavailability by acting on feed components prior to or after consumption, and by maximizing the performance of nutrients in feed. Also, these enzymes help to remove anti-nutrients, increase the digestibility of nutrients and fiber, and supplement the enzymes that animals already produce. Therefore, nowadays, different enzymes of microbial origins such as amylases, xylanases,  $\beta$ -glucanases, proteases, phytases and many others are added to feed (Beshay et al., 2003; Beshay et al., 2011; El Enshasy et al., 2013, El Enshasy et al., 2016a; El Enshasy et al., 2016b; Elsayed et al., 2016; Kandiyl et al., 2018). Hence, it is of general importance that the enzymes are not inactivated prior to ingestion and they function under the conditions of the gastrointestinal (GI) tract (Pariza and Cook, 2010). Recently, animal feeds are formulated with enzymes as close as possible to a target animal's nutritional requirements to avoid excesses. Nevertheless, some enzymatic predigestion of the feed substrate may also occur during storage depending on the exposure time prior to ingestion of the feed by the animal.

Furthermore, animal feed is known to be the largest cost item in livestock and poultry production, accounting for 60-70% of total expenses. Animal feed and nutrient utilisation are influenced by numerous factors, a major one being the wide variety of compounds present in the feed ingredients (Figure 1).

### Review

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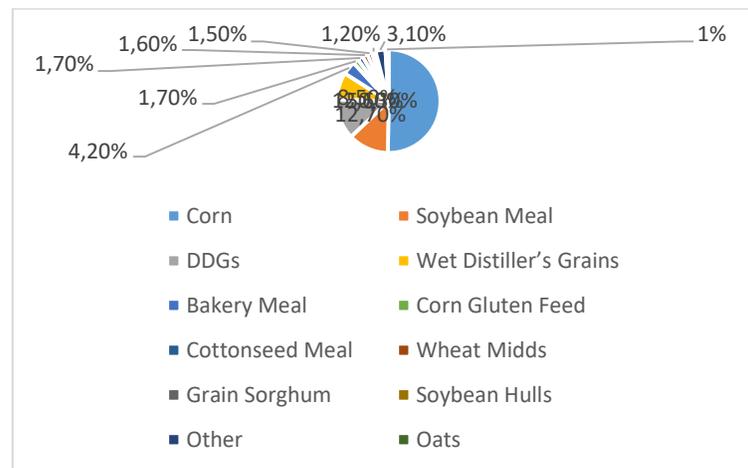
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Some of these tend to reduce diet quality, the feed efficiency and the growth rates of animals. The factors that influence the composition and utilization of the diets will thus have a substantial effect on the cost of poultry and other animal production (Acamovic, 2001). Producers of poultry and other animals always aim to provide high-quality products within a short span of time as possible at minimal cost. To save on costs, producers need to get smarter about optimizing animal production in a sustainable manner. The rapid growth achieved by monogastric animals generally necessitates

that they are fed on high-quality diets with readily available nutrients. Enzymes offer an opportunity to do the job which enable them to produce more meat per animal or to produce the same amount of meat cheaper and faster. Throughout this short growing period, maintenance of a high health is really important to yield a good quality product. During the growth of poultry there is also an increasing demand to minimize the amount of waste material requiring disposal and this has implications for pollution of the environment.



**Figure 1.** Total diet composition for top livestock and poultry in 2016

(Adapted from American Feed Industry Association, 2018)

### Enzymes in Animal Feed

Found in all living cells, enzymes catalyse chemical processes that convert nutrients into energy and new tissue. They do this by binding to substrates in the feed and breaking them down into smaller compounds. Enzymes' mode of actions are very specific towards their preferred substrates and eventually converting them into specific products. Each enzyme offers different characteristics that affect their dietary inclusion rate where they work within the animal's gut and their overall efficacy. Enzyme supplements are used widely in animal diets in an attempt to improve nutrient utilisation, the health and welfare, product quality and to reduce pollution as well as to increase the choice and content of ingredients. Commercially-

available enzymes can be derived from plants and animals as well as microorganisms. Considerable advances have been made during the last decade in the manufacture, activity, quality, thermostability and specificity of supplemental enzymes for use in animal diets (Acamovic 2001). There are various kind of enzymes currently used in the animal feed such as protease, which break down proteins into amino acids, carbohydrates, split carbohydrates into simple sugars, and lipases take apart lipids into fatty acids and glycerol. One of the most interesting and important factors of enzyme function is that each type has a very specific role within the animal.

Supplementation of diets for monogastric animals with enzymes has been progressively investigated and

applied as a means of enhancing production efficiency and increasing the effectiveness of nutrient utilization. Supplementation of poultry diets with enzymes frequently exerts beneficial effects rather than the bad effects. It is well documented that supplementation of diets with exogenous enzymes can reduce the adverse effects of some compounds, especially those produced by carbohydrates and proteins (Bedford and Schulze, 1998). Enzyme supplementation has also been shown to influence the absorption of fats and fatty acids as well as fat-soluble microconstituents contained in the animal diets (Danicke et al., 1999). The extent of the benefit depends on a number of factors, such as the nature of the dietary components, whether or not the diets have been processed and whether the appropriate enzymes have been included for the substrates contained in the diets (Acamovic, 2001).

Enzymes are also provided in various forms for supplementation of animal diets. They can be supplied as powders and added to the diet before mixing and pelleting, or they can be added as granules before the diets are mixed and pelleted. Both these procedures allow the enzymes to mix intimately with the dietary ingredients and this allows them, at least potentially, to react effectively with their substrates (Acamovic, 2001). Many enzymes, however, tend to be thermolabile, and thus processing at high temperatures may reduce their activity (Silversides and Bedford,

1999). The method which involves the greatest technological input has been applied to synthesize these enzymes for animal feed especially for phytases production. This review will discuss and focus on phytases that are typically considered for use in animal feeds.

### Classification and distribution of phytases

Phytases are enzymes categorized in the class of phosphatases (EC 3.1.3). These enzymes catalyze hydrolysis reaction to release inorganic phosphorus from phytate (Greiner, 2007). Phytases are widely distributed in plants, microorganisms, and animal (Humer et al., 2014). Phytases are internationally classified based on the enzymes stereospecificity of hydrolysis (Selle and Ravindran, 2007). Phytases that initiate hydrolysis of C3-position of phytic acid are classified as 3-phytases (EC 3.1.3.8). These hydrolysis reactions produce inorganic phosphate (Pi) and 1,2,4,5,6-pentakisphosphate. On the other hand, phytases that initiate hydrolysis of C6-position of phytic acid are classified as 6-phytases (EC 3.1.3.26), and this reaction produces Pi and 1,2,3,4,5-pentakisphosphate. Most phytases from microorganisms and filamentous fungi are 3-phytases, while plant phytases are 6-phytases (Reddy et al., 1982). Table 1 shows the production of phytases by different recombinant microorganisms.

**Table 1.** Production of phytases by recombinant microorganisms

Microorganism	Source of phy gene	Production process	Maximal yield	Reference
<i>Lactococcus lactis</i>	<i>Escherichia coli</i>	SmF	19 U mL <sup>-1</sup>	Pakbaten et al., 2018
<i>Escherichia coli</i>	<i>Theromotoga naphthophila</i>	SmF	1000 IU kg <sup>-1</sup>	Sabir et al., 2017
<i>Pichia pastoris</i>	<i>Shigella sp.</i>	SmF	967 U mg <sup>-1</sup>	Roy et al., 2016
<i>Escherichia coli</i>	<i>Shigella sp.</i>	SmF	2982 U mg <sup>-1</sup>	Roy et al., 2016
<i>Ogataea thermomethanolica</i>	<i>Aspergillus niger</i> BCC18081	SmF	134 U mL <sup>-1</sup>	Charoenrat et al., 2016
<i>Escherichia coli</i>	<i>Yersinia intermedia</i>	SmF	3849 U mg <sup>-1</sup>	Mirzaei et al., 2016
<i>Escherichia coli</i>	<i>Selenomonas ruminantium</i>	SmF	1204 U mL <sup>-1</sup>	Lan et al., 2014
<i>Pichia pastoris</i>	<i>Escherichia coli</i>	SmF	112 U mL <sup>-1</sup>	Tai et al., 2013
<i>Escherichia coli</i> DH5 $\alpha$	<i>Etrobacter sakazakii</i> ASUIA279	SSF	4194 U mL <sup>-1</sup>	Ariff et al., 2013
<i>Ogataea thermomethanolica</i>	<i>Aspergillus</i> thermostable phytase	SSF	134 U mL <sup>-1</sup>	Charoenrat et al., 2016

SmF; Submerged Fermentation, SSF: Solid State Fermentation.

Phytases are also classified based on their amino acid sequences, catalytic mechanisms, three-dimensional conformation and biochemical properties ( $\beta$ -propeller phytase [BPP], histidine acid phosphatases [HAP], purple acid phosphatases [PAP], or cysteine phosphatases [CP]) as well as optimum pH condition (alkaline or acid phytases) (Kumar et al. 2016). Acidic phytases have a pH optimum ranging from 3.0 to 5.5 while alkaline phytases from 7.0 to 8.0 (Yin et al., 2007; Vijayaraghavan et al., 2013). Like other proteins, most phytases are sensitive to high temperature and its optimum temperature is ranging from 50 to 60 °C (Igbasan et al., 2000). The variations of optimum condition for phytase activity and stability provide a wide choice of an enzyme to fit its application.

In plants, phytases are commonly found in oilseeds and nuts, legumes and in cereal pollen grains (Konietzny, 2002). Specifically, during seed germination, phytase activity increases to promote fast plant growth (Greiner, 2007). Phytases can also be found in plant roots, but with lower hydrolysis activity (Richardson et al., 2001). Many soil microorganisms, especially rhizosphere microorganisms species produce phytase as one of the mechanisms to increase phosphorus availability for plant growth, hence are used in biofertilizer. Some isolated phytate-degrading microorganisms are *Pseudomonas* sp. (Richardson et al., 2001, Jorquera et al., 2008, Singh et al., 2014, Sasirekha et al., 2012), *Azotobacter*, *Rhizobium* (Abd-Alla, 1994, Singh et al., 2014), *Azospirillum*, *Burkholderia* (Unno et al., 2005, Singh et al., 2014), *Enterobacter* (Yoon et al., 1996, Cao et al., 2007, Jorquera et al., 2008), *Pantoea* (Jorquera et al., 2008), *Serratia* (Cao et al., 2007), *Bacillus* sp. (Joseph and Raj, 2007), and some other fungi. Production of phytases from various microorganisms has been demonstrated in different studies and this has been reviewed previously (Kumar et al. 2016). Characterization studies revealed phytases from soil micromycetes, yeast, *Bacillus* sp. and *Enterobacter* sp. exhibit high specific activity and show a broad range of optimum pH (pH 3.5 to 7.5) and temperature (37°C - 70°C).

In animal, there is evidence of phytase activity in stomach and intestine of pigs and broiler (Humer et al.

2014). Low stomach mucosal phytase activity has been discovered from pigs (Hu et al. 1996) and broiler (Tamim et al., 2004). When fed low phosphorus diet, broilers have the adaptive ability by increasing intestinal phytase and phosphatase activity (McCuaig et al., 1972). Other than that, phytase are produced by gut microflora of the animal (Humer et al., 2014).

### Phytase in Animal Feed

The animal feed industry holds a great importance to ensure quality and continuous food supply for the ever-increasing world population. It plays a significant role in the food chain as it directly influences the quality of products like meat, milk, fish and eggs that people consume. The dependence on farm animals for human nutrition is huge around the globe, hence the animal feed should be highly nutritious and able to be absorbed by the animal effectively to produce animal's source foods with wholesome nutrition.

The major raw material for animal feed production are cereals and legumes-based ingredients such as soybean, corn, wheat and rapeseed meal. These plant-based protein meals contain 1-5% of phytic acid (PA; myo-inositol [MI] hexaphosphoric acid), which is an anti-nutritional factor (Nissar et al., 2017). Phytic acid is the major phosphate storage compound in seeds and it represents 50-80% of total phosphate in plant seeds (Dersjant-Li et al., 2015). This anti-nutrient factor interferes with the absorption of several minerals, namely zinc, iron, calcium, magnesium, manganese, copper, and it reduces the digestibility of protein as well (Nissar et al., 2017). Phytate can be eliminated by the enzyme phytase through hydrolysis into inositol, phosphate and other divalent metals such as calcium, iron and zinc (Lei and Porres, 2003). Phytase is the enzyme highly used in animal feed compared to other enzymes such as xylanase, cellulase, amylase, protease and lipases, because phytase, unlike other enzymes is linked to absorption of more than one nutrient or mineral as mentioned previously.

The first commercial phytase products were introduced into feed market in 1991 in which the swine, poultry, and aquaculture feeds benefit from the application of phytase as a feed additive (Greiner and

Konietzny, 2006). Moreover, phytase has a high marketing value which is estimated to surpass 300 million US dollars and continues to increase by 10% annually (Chen et al., 2015). Phytase enzymes work in multiple ways in an animal feed which renders benefit not only to the animal but also the environment and the feed manufacturers. For the animal nutrition, phytase aids in phosphate liberation from phytate which represents around 60-90% of total phosphate which otherwise would be wasted and pollutes the environment through the animal's excretion (Nissar et al., 2017).

Phosphorus is a key element in the animal cell biochemical reactions and bone mineralization. Phytase supplementation has been proven effective in bone mineralization and growth performance in broiler diets (Scholey et al., 2018). An improvement in phosphorus utilization also means that there is a reduction of phosphate waste excreted by the animal which is beneficial to the environment. Haefner et al. (2005) quoted that phosphorus excretion could be reduced to 50% with phytase application which is an important contribution towards environmental protection. In another study by Sugiura et al. (2001), phytase supplementation to rainbow trout diet has shown to increase nutrient digestibility and excretion of phosphorus was reduced to an extent of 95-98% compared with phosphorus excretion by fish consuming feeds without phytase thus reducing the occurrence of eutrophication. The feed manufacturers in another hand benefits from phytase enzyme because the addition of adequate amount of phytase reduces the dependency on phosphorus supplement which also reduces the feed production cost as well. Laboratory and field experiments have shown that 1g of inorganic phosphorus supplementation can be replaced by 500–1000 units of phytase and reduce total phosphorus excretion by 30–50% (Yao et al., 2012). In a nutshell, the ability of phytase to augment the efficiency of animal feed in those three areas makes phytase a necessity rather than a choice for feed manufacturers.

### **Phytases in poultry farming-functionality**

The poultry industry is one of the fastest and largest rising agro-based industries in the world. Broiler

production is increasing every year due to higher demand by local consumers and export markets. It is projected that the growth in both production and consumption of poultry meat in developing countries will remain at the rate of 3.6% and 3.5% y-o-y respectively, till 2030 due to economic firmness, changing lifestyle and diet patterns (Global Poultry Feed Market, 2017). Malaysia is among the highest poultry meat per capita consumption in the world with consumption of 1.8 million chickens and 2.8 million chicken eggs daily (Market Research Report, 2017).

Poultry diets are mainly composed of seed-based components and contain the great amount of phosphorus in the phytic acid form. Phosphorus is a crucial mineral in the growth and development of poultry. It is, therefore, necessary to supplement poultry with the adequate amount of phosphorus. However, this phytic acid often forms the complex with other cations such as calcium and proteins which hinder the efficiency of absorption. Defiance in phosphorus, therefore, hinder poultry growth which can further leads to birds losing appetites, becoming weak and die (Haque et al., 2012). Therefore, phytase, an exogenous enzyme supplement is added to commercial poultry diets to overcome this issue.

Previous researchers reported that supplementation of phytases improves the performance of phosphate, calcium, magnesium, and zinc used (Viveros et al., 2002). Poultry fed phytase had higher digestibility for Val, Ile, nonessential amino acid and total amino acid (Namkung and Leeson, 1999; Ravindan et al., 1999). Transgenic corn derived phytase (TCDP) improve laying performance and ileal P utilization in laying hens (Gao et al., 2013).

Phytase present in the feed ingredients themselves has been shown to increase daily feed consumption which proportionally increases body weight gain as well as the growth-rate of poultry (Zhamuer, 2013; Bradbury et al.; 2017, Moss et al, 2017). Study conducted by Moss et al. (2017) shows that protease supplementation alone increase weight gains of poultry, but in combination with phytase decrease weight gain. In addition to that, the pre and post pallet whole grain addition to non-supplemented diet significantly

improved apparent metabolizable energy (Moss et al 2017; Ravindran et al., 2000). Previous researchers also reported the efficacy of phytase on bone mineralization in poultry (Lalpanmawia et al.; 2014; Bradbury et al., 2017; Scholey et al., 2018). The benefit of phytase supplementation was greater in younger than older chicken (Li et al., 2018).

Interestingly, phytase has a great impact not only to the poultry itself but also to their laying performance. It was reported that phytase is able to improve both quantity and quality of eggs which is the laying performance, egg mass and eggshell quality (Liu et al., 2007; Gao et al., 2013). Supplementation of microbial

phytase to normal corn soybean diet can reduce Tricalcium Phosphate (TCP) level in the diet without affecting egg production, egg quality and ileal p utilization (Um and Paik, 1999). In addition to all the benefits mentioned, phytase was also found to be able to improve immunity measures in poultry (Ghosh et al., 2016). Combination of carbohydrates with phytase and acidifier decrease *E. coli* count and increase villus length in broiler chicken (Roofchaei et al., 2017). It was also reported that phytase improve immunity in broiler chicken to against New Castle Virus disease (Ghosh et al., 2016). Table 2 shows the summary of phytase functionality in poultry.

**Table 2.** Summary of phytase functionality in poultry

No.	Functionality	Reference
1	Enhance broiler body weight gain and performance	Scholey et al., 2018; Broch et al., 2018 Hamdi et al., 2018; Bradbury et al., 2017 Moss et al, 2017; Morgan et al, 2016 Chen et al., 2013; Sa et al., 2013; Dilger et al., 2004; Viveros et al., 2002; Zyla, 2001
2	Improve egg production	Gao et al., 2013; Cabuk et al., 2004; Jalal and Scheideler, 2001; Um and Paik, 1999;
3	Enhance egg quality	Gao et al., 2013; Cabuk et al., 2004 Um and Paik, 1999
4	Bone mineralization	Scholey et al., 2018; Hamdi et al., 2018; Li et al., 2018; Bradbury et al. (2017); Vieira et al., 2017; Lee et al, 2017; Gao et al., 2013; Sa et al., 2013; Dilger et al., 2004; Zyla, 2001
5	Improve ileal digestibility performance of Phosphorous, Calcium, Magnesium, Zinc or Sodium in broiler chicken diets	Moss et al., 2018; Kim et al., 2017; Viveros et al., 2002; Zyla, 2001; Um and Paik, 1999; Hamdi et al., 2018; Gao et al., 2013
6	Reduce liver weight	Viveros et al., 2002
7	Improve digestibility of protein and amino acid (Val, Ile, nonessential amino acid and total amino acid)	Moss et al., 2018; Sa et al., 2013; Zyla, 2001; Namkung and Leeson, 1999; Ravindran et al., 1999
8	Enhance Apparent Metabolizable Energy (AME)	Moss et al, 2017; Ravindran et al., 2000; Namkung and Leeson, 1999;
9	Improve immunity against New Castle virus	Ghosh et al., 2016
10	Decrease <i>E.coli</i> count and enhanced villus length	Roofchaei et al., 2017
11	Improved feed conversion ratio (FCR) or feed intake	Bradbury et al., 2017; Moss et al, 2017; Lee et al, 2017; Roofchaei et al., 2017; Sa et al., 2013

## Conclusion and future perspectives

This review showed clearly the positive effect of phytase supplementation that increase the growth performance, nutrients digestibility, optimized feed cost, improve sustainability of poultry production and reduce environmental pollution. Fundamental knowledge with respect to phytate and phytase is required in many aspects in order to produce an integrated form of understanding the correlation between phytase intake as poultry feed and minimizing removal in their manure. Investigation on genetically modified grains with reduced phytic acid content is another alternative that has potential in minimizing phosphorus lost.

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