Review Article

Microbial phytase in animal nutrition: Unlocking phytate for sustainable feed utilization

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Abstract

Phytase enzymes have earned significant importance in recent years, due to their critical role as biocatalysts in the hydrolysis of phytic acid, thereby enhancing the bioavailability of phosphorus in plant-based diets. In plants, phosphorus is predominantly stored in the form of phytic acid, which exists mainly as mineral salts such as phytate and phytin. Phytic acid is considered an anti-nutritional factor due to its strong chelating ability, which binds essential minerals such as phosphorus, calcium, iron, and zinc, reducing their bioavailability, especially for monogastric animals. To overcome this limitation, supplementation of the phytase enzyme along with raw feed or incorporating phytase into feed formulations has become a common strategy to enhance nutrient utilization efficiency of animal diets, by reducing the reliance on expensive inorganic phosphate supplements. In this review, we focus on the application of phytase enzyme in sustainable animal feed for monogastric animals. Numerous studies are ongoing in this field to develop advanced variants of phytase with superior catalytic performance, elevated thermal stability and broad pH activity range across various substrates, coupled with greater enzyme productivity. Moreover, recombinant phytases developed through various microbial expression systems have demonstrated superior performance in animal feed processing, to achieve maximal nutrient utilization while ensuring feed stability throughout processing. These advancements highlight the global potential of phytase in food processing, agriculture, human nutrition and health, development of transgenic plants, environmental protection, and various other industrial applications.

Keywords: Animal feed application, anti-nutrient, phosphorus recycling, phytase, phytate (phytic acid), recombinant phytase.

Introduction

Global demand for animal-derived food products is steadily increasing due to population growth and urbanization creating pressure on the livestock sector to enhance productivity while ensuring cost efficiency and environmental sustainability. Phytase has become one of the most important feed enzymes in modern livestock nutrition, offering a sustainable solution to unlock the nutritional potential of plant-based feed formulations. Phytase, an enzyme of the phosphatase family, is widely recognized for its ability to hydrolyse phytic acid, thereby releasing bound nutrients in the feed. Most feed ingredients used in livestock diets are of plant origin, primarily consisting of cereals, oilseeds, and their by-products derived from agro-industrial residues, such as wheat bran, soybean meal, and various types of oil cakes. These collectively serve as the major sources of energy and protein

for animal nutrition. However, phytic acid, the natural storage form of phosphorus in plants, is deposited in substantial amounts, particularly in cereals, legumes, nuts, and oilseeds.[1]

Phytic acid is poorly digested by monogastric animals such as poultry, swine, and fish because they lack sufficient endogenous phytase to hydrolyse phytate. As a result, the phosphorus bound to phytate remains unavailable, reducing overall nutrient utilization, while the excretion of undigested phytic acid in manure contributes to environmental pollution. Excessive phosphorus runoff from animal farming is a significant contributor to water eutrophication, resulting in ecosystem imbalance and posing risks to both the environment and human health.[2] Moreover, to compensate for the poor availability of phytate-bound phosphorus, farmers often add inorganic phosphate supplements to animal diets to meet the nutritional requirements, which in turn raises feed costs.

Supplementation with exogenous microbial phytase helps overcome these challenges by hydrolyzing phytate, thereby enhancing phosphorus and mineral bioavailability, in addition to reducing feed costs and minimizing phosphorus excretion into the environment. To achieve sustainable livestock production, it is essential to maximize nutrient utilization from plant-derived feed ingredients, thereby reducing reliance on non-renewable phosphate supplements and lowering nutrient waste.

The development of sustainable practices in animal nutrition through enzyme technology has been significantly advanced by supplementing diets with microbial phytase. This strategy has proven to be a practical and effective method for improving phytate digestibility in monogastric animal diets. The global feed industry spends billions of dollars annually on improving feed formulations by adding nutrient supplements, with inorganic phosphate being a major contributor. Recent advancements in enzyme technology and ongoing research have focused on engineering phytases with enhanced catalytic efficiency, broader substrate specificity, higher thermal stability, and improved resistance to proteolysis during feed processing. A key area of current development is the production of phytases with high thermal stability, as significant heat is generated during the feed pelleting process. This ensures consistent nutrient obtainability under commercial processing conditions, which would otherwise inactivate conventional enzymes. The development of next-generation phytases with enhanced catalytic activity and the ability to function across a broad pH range in the gastrointestinal tract has been achieved through site-directed mutagenesis and protein engineering.[3] These innovations have given a new dimension to the use of phytase in animal feed applications, as it not only improves phosphorus bioavailability and optimizes feed efficiency but also reduces reliance on inorganic phosphate supplementation, thus lowering feed costs and mitigating environmental impacts associated with phosphorus excretion. Collectively, these advancements place phytase as a cornerstone of modern feed enzyme technology, aligning economic, nutritional, and environmental objectives in sustainable livestock production.

Phytic acid- the anti-nutritional factor

Phytic acid, also known as D-myo-inositol (1,2,3,4,5,6)-hexakisphosphate (inositol hexaphosphate/IP6), consists of six phosphate groups connected to an inositol ring by ester bonds. It is the principal storage form of phosphorus in many plant-derived foods such as cereals, legumes, and oilseeds (Figure 1).[4] Phytic acid predominantly occurs as a complex by binding to divalent and monovalent metal cations such as calcium (Ca^{2+}), iron (Fe^{2+}), zinc (Zn^{2+}), magnesium (Mg^{2+}), potassium

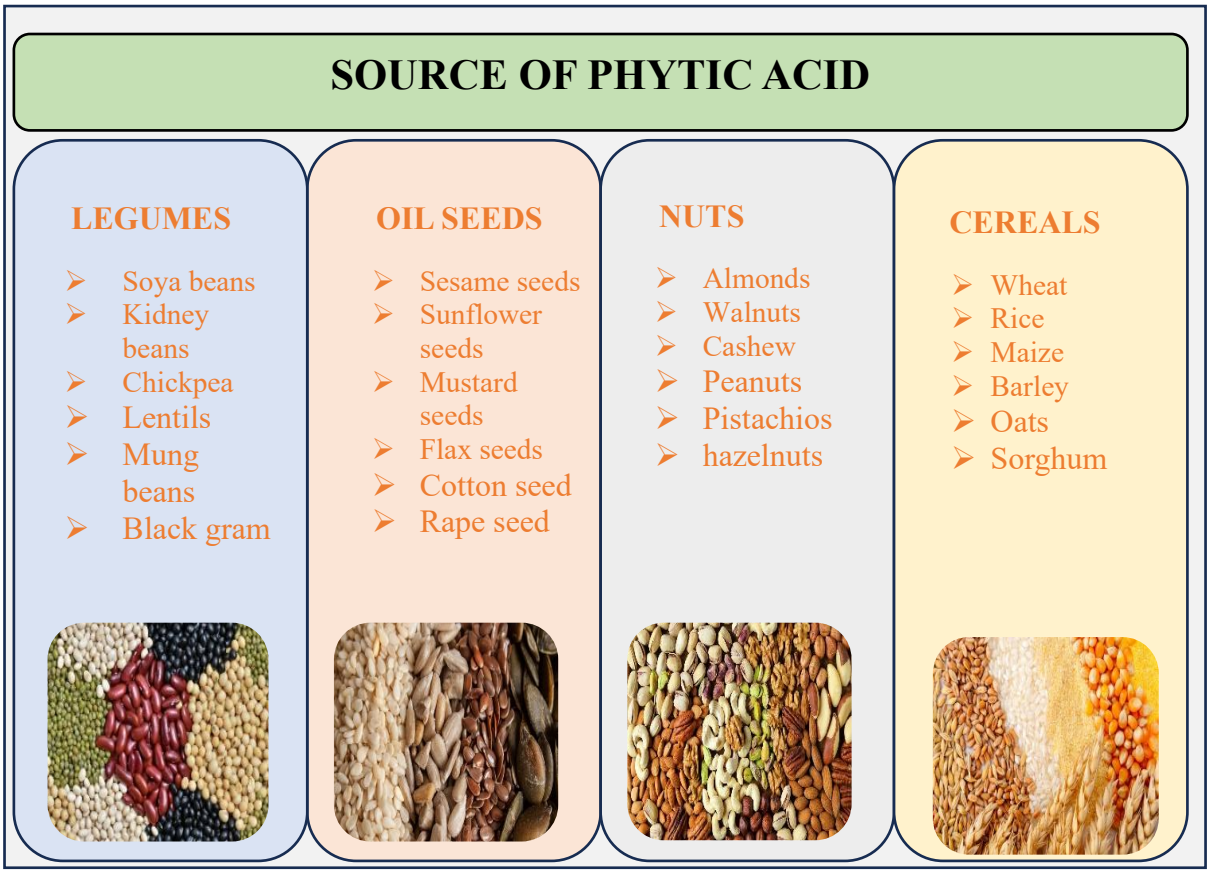

Figure 1: Different sources of phytic acid in feed ingredients [4,6]

(K^+), and manganese (Mn^{2+}), forming stable mineral–phytate complexes.[4] These salts of phytic acid are collectively referred to as *phytate*, and in plants they predominantly exist as mixed salts with calcium and magnesium, commonly termed *phytin*. In association with other nutrients, phytate can also form complexes known as "lipophytins" with lipids and their derivatives.[5] However, phytate phosphorus is poorly available to monogastric animals, reducing the bioavailability of essential nutrients such as calcium, iron, and zinc. Furthermore, it inhibits the activity of key digestive enzymes, including amylase, trypsin, and pepsin, in the gut. This inhibition arises from the nonspecific binding of phytate to proteins and the chelation of calcium ions, which are essential cofactors for the proper functioning of these enzymes.[6]

Phytic acid has traditionally been regarded as an antinutrient, as numerous studies have shown that its reactive phosphate groups, attached to the inositol ring, form insoluble complexes with cations, thereby reducing their intestinal absorption in humans and other monogastric animals. The primary reason for the antinutritional effects of phytic acid is its strong chelating effect, which arises from its six reactive, negatively charged phosphate groups.[7] These groups readily bind to positively charged minerals, such as calcium, iron, zinc, and magnesium, forming insoluble complexes that significantly reduce the bioavailability of these minerals. When phytate-containing plant-based food products, mainly derived from cereals, oilseeds and legumes, are consumed without being processed or cooked in large quantities, they can reduce the absorption of phosphorus and other important minerals in monogastric animals, which lack sufficient endogenous phytase activity to digest phytate. This results in poor phosphorus bioavailability. When the stomach pH rises above the isoelectric point of proteins,

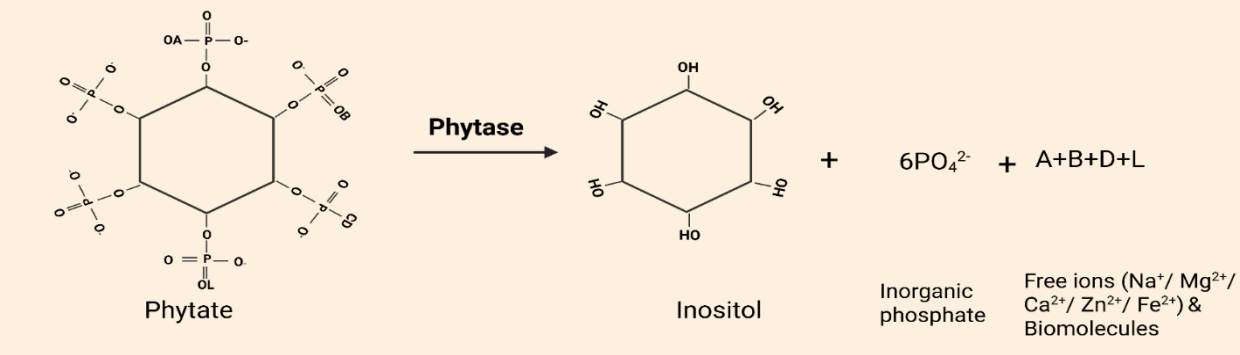

Figure 2: Breakdown of phytic acid by phytase [11]

phytate readily binds to protein–mineral complexes, generating insoluble aggregates that are resistant to enzymatic hydrolysis. This reduced solubility impairs the efficiency of protein digestion and limits the availability of nutrients. Moreover, these complexes can hinder the activity of endogenous proteases such as pepsin, which depend on substrate accessibility for optimal function, thereby decreasing the release and absorption of amino acids. Such interference not only reduces dietary protein utilization but also causes deficiency of amino acids, representing a hidden nutritional cost. The calcium–phytate complexes have been shown to adversely affect lipid digestion, as calcium bound to phytate promotes the precipitation of fatty acids into indigestible metallic soaps within the gut lumen. Collectively, these interactions highlight the multifaceted anti-nutritional role of phytate, impairing both protein and fat utilization in monogastric animals. [7,8]

To overcome this challenge, microbial phytases have been widely explored and utilised in both the animal feed and food processing industries. These enzymes, primarily produced by fungi but also by certain bacterial strains, play an important role in enhancing phosphorus bioavailability, reducing dependence on inorganic phosphate supplements, and minimizing environmental phosphate discharge. Beyond their established use in feed, phytases are increasingly recognized for their potential applications in food processing, agriculture, and human nutrition, making them a valuable tool in sustainable biotechnological practices.

Phytase

Phytase (myo-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.26) is an enzyme belonging to the phosphatase family that catalyses the hydrolysis of phytic acid (myo-inositol hexakisphosphate), primarily releasing inorganic phosphate, lower inositol phosphates, and essential minerals such as zinc, iron, and calcium that are bound within the phytic acid complex (Figure 2). It is a naturally occurring enzyme widely distributed across various biological systems, including microorganisms, plants, and certain animal tissues. Suzuki *et al*. (1907) first identified phytase in rice bran.[9] The pioneering research on phytase application in poultry feed was conducted by Nelson *et al*. (1968), in which soybean meal was treated with fermented *Aspergillus ficuum* for inclusion in chicken feed. Their findings showed improved utilization of phosphorus made available by the degradation of phytic acid in the substrate. This was indicated by a significant increase in bone ash content, which outperformed the results observed with inorganic phosphate supplementation.[10]

The breakdown of phytic acid or phytate is referred to as dephytinization, a process that plays a crucial role in enhancing the nutritional quality of plant-based foods and animal feeds by mitigating the anti-

nutritional effects of phytate. Several strategies can be employed to achieve dephytinization, including milling, soaking, germination, fermentation, thermal processing and application of exogenous microbial phytase. Soaking and germination are traditional approaches widely used to reduce phytate levels in plant-based foods. Extended soaking for 12–16 hours initiates hydration and activates endogenous enzymes, while germination induces metabolic changes that enhance phytase and associated phosphatase activity, leading to the stepwise hydrolysis of phytic acid into lower inositol phosphates and free phosphate groups. Enzymatic hydrolysis of phytate during soaking is strongly influenced by temperature and pH, with intrinsic plant phytases capable of degrading 26–100% of phytate when conditions are close to optimum (45–65 °C, pH 5.0–6.0).[12] According to Azeke *et al.* (2010), germination for 10 days led to an 81–88% reduction in phytate content across cereals, with maximum decreases observed between the 7th (Maize, Millet and Wheat) and 10th day (Sorghum) depending on the grain. This reduction corresponded with a significant ($P < 0.05$) increase in phytase activity, whereas minimal changes occurred within the first 24 hours.[13]

Fermentation and thermal processing are widely recognized as effective strategies to lower phytate levels. Fermentation promotes the production of microbial phytases and related phosphatases, which, in turn, cause hydrolysis of phytate, releasing bound minerals and improving nutrient absorption and protein digestibility. Thermal processes, such as boiling, cooking, and autoclaving, reduce phytate levels, although the extent of reduction depends on the temperature and duration, making them fast and scalable methods. Elhardallou and Walker (1994) reported that boiling reduced the phytic acid content of lentils by 60%, butter beans by 50%, and broad beans by only 11%.[14] Prolonged thermal treatments are not always optimal, as they can degrade heat-sensitive nutrients and lead to substantial nutrient losses. In comparison, fermentation offers a gentler means of reducing phytic acid while preserving nutritional quality, although it requires longer processing times, which can increase costs. Germination is also effective, as 6–10 days of sprouting significantly lowers phytate levels; however, since the process is time- and space-intensive, it can increase processing costs. While these methods are suitable for home-based food preparation, they are generally not cost-effective for large-scale industrial food production due to the extended time, labour, and resources required. Among the various dephytinization methods, the application of exogenous microbial phytase is considered one of the most effective approaches, as it directly catalyzes phytate hydrolysis and ensures a more efficient release of bound nutrients. Phytase is found naturally in many living organisms, including animals, plants, fungi, and bacteria (Table 1).[15]

Phytases are a structurally diverse group of enzymes classified according to their catalytic mechanism, sequence homology, and pH optima for enzymatic activity. Notably, phytase enzymes are classified into four types based on their structure and catalytic mechanism. Histidine acid phosphatases (HAPhy) are a common type of phytase found in filamentous fungi (*Aspergillus niger*, *Aspergillus fumigatus*) and yeast. They are optimally active at acidic pH (around 2.5–5.5) and have a wide range of applications in the feed industry for developing nutrient-rich feed for monogastric animals.[15] Purple acid phosphatases (PAPs), members of the calcineurin-type metallophosphoesterase superfamily, are named for their distinctive purple colour caused by a charge-transfer interaction between ferric iron (Fe^{3+}) and a tyrosine residue in the active site.[16,17] PAPs are mostly found in plants (soybean) and exhibit optimal activity at acidic pH.

Table 1: Phytase enzyme sources

PHYTASE SOURCE	
Animal Source	Calf liver, Small intestine mucosa, Gut microbes of ruminant animals (Sheep & Cattle)
Plant Source	Germinating seeds, Wheat, Barley, Rye, Soybean, Maize, Peas, Lily pollen, Millet
Bacterial Source	*Bacillus* sp., *E. coli*, *Pseudomonas*, *Enterobacter*, *Klebsiella* sp., *Bifidobacterium*, *Buttiauxella*, *Selenomonas ruminantium*, *Lactobacillus sanfranciscensis*, *Bacillus subtilis*, *Xanthomonas*, *Citrobacter braakii*, *Lactobacillus amylovorus*
Fungal Source	*Aspergillus niger*, *Aspergillus oryzae*, *Rhizomucor miehei*, *Penicillium* sp., *Trichoderma*, *Mucor racemosus*, *Rhizopus* sp., Yeasts – *Saccharomyces cerevisiae*, *Schwanniomyces castellii*, *Cryptococcus aureus*, *Candida tropicalis*, *Pichia anomala*, *Candida krusei*, *Arxula adeninivorans*

β-Propeller phytases (BPPs) are alkaline phytases primarily discovered in *Bacillus* species, characterized by a unique three-dimensional structure resembling a six-bladed propeller. BPPs hydrolyze phytic acid by specifically recognizing a Ca^{2+}-mediated bidentate chelation (P–Ca^{2+}–P) between two adjacent phosphate groups and play a crucial role in maintaining the phosphorus cycle in soil. This mechanism directs sequential cleavage, producing myo-Ins (2,4,6) P_3 (confirmed by ^1H- and ^{13}C NMR with the aid of 2D NMR) along with free phosphates as the final products.[18] Cysteine phosphatases represent a new class of phytase characterized by a cysteine-based active site, mainly found in anaerobic bacteria from the gut of ruminant animals. The active site cysteine is located within a highly conserved motif, HCxxGxxR, and these enzymes are optimally active at a pH range of approximately 4.5 to 6.0.[19]

In addition to structural classifications, phytases are also categorized based on their site of initial hydrolysis on the phytic acid molecule. This classification includes three main types: 3-phytase (EC 3.1.3.8), 5-phytase (EC 3.1.3.72), and 6-phytase (EC 3.1.3.26), which differ with respect to the specific position on the inositol ring where they first cleave the phosphate group.[20] Notably, based on their structure and functional diversity, these enzymes ensure efficient degradation of phytic acid under various physiological conditions and highlights their significance in animal nutrition and biotechnological applications.

Application of phytase in animal feed

The primary application of phytase is in animal nutrition, where it plays a vital role in enhancing phosphorus utilization from plant-based feed ingredients in monogastric animals such as poultry, swine, and fish. In such feeds, phytic acid (phytate) constitutes around 60–80% of the total phosphorus content; however, monogastric animals lack sufficient endogenous phytase to break it down, leading to poor mineral absorption. Microbial phytase supplementation facilitates the enzymatic hydrolysis of phytate, releasing bound essential minerals such as phosphorus, zinc, iron, and calcium, thereby significantly enhancing nutrient bioavailability.[15] This not only reduces the dependence on expensive inorganic phosphate supplements but also promotes more cost-effective and sustainable livestock production. Notably, several

Table 2: Commercially available Phytase enzyme

Trade name	Protein origin	pH optima	Thermal stability	Company	Country
Natuphos® E	*Aspergillus niger*	2.0-5.0 (5.5)	90°C	BASF Corporation	Germany
Ronozyme® P	*Peniophora lycii*	4.0-4.5	70-90°C	DSM-firmenich & Novozyme	Denmark
Ronozyme® Hiphos	*Citrobacter braakii*	-	80°C	DSM-firmenich & Novozyme	Denmark
Phyzyme® XP	*Escherichia coli*	5.5	-	DuPont Nutrition	USA
Finase® EC	*Escherichia coli*	5	70- 85°C	AB Vista	UK
Quantum® Blue	*Escherichia coli*	5.5	80–85°C	AB Vista	UK
Quantum®	*Escherichia coli*	4.5	85°C	AB Vista	UK
OptiPhos®	*Escherichia coli*	3.4,5.0	75- 90°C	Huvepharma	USA
Axtra® PHY 15 000 L	*Buttiauxeralla sp.*	3.5-4.5	-	Danisco Animal Nutrition (Dupont)	Denmark
Allzyme®SSF	*Aspergillus niger*	6.0	-	Alltech	USA

commercial phytase products are available in the market, as shown in Table 2.[21] Although many more commercial phytase enzymes are produced locally in various countries, the market is dominated by enzymes from developed nations, with European countries being key producers. The widespread use of these enzymes in feed for poultry, pigs, and fish underscores their importance as a microbial feed supplement.

Phytase enzymes can be incorporated into probiotic supplements for livestock to support gut microbiota and enhance nutrient utilization. Bandari *et al*. (2024) developed a biologically engineered probiotic by cloning the *phy* gene from *Bacillus subtilis* into *Lactococcus lactis*, enabling it to produce phytase. The engineered strain produced phytase in a granular form, enhancing phosphorus availability from plant-based feed ingredients, particularly benefiting monogastric animals.[22] Multi-enzyme formulations have become a prominent strategy in modern animal nutrition and feed development, especially for monogastric animals. Nowadays, phytase is commonly combined with other enzymes such as xylanase, protease, and amylase to target a broader range of anti-nutritional factors commonly present in plant-based feed ingredients, including phytate, non-starch polysaccharides (NSPs), and complex proteins. Multi-enzyme supplementation enhances nutrient availability, improves feed efficiency, and promotes gut health by breaking down complex feed components, thereby reducing the need for costly dietary nutrients without compromising animal growth and productivity.

The synergistic effect of exogenous multi-enzyme and phytase supplement was assessed by Kim *et al.* (2021) in corn, wheat and soybean-based diets for broiler

chickens. It was found that muti-enzyme phytase combination significantly improved body weight gain and feed conversion ratio during the finisher period. Birds receiving this combination also showed markedly higher digestibility of dry matter, crude protein, gross energy, calcium, and phosphorus, along with elevated serum Ca and P levels. Moreover, intestinal morphology was enhanced, with increased villus height, crypt depth, and villus height-to-crypt depth ratio. These improvements were superior to those achieved with phytase alone, while gut microflora remained unaffected.[23]

Further investigations are essential to optimize feed formulation strategies by examining the interactions between phytase and various feed components, including other enzymes, minerals, dietary fibres, and nutrients, as well as the influence of feed processing methods. The effect of phytase on the gut microbiota is also an important factor that collectively impacts nutrient absorption and overall animal health. Assessing both synergistic and antagonistic interactions will aid in optimizing phytase efficiency across diverse and unconventional feed formulations. These studies are vital not only for maximizing phytase utilization in animal feeds but also for promoting environmental sustainability by reducing phosphorus footprints resulting from animal waste, thereby improving the bioavailability of minerals and other nutrients, and enhancing overall feed efficiency.

Impact of phytase on animal performance and productivity

Animal feeds are primarily composed of cereals, legumes, and oilseeds, which are predominantly plant-based. In addition to breaking down phytate, phytase improves bone mineralization, enhances nutrient utilization, and promotes better digestion of proteins and fatty acids. Phytate forms electrostatic interactions with the terminal amino groups of proteins, resulting in phytate–mineral–protein complexes that lower the availability of essential amino acids (lysine and arginine) and reduce overall protein digestibility. Moreover, phytate inhibits key gastrointestinal enzymes (e.g., trypsin, pepsin, and α-amylase), thereby reducing protein and starch digestibility and diminishing overall nutrient utilization.[4,6,24,25] Phytate also reduces α-amylase activity through two mechanisms: direct noncompetitive inhibition of the enzyme and sequestration or interaction with essential divalent cations required for enzyme stabilization and activation.

Experimental studies by Deshpande and Cheryan (1984) demonstrated that in the presence of 6–30 mM phytate, calcium ions (Ca^{2+}) lowered α-amylase activity by 9–34%, whereas magnesium ions (Mg^{2+}) caused a much stronger inhibition, ranging from 24% to 49%. This indicates that Mg^{2+} amplifies the negative effects of phytate more significantly than Ca^{2+}.[25] The anti-nutritional effects of phytate are particularly pronounced in legumes such as soybean and chickpea, as well as in cereals. In soybeans and chickpeas, protein digestibility is reduced due to strong phytate-protein binding. [26] Collectively, these interactions emphasize the anti-nutritional role of phytate, posing major nutritional challenges in plant-based diets dominated by legumes and cereals. To overcome these effects, the application of phytase has been extensively studied in animal nutrition, especially in monogastric species such as poultry and swine.[27,28]

Adeshakin (2023) investigated the impact of varying phytase (PHY) levels, with or without the inclusion of multi-carbohydrase (MC), on growth performance, nutrient digestibility, and bone characteristics in nursery pigs fed phosphorus-deficient diets. The findings revealed that PHY supplementation, whether used alone or in combination with MC, significantly enhanced average daily gain (ADG), feed efficiency, and the apparent total tract

digestibility (ATTD) of ash and phosphorus release ($p < 0.05$). Phosphorus release refers to the enzymatic hydrolysis of phytate by phytase, resulting in the liberation of inorganic phosphorus (Pi) from phytate complexes. Phytase activity is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphorus per minute from 0.0015 mol/L sodium phytate at pH 5.5 and 37 °C, indicating the enzyme's efficiency in converting unavailable phytate-bound phosphorus into an absorbable form. Additionally, PHY improved bone mineralization, as indicated by increased ash and phosphorus content. While MC alone had limited influence, its combined use with PHY demonstrated a synergistic effect, further improving nutrient digestibility and bone quality.[27]

The study by Leyva-Jimenez *et al.* (2018) evaluated four commercially available phytase sources across different parameters by supplementing broilers at regular and super-dose levels to assess their effects on performance, bone mineralization, and apparent ileal digestible energy. Results show that broilers fed super-dose levels of phytase had significantly higher body weight, weight gain, and bone mineralization compared to birds on the negative control. Additionally, broilers fed super-dose phytase showed a 17% increase in apparent ileal energy digestibility at day 24, indicating improved nutrient utilization. Overall, high levels of phytase supplementation more effectively enhanced growth performance, bone characteristics, and energy utilization while compensating for phosphorus deficiency compared to broilers fed low levels of phytase.[28] It was found that supplementation of *Schizosaccharomyces pombe* expressed phytase (500–1,500 FTU/kg) in broiler diets from day 1 to 45 linearly improved body weight gain ($p = 0.001–0.018$) and feed conversion ratio ($p = 0.001–0.012$) throughout the trial. Additionally, ileal phosphorus digestibility ($p = 0.049$), toe ash content ($p < 0.001$), and carcass weight ($p = 0.035$) were increased, while footpad lesions decreased ($p = 0.040$), with no significant effects on organ indices, calcium content, crude protein digestibility, or meat quality.[29]

Supplementation of microbial phytase in low-phosphorus pig diets improved digestibility and growth performance, including feed intake, while enhancing overall mineral utilization. It also increased the retention of phosphorus, calcium, magnesium, and copper, reducing environmental excretion of P and Cu by 39% and 33%, respectively.[30] Babatunde and Adeola (2022) conducted two trials to investigate the impact of phytase supplementation on phosphorus utilization in growing and finishing pigs, assessing parameters such as growth performance, apparent total tract digestibility (ATTD) of nutrients, phosphorus excretion, and plasma mineral levels. In both experiments, pigs receiving the positive control diet exhibited greater body weight compared to those on the negative control diet. Inclusion of phytase in the diet significantly enhanced growth performance, with increases in body weight, average daily gain, and both linear and quadratic improvements in gain-to-feed ratio (G:F) ($P < 0.01$ for growing pigs; $P < 0.05$ for finishing pigs) relative to negative control. Phytase supplementation also improved nutrient digestibility, particularly ATTD of phosphorus and calcium, and markedly reduced water-soluble phosphorus excretion by 45% during the growing phase, 32% during finishing, and 35% across the full grow-finish period. Additionally, plasma phosphorus concentrations were elevated in pigs fed phytase, indicating its effectiveness in enhancing mineral utilization and overall growth performance.[31] Overall, phytase supplementation has been shown to improve growth performance in monogastric animals while enhancing nutrient digestibility, mineral absorption, bone mineralization, and energy utilization.

Optimisation of fermentation strategies for industrial scale phytase production

Optimisation of fermentation strategies is a key focus area for the industrial-scale production of phytase enzymes. Numerous ongoing studies aim to optimize phytase fermentation processes to achieve maximum yield at minimal cost. Submerged fermentation is commonly utilized for producing phytase from both bacterial and fungal sources, whereas solid-state fermentation is more suitable for filamentous fungi due to its reliance on low-moisture solid substrates.[32] Semi-solid fermentation offers an intermediate moisture environment, supporting specific microbial growth for specialized applications. Low cost agricultural and industrial residues such as wheat bran, soybean meal, molasses, corn steep liquor, various oil cakes, and other agro-industrial wastes are commonly used as effective substrates in fermentation processes to reduce production costs.[33] Moreover, each fermentation strategy must be carefully optimized for industrial-scale applications to ensure higher enzyme yields and reduce downstream processing costs. This includes fine-tuning media composition, pH, temperature and bioreactor design for efficient, scalable production.

Enzyme immobilization is a key industrial strategy used to reduce production costs and enhance enzyme efficiency, stability, and reusability. Immobilization of phytase enzyme from *Chenopodium album* onto chitosan-coated iron oxide (Fe_3O_4) nanoparticles was effective in reducing phytic acid in soymilk. The immobilized phytase exhibited broad substrate specificity towards various natural polymers and retained 70% of its catalytic activity over a 40-day storage period. Additionally, it demonstrated good operational stability with successful reuse over seven batch cycles, indicating strong potential for practical applications in food phytate reduction.[34] According to Coutinho *et al*. (2019) phytase immobilized on hydroxyapatite (HA) nanoparticles achieved complete adsorption with over 100% recovered activity. The immobilized enzyme also exhibited enhanced thermal stability at 80–90 °C, a broader pH activity range, and increased resistance to acidic conditions and proteolytic enzymes under simulated fish gastrointestinal conditions.[35] These properties highlight its strong potential for application in both aquatic and animal feed industry.

According to Khongkomolsakul *et al*. (2025) chitosan complexation markedly improved the thermal and gastrointestinal stability of phytase (phyA), increasing residual activity from 3% in the native enzyme to 40% in the 4:1 CS-phyA complex. Thermal stability was also significantly enhanced, rising from 20% to 74% at the same complex ratio. The optimized complexes retained up to 13-fold higher enzyme activity following exposure to heat and simulated gastric conditions. These results indicate that CS-phyA complexes hold substantial promise for expanding phytase applications in high-temperature food processing and plant-based dietary systems.[36] A thermostable phytase from *Mucor indicus*, optimized using black gram husk as a substrate, yielded the highest enzyme activity (92.10 U/ml). Further optimization of media and conditions enhanced activity to 184.03 U/ml. Immobilization significantly increased catalytic efficiency (17.26 mM/s vs. 5.68 mM/s for free enzyme). When applied to broiler and layer feed, the immobilized enzyme effectively released phosphorus (35.45 mg/g and 58.46 mg/g, respectively), highlighting its potential to enhance feed nutrition through sustainable utilization of agro-waste.[37] In industrial-scale phytase production, the integration of optimized fermentation strategies is essential to achieve high enzyme yields at minimal cost. Solid-state and submerged fermentation are the most effective approaches, depending on the microbial source and downstream processing

requirements. Efficient utilization of agro-industrial residues not only reduces production expenses but also promotes environmental sustainability. Optimization of media composition, process parameters, purification methods and enzyme stabilization techniques like immobilization are essential to enhance viability of phytase in animal nutrition and feed applications.[34]

Next-generation phytases in feed applications: Recombinant phytase production

The demand for efficient and sustainable animal nutrition has had a significant impact on global feed production, driving the development of next-generation phytases. Conventional phytases, although effective, exhibit several limitations, including a narrow pH activity range, low stability in the gastrointestinal tract, and poor thermostability at high temperatures, which is an essential requirement for feed pelleting and extrusion processes. To overcome these challenges, advanced molecular techniques such as genetic engineering and recombinant expression systems are being widely employed to enhance phytase yields and improve its functional properties. Significant progress has been made in the overexpression of phytase genes using host systems such as *Escherichia coli DH5α, Pichia pastoris X-33, Saccharomyces cerevisiae*, and *Yarrowia lipolytica*. This is a promising area of research, enabling the large-scale production of engineered enzymes with desired characteristics.[38-40] Attempts have also been made to express phytase genes directly in transgenic crops to develop self-sufficient feed sources. Cloning of novel phytase genes from bacteria, fungi, and even metagenomic sources has expanded the diversity of available phytases, offering enzymes with unique features like heat resistance, broader substrate activity, and wider pH optima, taking into consideration industrial-scale needs in animal feed processing, where high temperatures are required and the acidic gastrointestinal conditions in domesticated animals. Some recombinant phytases now exhibit dual pH optima, enabling the hydrolysis of phytate across a broad pH range with different substrates and increased resistance to proteolytic degradation, which helps them function more efficiently *in vivo*.

Many ongoing studies in this field aim to enhance phytase thermostability, broaden its pH activity range, and maximize production yield. For instance, phytase obtained from *Thermoascus aurantiacus* under submerged fermentation, using an optimized medium containing 3.75% (w/v) wheat bran particles and 2% Tween-20 as an additive, yielded a maximum thermostable phytase activity of 468.22 U/mL after 72 hours of incubation. The enzyme displayed excellent thermal stability, retaining nearly 80% of its activity at 70°C, underscoring its potential application as a feed enzyme.[38] Son *et al*. (2024) found that a novel phytase, TmPhy, isolated from *Turicimonas muris* and expressed in *Pichia*, had dual pH optima (3.0 and 6.8) with peak activity at 70°C. However, its native thermostability was inadequate for use in feed. Using genetic engineering and incorporating several mutation strategies, including random mutagenesis, disulfide bond introduction, and N-terminal modification, an improved mutant version of the enzyme, named TmPhyMD2, was produced, which exhibited the highest thermostability, retaining 74.1% of its activity at 80°C.[39] Xing *et al.* (2023) enhanced the thermostability of phytase by employing error-prone PCR and site-directed mutation in *E. coli*, followed by the expression of selected top mutants in *P. pastoris*. Five mutants demonstrated enhanced thermostability, retaining approximately 9.6%, 10.6%, 11.5%, 11.6%, and 12.2% more residual activity than the wild-type enzyme after exposure to 99 °C for 60 minutes. Notably, three mutants D7, E3,

and F8 also exhibited significant improvements in catalytic efficiency (kcat/Km), by 79.8%, 73.2%, and 92.6%, respectively, highlighting their potential as promising candidates for feed processing and other industrial applications.[40]

Another approach to improve phytase production involved Site-directed mutagenesis using Quick-Change PCR that introduced T312R and F260R mutations into the active site of *Yersinia intermedia* phytase gene. Then the mutated plasmids were introduced into *E. coli* DH5α using the heat shock method. Recombinant enzymes were expressed in the double mutant T312R/F260R which showed a 2.35-fold increase in activity over the wild-type. The lambda Red recombineering system is a modern genetic engineering technique used to create genetically modified organisms for the production of recombinant proteins.[41] The study by Arhar *et al.* (2025) effectively employed *Cupriavidus necator* H16 to integrate the *Escherichia coli* phytase gene (*appA*) into the *phaC1* locus using linear PCR products, thereby enabling efficient gene knockouts through electroporation. The resulting strain was marker-free, due to Cre/loxP-mediated marker recycling, and exhibited a complete loss of PHB granules as a result of disrupting the *phaC1* gene. Functional analysis confirmed the successful expression of phytase, with the engineered strain releasing approximately 8 µmol of phosphate per unit after 15 minutes of incubation with phytate.[42] These findings highlight the system's efficiency and establish *Cupriavidus necator* as a promising host for sustainable and stable production of recombinant phytase for industrial applications.

To improve phytase reusability, *Saccharomyces cerevisiae* was engineered using CRISPR/Cas9 to display a fusion of acid and alkaline phytases on its surface via the α-agglutinin-GPI system. Two marker-free strains were developed using MFα and Aga2p signal peptides, exhibiting high activity across a pH range of 1.0–7.0, with dual pH optima and efficient kinetics. The fusion enzyme showed 3.5–4 times higher activity than the native phytase.[43] A recombinant *Pichia pastoris* strain was engineered to secrete alkaline phytase from *Bacillus subtilis* for potential aquafeed applications. The phytase gene was cloned into the pPICZαA vector and integrated into the *P. pastoris* X33 genome, producing a methanol-utilizing (Mut^+) strain. Western blot confirmed phytase secretion, and enzyme assays showed activity at pH 7.5. This highlights the strain's potential for industrial-scale phytate degradation in aquafeed production.[44]

Strain improvement through genetic engineering is one of the most advanced approaches for developing recombinant organisms with desired traits. Extensive research is ongoing to refine techniques such as site-directed mutagenesis, random mutagenesis, and CRISPR-based systems, all of which have shown promising results.[40,42-44] These advancements pave the way for more efficient and sustainable phytase applications in the animal feed processing and food industries and nutrient enhancement of plant-based diets.

Phytase as a sustainable approach for environmental protection

Agriculture and livestock farming are major sources of phosphorus pollution. Undigested phytate from animal waste, either directly from farms or through manure applied to agricultural land, can exceed the crop's capacity to absorb it, leading to soil phosphorus accumulation. Excess phosphorus runoff contributes to eutrophication, causing algal blooms and oxygen depletion in aquatic ecosystems. This disrupts natural phosphorus cycling and creates environmental hazards.[45] Soil microorganisms can slowly degrade phytate and release phosphorus for plant uptake. Additionally, manure from cattle supplemented with microbial phytase has been shown to enhance the bioavailability

of phosphorus more efficiently than untreated manure. El Ifa *et al*. (2024) isolated three strains of phytase-producing rhizobacteria with the ability to enhance barley growth under phosphate-limited conditions. These strains should be considered in the development of eco-friendly biofertilizers as alternatives to conventional phosphorus fertilizers.[46] Therefore, incorporating phytase-producing microorganisms into biofertilizers could serve as an effective strategy to address the issue of phosphorus unavailability in soils.

For a sustainable environment, nutrient-rich soil serves as the main ingredient for the growth of microbes and plants, leading to the development of a healthy ecosystem. Soil is naturally formed through weathering caused by the disintegration of large rocks and minerals over centuries. As life forms begin to grow there, the soil becomes more nutrient-rich and adapted to recycling minerals through different cycles such as carbon cycle, nitrogen cycle, phosphorus cycle and so on. The phosphorus cycle is one of the most important cycles, playing a vital role in sustaining phosphorus levels in the environment and ensuring its availability. The phosphorus cycle lacks a gaseous phase, primarily involving the movement of phosphate from rocks to soil and water through the process of weathering. Plants absorb phosphate, which then moves through the food chain and returns to the environment *via* decomposition and excretion. Soil microbes play a crucial role in breaking down complex molecules, such as phytate, by producing phosphate-releasing enzymes, including phytase, thereby converting phosphorus into a form that plants can absorb. Human activities disrupt the natural phosphorus cycle through deforestation, excessive use of fertilizers and detergents, and the discharge of phosphate-rich wastes, causing adverse environmental impacts such as loss of soil fertility, water acidification, eutrophication, and consequent ecosystem imbalances.[47]

Phytase enzymes can also be used in industrial wastewater treatment processes, where they facilitate the breakdown of organic phosphorus compounds, thereby reducing phosphorus load and mitigating environmental pollution. The study by Dalas *et al.* (2025) highlights the biotechnological potential of phytase from *Bacillus subtilis* for wastewater treatment. The enzyme exhibited broad thermal stability (20–60°C) and pH stability (4–8), with optimal activity at 30°C (0.83 unit/mL) and pH 6. The application of the enzyme significantly reduced BOD (64.9% and 56.4%), COD (59.6% and 53%), nitrate, and metal levels in industrial wastewater.[48]

Several recent studies focus on the immobilization of phytase on biochar as a strategy to convert organic phosphorus (Org-P) in manure into plant-available inorganic phosphate. Immobilization of phytase into biochar by covalent grafting accomplished by the carbodiimide crosslinker method and physical sorption will help to withstand leaching, degradation, or denaturation. Physisorption was found to be as effective as grafting, with phytase loading positively correlated with the amount of biochar. Immobilized phytase was stable with minimal leaching but showed significantly reduced activity compared to free phytase; hence, further studies are needed to improve its efficiency and to enable industrial-scale usage. Studies using clay minerals (montmorillonite, kaolinite, and hematite) showed better phytase loading and activity than biochar.[49,50] Therefore, these methods need to be optimized for better application of microbial phytase and to reduce organic phosphorus content in the soil.

Conclusion

In recent decades, significant advancements have been made in phytase research, driven by the need for more efficient and

sustainable solutions in the development of animal feed. International efforts have pushed the boundaries of enzyme engineering to achieve superior stability and activity, by utilizing agricultural wastes for cost-effective production. Genetic engineering offers promising strategies for developing more efficient phytase-producing microorganisms through heterologous gene expression, aiming to enhance thermostability, broad pH activity, protease resistance, and other desirable traits. Ongoing research is increasingly focused on the structural and functional characterization of phytase enzymes, with particular emphasis on identifying novel sources and elucidating their catalytic mechanisms, active site architecture, and interactions with phytate. Beyond animal nutrition, phytase applications extend to food, nutrition and processing, development of transgenic plants incorporating microbial phytase, industrial production of value-added biochemicals such as myo-inositol, and eco-friendly technologies including the paper industry. As a significant number of phytases are produced using genetically modified organisms especially transgenic animals and plants, comprehensive safety evaluations are being conducted to ensure their safe use in food and animal feed, in accordance with regulatory standards and public health guidelines. The continued integration of molecular biology, fermentation technology, and protein engineering is anticipated to drive further advancements in the development of next-generation phytases with enhanced performance for a wide range of biotechnological applications.

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Conflicts of interest

There are no conflicts of interest.

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