

Research Progress on Phytase Superdosing in Feed: Postprint

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Abstract

Phytase, as a conventional feed additive, has been widely used in animal diets to degrade phytate in feed ingredients, release phosphorus, and reduce the usage of dicalcium phosphate or other ingredients for providing available phosphorus. As research progresses, it has been found that the current addition level of phytase is far from achieving its full efficacy; supra-nutritional supplementation of phytase can degrade over 90% of phytate, improve the utilization efficiency of protein, fat, starch, and mineral elements, enhance livestock and poultry production performance, reduce feed costs, and mitigate environmental pollution.

Full Text

Research Advances in the Superdosing of Phytase in Animal Feeds

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Abstract: As a conventional feed additive, phytase is widely used in animal feeds to degrade phytate in feed ingredients and release phosphorus, thereby reducing the need for inorganic phosphorus sources such as calcium hydrogen phosphate. However, further research has revealed that conventional phytase addition rates are far from optimal. Superdosing phytase can degrade over 90% of phytate, significantly improving the utilization of protein, fat, starch, and mineral elements, enhancing livestock and poultry growth performance, reducing feed costs, and mitigating environmental pollution.

Keywords: phytase; superdosing; potential nutritional value

1. Conventional Application of Phytase and the Concept of “Superdosing”

Phytase assists monogastric animals in degrading phytate—an indigestible compound—into inositol and phosphate. Phytate phosphorus represents the primary storage form of phosphorus in plant-based ingredients, with 60-90% of phosphorus in common cereals and legumes existing as phytate phosphorus. Phytase supplementation improves phytate phosphorus utilization in livestock and poultry, reducing the need for calcium hydrogen phosphate and lowering feed formulation costs. The conventional application rate is 500 FTU/kg in complete feeds for livestock and poultry, which releases 40-50% of phytate phosphorus, equivalent to replacing 0.1% available phosphorus from calcium hydrogen phosphate (containing 16% phosphorus), or approximately 6.25 kg/t of feed.

Since its introduction as a commercial feed additive in 1991 with a projected market value of €150 million, phytase has become ubiquitous in livestock feeds due to its low cost and clear benefits in improving phytate phosphorus utilization, production efficiency, and environmental protection. However, mounting evidence indicates that current inclusion rates substantially underestimate phytase’s potential, necessitating significant increases in dosage. This has given rise to the concept of phytase “superdosing.”

2. Potential Value of Phytase Superdosing

Superdosing refers to phytase inclusion rates exceeding the conventional 500 FTU/kg. Such elevated doses can release over 90% of phytate phosphorus from plant-based feedstuffs, thereby improving animal performance and reducing environmental pollution. Han et al. demonstrated that superdosing microbial phytase (4,000-8,000 FTU/kg) in low-phosphorus broiler diets (non-phytate phosphorus at 2.1 g/kg) increased total phosphorus utilization to over 85% and phytate phosphorus release to over 90%, while significantly improving growth performance, plasma and tibia metrics, and crude protein utilization to levels comparable with phosphorus-adequate controls (non-phytate phosphorus at 4.5 g/kg). Zeng et al. reported that adding 0, 500, 1,000, or 20,000 FTU/kg phytase to low-phosphorus diets for growing pigs (total phosphorus 0.38 g/kg) increased phytate apparent digestibility from 11.1% to 62.8%, 70.6%, and 90.5%, respectively. Compared with conventional doses, 20,000 FTU/kg significantly improved apparent digestibility of calcium, phosphorus, and phytate while substantially reducing ileal phytate content. Additional studies by Augspurger et al., Shirley et al., and Kies et al., using inclusion rates of 10,000, 12,000, and 15,000 FTU/kg respectively, all showed significant improvements in phytate degradation, nutrient absorption, growth performance, and feed efficiency.

Nevertheless, superdosing is not without limits. Han et al. found no significant differences in growth performance or physiological indices between 4,000-8,000 FTU/kg groups and phosphorus-adequate controls, suggesting 4,000 FTU/kg as the optimal dose in their study. Optimal phytase levels vary by species and

diet formulation. Shirley et al. achieved 83.8% phytate apparent digestibility in broilers with 12,000 FTU/kg, while Kies et al. reached 94.8% in weaned piglets with 15,000 FTU/kg. Augspurger et al. demonstrated that 1,000 FTU/kg could release approximately 0.2% phytate phosphorus. Zeng et al. also revealed that superdosing significantly improved ileal apparent digestibility of crude protein, dry matter, gross energy, and several amino acids including leucine, lysine, threonine, valine, aspartic acid, and serine. Furthermore, compared with high- and low-phosphorus controls, the 20,000 FTU/kg group showed improved utilization of crude fiber and neutral detergent fiber in the hindgut and significantly enhanced utilization of sodium, manganese, and zinc.

In summary, phytase superdosing improves nutrient utilization, though optimal superdose levels require further species-specific research. The available evidence also indicates that current superdose levels are far from producing negative effects in livestock and poultry.

3. Mechanisms of Action with Dietary Nutrient Levels and Interactions with Other Enzymes

The substantial underestimation of phytase's potential stems primarily from an incomplete understanding of its mechanisms of action with dietary nutrients and interactions with other feed enzymes.

3.1 Mechanisms of Action

While experimental data demonstrate numerous benefits from superdosing, the underlying mechanisms remain incompletely understood. Superdosing degrades over 90% of phytate and elicits various potential nutritional effects that directly or indirectly enhance animal performance. Two hypotheses have been proposed to explain these phenomena: First, phytate degradation yields inositol, which may promote growth performance. This could explain why superdosing shows significant effects on performance. Studies have detected high inositol concentrations in broiler gizzards when phytase was added at 1,000 or 1,500 FTU/kg compared with 500 FTU/kg, with near-complete phytate degradation. Inositol can stimulate GLUT4 translocation to cell membranes and promote glucose transport, gluconeogenesis, and protein deposition similarly to insulin. Second, Yu et al. reported that phytate binds to soy protein and β -casein, reducing protein utilization. Superdosing can completely degrade phytate, liberating more nutrients such as amino acids and minerals. Studies have confirmed improved utilization of calcium, magnesium, manganese, zinc, copper, and iron in pigs fed superdosed phytase, with effects appearing more pronounced in poultry than swine. The specific mechanisms are discussed below.

3.1.1 Improved Feed Efficiency Phytase enhances feed utilization through multiple pathways. First, phytase effects are minimal in phosphorus-adequate diets but substantially impact performance in low-phosphorus diets by increas-

ing available phosphorus. Second, phytate chelates metal cations (calcium, zinc, iron) and proteins into insoluble complexes; phytase addition disrupts these chelations, improving digestibility of minerals and proteins. Third, phytase supplementation improves starch and fat digestibility, indirectly enhancing energy utilization. For instance, phytase supplementation in low-phosphorus piglet diets improved feed efficiency by 3%.

3.1.2 Enhanced Amino Acid Utilization Amino acids are critical nutrients for animal growth, yet phytate impairs their absorption and utilization. Phytate directly affects sodium transport pathways in the intestine, influencing sodium pump activity and consequently sodium-dependent amino acid absorption. Ravindran et al. evaluated the relationship between lysine and phytase, demonstrating that phytase supplementation in lysine-deficient broiler diets could release lysine from phytate-protein complexes and improve growth performance. Other studies have reported varying degrees of improvement in other amino acid digestibilities.

3.1.3 Improved Protein Digestibility Phytate molecules in many crops are globular and stored in protein-rich tissues such as germ or aleurone layers. Due to similar solubility properties between proteins and phytate, these globules often chelate with proteins within plant cells, impairing protein digestibility. Phytase prevents extensive formation of protein-phytate complexes. Even after complex formation, phytase can synergize with pepsin to maximize protein degradation. While pepsin can partially hydrolyze proteins, its efficiency and extent improve dramatically with phytase addition. Pepsin only hydrolyzes small proteins from phytate-protein precipitates—those with molecular weights below 12 kDa can be released from the complex. When phytase and pepsin are added together, larger proteins are liberated and rapidly hydrolyzed into smaller fragments far more efficiently than without phytase. Since phytase cannot directly hydrolyze proteins, it must work in concert with pepsin to improve protein utilization.

3.1.4 Enhanced Mineral Utilization Superdosing phytase improves animal performance likely by increasing mineral availability. Rimbach et al. and Kies et al. demonstrated improved absorption of zinc, copper, and magnesium in weaned piglets fed phytase-supplemented diets. Calcium-phytate solubility decreases substantially above pH 6, and magnesium salts precipitate under high pH conditions. Therefore, adding 20,000 FTU/kg phytase in the upper small intestine effectively targets soluble phytate (magnesium-phytate complexes), significantly improving magnesium utilization and retention. Thus, superdosing at 20,000 FTU/kg indeed enhances mineral utilization compared with conventional 500 FTU/kg inclusion.

3.1.5 Improved Starch, Fat, and Energy Utilization Starch and fatty acids are primary energy sources, but phytate chelation indirectly reduces energy

utilization efficiency. Phytase significantly increased apparent metabolizable energy in sorghum-soybean diets from 12.8 to 13.1 MJ/kg DM and improved metabolizable energy in duck diets. Superdosing enhances apparent digestibility in low-phosphorus diets not only through improved protein/amino acid utilization but also by mitigating the negative effects of calcium-phytate complexes, which reduce apparent metabolizable energy. These complexes form insoluble soaps with fatty acids in the intestine, decreasing fat digestibility, and either bind directly to starch or inhibit α -amylase activity, reducing starch solubility and digestibility. Superdosing degrades phytate phosphorus, dissociates calcium-phytate complexes, and liberates fats and starches, thereby increasing apparent metabolizable energy.

3.1.6 Improved Gut pH and Microflora Phytase supplementation significantly improves intestinal pH. Walk et al. demonstrated that high phytase doses (5,000 FTU/kg) substantially affect gastrointestinal pH. In contrast, other studies showed no pH changes in 32 kg pigs fed 250–1,000 FTU/kg, 42-day-old broilers, or 21-day-old broiler ileum. However, 1,000 FTU/kg significantly reduced fecal pH in 53 kg pigs. High phytase doses can influence gastrointestinal pH by directly degrading phytate molecules, releasing calcium, and eliminating phytate's negative effects. *In vitro* simulations showed that adding phytate to corn-soybean diets significantly reduced gastric pH from 3.5 to 3.2 (approximately 9%). Phytate reduces dietary electrolyte balance and creates a more acidic gastrointestinal environment through chelation, increasing mineral excretion (sodium, calcium, phosphorus, sulfur, potassium). Ptak et al. found that low calcium and digestible phosphorus levels significantly affected crop and cecal pH, while phytase supplementation in low-phosphorus diets improved ileal pH, reduced calcium and digestible phosphorus concentrations, and increased total intestinal microflora populations. Low-phosphorus diets also reduced butyrate concentrations while increasing *Lactobacillus* populations.

3.2 Interactions with Other Exogenous Enzymes

Enzyme-enzyme interactions exist, with non-starch polysaccharide enzymes (typically xylanase) and phytase demonstrating mutual enhancement of protein and energy utilization and animal performance. In low-energy diets, individual supplementation of xylanase or phytase improved apparent ileal digestibility of amino acids from 0.802 to 0.839 (a 4.6% increase), while combined supplementation significantly improved digestibility further to 0.874 (a 9.0% increase), with particularly notable improvements in lysine, arginine, histidine, leucine, phenylalanine, tyrosine, alanine, and glycine. Low apparent metabolizable energy is often attributed to high soluble non-starch polysaccharide levels. In wheat-based broiler diets, one enzyme can enhance another's catalytic activity because phytate tightly binds water-soluble non-starch polysaccharides in low-energy diets, making phytase more accessible and improving phytate degradation. The complementary action allows both enzymes to work simultaneously on their respective substrates, increasing contact opportunities and reducing

the antinutritional effects of both phytate and non-starch polysaccharides.

Exogenous amylase activity is inhibited by phytate, and superdosing can alleviate this inhibition. Since 1983, research has shown that α -amylase activity is suppressed by phytate, which directly or indirectly inhibits amylase through calcium chelation (calcium being amylase's cofactor) or by complexing with proteins attached to starch granules. Studies on covalent phosphorus bonds in sorghum starch revealed that substantial phytate inhibits starch breakdown to glucose-3-phosphate and glucose-6-phosphate, suggesting phytate phosphorus may covalently bind glucose in starch molecules, forming starch-phytate complexes that reduce energy utilization. Theoretically, proteins can tightly bind starch, and phytate can indirectly chelate starch through proteins, forming ternary complexes in the small intestine (due to higher isoelectric points) from cereal-based dietary proteins. Combined amylase and phytase supplementation yields greater daily weight gain, starch utilization, and feed conversion ratio compared with phytase alone, demonstrating that exogenous amylase efficacy is phytase-dependent.

4. Considerations for Phytase Superdosing in Feeds

The aforementioned research demonstrates that superdosing phytase in low-phosphorus diets produces superior results, primarily because phytate degradation to inositol and phosphate is a reversible reaction subject to feedback inhibition. When excessive phosphorus from other sources is present, it inhibits the forward reaction catalyzed by phytase, preventing expected outcomes. Therefore, phytase use must be accompanied by reduced supplementation of other phosphorus sources, though the optimal reduction magnitude requires further investigation.

In conclusion, phytase not only reduces calcium hydrogen phosphate requirements but, when superdosed, also improves animal performance, feed efficiency, and utilization of protein, starch, fat, and minerals while optimizing gut pH and microflora structure. Additionally, phytase interacts synergistically with other exogenous enzymes to enhance animal production. However, commercial high-dose phytase products often fail to replicate experimental results because superdosing is not merely about high enzyme activity. Optimal phytase levels vary with species and diet formulation, requiring case-specific analysis rather than universal application—more is not always better. Current literature reports maximum phytase inclusion up to 20,000 FTU/kg (40 times the conventional rate) without negative effects. Therefore, further research must elucidate relationships between phytase, feed ingredients, and animal species to determine optimal inclusion rates and establish databases for scientifically formulated, cost-effective phytase applications. Moreover, interactions between phytase and other exogenous enzymes require deeper investigation to optimize enzyme cocktails and maximize efficacy.

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