

ANIMAL

# Evaluation of the impact of phytase supplementation on growth performance, nutrient digestibility, and fecal score of growing pigs

Shanshui Gao, Md Mortuza Hossain, In Ho Kim\*

Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea

\*Corresponding author: [inhokim@dankook.ac.kr](mailto:inhokim@dankook.ac.kr)

## Abstract

Phytase enhances phosphorus availability in pig diets by breaking down phytic acid, which is challenging for animals to digest. This study aimed to assess the impact of dietary phytase supplementation on the growth performance, nutrient digestibility, and fecal score of growing pigs. Sixty-four growing pigs ([Yorkshire × Landrace] × Duroc, average body weight 21.20 ± 0.18 kg) were randomly assigned to one of two treatment groups. The dietary treatments were CON, basal diet, and PHY, basal diet + 0.0025% phytase. Results indicated a tendency for increased average daily gain in the phytase-supplemented diet group ( $p < 0.10$ ) compared to the control diet group. The average daily feed intake and feed conversion ratio were not affected by the addition of 0.0025% phytase. Furthermore, dry matter digestibility, nitrogen content, and digestible energy were not influenced ( $p > 0.05$ ) by phytase supplementation. Fecal score remained similar ( $p > 0.05$ ) in growing pigs fed phytase-supplemented and control diets. These findings suggest that supplementing the diet of growing pigs with 0.0025% phytase may lead to improved average daily gain without adverse effects on nutrient digestibility and fecal score. This implies the potential to enhance growth performance without compromising overall health or digestion in pigs. Further investigations into the optimal levels of phytase supplementation for growing pigs are recommended.

**Keywords:** growing pig, growth performance, nutrient digestibility, phosphorus, phytase



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## Introduction

Phosphorus is an important element for various physiological functions such as growth, bone formation, and energy metabolism in pigs (Zhai et al., 2022). This mineral mainly enters their diet through plant-based food sources in a form known as phytate or phytic acid. However, swine and other monogastric animals have limited capabilities to digest phytic acid. Phosphorus often commands a high price due to its critical role in animal health and the cost of its digestible forms, making it typically the second or third most expensive component in dietary supplements, after

energy and protein supplementation (Satter et al., 2005; Moe, 2008). Because of the low phytase activity in digestive systems, monogastric animals cannot efficiently utilize or access phytate-bound phosphorus. As a result, this undigested phytate phosphorus is expelled, contributing to environmental pollution, notably in regions with intensive animal farming (Chen et al., 2007; Humer et al., 2015). Excess phosphorus from industrial effluents and agricultural runoff frequently enters lakes via sewage, acting as a significant contributor to eutrophication (Abbasi et al., 2019). The phenomena of eutrophication, which triggers harmful algal blooms, depletes oxygen, and causes nutrient over-saturation in water bodies (Heisler et al., 2008).

To solve the problem of phosphorus digestibility, phytase are used in animal diet. Phytase is an enzyme that breaks down phytate into simpler inorganic phosphates and inositol phosphates, for this reason it is incorporated into commercial pig diets (Hirvonen et al., 2019). Derived from various microorganisms like *Aspergillus* spp., *Buttiauxella* sp., *Citrobacter braakii*, *Escherichia coli*, *Hafnia* spp., and *Peniophora lycii*, distinct microbial phytase products are now commercially accessible. Notably, three commonly used phytase feed enzymes are sourced from *A. niger* (3-phytase - EC 3.1.3.8) and *Peniophora lycii* and *Escherichia coli* (6-phytases - EC 3.1.3.26) (Šefer et al., 2012). By increasing the bioavailability of phosphorus and other phytate-bound nutrients, phytase enhances nutrient utilization and mitigates the environmental impact of pig farming (Humer et al., 2015). It has been observed to improve the absorption of amino acids, energy, and other minerals like calcium in pig diets (Cowieson et al., 2017; Gallardo et al., 2020). However, Zhai et al. (2021) reported that phytase had no significant influence on the digestibility of dry matter, crude protein, and gross energy or the performance of the sows and their offspring. The impact of phytase appears to be more evident as dietary phytate levels increase, a trend observed in both poultry (Ravindran et al., 2006) and pigs (Selle et al., 2003). Thus, the supplementation of phytase is being increasingly recognized as a viable technique to boost the availability of phosphorus in plant ingredients with high phytate content (Selle and Ravindran, 2008).

Most of the available research affirms positive results with phytase supplementation exceeding 500 Phytase units (FTU)/kg feed for growing pigs (Madrid et al., 2013; Dersjant-Li et al., 2017; Grela et al., 2020). However, there are very few research that check the effectiveness of lower phytase doses. We set out to examine the potency of a low dose (250 FTU/kg) phytase supplementation, assessing its impact on pigs' growth performance, nutrient digestibility, and fecal score. If lower doses of phytase prove to be effective, farmers may be able to reduce their costs while also lessening the environmental footprint of pig farming. The objective of this study is to check the growth performance, nutrient digestibility and fecal score in growing pig fed 0.0025% phytase (250 FTU/kg).

## Materials and Methods

All procedures and protocols involving the care and use of animals in this research were carefully reviewed and formally approved by the Animal Care and Use Committee of Dankook University, South Korea (Ethical approval number: DK-2-2227).

### Information of phytase

The commercial name of the phytase used in this experiment was HiPhorius™, which was supplied by a commercial company (DSM Nutritional Products AG, Netherlands). HiPhorius™ is a fourth generation phytase. The active agent of the product (HiPhorius™) is a 6-phytase, produced from *Aspergillus oryzae*. According to the company, the concentration

of phytase in the product was 10,000 to 50,000 FTU/g for the solid product. Additionally, HiPhorius™ is intended to be included through premixtures or directly in feedstuffs to obtain a minimum activity of 200 FTU/kg feedstuffs for pigs. A single phytase unit, or FTU, is determined as the quantity of enzyme required to liberate one micromole of phosphate from phytate each minute, under specific conditions of 37°C and a pH level of 5.5 (Dang et al., 2022).

## Animals, experimental design, and feeding regimen

A total of 64 crossbred ([Landrace × Yorkshire] (F1), F1 × Duroc) growing pigs with an average body weight (BW) of  $21.20 \pm 0.18$  kg were divided into two groups in a complete random block design with 8 replicates and 4 pigs (2 gilts and 2 borrows) per pen. Dietary treatment diet were, CON, basal diet; and PHY, 0.0025% phytase. The basal diets used in this study were formulated in such a way to either meet or surpass the nutrient requirements recommended by the National Research Council (NRC, 2012) (Table 1). The experimental pens were equipped with self-feeders and nipple drinkers, enabling the growing pigs to have unrestricted access to feed and water throughout the entire duration of the trial. To maintain a consistent ambient temperature of approximately 25°C within the facility, a ventilation control system was installed.

**Table 1.** Ingredient composition of experimental diets as-fed basis.

Item	%
Wheat	19.00
Corn crushed	37.57
Dehulled soybean meal	15.11
Wheat bran	2.00
Rice bran	2.00
Soybean meal	3.00
Parm kernel meal	2.00
Brown rice	5.00
Rapeseed meal	4.00
Sesame meal	2.00
Animal fat	3.79
Molasses	2.00
Vitamin/mineral mixture <sup>2</sup>	0.40
Methionine 98%	0.01
Threonine 98%	0.02
Lysine 25%	0.50
Mono calcium phosphate	0.16
Salt	0.30
Choline chloride 50%	0.09
Limestone	1.05
Total	100.00
Calculated value	
Digestible energy (kcal/kg)	3,560
Metabolic energy (kcal/kg)	3,280
Crude protein (%)	17.50
Crude fat (%)	6.70
Crude ash (%)	4.40
Crude fiber (%)	3.80
Total lysine (%)	0.99
Calcium (%)	0.75
Phosphorus (%)	0.42

<sup>2</sup> Provided per kg diet: Fe, 100 mg as ferrous sulfate; Mn, 17 mg as manganese oxide; Cu, 17 mg as copper sulfate; I, 0.5 mg as potassium iodide; Zn, 100 mg as zinc oxide; and Se, 0.3 mg as sodium selenite. Provided per kilograms of diet: vitamin A, 10,800 IU; vitamin D3, 4,000 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin E, 40 IU; vitamin E, 40 IU; vitamin B2, 12 mg; vitamin B6, 6 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; vitamin B12, 0.05 mg; D-calcium pantothenate, 25 mg.

## Sampling and measurements

The body weight of individual growing pigs was measured at the starting day and the ending day of the experiment. Total feed intake per pen was calculated through deducting the remaining feed from the given feed. Average daily gain, average daily feed intake and feed conversion ratio was calculated from the data of total feed intake and body weight.

To determine the digestibility of dry matter, nitrogen and digestible energy, fresh excreta were taken on the last three days of week 1 and week 2. About seven days before the day of fecal sample collection, 0.2% chromic oxide ( $\text{Cr}_2\text{O}_3$ , 98.5%, Samchun Pure Chemical Co., Ltd., Korea) was mixed with treatment diet as an indigestible marker. Fecal sample was collected from every experimental pen and after collection they were dried at 60°C for 72 hours, and then subjected to AOAC-standard techniques for determining dry matter (methods 934.01), and nitrogen (methods 968.06) (AOAC, 2005). Gross energy was measured using bomb calorimeter (Parr Instrument Company, USA). An atomic absorption spectrophotometry was used to determine the amount of chromium (UV-1201, Shimadzu Corporation, Japan). Apparent total tract digestibility (%) =  $(1 - (\text{Nf} \times \text{Cd}) / (\text{Nd} \times \text{Cf}) \times 100)$ , where Nf represents nutrient concentration in feces (percent dry matter), Nd represents nutrient concentration in diet (percent dry matter), Cf represents chromium concentration in feces (percent dry matter), and Cd represents chromium concentration in diet (percent dry matter).

The fecal score of the growing pigs was assessed on a daily basis and calculated weekly. Two trained personnel checked the fecal score unbiasedly during the whole experimental period. The occurrence of diarrhoea was determined by employing a 5-grade scoring system. Grade 1 denoted the presence of hard, dry pellets in a compact, solid mass, while grade 2 indicated well-formed stool that remained firm yet soft. Grade 3 represented soft, formed, and moist stool that retained its shape. Grade 4 corresponded to soft, unformed stool that conformed to the shape of the container, and grade 5 indicated the presence of watery liquid stool that could be poured.

## Statistical analysis

Statistical analysis was performed using the Student's t-test in SAS software (version 9.4, SAS Institute Inc., USA). Variation in the data was referred to as standard error of mean (SEM). And  $p < 0.05$  is considered as significant difference whereas  $p < 0.10$  is considered as tendency.

## Results

The effect of dietary phytase at 0.0025% on the growth performance of growing pigs are shown in Table 2. Average daily gain on the phytase supplemented diet intended to increase ( $p < 0.10$ ) compared to the control diet. However, average daily feed intake and feed conversion ratio was not changed ( $p > 0.05$ ) through the supplementation of phytase in growing pig at 0.0025%.

Digestibility of dry matter, nitrogen and energy in different treatment group are shown in Table 3. Addition of phytase at 0.0025% did not showed any significant effect ( $p > 0.05$ ) on the digestibility in growing pigs.

Fecal score in growing pigs were not altered ( $p > 0.05$ ) through the supplementation of dietary phytase at 0.0025% (Table 4).

**Table 2.** Effect of dietary phytase supplementation on growth performance in growing pigs.

Item	CON	PHY	SEM	p-value
Overall				
ADG (g)	590	607	5.01	0.0885
ADFI (g)	1,344	1,377	14.57	0.2759
FCR	2.279	2.269	0.02	0.7640

CON, basal diet; PHY, 0.0025% phytase; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SEM, standard error of means.

**Table 3.** Effect of dietary phytase supplementation on nutrient digestibility in growing pigs.

Item	CON	PHY	SEM	p-value
Dry matter	81.88	82.03	0.19	0.7240
Crude protein	76.35	76.51	0.23	0.7512
Energy	82.05	82.22	0.17	0.6722

CON, basal diet; PHY, 0.0025% phytase; SEM, standard error of means.

**Table 4.** Effect of dietary phytase supplementation on fecal score in growing pigs.

Item	CON	PHY	SEM	p-value
Fecal score <sup>z</sup>				
Week 1	3.20	3.18	0.022	0.7053
Week 2	3.19	3.18	0.018	0.8177

CON, basal diet; PHY, 0.0025% phytase; SEM, standard error of means.

<sup>z</sup> Fecal scoring can be categorized as follows: 1, hard, dry pellet-like feces; 2, firm, shaped stool; 3, soft, moist stool that holds its form; 4, soft, shapeless stool that conforms to the shape of its container; 5, a watery, pourable liquid consistency.

## Discussion

### Growth

The present study investigated the effect of phytase at 0.0025% (250 FTU/kg) supplementation on the average daily gain in growing pigs, while examining whether feed intake and feed efficiency were affected. In a previous study, Guggenbuhl et al. (2016), Dersjant-Li et al. (2017), da Silva et al. (2019) reported that pigs fed a phytase supplemented had improved growth performance. The improvement in growth suggests that phytase supplementation can enhance the overall productivity and performance of growing pigs. The addition of microbial phytase to the diet works to break down the complex formed between phytate and other nutrient ingredients in the stomach. This action results in a heightened level of nutrients that can be absorbed, subsequently enhancing the growth performance of the animal (Dang et al., 2022). Moreover, in this study the observed feed intake and feed efficiency of the pigs were not significantly altered by phytase supplementation. This result suggests that the increased growth observed in the phytase group was not solely attributed to changes in feed consumption. It is possible that the enhanced availability of phosphorus and other key minerals facilitated by phytase supplementation played a crucial role in promoting growth without influencing feed intake or efficiency (Rizwanuddin et al., 2023). The level of breakdown of nutrient ingredient-phytate complexes can be gauged by tracking the variations in the digestibility of phosphorus (Jolliff and Mahan, 2012). By incorporating phytase at 0.0025% into growing pig diets, producers can potentially optimize growth performance without adversely affecting feed intake or efficiency. This offers an opportunity to improve profitability and sustainability in pig production systems by reducing the reliance on inorganic phosphorus sources, which

can be costly and environmentally detrimental. However, we suggest that higher dose of phytase in growing pig diet may show better results in growth performance and feed intake, which is also suggested by Dang and Kim (2021).

### **Nutrient digestibility**

In the present study phytase supplementation at 0.0025% (250 FTU/Kg) did not alter the digestibility of dry matter, nitrogen, and energy in growing pigs. Our findings align with the studies conducted by Rosenfelder-Kuon et al. (2020), where it was reported that phytase did not significantly alter the digestibility of dry matter, nitrogen, and energy. Similarly, Moita and Kim (2022) explained that 6-phytase supplemented above the traditional levels (500 - 1,000 FTU/kg feed) showed improvement in the nursery pigs. Contrarily, a few studies found improved nutrient digestibility growing or finishing pigs when phytase was supplemented at 1,000 to 3,000 FTU/kg diet (Thacker et al., 2006; Velayudhan et al., 2015). One possible explanation for this findings in our experiment could be that the level of phytase supplementation was insufficient to induce a noticeable improvement in the digestibility of these nutrients. By breaking down phytic acid, phytase not only makes phosphorus more available but also improves the bioavailability of other nutrients. Phytic acid can bind minerals such as calcium, zinc, and iron, and amino acids, making them less accessible for absorption. By degrading phytic acid, phytase liberates these nutrients, thus improving their absorption (Selle and Ravindran, 2007). The action of phytase and its effectiveness in improving nutrient absorption could be influenced by numerous factors, including diet composition, feed processing, animal age and physiological status, as well as pH conditions in the gastrointestinal tract (Dersjant-Li et al., 2015; Singh et al., 2018). Based on our results, supplementation of phytase at a low level (250 FTU/kg) might not be adequate to enhance the digestibility of these nutrients.

### **Fecal Score**

Phytase hydrolyzes phytate into available phosphorus and other nutrients (Rizwanuddin et al., 2023). This reaction occurs under favorable pH conditions, primarily in the stomach and small intestine of the pig (Hirvonen et al., 2019). If the digestion process experiences any abnormalities, these disruptions are likely to manifest through changes in the fecal score. The fecal score serves as a useful indicator of gut health and digestive efficiency (Vandeputte et al., 2016). A change in fecal score can indicate alterations in gut motility, microbial balance, and nutrient absorption (Wen et al., 2018). Our study found no alteration in fecal score in growing pigs supplemented with 0.0025% phytase (or 250 FTU/Kg). For instance, a study by Lagos et al. (2021) also reported no alteration in fecal score in pigs supplemented with phytase at various concentrations. It is possible that the amount of phytate in the diet was not high enough to create a measurable change in fecal score when phytase was added. Several studies have shown that the gut microbiota plays a significant role in fecal consistency and overall gut health (Vandeputte et al., 2016). As phytase can potentially change the available nutrients in the gut, it could influence the microbial composition. However, the fecal score remained unchanged may suggest that the phytase supplementation at the given level did not significantly impact the microbial balance. It is also noteworthy to consider that the host's physiological state, including the gut pH, enzymatic profile, and intestinal transit time, can significantly influence the efficacy of dietary phytase (Dersjant-Li et al., 2015). Therefore, future studies should consider these factors in understanding the interplay between phytase supplementation, nutrient absorption, and fecal score in pigs.

## Conclusion

In conclusion, this findings provide preliminary evidence that a 0.0025% phytase supplementation in the diet of growing pigs could potentially influence growth performance, specifically by exhibiting a trend toward improved average daily gain. However, there was no change to average daily feed intake, feed conversion ratio, digestibility nutrient, fecal score. This indicate the potential for phytase supplementation to enhance growth performance without posing adverse impacts on the overall health and digestion of the animals. However, we suggest that higher dose of phytase (more than 250 FTU) in growing pig diet for longer experimental period could show more noticeable outcome. Moreover, additional investigations are necessary to determine the optimal levels and effects of phytase supplementation for enhancing the growth and nutrient utilization in growing pigs.

## Conflict of Interests

No potential conflict of interest relevant to this article was reported.

## Authors Information

Shanshui Gao, <https://orcid.org/0000-0003-1491-2652>

Md Mortuza Hossain, <https://orcid.org/0000-0002-6732-286X>

In Ho Kim, <https://orcid.org/0000-0001-6652-2504>

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