

Dose of phytase from either *Aspergillus niger* or *Escherichia coli* on performance of nursery piglets

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Abstract

Supplementing swine diets with phytase increases phosphorus release by approximately 50% from cereal phytates. The increase in phosphorus availability allows for a reduction in dietary phosphorus supplementation from mineral sources and decreases the environmental impact of pork production through a decrease in phosphorus excretion. Superdosing phytase has been reported to boost swine productivity, improve the digestibility of other nutrients, and mitigate the antinutritional effects of phytates. However, there are significant cost differences among phytase products. Bacterial phytases are considered more modern, often with a higher cost of inclusion. A study was conducted with 288 piglets that were 21 d of age and weighed 6.43 \pm 0.956 kg. Pigs were divided into four groups. Each group of pigs was fed a different experimental diet varying in phytase source and level: fungal phytase (*Aspergillus niger*) at 500 FTU/kg of diet, fungal phytase at 2,000 FTU/kg, bacterial phytase (*Escherichia coli*) at 500 FTU/kg, and bacterial phytase at 2,000 FTU/kg. No differences were found for phytase sources or doses on productivity at 14 and 21 d postweaning. However, piglets supplemented with 2,000 FTU/kg of phytase in the diet during the first 21 d of nursery exhibited a 5.8% better feed conversion (P = 0.02). An interaction between phytase source and dose was observed for average live weight and daily weight gain over the 42-d nursery period (P < 0.05). Supplementing the diet with 2,000 FTU/kg of fungal phytase improved daily weight gain and live weight throughout the experimental period compared to piglets supplemented with 500 FTU/kg of the same phytase source. Additionally, it resulted in better final weights compared to piglets supplemented with 500 FTU/kg of bacterial phytase. Phytase inclusion at 2,000 FTU/kg improved feed conversion ratios were observed when supplementing the diet with fungal phytase at 2,000 FTU/kg.

Lay Summary

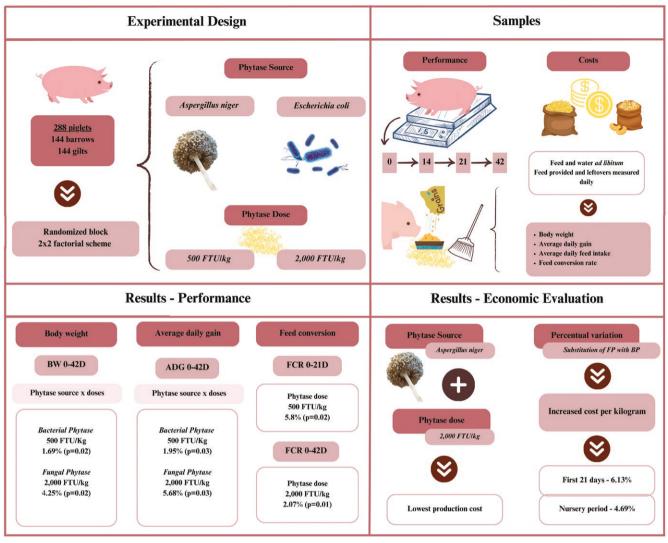
Our study delved into the effects of different phytase sources and doses on the growth and development of weaned piglets. The performance of piglets supplemented with either fungal phytase (from *Aspergillus niger*) or bacterial phytase (from *Escherichia coli*) at doses of 500 and 2,000 FTU/kg of diet was evaluated. Performance among the groups during the first 21 d was similar (P > 0.05). However, piglets receiving 2,000 FTUs/kg of phytase exhibited improved feed conversion rates during the first 21 d of the nursery phase. When analyzing the entire trial period (0-42 d), piglets supplemented with 2,000 FTUs/kg of fungal phytase demonstrated better growth and feed conversion compared to those receiving bacterial phytase. From an economic standpoint, the use of 2,000 FTUs/kg of fungal phytase emerged as the most cost-effective option. Our findings underscore the importance of considering both the source and dose of phytase when formulating piglet diets to optimize growth performance and minimize production costs.

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Graphical Abstract



Key words: bacterial, fungal, phytase, phosphorus, pig, swine

Introduction

Phosphorus, an essential mineral, is sourced from various organic and inorganic compounds. A significant portion of phosphorus in nonruminant diets exists as phytic phosphorus. Phosphorus in the phytic acid form can constitute 3% to 5% of the dry matter in cereals (Azeem et al., 2015; Bilal et al., 2019). The availability of phytic phosphorus in nonruminants can be improved through the addition of phytase enzymes (Dersjant-li et al., 2015; Valente Jr. et al., 2024).

Phytase acts on the phytate structure within grains, liberating phytic acid-bound phosphorus, thus increasing total phosphorus availability. This action allows for reduced supplementation from traditional sources such as dicalcium phosphate (Silva et al., 2019; Valente Jr. et al., 2024). Moreover, superdosing of phytase has been reported to exhibit an "extra-phosphoric" effect, enhancing the digestibility of other nutrients trapped in phytate complexes such as calcium, amino acids, and energy (Silva et al., 2019).

Phytases (myo-inositol-hexaphosphate phosphohydrolases) catalyze the hydrolysis of phytate, releasing phosphorus from plant structures. This activity is typically limited to the pigs' gastrointestinal tract when diets lack supplemental phytase (Rosenfelder-Kuon et al., 2019). Some fungi and bacteria genera produce phytase. The most common phytase has been derived from fungal sources, typically (EC 3.13.8), *Aspergillus* sp. (Tous et al., 2021). Recent studies also show the promise of bacterial phytase produced by *Escherichia coli* strains (Moita and Kim, 2023).

Over the years, positive effects have been observed with the intake of exogenous phytase at various levels from Aspergillus niger, E. coli, Peniophora, Citrobacter, or Buttiauxella spp., respectively. Phytase has been studied extensively and shown to improve performance, nutrient digestibility (ileal and fecal), and bone mineralization in piglets and growing-finishing pigs in a dose-responsive manner (Kühn et al., 2016; Torrallardona; Ader, 2016). The use of phytase not only promises productivity gains and sustainability but also enhances the profitability of the nursery phase. This is achieved by reducing the need for phosphate as a dietary source of phosphorus, which decreases the buffering effect of phosphate in the diet (Adeola and Cowieson, 2011). Additionally, phytase improves nutrient digestibility, making the digestive process easier for newly weaned piglets (Cuyper et al., 2020). Furthermore, the addition of phytase allows for

a reduction in the inclusion of inorganic phosphorus sources in diets (Grela et al., 2020).

The efficacy of phosphorus release by phytase depends on the inclusion level and specific product characteristics pathway of phytate phosphorus release. The usual dose of phytase, 500 FTU/kg, is commonly used in piglet diets and typically results in a 50% increase in the available phosphorus in the formulated diet (Gaffield et al., 2023). When higher doses, such as 2,000 to 2,500 FTU/kg, known as "superdosing" (Silva et al., 2019), are used, the primary benefits are not usually related to increasing phosphorus availability. Instead, the additional advantages of these higher doses are generally seen in improvements in piglet performance rather than the availability of phosphorus (Laird et al., 2018; Silva et al., 2019).

It is important to note that different phytase sources and doses may have varying potentials to enhance piglet performance and reduce production costs during the nursery phase. Therefore, the aim of this study was to evaluate the effects of two phytase sources at two inclusion levels in the diet on piglet performance and production costs in the nursery phase.

Material and Methods

The experimental procedures were conducted according to the directives of the Ethics Committee on the Animal Use (CEUA) from the Technology and Innovation Nucleus of Agroceres Multimix—Brazil, under protocol number 5087300720.

Animals and Experimental Design

The study was conducted on an experimental farm located in Patrocínio, Minas Gerais, Brazil. A total of 288 piglets weaned at 21 d with an average live weight of 6.43 ± 0.956 kg was used. The piglets included 144 barrows and 144 gilts of the commercial F2 lineage (Camborough X AGPIC 337, Agroceres PIC).

Four experimental treatments were tested:

- FP500: Fungal phytase at an inclusion level of 500 FTUs/ kg of feed.
- FP2,000: Fungal phytase at an inclusion level of 2,000 FTUs/kg of feed.
- BP500: Bacterial phytase at an inclusion level of 500 FTUs/kg of feed.
- BP2,000: Bacterial phytase at an inclusion level of 2,000 FTUs/kg of feed.

The fungal and bacterial phytases were derived from strains of the fungi *A. niger* and the bacteria *E. coli*, respectively.

The experimental design was a randomized block with a 2×2 factorial scheme (phytase source and dose), with the blocking factor being the initial weight (light, medium, and heavy). The piglets were distributed into 36 pens (experimental unit), with eight piglets in each pen (four barrows and four gilts), and the four dietary treatments were allocated across the pens, with nine replicates per treatment.

Each pen had an area of 2.6 m² with a fully slated floor, nipple drinkers, and linear feeders providing 19 cm of feeder space per piglet. Room temperature and humidity were manually controlled as needed to ensure the thermal comfort of the piglets throughout the experiment. The piglets had ad libitum access to feed and water for the 42-d duration of the trial.

Experimental Diets

The feeding protocol was designed to align with the experimental phase, following the phytase plan with nutritional adjustments based on the Nutrient Requirements of Swine from the National Research Council (NRC, 2012). The feeding plan consisted of three phases with decreasing lactose inclusion.

The feeding phases included prestarter 1 (0-14 d), prestarter 2 (14-21 d), and starter (21-42 d). In all phases and diets, ingredients derived from dairy products, corn, and soybean meal were used according to the NRC (2012) recommendation for the phase of production. The proximate composition and nutritional levels of the diets are presented in Tables 1 and 2, respectively.

Analyzed Variables

Performance.

Piglets were weighed individually at the beginning of the trial and on days 14, 21, and 42, corresponding to the end of the prestarter 1, prestarter 2, and starter phases, respectively. Average body weight (BW) and average daily weight gain (ADG) were calculated for each experimental unit using live pig weight. To obtain the average daily feed intake (ADFI) per experimental unit, feed leftover was subtracted from the total feed provided during the period and divided by the days on

 Table 1. Ingredients and proximate composition of the experimental diets provided to the nursery piglets during the prestarter 1 and prestarter 2 periods

Centesimal composition	Prestarter 1	Prestarter 2	Starter	
Corn	30.500	45.000	56.500	
Soybean meal	14.500	30.000	34.500	
Soybean oil	3.000	3.000	3.000	
Prestarter 1 premix ¹	50.000	0.000	0.000	
Prestarter 2 premix ²	0.000	20.000	0.000	
Starter premix [‡]	0.000	0.000	4.000	
Phytase	2.000	2.000	2.000	
Total, %	100.000	100.000	100.000	

¹Premixes composition: Cookie bran, degummed sovbean oil, textured soy protein, fish meal, dehydrated whey permeate, bovine plasma powder, partially demineralized whey, calcitic limestone, kaolin, sodium chloride (common salt), folic acid, fumaric acid, glutamic acid, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), biotin, choline chloride, silicon dioxide, DL-methionine, ethoxyquin, yeast extract, glutamine. calcium iodate, autolyzed sugarcane yeast, inactivated yeast, monosodium glutamate, L-lysine, L-threonine, L-tryptophan, L-valine, manganese monoxide, nicotinamide, zinc oxide, calcium pantothenate, sodium selenite, copper sulfate, ferrous sulfate, zinc sulfate, vitamin A, vitamin B12, vitamin B2, vitamin B6, vitamin D3, vitamin E, and vitamin K3. ²Soybean meal, ground whole corn, degummed soybean oil, textured soy protein, dehydrated whey permeate, calcitic limestone, kaolin, sodium chloride (common salt), dicalcium phosphate, folic acid, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), Biotin, choline chloride, silicon dioxide, DL-methionine, ethoxyquin, calcium iodate, autolyzed sugarcane yeast, L-lysine, L-threonine, L-tryptophan, L-valine, manganese monoxide, niacin (nicotinic acid), zinc oxide, calcium pantothenate, sodium selenite, copper sulfate, ferrous sulfate, zinc sulfate, vitamin A, vitamin B12, vitamin B2, vitamin B6, vitamin D3, vitamin E, and vitamin K3. [‡]Calcitic limestone, kaolin, sodium chloride (common salt), dicalcium phosphate, citric acid, folic acid, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), biotin, choline chloride, silicon dioxide, DLmethionine, ethoxyquin, calcium iodate, L-lysine, L-threonine, L-tryptophan, L-valine, niacin, manganese oxide, zinc oxide, calcium pantothenate, sodium selenite, copper sulfate, ferrous sulfate, vitamin A, vitamin B12, vitamin B2, vitamin B6, vitamin D3, vitamin E, and vitamin K3.

Table 2. Nutritional composition of the experimental diets provided to the nursery piglets during the prestarter 1, prestarter 2, and starter phases

Nutritional composition	Prestarter 1		Prestarter 2		Starter	
Metabolizable energy, kcal/kg	3496.250		3399.000		3390.000	
Crude protein, %	20.910		20.400		21.140	
Fat, %	7.660		6.330		5.940	
Lactose, %	20.000		8.000		0.000	
Crude fiber, %	1.500		2.370		2.660	
Digestible lysine, %	1.400		1.380		1.350	
Digestible methionine, %	0.440		0.490		0.460	
Digestible methionine + cystine, %	0.740		0.760		0.750	
Digestible threonine, %	0.890		0.930		0.910	
Digestible tryptophan, %	0.250		0.260		0.250	
Digestible valine, %	0.930		1.010		0.970	
Total calcium, %	0.820		0.910		0.770	
Total phosphorus, %	0.590		0.600		0.530	
Available phosphorus, %	0.550		0.490		0.400	
Sodium, %	0.370		0.300		0.210	
Iron, mg/kg	120.000		110.000		110.000	
Manganese, mg/kg	40.000		40.000		40.000	
Zinc, mg/kg	3000.000		2000.000		80.000	
Copper, mg/kg	25.000		75.000		75.000	
Iodine, mg/kg	0.300		0.220		0.230	
Selenium, mg/kg	0.300		0.220		0.230	
Vitamin A, IU/kg	8.760		6.600		6.620	
Vitamin D3, IU/kg	2.190		1.650		1.650	
Vitamin E, mg/kg	65.670		49.490		49.640	
Vitamin K, mg/kg	4.380		3.300		3.310	
Riboflavin, mg/kg	8.210					
Pyridoxine, mg/kg	2.190		1.650		1.650	
Vitamin B12, µg/kg	38.330		28.880		28.970	
Niacin, mg/kg	82.090		61.860		62.050	
Pantothenic acid, mg/kg	31.490		27.800		27.800	
Folic acid, mg/kg	1.890		1.720		1.720	
Biotin, mg/kg	0.280		0.270		0.270	
Choline, g/kg	0.600		0.480		0.400	
Phytase, FTU/kg Bacteri Fungal		2,000	500	2,000	500	2,000

trial. Feed conversion rate (FCR) was calculated as the ratio between ADFI and ADG.

Production cost analysis.

Cost per kilogram for prestarter 1, prestarter 2, and starter diets during respective time intervals (14, 7, and 21 d) was calculated using the ADFI and average daily weight gain for each phase using methods described by Alves et al. (2022). Equation (1) is used to calculate the cost per kilogram of piglet produced in each experimental phase:

$$Y_i \left(\frac{\$}{\text{kg}}\text{Piglet}\right) = \frac{X_i(\$/\text{kg}) * Z_i(\text{ADFI})}{W_i(\text{ADG})}$$
(1)

where Y_i is the cost per kilogram of piglet in phase *i*. X_i is the cost per kilogram of the experimental diet ($\frac{1}{kg}$) in phase *i*. Z_i is the average daily feed intake (ADFI) in phase *i*. W_i is the average daily weight gain (ADG) in phase *i*.

Statistical Analysis

The assumptions of normality and homogeneity of variance were tested using the Shapiro–Francia and Breusch–Pagan tests, respectively. The results were analyzed through analysis of variance using the factors "sources of phytase" (fungal or bacterial) and "dose of phytase" (500 or 2,000 FTU/kg), as well their interaction, as main effects (Equation (2)).

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk} \tag{2}$$

where Yijk is the response variable related to the treatments in each repetition. μ is the overall mean. αi is the effect of the source of phytase. βj é is the effect of the dose of phytase. γij is the effect of the interaction between the source and dose of phytase. εijk is the residual error related to observation Yijk.

The responses were considered significantly different when P < 0.05, and the results were presented as mean \pm standard

error (SEM). All statistical analyses were performed using R studio software (R Core Team, 2023).

Results

Performance

The average performance responses of piglets supplemented with different sources and doses of phytase are presented for each phase and over the 42-d nursery period (Table 3). No differences were observed between either phytase sources or doses on performance during the prestarter 1 and 2 phases (0 to 21 d postweaning, P > 0.05). However, piglets supplemented with 2,000 FTU/kg of phytase during the first 21 d of nursery showed a 5.8% better feed conversion (P = 0.02).

An interaction between phytase source and dose was observed for both final BW (42 d) and ADG from 0 to 42 d of the trials (P < 0.05). Piglets supplemented with 2,000 FTUs/kg of fungal phytase achieved a final BW that was 1 kg heavier than those supplemented with 500 FTUs/kg of the same fungal phytase, representing a 4.25% increase. By contrast, piglets receiving 500 FTUs/kg of bacterial phytase had a final BW of 0.4 kg higher than those supplemented with 2,000 FTUs/kg of bacterial phytase, a 1.69% improvement. Furthermore, the ADG was 5.68% greater in piglets supplemented with 2,000 FTUs/kg of fungal phytase compared to the group supplemented with 2,000 FTUs/kg of bacterial phytase. For piglets on 500 FTUs/kg of bacterial phytase, the ADG was 1.95% higher throughout the entire trial period (Table 3, P = 0.03).

Cumulatively, 42-d feed conversion was significantly impacted by phytase dose (P = 0.01). Supplementing the diet with 2,000 FTU/kg improved feed conversion by 2.07% compared to piglets supplemented with 500 FTUs/kg. ADFI did not differ among groups during the 42 d in the nursery, regardless of the dose or source of phytase provided.

Production Cost Analysis

The production costs per kilogram of gain using different sources and doses of phytase are presented in Table 4.

The lowest production cost was observed with a supplementation of 2,000 FTU/kg of fungal phytase, which was used as the reference for calculating the percentage cost variation for other combinations of sources and doses. Substituting fungal phytase with bacterial phytase increased the cost per kilogram of piglet produced by 4.69% over the total nursery period, with an increase of up to 6.13% during the first 21 d. This cost increase is attributed to the higher price of bacterial phytase compared to fungal phytase.

Discussion

This study was initiated to address the lack of scientific evidence regarding the use of different sources and doses of phytase and their effectiveness on the performance of postweaning piglets. The similar performance responses observed for the two different phytase sources and doses during the prestarter 1 phase can be attributed to the diet's complexity. Ingredients such as corn and soybean meal, which are rich in phytates, comprise less than 50% of the diet leading to a low phytate concentration. The remaining ingredients are of animal origin and are thus higher in phosphorus availability. Animal proteins are known for their greater phosphorus digestibility and lower potential to induce irritative or inflammatory processes in the intestinal mucosa of piglets introduced to solid diets postweaning (Zhai et al., 2022).

Consistent with the findings from this study, Gourley et al. (2018) observed no difference in piglet performance during the first 3 wk postweaning when applying superdose phytase (2,000, 3,000, or 4,000 FTUs/kg) compared to conventional doses (500 and 1,000 FTUs/kg) or a diet without phytase. Similarly, Moran et al. (2019) reported no effect of phytase superdosing (2,500 FTUs/kg) on piglet performance during the first 10 d postweaning.

The literature supports our results, indicating no immediate effect of phytase dose on postweaning piglet performance. However, when postweaning diets include higher proportions of plant-based ingredients, the phytate concentration in the digesta increases, making phytase supplementation important for phytate degradation (Melo et al., 2020). Phytate is considered an antinutritional factor as it is a complex structure that has the potential to sequester various nutrients, such as minerals and amino acids (Walk and Rama Rao, 2020; Valente Jr. et al., 2024).

Lee et al. (2021) reported supplementing piglet diets with high doses of phytase, 2,000 FTU/kg of feed produced by *E. coli*, reduces intestinal pH and increases plasma myo-inositol, compared to diets supplemented with traditional phytase dose, of 500 FTU, due to the greater degradation of phytates in the intestinal lumen, which correlates with better piglet performance, since myo-inositol is considered a conditionally important vitamin for high-performance animals, such as postweaning piglets. Rapid regulation of the gastric environment in young piglets can benefit pepsin activity, reduce the portion of chyme reaching the hindgut for fermentation, and act as a barrier for pathogenic bacteria (Lee et al., 2021).

Supplementation with 2,000 FTUs/kg of phytase improved feed conversion response during the prestarter 2 and starter phases, regardless of phytase source. These findings indicate that piglets benefit from the extra-phosphoric effects of phytase superdosing. Adeola and Cowieson (2011) described performance improvements in pigs fed with phytase superdosing not solely explained by increased phytic phosphorus availability but also by other nutrients sequestered in phytate complexes. Increased phytate degradation removes antinutritional effects, improves the digestibility of energy, amino acids, and minerals, and increases the expression of occlusion cells in the initial and middle portions of the small intestine (Lu et al., 2020; Lee et al., 2021).

At the end of the experimental period, the increase in body weight (BW 42 d) was primarily attributed to the superdosing of fungal phytase (2,000 FTUs/kg), resulting in a notable improvement in BW. However, the improvement in average cumulative daily gain (0-42 d) was more pronounced with 500 FTU/kg of bacterial phytase. This positive response to bacterial phytase at the traditional dose of 500 FTU/kg may be due to differences in nutrient release patterns by different phytate sources, with bacterial phytases having a wider pH activity range in the gastrointestinal tract and being more resistant to proteolytic degradation by pepsin, trypsin, and pancreatin compared to fungal phytases (Dersjant-Li et al., 2018; Moita and Kim, 2023). Our findings align with these characteristics, showing greater effectiveness of phytase produced by E. coli at traditional inclusion doses for weight gain, although this is not observed with the superdosing of fungal phytase.

Table 3. Cumulative average performance values for piglets supplemented with different phytase sources and doses in the nursery phase

Variables	Source Dose, FTU/kg		g	Source	SEM	P value		
		500	2,000			Source	Dose	S × E
Prestarter 1 phase (0 to 14 d of tr	ial)							
Initial body weight, kg	Fungal	6.428	6.428		0.160			
	Bacterial	6.426	6.426					
	Dose							
BW 14 d, kg	Fungal	8.601	8.651	8.626	0.180	0.550	0.980	0.63
	Bacterial	8.590	8.535	8.563				
	Dose	8.595	8.593					
ADG, kg	Fungal	0.155	0.163	0.159	0.004	0.380	0.780	0.43
	Bacterial	0.155	0.151	0.153				
	Dose	0.155	0.157					
ADFI, kg	Fungal	0.251	0.254	0.2525	0.004	0.180	0.720	0.570
	Bacterial	0.246	0.239	0.2425				
	Dose	0.249	0.247					
FCR	Fungal	1.630	1.540	1.585	0.030	0.770	0.440	0.490
	Bacterial	1.610	1.600	1.605				
	Dose	1.620	1.570					
Prestarter 1 and 2 phases (0 to 21	d of trial)							
BW (21 d), kg	Fungal	10.580	11.040	10.810	0.210	0.980	0.140	0.110
	Bacterial	10.820	10.800	10.810				
	Dose	10.700	10.920					
ADG, kg	Fungal	0.193	0.220	0.2065	0.004	0.840	0.060	0.090
	Bacterial	0.207	0.208	0.2075				
	Dose	0.200	0.214					
ADFI, kg	Fungal	0.322	0.333	0.328	0.005	0.810	0.520	0.390
	Bacterial	0.326	0.325	0.326				
	Dose	0.324	0.329					
FCR	Fungal	1.680	1.520	1.600	0.020	0.590	0.020	0.070
	Bacterial	1.590	1.570	1.580				
	Dose	1.635A	1.545B					
Prestarter 1, prestarter 2, and star	ter phase (0 to	42 d of trial)						
Final body weight (42 d), kg	Fungal	23.540 Ab	24.540 Aa	24.040	0.380	0.610	0.310	0.020
	Bacterial	24.090 Aa	23.690 Ba	23.890				
	Dose	23.815	24.115					
ADG, kg	Fungal	0.405 Bb	0.428 Aa	0.417	0.006	0.830	0.280	0.030
	Bacterial	0.419 Aa	0.411 Ab	0.415				
	Dose	0.412	0.420					
ADFI, kg	Fungal	0.61	0.617	0.614	0.008	0.690	0.820	0.320
	Bacterial	0.621	0.603	0.612				
	Dose	0.616	0.610					
FCR	Fungal	1.510	1.440	1.475	0.010	0.910	0.010	0.06
	Bacterial	1.440	1.470	1.455				
	Dose	1.475 A	1.455 B					

BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion rate; d, day of trial; SEM, standard error of the mean; $S \times D$, interaction between phytase source and dose. Means denoted by a different letter indicate significant differences between treatments (P < 0.05).

Incorporating these findings into nursery piglet diet formulations, the economic benefit of different phytase sources becomes a crucial factor. The 2,000 FTU/kg dose of fungal phytase emerges as the most cost-effective option, balancing both performance improvements and production costs. This insight is important for optimizing diet formulations in terms of both economic and zootechnical aspects, ensuring that the chosen phytate source aligns with the desired outcomes for growth and cost-efficiency of piglet nutrition.

 Table 4. Production cost per kilogram and percentual variation for piglets

 supplemented with different sources and doses of phytase

Phase	Fungal	phytase	Bacterial phytase		
	500	2,000	500	2,000	
Production cost per kilogra	.m, \$/kg				
Prestarter 1 (0-14 d)	1.44	1.38	1.41	1.42	
Prestarter 2 (14-21 d)	0.84	0.77	0.79	0.81	
Starter (21-42 d)	0.53	0.51	0.54	0.54	
Entire trial (0-42 d)	0.68	0.65	0.68	0.69	
Percentual variation, %*					
Prestarter 1 (0-14 d)	3.89	0	2.12	2.35	
Prestarter 2 (14-21 d)	8.95	0	2.77	4.77	
Starter (21-42 d)	3.01	0	4.69	6.13	
Entire trial (0-42 d)	3.86	0	3.34	4.69	

*Percentual variation, %—the ratio between the lowest cost per kilogram in each nursery phase and the costs per kilogram of other treatments in the same nursery phase. A value other than zero indicates an increase in cost for the different treatments.

Conclusions

Supplementing postweaning piglet diets with phytases from *A. niger* or *E. coli* at doses of 500 or 2,000 FTUs/kg results in similar performance outcomes during the immediate postweaning phase. However, piglets receiving 2,000 FTUs/kg of fungal phytase exhibit notable FCRs, at 42 d of age and enhanced average daily gain by 63 d of age, indicating the benefits of using a higher dose of fungal phytase. The economic analysis supports this observation, demonstrating that 2,000 FTUs/kg of fungal phytase is the most cost-effective strategy, optimizing production costs per kilogram of piglet produced. This suggests that while both fungal and bacterial phytases are effective, the higher dose of fungal phytase offers the best balance of economic return and performance benefits in nursery piglet's nutrition.

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Conflict of interest statement

None declared.

Literature Cited

- Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218. doi: 10.2527/jas.2010-3715
- Alves, L. K. S., A. H. Gameiro, A. P. Schinckel, and C. A. P. Garbossa. 2022. Development of a swine production cost calculation model. Animals (Basel). 12:2229. doi: 10.3390/ani12172229
- Azeem, M., A. Riaz, A. N. Chaudhary, R. Hayat, Q. Hussain, M. I. Tahir, and M. Imran. 2015. Microbial phytase activity and their

role in organic P mineralization. Arch. Agron. Soil Sci. 61:751–766. doi: 10.1080/03650340.2014.963796

- Bilal, H. M., T. Aziz, M. A. Maqsood, and M. Farooq. 2019. Grain phosphorus and phytate contents of wheat genotypes released during last 6 decades and categorization of selected genotypes for phosphorus use efficiency. Arch. Agron. Soil Sci. 65:727–740. doi: 10.1080/03650340.2018.1521957
- Cuyper, C., L. Nollet, M. Aluwe, J. De Boever, K. Douidah, E. Vanderbeke, N. Outchkourov, S. Petkov, and S. Millet. 2020. Effect of supplementing phytase on piglet performance, nutrient digestibility and bone mineralisation. J. Appl. Anim. Nutr. 8:3–10. doi: 10.3920/JAAN2019.0003
- Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95:878–896. doi: 10.1002/jsfa.6998
- Dersjant-Li, Y., C. Evans, and A. Kumar. 2018. Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean mealbased diets with reduced nutrient density. Animal Feed Science and Technology 242:95–110. doi: 10.1016/j.anifeedsci.2018.05.013
- Gaffield, K. N., H. R. Williams, L. L. Becker, J. M. DeRouchey, J. C. Woodworth, M. Tokach, R. D. Goodband, J. T. Gebhardt, and J. M. Faser. 2023. Determining the phosphorus release curve for Smizyme TS G5 2,500 phytase from 500 to 2,500 FTU/kg in nursery pig diets. Transl. Anim. Sci. 7:txad090. doi: 10.1093/tas/txad090
- Gourley, K. M., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, and R. D. Goodband. 2018. Effect of high doses of Natuphos E 5,000 G phytase on growth performance of nursery pigs. J. Anim. Sci. 96:570–578. doi:10.1093/jas/sky001
- Grela, E. R., S. Muszyński, A. Czech, J. Donaldson, P. Stanisławski, M. Kapica, O. Brezvyn, V. Muzyka, I. Kotsyumbas, and E. Tomaszewska. 2020. Influence of phytase supplementation at increasing doses from 0 to 1500 FTU/kg on growth performance, nutrient digestibility, and bone status in grower–finisher pigs fed phosphorus-deficient diets. Animals (Basel). 10:847. doi: 10.3390/ani10050847
- Kühn, I., M. Schollenberger, and K. Manner. 2016. Effect of dietary phytase level on intestinal phytate degradation and bone mineralization in growing pigs. J. Anim. Sci. 94:264–267. doi: 10.2527/jas.2015-9771
- Laird, S., I. Kühn, and H. M. Miller. 2018. Super-dosing phytase improves the growth performance of weaner pigs fed a low iron diet. Animal Feed Science and Technology 242:150–160. doi: 10.1016/j.anifeedsci.2018.06.004
- Lee, S. A., E. Febery, P. Wilcock, and M. R. Bedford. 2021. Application of creep feed and phytase super-dosing as tools to support digestive adaptation and feed efficiency in piglets at weaning. Animals (Basel). 11:2080. doi: 10.3390/ani11072080
- Lu, H., S. Shin, I. Kuehn, M. Bedford, M. Rodehutscord, O. Adeola, and K. M. Ajuwon. 2020. Effect of phytase on nutrient digestibility and expression of intestinal tight junction and nutrient transporter genes in pigs. J. Anim. Sci. 98:skaa206. doi: 10.1093/jas/skaa206
- Melo, A. D. B., A. C. F. de Oliveira, P. da Silva, J. B. Santos, R. de Morais, G. R. de Oliveira, B. Wernick, P. L. Carvalho, S. M. B. Artoni, and L. B. Costa. 2020. 6-phytase and/or endo-β-xylanase and -glucanase reduce weaner piglet's diarrhea and improve bone parameters. Livestock Sci. 238:104034. doi: 10.1016/j.livsci.2020.104034
- Moita, V. H. C., and S. W. Kim. 2023. Efficacy of a bacterial 6-phytase supplemented beyond traditional dose levels on jejunal mucosaassociated microbiota, ileal nutrient digestibility, bone parameters, and intestinal health, and growth performance of nursery pigs. J. Anim. Sci. 101:skad134. doi: 10.1093/jas/skad134
- Moran, K., P. Wilcock, A. Elsbernd, C. Zier-Rush, R. D. Boyd, and E. van Heugten. 2019. Effects of super-dosing phytase and inositol on growth performance and blood metabolites of weaned pigs housed under commercial conditions. J. Anim. Sci. 97:3007–3015. doi: 10.1093/jas/skz156
- National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press.

- R Core Team. 2023. *R: A language and environment for statistical computing* (Version 4.4.0). R Foundation for Statistical Computing. https://www.r-project.org/
- Rosenfelder-Kuon, P., W. Sieger, and M. Rodehutscord. 2019. Effect of microbial phytase supplementation on P digestibility in pigs: a meta-analysis. Arch. Anim. Nutr. 74:1–18. doi: 10.1080/1745039X.2019.1687249
- Silva, C. A., M. A. Callegari, C. P. Dias, A. M. Bridi, C. R. Pierozan, L. Foppa, and R. Hermes. 2019. Increasing doses of phytase from Citrobacter braakii in diets with reduced inorganic phosphorus and calcium improve growth performance and lean meat of growing and finishing pigs. PLoS One 14:e0217490. doi: 10.1371/journal.pone.0217490
- Torrallardona, D., and P. Ader. 2016. Effects of a novel 6-phytase (EC 3.1. 3.26) on performance, phosphorus and calcium digestibility, and bone mineralization in weaned piglets. J. Anim. Sci. 94:194– 197. doi: 10.2527/jas.2015-9746
- Tous, N., J. Tarradas, M. Francesch, M. Font-i-Furnols, P. Ader, and D. Torrallardona. 2021. Effects of exogenous 6-phytase (Ec 3.1. 3.26) supplementation on performance, calcium and phosphorous digestibility, and bone mineralisation and density in weaned piglets. Animals (Basel). 11:1787. doi: 10.3390/ani11061787
- Valente Junior, D. T., J. L. Genova, S. W. Kim, A. Saraiva, and G. C. Rocha. 2024. Carbohydrases and phytase in poultry and pig nutrition: a review beyond the nutrients and energy matrix. Animals (Basel). 14:226. doi: 10.3390/ani14020226
- Walk, C. L., and S. V. Rama Rao. 2020. Increasing dietary phytate has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations in broilers. Poult. Sci. 99:290–300. doi: 10.3382/ps/pez489
- Zhai, H., O. Adeola, and J. Liu. 2022. Phosphorus nutrition in growing pigs. Anim. Nutr. 9:127–137. doi: 10.1016/j.aninu.2022.01.010