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Original Research Article

Effect of phytase on phosphorous balance in 20-kg barrows fed low or adequate phosphorous diets

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ABSTRACT

The effects of phytase on phosphorus (P) digestibility are well established. However, there are few studies that report P balance, particularly when phytase is used in diets that have adequate or deficient P. The main objective of the study was to determine the effect of dietary P levels and exogenous phytase on P balance in growing pigs. The first part of the experiment was a 14-d metabolism study conducted with 80 barrows (initial body weight 18.5 ± 0.5 kg) with a 2 × 5 factorial arrangement of treatments and main effects of available P (0.13% available P, low P [Low-P] diet; 0.35% available P, adequate P [Adeq-P] diet) and phytase (0, 250, 500, 2,500, and 12,500 U/kg). A portion of the pigs ($n = 24$) fed the Low-P diet, with 0, 500, 2,500, 12,500 U/kg phytase, and those fed the Adeq-P diet, with 0 and 12,500 U/kg phytase, remained on test diets for another 4 d, and tissues were collected for determination of bone characteristics and tissue P concentration. There was a P × phytase interaction for P retention that was accounted for by a lack of response to phytase in pigs fed the Adeq-P diet. Retention of P was greater with incremental levels of phytase in pigs fed Low-P diets as compared to those fed Adeq-P diets (P level × phytase, $P < 0.01$), but calcium (Ca) retention was greater in pigs fed Adeq-P diets (P level × phytase $P < 0.01$). Apparent total tract digestibility (ATTD) of P was improved by phytase ($P < 0.001$) and was greater in pigs fed Adeq-P diets as compared to those fed Low-P diets ($P = 0.006$). Metatarsal bone ash (quadratic, $P = 0.01$) and strength (linear, $P = 0.03$) was increased by phytase addition to the Low-P diets. There were no phytase or dietary P effects on P concentrations of the heart, kidney, liver, muscle, and spleen. These results suggest that as compared to the effects in an Adeq-P diet, adding phytase to a Low-P diet was more effective at reducing the P and Ca excretion and restoring average daily gain (ADG). The P released by phytase is absorbed and contributes to improved bone growth, greater rates of tissue accretion, and increased body weight, but does not change tissue P concentrations. There is, however, a threshold for P retention, beyond which it is excreted in the urine.

1. Introduction

The benefits of exogenous phytases in swine and poultry diets have been well established (Greiner and Konietzny, 2011; Lei et al., 2013; Humer et al., 2015). Studies suggest that *Escherichia coli*-derived phytases have a greater benefit on nutrient digestibility and reduction of phosphorus (P) excretion than fungal phytase in broiler chickens (Silversides et al., 2004) and in pigs (Augspurger et al., 2003). While it is clear that phytase improves P digestibility, the effects on P retention are less well established, particularly in cases where P absorption is above the requirement. A greater understanding of P utilization and balance with phytase supplementation in the diet can maximize the phytase benefits of

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growth performance and the reduction of environmental contamination. The objective of this work was to determine the benefit of *E. coli* phytase supplementation in young pigs and to determine P retention in pigs fed diets that were adequate (Adeq-P, 0.35% available P) or deficient (Low-P, 0.13% available P) in P and supplemented with phytase.

2. Materials and methods

2.1. Ethics statement

The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

2.2. Animals

A total of 80 barrows (7 wk of age, average weight 18.5 ± 0.5 kg, C42 maternal × 280 sire line, Pig Improvement Company, Hendersonville, TN, USA) were randomly selected from the Animal & Dairy Science Department Swine Unit at the University of Georgia. Pigs used for this research were weaned at approximately 21 d of age and fed a common diet for 4 wk before the experiment started. The study was conducted in 4 replicate trials of 20 pigs per trial. Pigs were housed in an environmentally regulated room. Room temperature was set at 23 °C, and a 12 h light/dark (07:00/19:00) cycle was set.

2.3. Digestibility phase

The digestibility phase of the study was conducted in a 2 × 5 factorial arrangement of treatments with main effects of available P (Low-P, 0.13% available P vs. Adeq-P, 0.35% available P) and phytase (0, 250, 500, 2,500, and 12,500 U/kg phytase; Quantum phytase, *E. coli* phytase modified for thermo-tolerance 2,500 FTU/g). There were 8 pigs per treatment and 80 total pigs. A corn-soybean basal diet was used, and each treatment met or exceeded all nutrient requirements from the NRC (National Research Council, 1998, 2012), except for the P content in the Low-P group. The corn-soybean meal based diet contained 1.15% of lysine and 3,400 kcal/kg metabolizable energy in both Low-P and Adeq-P groups. The ratio of Ca to total P was 1.3:1 in Adeq-P and 2:1 in Low-P. The Low-P (0.13% available P) diets contained no inorganic P sources and were supplemented with 0, 250, 500, 2,500, 12,500 U/kg phytase at the expense of corn. The Adeq-P (0.35% available P) diets were formulated with the addition of dicalcium phosphate and supplementation of phytase at the same levels as in the deficient group. Diets contained 0.1% titanium dioxide as a digestibility marker. The diet composition is shown in Table 1.

Pigs were weighed at d 0 and placed into individual stainless-steel metabolism cages (0.71 m × 0.81 m), which were equipped with a water nipple, feeding bowl holder, and plastic-coated expanded metal floors. The pigs were allowed to adapt to the environment for 10 d and were trained to meal feeding twice each day at approximately 08:00 and 16:00 at the rate of 4% to 5% of body weight per day. Feeding time was 45 min. Water was available *ad libitum*. Feed consumption was recorded each meal, and feed refusals and spillage were collected. On d 10, pigs were weighed, and cages were cleaned and set up for the 4-d sequential total collection period.

During the 4-d collection period, the total fecal output was collected twice daily from each pig. Urine was collected twice daily into containers with 25 mL 3 mol/L HCl. The individual pig urine total volume was recorded, and 10% of the total was reserved in a 1-L bottle. Fecal and urine samples were stored at −20 °C until further analysis. The screens and trays were washed after every collection. At the end of the digestibility phase of the trial, 24 (of 80) pigs were transferred to larger pens and fed *ad libitum* for an additional 4 d to determine tissue and bone P as described below.

Table 1
Diet composition (%, as-fed basis)[1].

Item	Low-P (0.13% available P)	Adeq-P (0.35% available P)
Ingredients		
Corn	64.75	64.15
Soybean meal (48% CP)	29.53	29.62
Poultry fat	3.0	3.0
Common salt	0.35	0.35
Limestone	1.73	1.08
Dicalcium phosphate	0.0	1.15
Vitamin premix [2]	0.25	0.25
Mineral premix [3]	0.15	0.15
Lysine-HCl	0.14	0.14
Titanium dioxide [4]	0.10	0.10
Calculated analysis		
ME, kcal/kg	3,420	3,400
CP	20.16	20.15
Lysine	1.15	1.15
SID	1.01	1.01
Ca	0.75	0.75
Total P	0.36	0.58
Available P	0.13	0.35

Adeq-P = adequate P; ME = metabolizable energy; SID = standardized ileal digestible.
[1] Both Low-P diet and Adeq-P diet supplemented with 0, 250, 500, 2,500, and 12,500 U/kg *E. coli* phytase formed 10 treatments.
[2] Supplied per kilogram of premix: vitamin A 4,400 IU; vitamin D 660,000 IU; vitamin E 17,600 IU; vitamin K 1,760 IU; riboflavin 3,960 mg; niacin 22,000 mg; vitamin B_{12} 17,600 μg.
[3] Supplied per kilogram of premix: iron 110,000 mg; copper 11,000 mg; manganese 26,400 mg; zinc 110,000 mg; iodine 198 mg; selenium 198 mg.
[4] A total of 0.10% titanium dioxide was added as a marker for digestibility determination.

2.4. Bone and tissue phosphorous phase

Following the digestibility portion of the experiment, pigs from selected treatments in 2 trials were maintained on the same test diets an additional 4 d. Pigs from the 4 deficient P (0.13% available P) groups (0, 500, 2,500, and 12,500 U/kg phytase) and pigs from 2 Adeq-P (0.35% available P) groups (0 and 12,500 U/kg phytase) were transferred to larger pens (1.8 m × 0.9 m) with 2 pigs of the same treatment, housed together, and fed the same test diets *ad libitum* for an additional 4 d after the digestibility phase was finished. The total number of pigs for this portion was 24 (4 pigs per treatment). Feed consumption and body weight were determined on the last day. However, since there was only one pen per dietary treatment in each replicate, intake data was not analyzed.

Blood samples were drawn from each pig at the end of the feeding period. Pigs were then euthanized by CO_2 inhalation. Both rear legs and 10th ribs were removed and frozen (−20 °C) for later bone isolation and determination of bone ash and bone strength. The internal organs (liver, heart, kidney, spleen, muscle) were collected and stored at −20 °C.

2.5. Sample analysis

Total fecal samples collected from each pig were thawed and then homogenized by blender. The total sample weight was recorded. A sub-sample was freeze-dried (Labconco, Co., Kansas City, MO), and the freeze-dry matter weight was recorded. Diet and

freeze dried fecal samples were ground through a 1-mm screen by a Wiley mill (Thomas Scientific, Swedesboro, NJ) and used for determination of minerals, CP, and gross energy. The sub-samples of urine were thawed and pooled into large measuring container. After mixing, triplicate 100-mL samples were transferred to plastic cups for other analysis. Diet, fecal, and urine CP was analyzed by a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Diet and fecal gross energy were determined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). Titanium concentration in feed and fecal samples was determined using a modification of the procedure of Short et al. (1996) as described previously (Tsai et al., 2017). Diet, and freeze-dried fecal and urine samples were sent to the Agricultural and Environmental Services Laboratories (AESL) at the University of Georgia, and P and Ca content was determined by inductively coupled atomic emission spectroscopy (ICP-AES). Apparent total digestibility (ATTD) was calculated by following equation: ATTD = 1 − [($Marker_{feed}$ × $Nutrient_{feces}$)/($Marker_{feces}$ × $Nutrient_{feed}$)], where marker was the titanium concentration in feed and feces and nutrient was the energy, CP, phosphorus, and calcium in feed and feces.

Two metatarsal and 10th rib bones from each pig were cleaned and weighed. Bones were oven dried at 177 °C for 72 h, and dry weight was recorded for dry matter determination. Bone length and width were measured. One dry metatarsal bone from each pig was used to determine bone strength (Instron Universal Testing Machine, Model 1122 with a 5500R Series system interface), and the other one was ashed at 600 °C for 72 h for ash weight determination by Isotemp Muffle Furnace (Fisher Scientific, Suwanee, GA). Tissue samples were freeze dried. Whole blood and tissue samples were sent to the AESL at the University of Georgia, and P content was determined by inductively coupled atomic emission spectroscopy (ICP-AES).

2.6. Statistical analysis

All data were analyzed using the PROC Mixed of SAS (SAS Inst. Inc., Cary, NC). The 10-treatment combination in the digestibility phase (d 0 to 14) comprised of 2 levels of P (0.13% and 0.35% available P) and 5 levels of phytase (0, 250, 500, 2,500, 12,500 U/kg) and in a 2 × 5 factorial arrangement for growth performance, Ca and P balance, and nutrient digestibility. The pig was considered the experimental unit, and main effects of dietary P and phytase and their interaction, as well as trial, were included in the model. Initial body weight was used as a covariate for analysis of the growth data. Results are presented as least square means for the phytase and P level interaction. A *P*-value of $P < 0.05$ was considered statistically significant.

The results for bone characteristics, tissue and blood P concentration were analyzed by using PROC Mixed of SAS with dietary treatment as main effect, and pigs' initial body weight was used as a covariant. Each pen was used as an experiment unit in ANOVA from d 14 to 18 growth data analysis, whereas each pig was used as experiment unit in ANOVA for bone, tissue, and blood results determination.

3. Results

3.1. Growth performance

Growth performance is shown in Table 2. Higher dietary P ($P < 0.001$) and the addition of phytase ($P = 0.002$) resulted in improved average body weight gain (AWG). There was a P × phytase interaction ($P = 0.03$) that was accounted for by the greater improvement in average daily gain (ADG) in phytase supplemented pigs on the Low-P diet.

Average daily feed intake was greater in pigs fed the Adeq-P diets as compared to those fed the Low-P ($P = 0.013$), but there was no effect of phytase on AWG. Feed efficiency (G:F ratio) was improved in pigs fed the Adeq-P diet ($P < 0.001$) and with the addition of phytase ($P < 0.001$). There was a P × phytase interaction that was accounted for by the greater magnitude improvement in G:F ratio with phytase in pigs fed Low-P ($P = 0.03$).

3.2. Phosphorous balance

Phosphorous intake was higher in the Adeq-P diet group (Table 3, 6.31 vs. 4.50 g/d, $P < 0.001$), but was not affected by phytase. Pigs fed Adeq-P diet had greater fecal (1.65 vs. 1.26 g/d, $P < 0.001$), urinary (0.30 vs. 0.05 g/d, $P < 0.001$), and total P (1.96 vs. 1.31 g/d, $P < 0.001$) excretion and greater total retention (4.36 vs. 3.19 g/d, $P < 0.001$) compared to those fed Low-P. Phytase reduced fecal P loss ($P < 0.001$) and improved P retention ($P = 0.005$). There was a P × phytase interaction ($P < 0.001$) on urinary P excretion that was accounted for by an increased urinary P loss in pigs fed the Adeq-P diets. Total P excretion was reduced with phytase and there was a P × phytase interaction ($P = 0.006$) that was accounted for by the greater effect in the pigs fed Low-P. Overall, there was a P × phytase interaction ($P < 0.001$) on percent P retention that was due to the greater improvement in retention in pigs fed the Low-P diets.

Table 2
Effect of dietary phosphorous (P) level and phytase on growth performance (0 to 14 d).[1]

Item	P level	SEM	Phytase					SEM	*P*-value		
			0	250	500	2,500	12,500		P level	Phytase	Inter[2]
ADG, kg/d											
Low-P	0.47[a]	0.012	0.38[a]	0.45[b]	0.44[ab]	0.54[c]	0.53[c]	0.03	<0.001	0.002	0.03
Adeq-P	0.55[b]		0.53	0.52	0.59	0.56	0.56				
ADFI, kg/d											
Low-P	0.93[a]	0.013	0.91	0.91	0.90	0.97	0.94	0.03	0.013	0.538	0.25
Adeq-P	0.97[b]		0.96	0.95	1.03	0.98	0.95				
G:F ratio											
Low-P	0.50[a]	0.01	0.41[a]	0.49[b]	0.49[b]	0.55[c]	0.55[c]	0.02	<0.001	<0.001	0.03
Adeq-P	0.56[b]		0.54	0.55	0.57	0.56	0.58				

ADG = average daily gain; Low-P = 0.13% available P; Adeq-P = 0.35% available P; AFDI = average daily feed intake.
[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$).
[1] There were a total of 80 pigs, with 4 pigs per treatment. Initial body weight = 18.5 ± 0.5 kg. Results are LS Means for the digestibility phase of the trial with 2 P levels (0.13% and 0.35% available P) and 5 phytase levels (0, 250, 500, 2,500, and 12,500 U/kg).
[2] Phosphorus levels and phytase levels interaction.

Table 3
Effect of dietary phosphorous (P) level and phytase on P balance.[1]

Item	P level	SEM	Phytase, U/kg						*P*-value		
			0	250	500	2,500	12,500	SEM	P level	Phytase	Inter[2]
Intake, g/d											
Low-P	4.50[a]	0.52	4.19	4.66	4.42	4.68	4.47	0.27	<0.001	0.262	0.986
Adeq-P	6.31[b]		6.07	6.61	6.07	6.54	6.27				
Fecal excretion, g/d											
Low-P	1.26[a]	0.098	1.50[b]	1.40[b]	1.54[b]	0.96[a]	0.81[a]	0.14	<0.001	<0.001	0.135
Adeq-P	1.65[b]		2.13[y]	1.66[x]	1.56[x]	1.43[x]	1.48[x]				
Urinary excretion, g/d											
Low-P	0.05[a]	0.034	0.07	0.04	0.05	0.05	0.04	0.05	<0.001	<0.001	<0.001
Adeq-P	0.31[b]		0.09[x]	0.19[y]	0.22[y]	0.37[z]	0.66[w]				
Total excretion[3], g/d											
Low-P	1.31[a]	0.11	1.57[b]	1.45[b]	1.59[b]	1.01[a]	0.85[a]	0.15	<0.001	0.019	0.006
Adeq-P	1.96[b]		2.22[y]	1.85[xy]	1.78[x]	1.80[x]	2.13[xy]				
Retention, g/d											
Low-P	3.19[a]	0.46	2.63[a]	3.21[ab]	2.83[a]	3.67[b]	3.61[b]	0.26	<0.001	0.005	0.312
Adeq-P	4.36[b]		3.85[x]	4.76[y]	4.29[xy]	4.75[y]	4.14[xy]				
Retention, %											
Low-P	70.45	2.27	61.14[a]	68.97[b]	63.42[ab]	78.36[c]	80.82[c]	2.69	0.155	<0.001	<0.001
Adeq-P	68.12		62.63[x]	70.70[yz]	70.18[yz]	72.61[z]	64.48[xy]				

[a,b,c] Within a row, means without a common superscript differ significantly ($P < 0.05$).
[x,y,z] Within a row, means without a common superscript differ significantly ($P < 0.05$).
[1] Results are LS Means for the interaction between 2 P levels (0.13% and 0.35% available P) and 5 phytase levels (0, 250, 500, 2,500, and 12,500 U/kg). There were a total of 80 pigs in the study which was conducted in 4 replicates of 20 pigs each.
[2] Phosphorus and phytase levels interaction.
[3] Total excretion = Fecal excretion + Urine excretion.

3.3. Calcium balance

Calcium intake was not affected by diet (Table 4). Fecal Ca excretion was greater in pigs fed the Adeq-P diets ($P = 0.029$) and there was no effect of phytase. Urinary Ca excretion was greater in pigs fed the Low-P diet (1.58 vs. 0.50 g/d, $P < 0.001$) and was reduced by phytase ($P < 0.001$) in both levels of dietary P. Total Ca excretion was greater in pigs fed the Low-P diets (3.29 vs. 2.46 g/d, $P < 0.001$) and was reduced by phytase in pigs fed both diets ($P < 0.001$). Percent Ca retention was greater in pigs fed the Adeq-P diet (64.76 vs. 55.43%, $P < 0.001$) and retention was improved with phytase ($P < 0.001$) with a greater improvement in pigs fed Adeq-P (P × phytase, $P = 0.009$).

3.4. Nutrient digestibility

Apparent total tract digestibility of GE, CP, P and Ca was not affected by dietary P or phytase (Table 5). Apparent total tract digestibility of P was greater in pigs fed the Adeq-P diet (58.55% vs. 50.56%, $P = 0.006$) and improved with phytase ($P < 0.001$) independent of diet.

Table 4
Effect of dietary phosphorous (P) level and phytase on calcium (Ca) balance.[1]

Item	P level	SEM	Phytase, U/kg						*P*-value		
			0	250	500	2500	12500	SEM	P level	Phytase	Inter[2]
Intake, g/d											
Low-P	7.71	0.84	7.99	7.36	6.64	8.17	8.20	0.57	0.856	0.464	0.468
Adeq-P	7.61		7.05	7.51	7.73	7.82	7.91				
Fecal excretion, g/d											
Low-P	1.71[a]	0.11	1.67	1.88	1.93	1.50	1.47	0.19	0.029	0.275	0.174
Adeq-P	1.96[b]		2.32	1.92	1.80	1.69	2.06				
Urinary excretion, g/d											
Low-P	1.58[b]	0.09	2.06[c]	1.80[c]	1.59[b]	1.38[ab]	1.12[a]	0.14	<0.001	<0.001	0.353
Adeq-P	0.50[a]		1.09[y]	0.41[x]	0.47[x]	0.23[x]	0.29[x]				
Total excretion[3], g/d											
Low-P	3.29[b]	0.17	3.73[c]	3.69[c]	3.53[bc]	2.88[ab]	2.59[a]	0.26	<0.001	<0.001	0.063
Adeq-P	2.46[a]		3.42[y]	2.33[x]	2.27[x]	1.91[x]	2.35[x]				
Retention, g/d											
Low-P	4.42[a]	0.78	4.25[ab]	3.67[a]	3.12[a]	5.29[b]	5.62[b]	0.53	0.024	0.003	0.045
Adeq-P	5.15[b]		3.64[x]	5.18[y]	5.46[y]	5.91[y]	5.56[y]				
Retention, %											
Low-P	55.43[a]	3.97	48.37[a]	49.15[a]	43.04[a]	64.28[b]	68.73[b]	4.18	<0.001	<0.001	0.009
Adeq-P	64.76[b]		49.02[x]	62.12[y]	69.77[yz]	75.28[z]	67.64[yz]				

[a,b,c] Within a row, means without a common superscript differ significantly ($P < 0.05$).
[x,y,z] Within a row, means without a common superscript differ significantly ($P < 0.05$).
[1] Results are LS Means for the interaction between 2 P levels (0.13% and 0.35% available P) and 5 phytase levels (0, 250, 500, 2,500, and 12,500 U/kg). There were a total of 80 pigs in the study which was conducted in 4 replicates of 20 pigs each.
[2] Phosphorus and phytase level interaction.
[3] Total excretion = Fecal excretion + Urine excretion.

Table 5
Effect of dietary phosphorous (P) level and phytase on apparent total tract digestibility (ATTD) in grower pigs (%).[1]

Item	Phytase, U/kg					SEM	*P*-value		
	0	250	500	2,500	12,500		P level	Phytase	Inter[2]
Energy									
Low-P	86.02	84.58	83.79	83.39	85.22	0.87	0.972	0.296	0.249
Adeq-P	83.37	85.11	84.40	84.18	85.83				
CP									
Low-P	84.18	83.44	81.49	82.04	82.90	1.21	0.777	0.453	0.427
Adeq-P	82.08	82.31	83.05	81.02	84.49				
P									
Low-P	37.77^{a}	50.73^{b}	44.20^{ab}	55.76^{bc}	65.79^{c}	4.26	0.006	<0.001	0.264
Adeq-P	43.73^{x}	62.26^{y}	60.67^{y}	62.42^{y}	63.68^{y}				
Ca									
Low-P	59.96^{ab}	57.59^{ab}	53.44^{a}	60.95^{ab}	67.43^{b}	3.73	0.55	0.106	0.015
Adeq-P	46.69^{x}	59.76^{y}	64.51^{y}	62.82^{y}	58.65^{y}				

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).
^{x,y,z} Within a row, means without a common superscript differ significantly ($P < 0.05$).
[1] Results are LS Means for the interaction between 2 P levels (0.13% and 0.35% available P) and 5 phytase levels (0, 250, 500, 2,500, and 12,500 U/kg).
[2] Phosphorus and phytase level interaction.

3.5. Tissue P concentration

Pigs from selected treatments in the balance portion of the study were maintained on test diets an additional 4 d for determination of tissue and bone mineral content. Growth rate of pigs fed the Low-P diet was less than that of those fed the Adeq-P and there was a linear effect of phytase to improve ADG ($P = 0.005$). There was no significant effect of diet on P concentration in the selected soft tissues or blood (Table 6).

3.6. Metatarsal bone and 10th rib bone characteristics

The results of metatarsal and 10th rib bone characteristics are shown in Table 7. Pigs fed the un-supplemented Low-P diet (0.13% available P + 0 U/kg phytase) had the lowest bone weight, bone dry matter (g), and ash (% and g) for both metatarsal bone and 10th rib bone. In contrast, pigs fed 12,500 U/kg phytase in Adeq-P diet had the greatest metatarsal and 10th rib bone characteristics. Phytase supplementation in the Low-P diet resulted in increased ash (g and %) content in metatarsal and bone weight, DM (g), and ash (g and %) in 10th rib bone. Moreover, 2,500 U/kg phytase addition in the low P diet normalized both metatarsal and 10th rib bone weight and bone ash (g) to that seen in pigs fed the Adeq-P diet. Supplementing the Adeq-P diet with phytase (12,500 U/kg phytase) resulted in numerically heavier metatarsal (8.82 vs. 10.2 g) and 10th rib (4.73 vs. 562 g) bone weight and statistically greater metatarsal bone ash (1.92 vs. 2.17 g). Pigs fed the Low-P diet devoid of phytase had the lowest metatarsal bone strength. Supplementation of the low P diet with 2,500 and 12,500 U/kg phytase not only showed 55.7% and 61.6% improvement in metatarsal bone strength when compared to the un-supplemented Low-P diet, but it also restored bone strength to that of the Adeq-P diet.

4. Discussion

The effects of phytase on growth performance, reducing P excretion, and improving bone strength has been widely discussed (Cromwell et al., 1993; da Silva et al., 2019; Jongbloed et al., 1992; Kornegay and Qian, 1996; Lei et al., 1993; Nelson et al., 1968; Simons et al., 1990). The levels of phytase used in the present study represent both the standard levels used in the industry (250 and 500 U/kg; Goncalves et al., 2016) and "super-dosing" levels (2,500 and 12,500 U/kg; Lu et al., 2019; Moran et al., 2019). Historically, inorganic P was used in diets to provide sufficient available P (Harper et al., 1997). In the present work, an Adeq-P diet was made by adding dicalcium phosphate to the Low-P diet. This resulted in improved ADG (21%) and G:F (16%) as compared to that of pigs fed the Low-P diet. However, this addition of inorganic P

Table 6
Effect of dietary phosphorous (P) level and phytase on tissue P concentration in grower pigs.[1]

Item	Diet						SEM	*P*-value [2]				
	1	3	4	5	6	10						
	Low-P (0.13%)				Adeq-P (0.35%)			Treatment	Linear Low-P	Quad. Low-P	6 vs. 10	1 vs. 6
	0 U/kg	500 U/kg	2,500 U/kg	12,500 U/kg	0 U/kg	12,500 U/kg						
ADG, kg/d, 0 to 18 d	0.47^{a}	0.54^{ab}	0.62^{bc}	0.64^{c}	0.67	0.72	0.03	0.002	0.005	0.0195	0.001	0.001
Tissue P, mg/g												
Blood	0.095	0.097	0.109	0.106	0.109	0.119	0.007	0.167	0.255	0.118	0.389	0.190
Heart	0.271	0.258	0.335	0.273	0.283	0.261	0.033	0.538	0.938	0.086	0.657	0.800
Kidney	0.317	0.271	0.327	0.303	0.328	0.332	0.022	0.467	0.920	0.427	0.899	0.735
Liver	0.435	0.461	0.451	0.398	0.442	0.429	0.037	0.907	0.311	0.675	0.824	0.891
Muscle	0.280	0.313	0.305	0.277	0.282	0.277	0.015	0.384	0.204	0.251	0.836	0.919
Spleen	0.362	0.356	0.351	0.372	0.408	0.372	0.031	0.906	0.679	0.738	0.478	0.378

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).
[1] Pigs from treatments 1, 3, 4, 5, 6, and 10 of the balance experiment were maintained on test diets for an additional 4 d. The internal organs (liver, heart, kidney, spleen, muscle) were collected and stored at −20 °C. Tissues were freeze dried before determination of mineral content by inductively coupled atomic emission spectroscopy (ICP-AES).
[2] Treatment effect. Significance level set at $P < 0.05$.

Table 7
Effect of dietary phosphorous (P) level and phytase on bone characteristics in grower pigs.[1]

Item	Diet						SEM	*P*-value[2]				
	1	3	4	5	6	10						
	Low-P (0.13%)				Adeq-P (0.35%)			Treatment	Linear Low-P	Quad. Low-P	6 vs. 10	1 vs. 6
	0 U/kg	500 U/kg	2,500 U/kg	12,500 U/kg	0 U/kg	12,500 U/kg						
Metatarsal bone												
Length, mm	46.43	47.87	49.11	48.83	46.91	48.28	0.70	0.106	0.112	0.037	0.182	0.651
Width, mm	11.57	12.61	11.81	12.11	12.21	12.09	0.40	0.587	0.837	0.862	0.844	0.293
Bone wt, g	8.19	9.02	9.37	8.60	9.10	10.21	0.43	0.084	0.838	0.088	0.081	0.170
DM, g	5.09	5.37	5.48	5.32	5.42	5.84	0.24	0.494	0.847	0.337	0.230	0.373
DM, %	71.47^{b}	70.31^{ab}	68.18^{a}	69.51^{ab}	70.72	65.78	0.96	0.010	0.404	0.033	0.002	0.603
Ash, g	1.42^{a}	1.76^{b}	1.86^{b}	1.72^{b}	1.92	2.18	0.08	<0.001	0.356	0.010	0.043	<0.001
Ash, %	28.19^{a}	32.89^{b}	34.02^{b}	32.16^{b}	35.64	37.40	1.17	0.002	0.398	0.010	0.293	<0.001
Load at peak, kg	44.59^{a}	54.69 ^{ab}	67.44^{b}	69.50^{b}	68.82	60.33	5.77	0.081	0.030	0.042	0.330	0.016
Rib Bone												
Bone wt, g	3.41^{a}	3.73^{ab}	4.59^{b}	4.08^{ab}	4.74	5.64	0.36	0.010	0.418	0.036	0.094	0.025
DM, g	2.81^{a}	3.12^{a}	3.73^{a}	3.33^{a}	3.87	4.63	0.33	0.029	0.537	0.074	0.123	0.047
DM, %	81.82	82.57	81.26	81.82	81.61	81.59	2.01	0.998	0.934	0.766	0.993	0.946
Ash, g	1.27^{a}	1.62^{a}	1.79^{a}	1.62^{a}	1.94	2.39	0.19	0.029	0.565	0.115	0.113	0.032
Ash, %	45.19	50.40	47.98	48.29	50.08	51.66	1.44	0.110	0.723	0.538	0.445	0.036

^{a,b,c} Within a row, means without a common superscript differ significantly ($P < 0.05$).
[1] Pigs from treatments 1, 3, 4, 5, 6, and 10 from the balance experiment were maintained on test diets for an additional 4 d. Metatarsal and rib bones from each pig were cleaned and weighed. Bones were oven dried at 177 °C for 72 h, and dry weight was recorded for dry matter determination. Bone length and width were measured. One dry metatarsal bone from each pig was used to determine bone strength (Instron Universal Testing Machine, Model 1122 with a 5500R Series system interface), and the other one as ashed at 600 °C for 72 h for ash weight determination.
[2] Treatment effect. Significance level set at $P < 0.05$.

resulted in 46% more fecal P excretion than Low-P diets. In contrast, supplementation of the Low-P diet with phytase improved growth performance and bone characteristics to a similar extent as seen with inorganic P, but decreased fecal P excretion.

In addition to the effects of phytase on body weight gain, higher metatarsal bone dry weight, bone ash, and bone strength were also observed in pigs fed phytase, which is in agreement with other reports (Harper et al., 1997; Buhler et al., 2010; Veum et al., 2006). Bone strength was normalized to that of the pigs fed an Adeq-P diet when pigs were fed a low P diet supplemented with 2,500 or 12,500 U/kg phytase, which is in agreement with (Veum et al., 2006). Similar improvements in 10th rib bone's wet weight, dry matter, and ash weight were also noted in response to phytase feeding when compared to no phytase Low-P diet. Harper et al. (1997) found that increased available P, by either adding inorganic P or phytase addition, resulted in linear increases in 10th rib bone ash and strength.

Addition of 12,500 U/kg phytase to the Adeq-P diet resulted in increased bone weight and percent ash for both the metatarsal and 10th rib bones, suggesting that P requirements of the pig are greater than assumed in the NRC (1998, 2012).

A novel finding of the present work was that in contrast to the effects of phytase and dietary P on bone, there was no effect of these factors on the P concentration in any of the soft tissues (heart, kidney, liver, muscle or spleen). Previously, Shelton et al. (2005) examined tissue levels of P in pigs fed diets with and without one level (500 U/kg) of a fungal phytase and showed no changes in tissue P concentrations. However, in that study, there was no effect of phytase on growth performance, or bone parameters. In the present study, phytase improved growth performance and bone characteristics, but did not change soft tissue concentration of P. Thus, the improved retention of P in response to phytase addition to the Low-P diets can be accounted for by accumulation of P in bone and not in soft tissues. Furthermore, the addition of phytase to an Adeq-P diet, while improving P digestibility (and lowering fecal loss of P) did not alter P retention. Overall, the results suggest there is a threshold for P retention. Once bone mineralization is maximized, P supplied over the requirement is excreted in the urine.

Although pigs fed the Adeq-P diet did not respond to phytase as dramatically as pigs fed the Low-P diet, there were nevertheless examples of numerical improvements in growth, feed efficiency, and bone characteristics that suggest an effect of phytase even when pigs are apparently meeting their P requirement. The effects of phytase in Adeq-P diets are similar to those of Olsen et al. (2019) who also reported that phytase resulted in increased urinary P when used in Adeq-P diets.

Most (95.5%) of the P excreted in pigs fed the Low-P (0.13% available P) without phytase was fecal, with minimal loss (4.5%) in the urine and no change in urinary loss with the addition of phytase. In the Low-P diet, most of the P is in phytate form which is largely unavailable to pigs. Phytase addition to the Low-P diet reduced fecal loss of P, but had little or no effect on urinary loss. Similarly, most (95.9%) P excretion was fecal in pigs fed the Adeq-P diet without phytase. Phytase addition to the Adeq-P diet also reduced fecal P loss, but had little effect on total excretion due to the increase in urinary P loss. Urinary P loss increased from approximately 4% of the total P in the control group (no added phytase) to 31% in pigs fed the high phytase diet in the Adeq-P group. Excretion of Ca was more evenly split between fecal and urinary. Total excretion was greater in pigs fed the Low-P diet and more of the excretion (55%) was urinary. In pigs fed the Adeq-P diet, most (67%) of the Ca excretion was fecal. In both diets, the addition of phytase reduced total Ca excretion and most of this effect was due to a reduction of urinary Ca loss. This reduction in urinary Ca loss and therefore greater Ca retention is likely accounted for by the greater bone mineralization due to improved P availability.

5. Conclusion

The benefits of supplemental phytase in swine and poultry diets are clearly established. The results of this study demonstrate that in low available P diets, phytase supplementation increased growth, nutrient digestibility, bone mineralization, and more importantly, decreased total P and Ca excretion. Supplementation of 2,500 U/kg phytase to a low P diet (0.13% available P) can totally replace inorganic P. In Adeq-P diets (0.35% available P), growth performance

was not affected by phytase, but phytase improved bone characteristics. Tissue P concentration was not affected by dietary P or phytase levels. Overall, the effects of phytase on P retention were accounted for by bone mineralization. Furthermore, the results indicate that there is a threshold for P retention and once this is met, urinary excretion of P is increased.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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