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Evaluating the Effects of HiPhorius Phytase Added in Diets at or Below the P Requirement on Nursery Pig Growth Performance and Bone Mineralization[1]

Macie E. Reeb, Jason C. Woodworth, Joel M. DeRouchey, Mike D. Tokach, Robert D. Goodband, Jordan T. Gebhardt,[2] and Jon R. Bergstrom[3]

Summary

A total of 297 pigs (DNA 241 × 600; initially 8.64 ± 0.181 kg) were used in a 21-d trial to determine the effects of HiPhorius phytase on growth performance and bone characteristics. Pens of pigs were assigned to 1 of 5 treatments in a randomized complete block design with 5 pigs per pen and 12 replications per treatment. The first three diets were formulated to contain 0.09% aP; without added phytase (control), or the control diet with 600 or 1,000 FYT/kg of added phytase (considering a release of 0.15 or 0.18% aP, respectively). The remaining two diets were formulated to contain 0.27% aP, one without added phytase and the other with 1,000 FYT/kg. From d 0 to 21, pigs fed diets with increasing phytase containing 0.09% aP had increased (linear $P \leq 0.002$) ADG, ADFI, and better F/G but phytase added to the 0.27% aP diet did not impact growth performance. Increasing phytase in diets containing 0.09% aP increased percentage bone ash in metacarpals and 10th ribs (linear, $P < 0.001$; quadratic, $P = 0.004$, respectively), and increased grams of Ca and P in all three bones (linear, $P \leq 0.027$). An increase in percentage bone ash ($P \leq 0.038$) and increased grams of Ca and P in fibulas and 10th ribs ($P \leq 0.023$) was observed when adding 1,000 FYT/kg phytase in diets with 0.27% aP compared with pigs fed 0.27% aP without added phytase. Increasing aP from 0.09% to 0.27% in diets without added phytase increased ($P < 0.001$) ADG, ADFI, and improved F/G. Increasing aP from 0.09% to 0.27% in diets without added phytase increased bone density ($P \leq 0.002$) in fibulas and metacarpals, percentage bone ash in all bones ($P \leq 0.074$), and increased ($P < 0.05$) grams of Ca and P in fibulas and 10th ribs. Pigs fed diets containing 0.27% aP with 1,000 FYT phytase had increased ADFI ($P \leq 0.047$), bone density ($P \leq 0.008$) in fibulas and metacarpals, percentage bone ash in all bones ($P \leq 0.002$), and increased ($P < 0.05$) grams of Ca and P in fibulas and 10th ribs compared to those fed 0.09% aP with 1,000 FYT phytase. For growth performance (average of ADG and F/G), aP release was calculated to be 0.170% for

[1] The authors appreciate DSM Nutritional Products, North America, (Parsippany, NJ) for their support in this study.

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diets with 600 FYT/kg and 0.206% for 1,000 FYT/kg. For the average of all bone measurements (average of 3 bones for bone density and percentage bone ash), aP release was calculated to be 0.120 and 0.125% for diets with 600 and 1,000 FYT/kg, respectively. In conclusion, increasing phytase in diets formulated with less than the pig's phosphorus requirement improved growth performance and bone mineralization. These data help to confirm the efficacy of HiPhorius phytase in making P more available in aP deficient diets, resulting in improved growth performance and bone mineralization. Furthermore, these data show that adding high levels of phytase in diets adequate in aP did not further improve growth performance, but increased bone mineralization.

Introduction

Phytase is an enzyme commonly added to swine diets to improve the digestibility of phytate-bound phosphorus. Phytate or phytic acid is a six-fold dihydrogen phosphate ester of inositol that is the major storage form of phosphorus (P) found in feedstuffs of plant origin. Pigs and other monogastric animals do not synthesize adequate levels of endogenous phytase to effectively cleave the phosphates from the phytate. The P found in corn-soybean meal-based diets has limited availability, requiring nutritionists to add dietary P from inorganic sources—such as monocalcium phosphate—to optimize growth and ensure normal bone formation. Consequently, adding phytase to a swine diet will improve the hydrolysis of phytic acid, making P more available. This decreases the added level of inorganic P required, lowers diet cost, and reduces the amount of P excreted in swine waste.

Because of various intrinsic differences between phytase sources, when a new phytase enters the marketplace, feeding studies are required to determine its efficacy. HiPhorius 10 (DSM Nutritional Products, Parsippany, NJ) phytase is classified as a 6-phytase and is a modified *Citrobacter braakii* phytase expressed from a strain of *A. oryzae*. In recent studies, HiPhorius phytase improved growth performance and bone mineralization when fed to nursery pigs.[4,5] However, these previous trials were not conducted in the U.S. and utilized diet formulation strategies not typical of U.S. production. In addition, their approach to bone mineralization was limited to analyzing one bone from each pig and specifically focused on determining bone ash as the primary response criterion to assess bone mineralization. In the current study, multiple bones were collected and analyzed for bone density, bone ash, and Ca and P content while utilizing diet formulation strategies comparable to those used by U.S. swine nutritionists. Thus, the objective of the current study was to determine the performance and bone mineralization of nursery pigs fed various levels of HiPhorius phytase in diets with deficient and adequate (NRC, 2012)[6] levels of P.

[4] Thorsen, M., Nielsen, L. A., Zhai, H. X., Zhang, Q., Wulf-Andersen, L., & Skov, L. K. 2021. Safety and efficacy profile of a phytase produced by fermentation and used as a feed additive. Heliyon. *7*(6), e07237. doi:10.1016/j.heliyon.2021.e07237.

[5] Zhai, H., Zhang, J., Wang, Z., Wang, S., Prasad, S., Stamatopoulos, K., & Duval, S. 2023. Comparison of digestible and available phosphorus release values for a novel phytase determined with fecal phosphorus digestibility and bone mineralization in weaner pigs. Animal Feed Science and Technology, 115580. doi:10.1016/j.anifeedsci.2023.115580.

[6] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Research and Teaching Center in Manhattan, KS.

Animals and diets

A total of 297 pigs (DNA 201 × 600; initially 19.1 ± 0.40 lb) were used in a 21-d growth trial to determine the effect of HiPhorius phytase on growth performance and bone mineralization in nursery pigs. Pigs were given *ab libitum* access to feed and water with each pen containing a 4-hole, dry self-feeder, and nipple waterer. At approximately 21 d of age, pigs were weaned and fed common phase 1 and 2 diets for 14 d. All pigs were fed a phosphorus depletion diet (0.09% aP) for a 3-d period before the start of the study and were then assigned to 1 of 5 treatments in a randomized complete block design with 5 pigs per pen and 12 replications per treatment. The first three treatment diets were formulated with a basal diet containing 0.09% aP, one without added phytase, another with 600 FYT/kg of added phytase, and one diet with 1,000 FYT/kg of phytase added on top of the basal diet formulation. The remaining two treatments consisted of 2 diets formulated to the NRC requirement estimate for STTD P which equated to 0.27% aP, one diet without added phytase and the other with 1,000 FYT/kg added. The source of phytase was HiPhorius 10 (DSM Nutritional Products, Parsippany, NJ). The phytase was analyzed to determine its inclusion rate prior to diet manufacturing and was found to contain 11,913 FYT/g (DSM Laboratory, Belvidere, NJ). Monocalcium phosphate and calcium carbonate were analyzed in duplicate for Ca and P (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS), to determine nutrient loading values used in diet formulation. Limestone contained 40.11% and 0.04% Ca and P, respectively. Monocalcium phosphate contained 16.81% and 21.16% Ca and P, respectively. Sand was used in diets to equalize the batch size and dietary NE among all treatments. All diets were formulated to contain a Ca:P ratio of 1.20:1 with no allowance for Ca release by phytase. All diets were corn-soybean meal-based and were manufactured at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS. Except for the intended levels of Ca and P, all diets were formulated to meet or exceed the NRC requirement estimates for all other nutrients (Table 1). Available P values were derived from NRC (1998).[7] Complete diet samples were taken during bagging of experimental diets with a subsample collected from every third bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -40°F until two samples from each dietary treatment were analyzed in duplicate for Ca and P (the average was taken of 4 results for both Ca and P; K-State soils lab).

During the experiment, pig and feeder weights were measured on d 0, 10, and 21 to determine ADG, ADFI, and F/G. On d 21, 1 pig from each pen (weighing closest to the mean weight of pigs in the pen) was euthanized. The right fibula, metacarpal, and 10th rib from each pig were collected for determination of bone density, bone ash, and Ca and P. After collection, bones were individually placed in plastic bags with pen identification and stored at -40°F until analysis. To determine bone density, bones were cleaned of extraneous soft tissue, and the weight of each bone was recorded and then placed in ultrapure water for 4 h under a negative pressure vacuum at 1.06 kg per

[7] National Research Council. 1998. Nutrient Requirements of Swine: Tenth Revised Edition. Washington, DC: The National Academies Press.

cubic centimeter. Bones were then submerged in water and weighed again. Archimedes principle was used to determine bone density. The weight or volume of the liquid was assumed equal to the weight of the initial bone minus the submerged bone weight. The initial bone weight was then divided by the volume of the liquid to determine bone density. Next, bones were dried at 105°C for 7 d in a drying oven, weighed, and then ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and percentage ash relative to dried bone weight. Subsequently, Ca and P content within each bone was measured. Between 0.025 and 0.040 g of bone ash from each bone was added to 100 uL nitric acid. Tubes were placed in a hot block at 16°C for 6 h, diluted 1:10 with ultrapure water and then sent to the K-State soils lab where Ca and P quantity was determined.

Using performance and bone analysis data, calculations were made to estimate the aP release values of the phytase for different response criteria. Using the aP levels of the 0.09 and 0.27% aP diets, standard release curves were constructed for each criterion. Marginal intake of aP was then estimated using this curve for both the 600 and 1,000 FYT/kg phytase treatments added to the low aP diet, and then using ADFI, the aP release could be estimated for each response criterion. Release estimates were generated based on growth performance (ADG and G:F) as well as measures of bone mineralization including bone density and percentage bone ash for the fibula, metacarpal, and 10th rib.

Statistical analysis

Pens of pigs were randomly allotted to treatments using initial pen weight as a blocking factor. Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.2 (2-07-2018) with pen as the experimental unit, treatment considered as a fixed effect, and weight block as a random effect. For all growth performance and bone mineralization analysis, treatments were analyzed to determine the linear and quadratic responses of phytase dose within the diets containing 0.09% aP. Contrasts were made to compare diets with no phytase and increasing aP (0.09 % vs 0.27%), diets with 1,000 FYT/kg of phytase and increasing aP (0.09 % vs 0.27%), and treatments with 0 versus 1,000 FYT/kg phytase in the diets containing 0.27% aP. Results were considered significant with P-values ≤ 0.05 and were considered marginally significant with P-values $0.05 < P \leq 0.10$.

Results and Discussion

For the experimental period (d 0 to 21), a linear increase ($P \leq 0.002$) in ADG, ADFI, and d 21 BW and improvement in F/G was observed for pigs fed increasing phytase in 0.09% aP diets (Table 2). When aP was increased from 0.09 to 0.27%, the ADG, ADFI, and d 21 BW increased ($P < 0.001$) and F/G improved ($P < 0.001$), when no phytase was included in the diet, but when 1,000 FYT/kg of phytase was added, ADFI was increased ($P = 0.047$) without any change in ADG or F/G.

Pigs fed increasing phytase within the 0.09% aP diet had a tendency ($P \leq 0.074$) for increased fibula and 10th rib bone density and had increased (linear, $P = 0.019$) metacarpal bone density. There was a tendency ($P = 0.081$) for pigs fed 1,000 FYT/kg phytase added to the diet with 0.27% aP to have increased metacarpal bone density compared to pigs fed a 0.27% aP diet with no added phytase. When aP was increased from 0.09 to 0.27% in diets without added phytase, bone density was increased ($P \leq 0.002$) in fibulas and metacarpals, and 10th rib bone density tended to be increased ($P = 0.084$). Pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase had increased bone density ($P \leq 0.008$) in fibulas and metacarpals as aP increased.

For all three bones, bone ash weight increased linearly ($P < 0.006$) when phytase was increased in diets with 0.09% aP as well as when 1,000 FYT/kg phytase was added in the diet containing 0.27% aP. For all three bones, there was an increase ($P < 0.001$) in bone ash weight when increasing aP from 0.09 to 0.27% when diets did or did not contain phytase.

Percentage bone ash tended to increase (linear, $P = 0.100$) in fibulas, and increased in metacarpal (linear, $P < 0.001$) and 10th rib (quadratic, $P = 0.004$) when increasing phytase in diets containing 0.09% aP. All bones showed an increase in percentage bone ash ($P \leq 0.038$) when adding 1,000 FYT/kg phytase in diets with 0.27% aP compared to pigs fed a 0.27% diet with no added phytase. An increase in aP from 0.09% to 0.27%, increased the percentage bone ash in all bones in diets without added phytase ($P \leq 0.074$) and with added phytase ($P \leq 0.002$).

Increasing phytase in diets containing 0.09% aP increased the grams of Ca and P in all three bones (linear, $P \leq 0.027$). Grams of Ca and P increased ($P \leq 0.023$) in fibulas and 10th ribs and marginally increased ($P \leq 0.089$) in metacarpals when 1,000 FYT/kg was added to the diet containing 0.27% aP compared to pigs fed a 0.27% aP diet without added phytase. Increasing aP from 0.09% to 0.27% aP in diets without added phytase increased ($P \leq 0.007$) grams of Ca and P in fibulas and 10th ribs and increased ($P = 0.049$) P in metacarpals. Comparing pigs fed diets containing either 0.09 or 0.27% aP with 1,000 FYT phytase, increasing the aP level also increased ($P < 0.001$) grams of Ca and P in fibulas and 10th ribs and tended to increase ($P \leq 0.070$) Ca and P in metacarpals.

There was a quadratic response ($P \leq 0.034$) in metacarpals for both Ca and P percentage where pigs fed 600 FYT/kg phytase in the 0.09% aP diet had reduced Ca and P percentage. There was also a greater percentage ($P = 0.044$) of Ca in metacarpals when pigs were fed a P deficient (0.09% aP) diet with no added phytase versus an adequate P (0.27% aP) diet when no phytase was added.

Using growth performance (ADG and G:F), aP release attributed to phytase was estimated to be 0.170% for 600 FYT/kg and 0.206% for 1,000 FYT/kg (Table 3). For bone density, 600 FYT/kg provided a 0.097% release and 1,000 FYT/kg provided a 0.110% aP release. For percentage bone ash, the estimated aP release value for 600 FYT/kg was 0.142% and for 1,000 FYT/kg the aP release value was 0.140% for the average of the three bones. The magnitude of aP release at different FYT/kg inclusion rates depended on the response criteria measured; however, the calculated release values were comparable to the release values for aP of each inclusion of HiPhorius phytase estimated by Zhai et al. (2023).

In conclusion, increasing phytase in diets formulated below the pig's phosphorus requirement improved the growth performance and bone mineralization. Increasing phytase in diets with 0.27% aP did not influence growth performance, but improved bone mineralization. These data help to confirm the efficacy of HiPhorius phytase and aP release values determined from different growth and bone measurement criteria.

Table 1. Composition of experimental diets (as-fed basis)[1]

aP, %:	0.09			0.27	
Phytase, FYT/kg:	**0**	**600**	**1,000**	**0**	**1,000**
Ingredient, %					
Corn	63.26	63.26	63.26	63.26	63.26
Soybean meal	33.65	33.65	33.65	33.65	33.65
Limestone	0.26	0.26	0.26	0.44	0.44
Monocalcium P	0.06	0.06	0.06	0.91	0.91
Salt	0.60	0.60	0.60	0.60	0.60
L-Lys-HCl	0.30	0.30	0.30	0.30	0.30
DL-Met	0.12	0.12	0.12	0.12	0.12
L-Thr	0.12	0.12	0.12	0.12	0.12
L-Val	0.01	0.01	0.01	0.01	0.01
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Sand[2]	1.23	1.22	1.22	0.19	0.18
Phytase[3]	---	0.006	0.010	---	0.010

continued

Table 1. Composition of experimental diets (as-fed basis)[1]

aP, %:	**0.09**			**0.27**	
Phytase, FYT/kg:	**0**	**600**	**1,000**	**0**	**1,000**
Calculated analysis					
Standard ileal digestible (SID) amino acids, %					
Lys	1.24	1.24	1.24	1.24	1.24
Ile:Lys	63	63	63	63	63
Leu:Lys	129	129	129	129	129
Met:Lys	33	33	33	33	33
Met and Cys:Lys	57	57	57	57	57
Thr:Lys	63	63	63	63	63
Trp:Lys	18.7	18.7	18.7	18.7	18.7
Val:Lys	69	69	69	69	69
His:Lys	41	41	41	41	41
Total Lys, %	1.39	1.39	1.39	1.39	1.39
NE, kcal/lb	1,488	1,488	1,488	1,488	1,488
SID Lys:NE, g/Mcal	1,074	1,074	1,074	1,074	1,074
aP, %	0.09	0.09	0.09	0.27	0.27
Ca, %	0.43	0.43	0.43	0.64	0.64
P, %	0.36	0.36	0.36	0.54	0.54
Ca:P ratio	1.20	1.20	1.20	1.20	1.20
Analyzed composition[4]					
Ca, %	0.36	0.46	0.36	0.68	0.59
P, %	0.36	0.38	0.41	0.57	0.55
Ca:P ratio	0.98	1.19	0.89	1.19	1.07

[1]Diets were fed for 21 d from approximately 20 to 40 lb.
[2]To form the treatments, a hand-add of sand, limestone, monocalcium P, and phytase was used to equalize the dietary contribution of all other ingredients.
[3]HiPhorius 10 2400 phytase was analyzed to contain 11,914 FYT/g and was provided by DSM Nutritional Products, Parsippany, NJ.
[4]Complete diet samples were taken during the bagging of experimental diets with a sub-sample collected from every third bag and pooled into one homogenized sample per dietary treatment. After homogenization, samples were stored at -20°C until analysis (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS) for Ca and P. Two samples of each dietary treatment were analyzed in duplicate to obtain average Ca and P values.

Table 2. Effects of HiPhorius phytase on growth performance and bone mineralization in nursery pigs[1]

aP, %:	0.09			0.27			*P* =				
							Phytase in 0.09% aP				
Phytase, FYT/kg:	0	600	1,000	0	1,000	SEM	Linear	Quadratic	Phytase in 0.27% aP[2]	P level in 0 FYT/kg[3]	P level in 1,000 FYT/kg[4]
BW, lb											
d 0	19.1	19.1	19.1	19.1	19.1	0.40	0.990	0.985	0.999	0.999	0.998
d 21	37.5	42.5	44.0	44.8	45.7	0.89	0.151	0.604	0.941	0.070	0.254
d 0 to 21											
ADG, lb	0.88	1.12	1.16	1.20	1.25	0.031	<0.001	0.051	0.754	<0.001	0.153
ADFI, lb	1.48	1.66	1.66	1.76	1.83	0.046	0.002	0.184	0.779	<0.001	0.047
F/G	1.69	1.49	1.44	1.47	1.46	0.024	<0.001	0.094	0.997	<0.001	0.973
Bone density, g/mL											
Fibula	1.17	1.23	1.21	1.27	1.30	0.171	0.068	0.156	0.812	0.001	0.008
Metacarpal	1.16	1.17	1.18	1.20	1.23	0.008	0.019	0.419	0.081	0.002	0.002
10th rib	1.27	1.35	1.37	1.41	1.46	0.037	0.074	0.606	0.871	0.084	0.413
Bone ash, g											
Fibula	0.41	0.67	0.66	0.72	1.00	0.044	< 0.001	0.057	< 0.001	< 0.001	< 0.001
Metacarpal	0.70	0.95	1.04	1.12	1.34	0.046	< 0.001	0.405	0.006	< 0.001	< 0.001
10th rib	0.48	0.77	0.86	1.00	1.31	0.056	< 0.001	0.370	0.003	< 0.001	< 0.001
Bone ash, %											
Fibula[5]	38.3	43.8	42.5	45.6	53.7	0.02	0.100	0.229	0.038	0.074	0.002
Metacarpal	30.1	34.0	35.3	36.3	40.4	0.01	< 0.001	0.384	0.001	< 0.001	< 0.001
10th rib[6]	44.3	49.1	48.7	51.2	54.3	0.01	< 0.001	0.004	0.004	< 0.001	< 0.001
Bone Ca, g											
Fibula	0.17	0.27	0.28	0.28	0.43	0.022	< 0.001	0.212	< 0.001	0.007	< 0.001
Metacarpal	0.30	0.32	0.38	0.29	0.47	0.024	0.027	0.343	0.089	0.110	0.070
10th rib	0.18	0.29	0.33	0.39	0.51	0.028	< 0.001	0.526	0.023	< 0.001	< 0.001
Bone Ca, %											
Fibula	41.1	40.5	43.0	38.3	41.5	1.95	0.528	0.458	0.742	0.837	0.983
Metacarpal	41.3	33.9	36.8	34.8	35.5	1.60	0.027	0.020	0.998	0.044	0.979
10th rib	37.0	37.6	37.5	38.5	38.8	1.46	0.756	0.877	0.999	0.929	0.958

continued

Table 2. Effects of HiPhorius phytase on growth performance and bone mineralization in nursery pigs[1]

aP, %:	0.09			0.27			*P* =				
							Phytase in 0.09% aP		Phytase in 0.27% aP[2]	P level in 0 FYT/kg[3]	P level in 1,000 FYT/kg[4]
Phytase, FYT/kg:	0	600	1,000	0	1,000	SEM	Linear	Quadratic			
Bone P, g											
Fibula	0.07	0.12	0.13	0.13	0.20	0.01	< 0.001	0.189	< 0.001	0.003	< 0.001
Metacarpal	0.13	0.15	0.18	0.18	0.22	0.01	0.011	0.388	0.075	0.049	0.059
10th rib	0.09	0.15	0.17	0.20	0.26	0.01	< 0.001	0.472	0.020	< 0.001	< 0.001
Bone P, %											
Fibula	17.8	18.3	19.5	17.5	18.9	0.91	0.204	0.619	0.773	0.998	0.989
Metacarpal	18.4	15.6	17.1	16.1	16.6	0.75	0.140	0.034	0.994	0.218	0.986
10th rib	18.2	19.1	18.9	19.5	19.7	0.74	0.391	0.614	0.999	0.626	0.921

[1]A total of 297 pigs (DNA 241 × 600; initially 19.1 ± 0.40 lb) were used in a 21-d growth study. There were 5 pigs per pen and 12 pens per treatment. HiPhorius 10 2400 phytase was provided by DSM Nutritional Products (Parsippany, NJ). One pig per pen was euthanized and the right fibula, 10th rib, and metacarpal were collected to determine bone density, bone ash, and bone Ca and P.

[2]Phytase in 0.27% aP: this contrast compares pigs fed diets containing 0.27% aP with no added phytase to pigs fed diets containing 0.27% aP with an additional 1,000 FYT/kg of added phytase.

[3]P level in 0 FYT/kg phytase: this contrast compares pigs fed 0.09% aP with no added phytase to pigs fed 0.27% aP with no added phytase.

[4]P level in 1,000 FYT/kg phytase: this contrast compares pigs fed 0.09% aP with 1,000 FYT/kg added phytase to pigs fed 0.27% aP with 1,000 FYT/kg of added phytase.

[5]Pigs fed 0.27% aP with no phytase had increased ($P = 0.018$) fibula percentage bone ash compared to pigs fed 0.09 aP and 1000 FYT/kg phytase.

[6]Pigs fed 0.27% aP with no phytase had increased ($P = 0.028$) 10th rib percentage bone ash compared to pigs fed 0.09 aP and 1000 FYT/kg phytase.

Table 3. Estimated aP % release of HiPhorius phytase[1]

	FYT/kg	
Response	600	1,000
ADG	0.154	0.180
F/G	0.187	0.233
Average of growth performance	0.170	0.206
Bone density		
Fibula	0.123	0.082
Metacarpal	0.051	0.103
10th rib	0.117	0.147
Average of 3 bones	0.097	0.110
Percentage bone ash		
Fibula	0.155	0.118
Metacarpal	0.129	0.172
10th rib	0.143	0.131
Average of 3 bones	0.142	0.140
Average of all bone measures	0.120	0.125

[1]Release estimates were calculated using the aP levels of the 0.09% and 0.27% aP diets to develop a standard curve with estimated marginal intake of aP above that of pigs fed the diet with 0.09% aP as the independent variable and response as the dependent variable. Response criteria including growth and measures of bone mineralization for the 0.09% aP + 600 FYT/kg and 0.09% aP + 1,000 FYT/kg treatment groups were then used to estimate the marginal aP intake, and thus estimate the aP release for each phytase dose for each response criterion.