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The Effect of Different Bone and Analytical Methods on the Assessment of Bone Mineralization to Dietary Phosphorus, Phytase, and Vitamin D in Finishing Pigs

Hadley R. Williams
Kansas State University, hadley1@k-state.edu

Jordan T. Gebhardt
Kansas State University, jgebhardt@k-state.edu

Mike D. Tokach
Kansas State University, mtokach@k-state.edu

See next page for additional authors

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The Effect of Different Bone and Analytical Methods on the Assessment of Bone Mineralization to Dietary Phosphorus, Phytase, and Vitamin D in Finishing Pigs

Abstract

Eight hundred eighty-two pigs (initially 73.2 ± 0.7 lb) were used to evaluate the effects of different bones and analytical methods on the assessment of bone mineralization response to dietary P and vitamin D in growing-finishing pigs. Pens of pigs (20 pigs per pen) were randomized to 1 of 5 dietary treatments in a completely randomized design with 9 pens per treatment. Treatments were formulated to have varying levels of P, phytase, and vitamin D to potentially provide wide differences in bone characteristics. After feeding diets for 112 d, nine pigs per treatment were euthanized for bone, blood, and urine analysis. There were no significant differences for final BW, ADG, ADFI, F/G ($P > 0.10$), or bone ash (bone ash × bone interaction, $P > 0.10$) regardless of the ashing method. The response to treatment for bone density and bone mineral content was dependent upon the bone (density interaction, $P = 0.053$; mineral interaction, $P = 0.078$). There were no treatment differences for bone density and bone mineral content for metacarpals, fibulas, and 2nd rib ($P > 0.05$). For 10th rib bone density, pigs fed industry levels of P and vitamin D had increased ($P < 0.05$) bone density compared to pigs fed NRC levels with phytase, with pigs fed deficient P, NRC levels of P with no phytase, and extra 25(OH)D_3 vitamin D (HyD) intermediate. Pigs fed extra vitamin D from HyD had increased ($P < 0.05$) 10th rib bone mineral content compared to pigs fed deficient P and NRC levels of P with phytase, with pigs fed industry P and vitamin D, and NRC P with monocalcium intermediate. In summary, bone density and bone mineral content responses varied depending on the bone. The difference between bone ash procedures was more apparent than the differences between diets. Differences in bone density and mineral content in response to P and vitamin D were most apparent with the 10th ribs.

Keywords

bone mineralization, finishing pigs, growth performance

Cover Page Footnote

Appreciation is expressed to the Minnesota Pork Board, Iowa Pork Board, and DSM Nutritional Products (Parsippany, NJ) for partial financial support of this project.

Authors

Hadley R. Williams, Jordan T. Gebhardt, Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Joel M. DeRouchey, Jon R. Bergstrom, Chad W. Hastad, Zach B. Post, Michael C. Rahe, Christopher L. Siepker, Panchan Sitthicharoenchai, and Steve M. Ensley

K-STATE Research and Extension

SWINE DAY 2022

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2022

The Effect of Different Bone and Analytical Methods on the Assessment of Bone Mineralization to Dietary Phosphorus, Phytase, and Vitamin D in Finishing Pigs[1]

Hadley R. Williams, Jordan T. Gebhardt,[2] Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Joel M. DeRouchey, Jon R. Bergstrom,[3] Chad W. Hastad,[4] Zach B. Post,[4] Michael C. Rahe,[5] Christopher L. Siepker,[5] Panchan Sitthicharoenchai,[5] and Steve M. Ensley[6]

Summary

Eight hundred eighty-two pigs (initially 73.2 ± 0.7 lb) were used to evaluate the effects of different bones and analytical methods on the assessment of bone mineralization response to dietary P and vitamin D in growing-finishing pigs. Pens of pigs (20 pigs per pen) were randomized to 1 of 5 dietary treatments in a completely randomized design with 9 pens per treatment. Treatments were formulated to have varying levels of P, phytase, and vitamin D to potentially provide wide differences in bone characteristics. After feeding diets for 112 d, nine pigs per treatment were euthanized for bone, blood, and urine analysis. There were no significant differences for final BW, ADG, ADFI, F/G ($P > 0.10$), or bone ash (bone ash × bone interaction, $P > 0.10$) regardless of the ashing method. The response to treatment for bone density and bone mineral content was dependent upon the bone (density interaction, $P = 0.053$; mineral interaction, $P = 0.078$). There were no treatment differences for bone density and bone mineral content for metacarpals, fibulas, and 2nd rib ($P > 0.05$). For 10th rib bone density, pigs fed industry levels of P and vitamin D had increased ($P < 0.05$) bone density compared to pigs fed NRC[7] levels with phytase, with pigs fed deficient P, NRC levels of P with no phytase, and extra 25(OH)D_3 vitamin D (HyD) intermediate. Pigs fed extra vitamin D from HyD had increased ($P < 0.05$) 10th rib bone mineral content compared to pigs fed deficient P and NRC levels of P with phytase, with pigs fed industry P and vitamin

[1] Appreciation is expressed to the Minnesota Pork Board, Iowa Pork Board, and DSM Nutritional Products (Parsippany, NJ) for partial financial support of this project.

[2] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

[3] DSM Nutritional Products, Parsippany, NJ.

[4] New Fashion Pork, Jackson, MN.

[5] Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA.

[6] Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University.

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

D, and NRC P with monocalcium intermediate. In summary, bone density and bone mineral content responses varied depending on the bone. The difference between bone ash procedures was more apparent than the differences between diets. Differences in bone density and mineral content in response to P and vitamin D were most apparent with the 10th ribs.

Introduction

In recent years, the occurrence of lameness in growing pigs has increased, leading to an increased incidence of removals and mortality. Lameness is defined as impaired movement or deviation from normal gait. There are many factors that can contribute to lameness, including infectious disease, genetic and conformational deficiencies, impaired physiological development of articular surfaces within joints, and skeletal mineralization. Metabolic bone disease is a common cause of lameness in swine production and can be caused by inappropriate levels of essential vitamins or minerals.

An important component of lameness evaluation is the histopathological examination of tissues including articular surfaces, synovium, and growth plates. While these tissues can provide an indication of a variety of pathological processes, such as metabolic bone disease induced by vitamin D deficiency (Madson et al., 2012[8]), they do not always result in a definitive diagnosis. Ancillary diagnostic tests that can be used in a workup of clinical lameness include measures of bone mineralization such as bone ash and serum concentrations of Ca, P, and vitamin D.

Serum 25(OH)D has been the standard for the determination of vitamin D status within swine; however, there has been recent speculation that the activated form, $1,25(OH)_2D$, may provide additional benefits in assessing vitamin D status (Hurst et al., 2020[9]). There is limited information regarding serum $1,25(OH)_2D$ levels in swine under a variety of feeding conditions.

Bone ash is an established method for measuring bone mineralization. However, questions remain about whether defatted or non-defatted bone ash is the more accurate method. Wensley et al. (2020[10]) compared defatted and non-defatted processing methods to determine their effects on the ability to detect treatment differences. The authors observed that either non-defatted or defatted bone processing methods can be used to determine the bone ash weight and percentage bone ash to assess bone mineralization in nursery pigs although bone ash percentage is greater for the defatted method compared to the non-defatted method.

A non-invasive technique for evaluating mineralization status is the collection and analysis of urine samples. Urinary Ca and P can be measured, and when put in a ratio to creatinine to standardize for potential differences in urine volume, has been shown to be a promising indicator of mineral status. On their own, the presented assays are

[8] Madson, D. M., S. M. Ensley, P. C. Gauger, K. J. Schwartz, G. W. Stevenson, V. L. Cooper, B. H. Janke, E. R. Burrough, J. P. Goff, and R. L. Horst. Rickets: case series and diagnostic review of hypovitaminosis D in swine. J. Vet. Diagn. Invest. 24(6):1137-1144. doi:10.1177/1040638712461487.

[9] Hurst, E. A., N. Z. Homer, and R. J. Mellanby. 2020. Vitamin D metabolism and profiling in veterinary species. Metabolites. 10:371. doi: 10.3390/metabo10090371.

[10] Wensley, M. R., C. M. Vier, J. T. Gebhardt, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. M. DeRouchey. 2020. Technical note: assessment of two methods for estimating bone ash in pigs. J. Anim. Sci. 98:1-8. doi: 10.1093/jas/skaa251.

limited in their ability to diagnose metabolic bone disease and identify the cause. However, when evaluated collectively, the assays can result in a better diagnosis and can lead to intervention. Therefore, the objective of this study was to evaluate the effect of different bone and analytical methods on the assessment of bone mineralization responses to dietary P, phytase, and vitamin D in finishing pigs.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The trial was conducted at a commercial research facility owned and operated by New Fashion Pork (Jackson, MN).

Animal and diets

A total of 882 pigs (PIC TR4 × (Fast LW × PIC L02); initially 73.2 ± 0.7 lb) were used in a 112-d trial. Pens of pigs were fed common diets from weaning until the start of the study. Calcium and P levels for all common diets were at or above NRC requirement estimates. Pens of pigs (20 pigs per pen) were then randomized to 1 of 5 dietary treatments in a completely randomized design with 9 pens per treatment. The dietary treatments were: 1) STTD P fed at 80% of the NRC requirement estimate (deficient); 2) STTD P fed at 100% of the requirement (NRC requirement) using monocalcium phosphate; 3) STTD P fed at 100% of the NRC requirement with phytase included; 4) STTD P fed at 128% of the NRC requirement (industry level) including 0.14% release from phytase; and 5) diet 4 with additional 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 (HyD). All diets were manufactured in meal form at the New Fashion Pork feed mill in Estherville, IA (Table 1 and 2). For treatments 1 and 2, P levels were met by only using monocalcium phosphate. All other treatment diets had 2,000 FYT/kg of phytase included in the diet to meet the desired STTD P levels, with an assumed STTD P release of 0.14%. Vitamin D was included in all diets via the vitamin premix at a rate of 992 IU/kg. Treatment 5 had an additional 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 HyD, (DSM Nutritional Products, Parsippany, NJ). All diets were formulated to an analyzed Ca:analyzed P ratio of 1.20:1. Experimental diets were fed for 112 d.

Pens of pigs were weighed, and feed disappearance was measured every 14 d to determine ADG, ADFI, and F/G. On d 112, 1 pig per pen (9 pigs per treatment) were euthanized and used for the analysis of bones, blood, and urine. The right and left metacarpal, fibula, 2nd rib, and 10th rib were collected from each pig for a total of 8 bones per pig. All bones were analyzed using dual-energy X-ray absorptiometry (DEXA) scans, then bone density, breaking strength, bone ash, and bone Ca and P were determined. Histologic evaluation of hematoxylin and eosin (H&E) stained sections of the 2nd rib, 10th rib, and fibula was performed by three diagnostic pathologists. Hematoxylin stains the cell nuclei a purplish blue and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors. Bones were scored for lesions of failure of endochondral ossification of the physis and microscopic fractures (infractions). Medullary trabeculae and cortical bone thickness was measured. Ten mL of blood was collected to measure serum chemistry, and 10 mL of urine was collected directly from the bladder to measure Ca, P, and creatinine.

Statistical analysis

Growth performance, blood, and urine analysis were analyzed as a completely randomized design for one-way ANOVA using the lm function from the lm package in R version 3.5.1 (07-02-2018) with pen considered as the experimental unit and treatment as a fixed effect. A Tukey multiple comparison adjustment was used when appropriate. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

For the statistical analysis for bones, data were analyzed as a completely randomized design for two-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria), with pen considered as the experimental unit, pen as a random effect, and treatment, bone, and the associated interaction included as fixed effects. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Growth performance

For final body weight and overall growth performance, there were no differences between the dietary treatments ($P > 0.10$; Table 3).

Serum analysis

For serum Ca, pigs fed 80% of the NRC requirement for P had increased circulating Ca compared to pigs fed NRC P levels with phytase ($P < 0.05$), with pigs fed industry levels of P, and NRC levels of P with no phytase intermediate. Pigs fed NRC levels of P with no phytase had increased serum P levels compared to pigs fed industry levels of P with HyD included in the diet ($P < 0.05$), with the other 3 treatments intermediate. Pigs fed the diets containing 25-hydroxyvitamin D_3 from HyD had increased ($P < 0.05$) circulating 25-hydroxyvitamin D_3 compared to pigs fed industry levels of vitamin D in the diet. 25-hydroxyvitamin D_3 is the precursor to the active form of vitamin D and undergoes a bioconversion process within the kidney to form the active metabolite, 1,25-dihydroxyvitamin D_3. There was no difference between treatments for circulating 1,25-dihydroxyvitamin D_3 ($P = 0.147$). For urine Ca:creatinine, there was a tendency for a difference between treatments ($P = 0.081$), but no significant mean separation was observed between treatments ($P > 0.05$). For urine P:creatinine levels, there was no difference between treatments ($P = 0.378$).

Bone analysis

There were no treatment differences for defatted bone ash percent, percentage non-defatted bone ash, non-defatted bone ash grams, or percentage and grams of bone ash Ca and P ($P > 0.10$). For defatted bone ash grams, pigs fed industry levels of P had numerically increased bone ash grams compared to pigs fed the other diets, but no significant mean separation was observed ($P > 0.05$). The fibula had greater percentage defatted bone ash compared to the 2nd and 10th ribs ($P < 0.05$), while the metacarpal had the lowest ($P < 0.05$) defatted bone ash percentage (Table 4). In non-defatted bones, percentage bone ash of the fibulas were greater compared to the 2nd rib and metacarpal, and the 2nd rib and 10th rib both had greater ($P < 0.05$) percentage bone ash than the metacarpal.

The response to treatment for bone density and bone mineral content was dependent upon the bone analyzed (density interaction, $P = 0.053$; mineral interaction, $P = 0.078$; Table 5). There were no treatment differences for bone density and bone mineral content for metacarpals, fibulas, and 2nd rib ($P > 0.05$). For 10th rib bone density, pigs fed industry levels of P and vitamin D had increased ($P < 0.05$) bone density compared to pigs fed NRC levels with phytase, with pigs fed deficient P, NRC P with no phytase, and extra vitamin D from HyD intermediate. Pigs fed extra vitamin D from 25-hydroxyvitamin D_3 (from HyD) had increased bone mineral content compared to pigs fed deficient P and NRC levels of P with phytase, with pigs fed industry P and vitamin D, and NRC P with monocalcium intermediate.

For histopathological analysis of bones, there were no treatment differences for infraction score, trabeculae bone thickness, and cortical bone thickness ($P > 0.10$). For physeal score, there was a significant difference between treatments ($P = 0.040$), but no significant mean separation was observed. Numerically, pigs fed 80% of the NRC P requirement and those fed industry levels of P with industry levels of vitamin D had an increased physeal score, representing more lesions of endochondral ossification.

In summary, bone density and bone mineral content responses varied depending on the bone. The difference between bone ash procedures was more apparent than the differences between treatments. Differences in bone density and mineral content in response to P and vitamin D were most apparent with the 10th ribs.

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Table 1. Diet composition, phases 1 and 2 (as-fed basis)[1]

	Phase 1					Phase 2				
STTD P, %:	**0.23**	**0.29**	**0.29**	**0.37**	**0.37**	**0.21**	**0.26**	**0.26**	**0.33**	**0.33**
Vitamin D, IU/kg:	**992**	**992**	**992**	**992**	**992 + HyD**	**827**	**827**	**827**	**827**	**827 + HyD**
Phytase, FYT/kg[3]:	**0**	**0**	**2,000**	**2,000**	**2,000**	**0**	**0**	**770**	**2,000**	**2,000**
Ingredients, %										
Corn	70.15	69.75	70.60	70.05	70.05	74.85	74.50	75.25	74.85	74.85
Soybean meal, 46.5% CP	27.45	27.45	27.45	27.45	27.45	22.95	22.95	22.95	22.95	22.95
Calcium carbonate	0.65	0.71	0.56	0.65	0.65	0.66	0.72	0.59	0.65	0.65
Monocalcium phosphate	0.45	0.75	---	0.45	0.45	0.35	0.65	---	0.28	0.28
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Feed grade amino acids	0.52	0.52	0.52	0.52	0.52	0.44	0.44	0.44	0.44	0.44
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15	0.13	0.13	0.13	0.13	0.13
Phytase[3]	---	---	0.08	0.08	0.08	---	---	0.03	0.08	0.08
HyD[4]	---	---	---	---	0.04	---	---	---	---	0.03
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis										
SID Lys, %	1.03	1.03	1.03	1.03	1.03	0.91	0.91	0.91	0.91	0.91
NE, kcal/lb	1,118	1,114	1,124	1,117	1,117	1,131	1,127	1,136	1,131	1,131
Analyzed Ca, %	0.54	0.62	0.43	0.54	0.54	0.50	0.57	0.41	0.48	0.48
Analyzed P, %	0.45	0.52	0.36	0.45	0.45	0.41	0.48	0.34	0.40	0.40
STTD P with phytase, %	0.23	0.29	0.29	0.37	0.37	0.20	0.26	0.26	0.33	0.33
Analyzed Ca:analyzed P	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20

[1]Phase 1 was fed from 80 to 120 lb. Phase 2 was fed from 120 to 160 lb.

[2]Vitamin premix contained 992 IU/kg of vitamin D_3 in the diet when the premix was included in the diet at 0.15% and 827 IU/kg of vitamin D_3 in the diet when the premix was included in the diet at 0.13%.

[3]Ronozyme HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) was included at 2,000 FYT/kg with an assumed release of 0.14 STTD P when included in the diet at 0.08%. When phytase was provided in the diet at 0.03% it included 770 FYT/kg with an assumed release of 0.12 STTD P.

[4]HyD provided 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 to the diet (DSM Nutritional Products, Parsippany, NJ).

Table 2. Diet composition, phases 3 and 4 (as-fed basis)[1]

	Phase 3					Phase 4				
STTD P, %:	**0.20**	**0.24**	**0.24**	**0.29**	**0.29**	**0.18**	**0.22**	**0.22**	**0.26**	**0.26**
Vitamin D, IU/kg:	**661**	**661**	**661**	**661**	**661 + HyD**	**496**	**496**	**496**	**496**	**496 + HyD**
Phytase, FYT/kg[3]:	**0**	**0**	**770**	**2,000**	**2,000**	**0**	**0**	**490**	**2,000**	**2,000**
Ingredients, %										
Corn	82.65	82.40	83.15	82.90	82.85	85.30	85.00	85.70	85.60	85.60
Soybean meal, 46.5% CP	14.95	14.95	14.95	14.95	14.95	12.50	12.50	12.50	12.50	12.50
Calcium carbonate	0.71	0.75	0.63	0.65	0.65	0.73	0.76	0.65	0.65	0.65
Monocalcium phosphate	0.42	0.63	---	0.15	0.15	0.35	0.57	---	---	---
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Feed grade amino acids	0.58	0.58	0.58	0.58	0.58	0.51	0.51	0.51	0.51	0.51
Trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.08	0.08	0.08	0.08	0.08
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.08	0.08	0.08	0.08	0.08
Phytase[3]	---	---	0.03	0.08	0.08	---	---	0.02	0.08	0.08
HyD[4]	---	---	---	---	0.02	---	---	---	---	0.02
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis										
SID Lys, %	0.79	0.79	0.79	0.79	0.79	0.72	0.72	0.72	0.72	0.72
NE, kcal/lb, %	1,152	1,149	1,158	1,155	1,155	1,160	1,157	1,165	1,164	1,164
Analyzed Ca, %	0.48	0.53	0.37	0.41	0.41	0.45	0.51	0.36	0.36	0.36
Analyzed P, %	0.40	0.44	0.31	0.34	0.34	0.38	0.42	0.30	0.30	0.30
STTD P, with phytase, %	0.20	0.24	0.24	0.29	0.29	0.18	0.22	0.21	0.25	0.25
Analyzed Ca:analyzed P	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20

[1]Phase 3 was fed from 160 to 220 lb. Phase 4 was fed from 220 to 280 lb.

[2]Vitamin premix contained 661 IU/kg of vitamin D_3 in the diet when the premix was included in the diet at 0.10% and 496 IU/kg of vitamin D_3 in the diet when the premix was included in the diet at 0.08%.

[3]Ronozyme HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) was included at 2,000 FYT/kg with an assumed release of 0.14 STTD P when included in the diet at 0.08%. When phytase was provided in the diet at 0.03% it included 770 FYT/kg with an assumed release of 0.12 STTD P. When phytase was provided in the diet at 0.02% it included 490 FYT/kg with an assumed release of 0.10 STTD P.

[4]HyD provided 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 to the diet (DSM Nutritional Products, Parsippany, NJ).

Table 3. Effect of STTD P, phytase, and vitamin D on growth performance, serum, and bone analysis of finishing pigs[1,2]

STTD P, %:	80% NRC[3]	NRC	NRC	Industry	Industry		
Vitamin D:	Industry	Industry	Industry	Industry	Industry + HyD[4]		
Phytase:	No	No	Yes	Yes	Yes	SEM	*P*-value
Body weight, lb							
d 0	73.1	73.2	73.1	72.9	72.9	0.70	0.994
d 112	287.1	287.2	286.6	287.4	291.9	4.13	0.891
Overall							
ADG, lb	1.97	1.97	1.99	1.99	2.02	0.032	0.810
ADFI, lb	5.17	5.17	5.24	5.17	5.26	0.101	0.945
F/G	2.62	2.62	2.63	2.60	2.60	0.004	0.782
Serum[5]							
Ca, mg/dL	11.6^{a}	11.0^{ab}	10.9^{b}	11.4^{ab}	11.0^{ab}	0.17	0.030
P, mg/dL	8.4^{ab}	9.4^{a}	8.9^{ab}	9.0^{ab}	8.0^{b}	0.33	0.036
25-hydroxyvitamin D_3, ng/mL	19.3^{b}	18.1^{b}	16.6^{b}	20.7^{b}	37.8^{a}	1.80	< 0.001
1,25-dihydroxyvitamin D_3, pg/mL	213	151	160	161	133	23.6	0.147
Urine							
Calcium:creatinine	0.38	0.16	0.13	0.08	0.18	0.078	0.081
Phosphorus:creatinine	0.02	0.09	0.04	0.06	0.03	0.026	0.378

continued

Table 3. Effect of STTD P, phytase, and vitamin D on growth performance, serum, and bone analysis of finishing pigs[1,2]

STTD P, %:	80% NRC[3]	NRC	NRC	Industry	Industry		
Vitamin D:	Industry	Industry	Industry	Industry	Industry + HyD[4]		
Phytase:	No	No	Yes	Yes	Yes	SEM	*P*-value
Defatted bone ash[6]							
Bone ash, g	6.26	6.61	6.33	6.98	6.90	0.260	0.017
Bone ash, %	62.8	63.9	63.1	63.3	63.4	0.26	0.219
Non-defatted bone ash[7]							
Bone ash, g	6.88	7.39	6.79	7.46	7.53	0.328	0.168
Bone ash, %	48.0	49.8	47.1	49.9	49.7	0.94	0.327
Dual-energy X-ray absorptiometry							
Bone mineral density, g/cm^2	0.18	0.19	0.19	0.20	0.21	0.007	0.028
Bone mineral content, g	2.07	2.15	2.17	2.31	2.39	0.111	0.002
Bone ash content[8]							
Ca, g	2.60	2.54	2.39	2.51	2.71	0.131	0.119
P, g	1.25	1.23	1.16	1.21	1.31	0.064	0.115
Ca, %	39.5	39.1	38.4	37.2	39.4	1.51	0.758
P, %	19.1	18.9	18.7	18.1	19.1	0.75	0.803
Histopathology[9]							
Physeal score	0.69	0.24	0.05	0.78	0.20	0.275	0.040
Infraction score	0.43	0.08	0.08	0.52	0.00	0.288	0.868
Trabeculae bone thickness, mm	46.8	48.6	48.3	55.5	48.4	2.16	0.244

[abc]Means within a row with different superscripts differ ($P < 0.05$).

[1]A total of 882 pigs were used in a 112-d finishing trial with 20 pigs per pen and 9 pens per treatment. Pigs were placed on experimental diets at approximately 73 lb.

[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake; F/G = feed efficiency.

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

[4]HyD provided 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 to the diet (DSM Nutritional Products, Parsippany, NJ).

[5]Serum Ca and P were measured at the Iowa State University Veterinary Diagnostic Lab (Ames, IA) as a part of the large animal complete profile. The vitamin D serum analysis was conducted at Heartland Assays (Ames, IA).

[6]One pig per pen (9 pigs per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 days as a means of removing water and fat. Bones were then dried at 221°F for 7 days and then ashed in a muffle furnace at 1,112°F for 24 h.

[7]One pig per pen (9 pens per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then dried at 221°F for 7 days and then ashed in a muffle furnace at 1,112°F for 24 h.

[8]After bone ash was completed, the ash was digested and sent to the K-State Research and Extension Soil Testing Laboratory, Manhattan, KS, for analysis of Ca and P via ICP.

[9]The left fibula, 2nd rib, and 10th rib were taken to Iowa State University VDL for analysis of histopathology. Each bone was scored on a scale of 0 to 3 on the severity of fractures on the physis of each growth plate.

Table 4. Effect of STTD P, phytase, and vitamin D on bone analysis of finishing pigs[1]

	Metacarpal	Fibula	2nd rib	10th rib	SEM	*P*-value
Defatted bone ash[2]						
Bone ash, g	8.82[a]	4.45[b]	4.34[b]	8.85[a]	0.152	< 0.001
Bone ash, %	60.9[c]	66.7[a]	62.6[b]	63.0[b]	0.208	< 0.001
Non-defatted bone ash[3]						
Bone ash, g	8.83[b]	5.15[c]	5.34[c]	9.52[a]	0.190	< 0.001
Bone ash, %	41.8[c]	52.9[a]	50.4[b]	51.3[ab]	0.57	< 0.001
Dual-energy X-ray absorptiometry						
Bone mineral density, g/cm^2	0.35[a]	0.13[c]	0.13[c]	0.16[b]	0.005	< 0.001
Bone mineral content, g	5.29[d]	0.80[b]	0.17[a]	2.62[c]	0.090	< 0.001
Bone ash content[4]						
Ca, g	3.29[a]	1.80[b]	1.67[b]	3.43[a]	0.108	< 0.001
P, g	1.56[a]	0.88[b]	0.82[b]	1.67[a]	0.053	< 0.001
Ca, %	37.1	40.4	38.6	37.8	1.34	0.482
P, %	17.6	19.7	18.9	18.9	0.67	0.472
Histopathology[5]						
Physeal score	---	0.29	0.49	0.40	0.216	0.179
Infraction score	---	0.30	0.10	0.27	0.219	0.726
Trabeculae bone thickness, mm	---	65.2[a]	41.4[b]	42.0[b]	1.34	< 0.001
Cortical bone thickness, mm	---	167.1[a]	130.7[b]	131.4[b]	3.27	0.001

[abc]Means within a row with different superscripts differ ($P < 0.05$).

[1]A total of 882 pigs were used in a 112-d finishing trial with 20 pigs per pen and 9 pens per treatment. Pigs were placed on experimental diets at approximately 73 lb.

[2]One pig per pen (9 pigs per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 days as a means of removing water and fat. Bones were then dried at 221°F for 7 days and then ashed in a muffle furnace at 1,112°F for 24 h.

[3]One pig per pen (9 pens per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then dried at 221°F for 7 days and then ashed in a muffle furnace at 1,112°F for 24 h.

[4]After bone ash was completed, the ash was digested and sent to the K-State Research and Extension Soil Testing Laboratory (Manhattan, KS) for analysis of Ca and P via ICP.

[5]The left fibula, 2nd rib, and 10th rib were taken to the Iowa State University Veterinary Diagnostic Lab (Ames, IA) for analysis of histopathology. Each bone was scored on a scale of 0 to 3 on the severity of fractures on the physis of each growth plate.

Table 5. Interactive effects of STTD P, phytase, and vitamin D on bone analysis

STTD P, %:	80% NRC[1]	NRC	NRC	Industry	Industry	
Vitamin D:	Industry	Industry	Industry	Industry	Industry + HyD[2]	
Phytase:	No	No	Yes	Yes	Yes	SEM
Bone density, g/mL[3]						0.016
Metacarpal	1.28	1.31	1.30	1.32	1.31	
Fibula	1.43	1.46	1.45	1.48	1.49	
2nd rib	1.30	1.33	1.30	1.34	1.30	
10th rib	1.37^{ab}	1.37^{ab}	1.33^{b}	1.40^{a}	1.38^{ab}	
Non-defatted bone ash, %[4]						1.28
Metacarpal	39.8	42.9	41.1	42.6	42.8	
Fibula	51.2	53.0	52.6	53.4	54.3	
2nd rib	49.2	51.0	50.0	51.9	49.6	
10th rib	51.8	52.3	48.9	51.5	52.1	
Non-defatted bone ash, g[5]						0.421
Metacarpal	8.50	9.01	8.50	9.04	9.08	
Fibula	4.86	5.37	4.78	5.32	5.42	
2nd rib	4.91	5.36	5.16	5.77	5.50	
10th rib	9.24	9.81	8.74	9.71	10.12	
Defatted bone ash, %[6]						0.488
Metacarpal	60.8	61.0	60.6	60.5	61.6	
Fibula	66.3	67.1	66.5	66.9	66.8	
2nd rib	62.1	63.7	62.5	62.9	62.1	
10th rib	62.2	63.7	62.8	63.1	63.2	
Defatted bone ash, g[5]						0.337
Metacarpal	8.54	8.62	8.82	9.08	9.07	
Fibula	4.26	4.56	4.18	4.76	4.49	
2nd rib	4.01	4.27	4.11	4.84	4.49	
10th rib	8.22	8.98	8.23	9.25	9.55	
Histopathology physeal score[7]						0.382
Fibula	0.50	0.38	0.00	0.11	0.44	
2nd rib	1.39	0.32	0.01	0.58	0.15	
10th rib	0.18	0.01	0.14	1.66	0.01	
Dual-energy X-ray absorptiometry						
Bone mineral content, g[8]						0.200
Metacarpal	5.09	5.10	5.69	5.33	5.26	
Fibula	0.69	0.82	0.77	0.77	0.93	
2nd rib	0.12	0.16	0.12	0.23	0.21	
10th rib	2.39^{bc}	2.53^{abc}	2.09^{c}	2.92^{ab}	3.18^{a}	

continued

Table 5. Interactive effects of STTD P, phytase, and vitamin D on bone analysis

STTD P, %:	80% NRC[1]	NRC	NRC	Industry	Industry	
Vitamin D:	Industry	Industry	Industry	Industry	Industry + HyD[2]	
Phytase:	No	No	Yes	Yes	Yes	SEM
Bone mineral density, g/cm[5]						0.012
Metacarpal	0.33	0.34	0.37	0.36	0.34	
Fibula	0.12	0.13	0.13	0.12	0.15	
2nd rib	0.11	0.13	0.14	0.14	0.14	
10th rib	0.15[ab]	0.16[ab]	0.13[b]	0.17[ab]	0.19[a]	
Bone ash content						
Phosphorus, g[5]						0.118
Metacarpal	1.71	1.57	1.50	1.37	1.66	
Fibula	0.90	0.86	0.67	0.95	0.81	
2nd rib	0.83	0.87	0.72	0.86	0.82	
10th rib	1.56	1.61	1.57	1.67	1.95	
Calcium, g[5]						0.254
Metacarpal	3.61	3.29	3.14	2.89	3.50	
Fibula	1.84	1.79	1.76	1.97	1.66	
2nd rib	1.69	1.79	1.46	1.76	1.66	
10th rib	3.25	3.31	3.20	3.40	4.00	
Phosphorus, %[5]						1.57
Metacarpal	18.4	18.6	17.1	15.3	18.6	
Fibula	21.1	19.0	20.4	20.0	17.8	
2nd rib	18.1	20.1	18.2	18.7	19.5	
10th rib	18.7	18.0	19.1	18.2	20.4	
Calcium, %[5]						3.15
Metacarpal	38.9	39.0	35.9	32.4	39.3	
Fibula	43.3	39.5	41.3	41.3	36.8	
2nd rib	36.9	41.1	37.1	38.3	39.6	
10th rib	39.0	36.9	39.2	36.9	41.8	

[abc]Means within a row with different superscripts differ ($P < 0.05$).

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

[2]HyD provided 2,000 IU/kg of vitamin D equivalency from 25(OH)D_3 to the diet (DSM Nutritional Products, Parsippany, NJ).

[3]Bone density was measured on each bone based on Archimedes' principle. Bone × treatment, $P = 0.053$.

[4]One pig per pen (9 pens per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then dried at 221°F for 7 days and then ashed in a muffle furnace at 1,112°F for 24 h. Bone × treatment, $P = 0.542$.

[5]Bone × treatment, $P > 0.10$.

[6]One pig per pen (9 pigs per treatment) were euthanized and the left and right metacarpal, fibula, 2nd rib, and 10th rib were collected to determine bone ash weight and percentage bone ash. All bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 days as a means of removing water and fat. Bones were then dried at 221°F for 7 days, and then ashed in a muffle furnace at 1,112°F for 24 h. Bone × treatment, $P = 0.702$.

[7]The left fibula, 2nd rib, and 10th rib were taken to the Iowa State University Veterinary Diagnostic Lab (Ames, IA) for analysis of histopathology. Each bone was scored on a scale of 0 to 3 on the severity of fractures on the physis of each growth plate. Bone × treatment, $P = 0.174$.

[8]Bone × treatment, $P = 0.078$.