Kansas Agricultural Experiment Station Research Reports

Volume 10
Issue 6 *Swine Day*

Article 15

2024

Effects of Added 25(OH)D3 With Varying Standardized Total Tract Digestibility of Phosphorus Levels on Nursery Pig Performance, Bone and Urine Characteristics, and Serum Vitamin D

Larissa L. Becker
Kansas State University

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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Funding Source

Appreciation is expressed to dsm-firmenich for financial support of this trial.

Authors

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Effects of Added 25(OH)D_3 With Varying Standardized Total Tract Digestibility of Phosphorus Levels on Nursery Pig Performance, Bone and Urine Characteristics, and Serum Vitamin D

Larissa L. Becker, Jordan T. Gebhardt,[1] Mike D. Tokach, Robert D. Goodband, Joel M. DeRouchey, Jason C. Woodworth, and Jon R. Bergstrom[2]

Summary

A total of 360 pigs (DNA 600 × 241; initially 12.8 ± 1.18 lb) were used in a 45-d growth study to evaluate the effects of added 25(OH)D_3 with three levels of STTD P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D. Pigs were weaned at approximately 19 d of age and randomly allotted to one of six dietary treatments. A total of 72 pens were used with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 (0 or 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3; Hy-D, dsm-firmenich, Plainsboro, NJ) and STTD P (70, 100, or 130% of the NRC requirement estimate on a dietary percentage basis). All diets contained 1,653 IU/kg of vitamin D_3 provided by the vitamin premix, but the premixes did not contain additional 25(OH)D_3. Diets were corn-soybean meal-based and fed in three phases. On d 45, one pig per pen was euthanized to collect the right fibula, metacarpal, and second and tenth ribs. Overall, increasing STTD P increased (quadratic, $P < 0.003$) BW, ADG, ADFI, and improved F/G with minimal improvement above 100% of the NRC STTD P requirement. Added 25(OH)D_3 had no effect ($P > 0.10$) on growth performance. Pigs fed increasing STTD P had decreased urinary Ca concentration (linear, $P < 0.001$ and quadratic, $P = 0.078$) and increased urinary P concentration (quadratic, $P < 0.001$), with all pigs fed 70% of the NRC STTD P requirement having non-detectable levels of P in the urine. Added 25(OH)D_3 had no effect ($P > 0.10$) on urine Ca and P concentrations. A quadratic 25(OH)D_3 × STTD P interaction ($P = 0.032$) was observed for serum 25(OH)D_3. When pigs were fed added 25(OH)D_3, serum 25(OH)D_3 increased (quadratic, $P = 0.005$) as STTD P increased but no differences ($P > 0.10$) were observed when no 25(OH)D_3 was added and STTD P increased. A quadratic STTD P × 25(OH)D_3 interaction was observed for 1,25(OH)$_2$$D_3$ concentrations ($P = 0.002$). When pigs were fed added 25(OH)D_3, serum 1,25(OH)$_2$$D_3$ decreased

[1] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.
[2] dsm-firmenich, Plainsboro, NJ

(quadratic, $P < 0.001$) as STTD P increased. When no $25(OH)D_3$ was included, no differences were observed ($P > 0.10$). No STTD P × $25(OH)D_3$ interaction was observed for $24,25(OH)_2D_3$. Pigs fed diets with added $25(OH)D_3$ had increased ($P < 0.001$) serum $24,25(OH)_2D_3$ concentration. Added $25(OH)D_3$ had no effect ($P > 0.10$) on serum $1,25(OH)_2D_3$ but concentrations decreased (quadratic, $P = 0.001$) as STTD P increased. Bone density of metacarpals increased (linear, $P = 0.001$) as STTD P increased, as did the fibulas and ribs (quadratic, $P \leq 0.055$). The percentage bone ash and bone ash weight increased (quadratic, $P \leq 0.065$) for all bones as STTD P increased. Added $25(OH)D_3$ had no effect on bone density or bone ash weight for any of the bones ($P > 0.10$); however, the reduction in bone ash observed with reducing STTD P level tended to be less when $25(OH)D_3$ was provided (linear interaction, $P = 0.098$). In summary, added $25(OH)D_3$ had limited effect on growth performance, urine Ca and P, or bone characteristics; however, added $25(OH)D_3$ increased serum concentrations of $25(OH)D_3$ and $24,25(OH)_2D_3$. Increasing STTD P to 100% of NRC (2012) requirement estimate increased growth and 130% of NRC maximized bone ash.

Introduction

Vitamin D_3 must be activated through a two-step hydroxylation process. After absorption in the small intestine, vitamin D_3 is stored in the liver where it is hydroxylated to produce 25-hydroxyvitamin D_3 [$25(OH)D_3$], which is the major circulating metabolite of vitamin D. After hydroxylation in the liver, $25(OH)D_3$ is transported to the kidney and undergoes a second hydroxylation process to become 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$], which is the most active form of vitamin D in the body. The kidney also converts $25(OH)D_3$ into other vitamin D metabolites including $24,25(OH)_2D_3$, which is important for bone mineralization.

Research has observed that $1,25(OH)_2D_3$ affects bone tissue growth by controlling the differentiation and proliferation of the elements in bone.[3] Additionally, $1,25(OH)_2D_3$ and parathyroid hormone function metabolically to control Ca and P metabolism. When diets are deficient in Ca or P, an increase in circulating $1,25(OH)_2D_3$ occurs. Recent research by Zhao et al.[4] observed that when pigs were fed Ca and P deficient diets, supplementation of $25(OH)D_3$ and phytase improved growth performance and bone development of weaned pigs. Furthermore, Williams et. al[5] observed increased levels of serum $1,25(OH)_2D_3$ when pigs were fed P-deficient diets indicating that metabolic processes occur to increase circulating $1,25(OH)_2D_3$ when insufficient levels of P are consumed. The authors also observed a decrease in grams of P in bone ash when fed P-deficient diets, which means that bone mineralization is only optimized when

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[3] Lee, S. S. A. Clark, R. K. Gill, and S. Christakos. 1994. 1,25-Dihydroxyvitamin D3 and pancreatic Beta-cell function: vitamin D receptors, gene expression, and insulin secretion. Endocrinology. 134:1602-1610. doi: 0013-7227/94/1344-1602$03.00/O

[4] Zhao, Y., X. Wen, H. Xiao, L. Hou, X. Wang, Y. Huang, Y. Lin, C. Zheng, L. Wang, and Z. Jiang. 2021. Effects of phytase and 25-hydroxyvitamin D3 supplementation on growth performance and bone development in weaned piglets in Ca- ad P-deficient dietary. J. Sci. Food Agric. 102: 940-948. doi:10.1002/jsfa.11426

[5] Williams, H. R., T. E. Chin, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, J. R. Bergstrom, M. C. Rahe, C. L. Siepker, P. Sitthicharoenchai, S. L. Radke, S. M. Ensley, and J. T. Gebhardt. 2023. The effect of bone and analytical methods on the assessment of bone mineralization response to dietary phosphorus, phytase, and vitamin D in nursery pigs. J. Anim. Sci 101:1-15 doi: 10.1093/jas/skad353

adequate levels of Ca and P are in the diet. Research with 25(OH)D_3 in diets with adequate P and vitamin D_3 has not altered bone ash in most studies. Our hypothesis was that supplementing 25(OH)D_3 would increase growth performance and bone development when pigs are fed diets deficient or marginally deficient in P compared to pigs fed P-deficient diets without supplementation of 25(OH)D_3. The objective of this study was to determine the effects of added 25(OH)D_3 with three levels of STTD P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D.

Materials and Methods

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 Teaching and Research Center in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder, and nipple waterer for ad libitum access to feed and water.

Animals and diets

A total of 360 pigs (DNA 600 × 241; initially 12.8 ± 1.18 lb) were used in a 45-d growth study to evaluate the effects of added 25(OH)D_3 with three levels of STTD P on nursery pig growth performance, bone and urine characteristics, and serum vitamin D. Pigs were weaned at approximately 19 d of age and randomly allotted to one of six dietary treatments with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 (0 or 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3; Hy-D, dsm-firmenich, Plainsboro, NJ) and standardized total tract digestibility (STTD) P (70, 100, or 130% of the NRC[6] requirement estimate on a dietary percentage basis). Diets were corn-soybean meal-based and fed in three phases (Tables 1 and 2). All diets contained 1,653 IU/kg of vitamin D_3 provided by the vitamin premix, but the premixes did not contain additional 25(OH)D_3. Pigs were weighed on d 0, 10, 17, 24, 31, 38, and 45 to determine ADG, ADFI, and feed efficiency.

Fecal characteristics

On d 45 of the experiment, fecal samples were collected from two pigs per pen. Samples were collected into individual sealable plastic bags for each pig. Fecal dry matter (DM) was analyzed independently on all samples. Fecal DM was determined by drying the fecal sample at 131°F (55°C) for 48 h.

Bone, blood, and urine analyses

On d 45, one pig per pen (72 pigs total) was euthanized and used for the analysis of bones, blood, and urine. The right metacarpal, fibula, second rib, and tenth rib were collected from each pig to determine bone density and percentage bone ash. After removal, bones were stored at -4°F until analysis. Bone density was measured on each bone based on the Archimedes principle. For the bone ash that was measured using the de-fatted processing method, bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 221°F for 7 d in a drying oven and then ashed in a muffle furnace at 1,112°F for 24 h to determine the percentage of ash relative to dried bone weight. Ten mL of blood was

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

collected to measure serum $25(OH)D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$. Approximately 10 mL of urine was collected directly from the bladder to measure Ca, P, and creatinine.

Statistical analysis

Growth performance, vitamin D status in blood, urine concentrations, and bone characteristic data were analyzed as a randomized complete block design. Pen was considered the experimental unit. Treatment was used as the fixed effect and block was used as the random effect. Pig BW was incorporated within the blocking structure. Linear and quadratic contrasts were evaluated within increasing STTD P treatments. Fecal dry matter data were analyzed as repeated measures representing multiple observations on each pen, and pen nested within treatment was included in the model as a random intercept to account for subsampling attributed to the multiple observations for each experimental unit. For bone characteristics, treatment, bone, and the associated interactions were considered fixed effects, with block and pig serving as random effects. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

No added $25(OH)D_3$ × STTD P interactions were observed ($P > 0.10$) for growth performance (Table 3).

For phase 1 (d 0 to 10), increasing STTD P increased (linear, $P \leq 0.031$; Table 4) BW, ADG, and improved F/G. No differences ($P > 0.10$) were observed for ADFI. Added $25(OH)D_3$ had no effect on growth performance ($P > 0.10$).

For phase 2 (d 10 to 22), increasing STTD P increased (quadratic, $P \leq 0.015$) BW, ADG, ADFI, and improved F/G with pigs fed 100% of the NRC STTD P requirement estimate having the greatest performance. Added $25(OH)D_3$ had no effect on growth performance ($P > 0.10$).

For phase 3 (d 22 to 45), increasing STTD P increased (quadratic, $P \leq 0.022$) BW, ADG, ADFI, and improved F/G with the greatest response as STTD P increased from 70 to 100% of the NRC requirement estimate with a smaller improvement in ADG and F/G as STTD P increased from 100 to 130% of the NRC STTD P requirement estimate. Added $25(OH)D_3$ had no effect on BW, ADG, or ADFI ($P > 0.10$), but did result in poorer ($P < 0.036$) F/G.

Overall (d 0 45), pigs fed increasing STTD P from 70 to 130% of the NRC requirement estimate had increased (quadratic, $P < 0.003$) BW, ADG, ADFI, and improved F/G with minimal improvement above 100% of the NRC STTD P requirement estimate. Added $25(OH)D_3$ had no effect on growth performance ($P > 0.10$). The growth performance data does not support our hypothesis that added $25(OH)D_3$ would increase growth performance when pigs were fed diets deficient or marginally deficient STTD P.

No treatment differences were observed for fecal DM ($P > 0.10$).

Blood and urine analysis

No added 25(OH)D_3 × STTD P interactions were observed ($P > 0.10$) for blood or urine concentrations.

No treatment differences were observed ($P > 0.10$) for creatinine. Pigs fed increasing STTD P tended to have decreased urinary Ca excretion (quadratic, $P = 0.078$) and increased urinary P excretion (quadratic, $P < 0.001$), with all pigs fed 70% of the NRC STTD P requirement having non-detectable levels of P in the urine. For pigs fed 130% of the NRC STTD P requirement estimate, eight out of 22 samples had non-detectable levels of Ca. Added 25(OH)D_3 had no effect on urine Ca and P concentrations ($P > 0.10$).

For serum 25(OH)D_3, a quadratic 25(OH)D_3 × STTD P interaction was observed ($P = 0.032$). When pigs were fed added 25(OH)D_3, serum 25(OH)D_3 increased as STTD P increased (quadratic, $P = 0.005$). However, when no 25(OH)D_3 was added, no differences were observed for 25(OH)D_3 concentrations ($P > 0.10$). A quadratic 25(OH)D_3 × STTD P interaction ($P = 0.002$) was also observed for 1,25(OH)$_2D_3$ concentrations. When pigs were fed added 25(OH)D_3, serum 1,25(OH)$_2D_3$ decreased (quadratic, $P < 0.001$) as STTD P increased. However, when no 25(OH)D_3 was added, no differences were observed for 1,25(OH)$_2D_3$ concentrations ($P > 0.10$). Pigs fed diets with added 25(OH)D_3 had increased ($P < 0.001$) 24,25(OH)$_2D_3$ concentrations. Added 25(OH)D_3 had no effect on 1,25(OH)$_2D_3$ concentrations ($P > 0.10$). No added 25(OH)D_3 × STTD P interaction was observed for 24,25(OH)$_2D_{3.}$ Serum 1,25(OH)$_2D_3$ decreased (quadratic, $P = 0.001$) as STTD P increased.

Bone characteristics

For bone density, a marginally significant linear 25(OH)D_3 × STTD P × bone interaction was observed ($P = 0.058$; Table 5). Bone density of metacarpals increased as STTD P increased (linear, $P = 0.001$). For fibulas, second ribs, and tenth ribs, bone density increased as STTD P increased (quadratic, $P \leq 0.055$). Added 25(OH)D_3 had no effect on bone density for any of the bones ($P > 0.10$). Additionally, a main effect of bone was observed with fibulas having the greatest bone density followed by tenth and second ribs with metacarpals having the lowest bone density ($P < 0.001$).

A marginally significant 25(OH)D_3 × STTD P interaction (linear, $P < 0.10$; Table 6; Figure 1) was observed for percentage bone ash, where percentage bone ash increased numerically as 25(OH)D_3 was added to diets with 70 or 100% the STTD P requirement estimate, but not when added to diets at 130% of NRC requirement estimate. Unlike growth performance responses, for bone characteristics this numeric improvement in percentage bone ash does support our hypothesis of 25(OH)D_3 supplementation increasing bone development when pigs are fed diets deficient or marginally deficient in P. Additionally, percentage bone ash increased (quadratic, $P \leq 0.006$) as STTD P increased with pigs fed 130% of the NRC STTD P requirement estimate having the greatest bone ash for all bones. Added 25(OH)D_3 had no effect on percentage bone ash for any of the bones ($P > 0.10$). A main effect of bone was observed with fibulas, metacarpals, and second ribs having greater percentage bone ash compared to tenth ribs ($P < 0.001$).

No added 25(OH)D_3 × STTD P × bone interactions were observed ($P > 0.10$) for bone ash weight. For all bones, bone ash weight increased (quadratic, $P \leq 0.065$) as STTD P increased with pigs fed 130% of the NRC STTD P requirement estimate having the greatest bone ash weight for all bones. Added 25(OH)D_3 had no effect on bone density or bone ash weight for any of the bones ($P>0.10$); however, percentage bone ash tended to increase at 70 and 100% STTD P, but not at 130% (linear interaction, $P = 0.098$). Additionally, no added 25(OH)D_3 × bone interaction was observed ($P > 0.10$). A main effect of bone was observed with metacarpals having the heaviest bone ash weight followed by tenth ribs, fibulas, and second ribs having the lightest bone ash weight ($P < 0.001$).

In summary, added 25(OH)D_3 had limited effect on growth performance and urine Ca and P; however, added 25(OH)D_3 increased serum concentrations of 25(OH)D_3 and 24,25$(OH)_2D_3$. The addition of 25(OH)D_3 to P-deficient diets increased percentage bone ash. Increasing STTD P increased growth to 100% of NRC requirement estimate and bone ash to 130% of NRC requirements.

Acknowledgements

Appreciation is expressed to dsm-firmenich for financial support of this trial.

Table 1. Diet composition for experimental diets (as-fed basis)[1]

	Phase		
Item	**1[2]**	**2[3]**	**3[4]**
Ingredient, %			
Corn	41.05	54.72	64.43
Soybean meal (46.5% CP)	22.59	27.10	31.36
Whey powder	25.00	10.00	---
Enzymatically treated soybean meal[5]	5.00	2.50	---
Soybean oil	2.00	1.00	---
Calcium carbonate	0.49	0.58	0.64
Monocalcium P (21% P)	0.14	0.32	0.35
Salt	0.34	0.55	0.60
L-Lys-HCl	0.40	0.48	0.45
DL-Met	0.22	0.22	0.20
L-Thr	0.19	0.23	0.22
L-Trp	0.03	0.04	0.03
L-Val	0.14	0.17	0.13
Vitamin and trace mineral premixes[6]	0.40	0.40	0.40
Zinc oxide	0.41	0.27	---
25(OH)D_3[7]	+/-	+/-	+/-
Sand[8]	+/-	+/-	+/-
Total	100	100	100

continued

Table 1. Diet composition for experimental diets (as-fed basis)[1]

Item	Phase		
	1[2]	2[3]	3[4]
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.30
Ile:Lys	59	57	57
Leu:Lys	113	113	118
Met:Lys	36	37	37
Met and Cys:Lys	58	58	58
Thr:Lys	65	65	65
Trp:Lys	19.4	19.6	19.1
Val:Lys	72	72	72
Total Lys, %	1.48	1.49	1.44
ME, kcal/lb	1,537	1,506	1,475
NE, kcal/lb	1,154	1,121	1,088
SID Lys:ME, g/Mcal	5.31	5.46	5.42
CP, %	20.6	20.9	21.1
Ca, %	0.56	0.54	0.51
STTD P, %	0.32	0.28	0.23
Total P, %	0.51	0.49	0.47
Total Ca:Total P	1.10	1.10	1.10

[1]Dietary treatments were arranged in a 2 × 3 factorial with main effects of 25(OH)D_3 and standardized total tract digestibility (STTD) P (70, 100, or 130% of NRC requirement estimate on a dietary percentage basis). Diet composition is reported for the 70% STTD P treatment diets.

[2]Phase 1 diets were fed from approximately d 0 to 10 (13 to 17 lb). Calcium carbonate increased to 0.59 and 0.69% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.82 and 1.56% for 100 and 130% STTD P treatments, respectively.

[3]Phase 2 diets were fed from approximately d 10 to 22 (17 to 27 lb). Calcium carbonate increased to 0.67 and 0.76% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.95 and 1.58% for 100 and 130% STTD P treatments, respectively.

[4]Phase 3 diets were fed from approximately d 22 to 45 (27 to 58 lb). Calcium carbonate increased to 0.72 and 0.78% for 100 and 130% STTD P treatments, respectively. Monocalcium P increased to 0.90 and 1.40% for 100 and 130% STTD P treatments, respectively.

[5]HP 300; Hamlet Protein; Findlay, OH.

[6]No phytase was added in any treatment diets.

[7]Rovimix Hy-D (dsm-firmenich, Parsippany, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D3.

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

Added 25(OH)D_3[2]:	No			Yes		
STTD P, % of NRC:	70	100	130	70	100	130
Phase 1						
Calculated analysis						
Ca, %	0.56	0.72	0.89	0.56	0.72	0.89
P, %	0.51	0.65	0.81	0.51	0.65	0.81
Total Ca:Total P	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition						
Ca, %	0.61	0.65	0.87	0.50	0.75	0.86
P, %	0.50	0.60	0.78	0.48	0.68	0.82
Phase 2						
Calculated analysis						
Ca, %	0.54	0.69	0.84	0.54	0.69	0.84
P, %	0.49	0.63	0.76	0.49	0.63	0.76
Total Ca:Total P	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition						
Ca, %	0.53	0.66	0.83	0.50	0.64	0.83
P, %	0.47	0.65	0.76	0.52	0.67	0.80
Phase 3						
Calculated analysis						
Ca, %	0.51	0.64	0.76	0.51	0.64	0.76
P, %	0.46	0.58	0.69	0.46	0.58	0.69
Total Ca:Total P	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition						
Ca, %	0.64	0.63	0.70	0.46	0.65	0.83
P, %	0.45	0.54	0.68	0.46	0.58	0.67

[1]Complete diet samples were collected during bagging of experimental diets and pooled into one homogenized sample per dietary treatment for each phase. Samples were stored at -4°F until they were submitted for analysis of Ca and P in triplicate (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS).
[2]Rovimix Hy-D (dsm-firmenich, Parsippany, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

Table 3. Effects of added 25(OH)D_3 and STTD P on nursery pig performance[1]

								P =[2]		
Added 25(OH)D_3[3]:	**No**			**Yes**					**STTD P**	
STTD P, % of NRC[4]:	**70%**	**100%**	**130%**	**70%**	**100%**	**130%**	**SEM**	**25(OH)D_3**	**Linear**	**Quadratic**
BW, lb										
d 0	12.8	12.8	12.8	12.8	12.8	12.8	1.18	0.984	0.872	0.710
d 10	16.2	16.5	16.8	16.5	16.8	16.8	1.30	0.232	0.029	0.671
d 22	25.8	27.3	26.5	25.9	27.8	26.6	1.82	0.471	0.051	< 0.001
d 45	51.2	61.2	60.9	51.5	60.1	60.8	3.04	0.682	< 0.001	< 0.001
d 0 to 10 (phase 1)										
ADG, lb	0.34	0.38	0.41	0.38	0.40	0.40	0.021	0.233	0.031	0.707
ADFI, lb	0.42	0.42	0.44	0.44	0.45	0.44	0.025	0.413	0.803	0.930
F/G	1.24	1.12	1.08	1.18	1.12	1.08	0.022	0.324	< 0.001	0.297
d 10 to 22 (phase 2)										
ADG, lb	0.78	0.89	0.80	0.77	0.89	0.82	0.050	0.841	0.300	0.001
ADFI, lb	1.10	1.15	1.07	1.10	1.15	1.07	0.063	0.952	0.452	0.015
F/G	1.40	1.31	1.35	1.43	1.29	1.32	0.013	0.732	0.002	0.001
d 22 to 45 (phase 3)										
ADG, lb	1.10	1.47	1.46	1.11	1.41	1.46	0.063	0.455	< 0.001	< 0.001
ADFI, lb	1.83	2.25	2.19	1.92	2.20	2.23	0.117	0.522	< 0.001	< 0.001
F/G	1.66	1.53	1.50	1.73	1.57	1.53	0.011	0.036	< 0.001	0.022
d 0 to 45										
ADG, lb	0.85	1.07	1.04	0.86	1.04	1.05	0.046	0.879	< 0.001	< 0.001
ADFI, lb	1.32	1.55	1.49	1.37	1.52	1.52	0.079	0.422	< 0.001	< 0.001
F/G	1.55	1.45	1.43	1.60	1.46	1.44	0.009	0.111	< 0.001	0.003

continued

Table 3. Effects of added 25(OH)D_3 and STTD P on nursery pig performance[1]

Added 25(OH)D_3[3]:	No			Yes				P =[2]		
									STTD P	
STTD P, % of NRC[4]:	70%	100%	130%	70%	100%	130%	SEM	25(OH)D_3	Linear	Quadratic
Fecal DM, %[5]	24.2	21.5	22.0	23.8	23.5	23.4	0.83	0.133	0.123	0.255
Urine										
Creatinine										
Count of samples > LOD[6]	11	12	11	12	12	11	---	---	---	---
Concentration, mg/dL	64.4	89.6	80.7	75.1	71.4	85.8	9.96	0.922	0.175	0.635
Calcium										
Count of samples > LOD[7]	10	11	7	12	12	7	---	---	---	---
Concentration, mg/dL	103.9	37.7	7.4	126.0	47.9	12.0	13.00	0.242	< 0.001	0.078
Phosphorus										
Count of samples > LOD[8]	0	5	10	0	2	11	---	---	---	---
Concentration, mg/dL	< 5.5	8.0	76.6	< 5.5	6.1	84.9	9.18	0.770	< 0.001	< 0.001
Serum										
25(OH)D_3, ng/mL[9]	7.2	7.2	8.2	18.7	29.2	25.0	2.07	< 0.001	0.082	0.057
24,25$(OH)_2D_3$, ng/mL	1.6	1.3	2.0	9.8	10.0	8.6	0.52	< 0.001	0.455	0.777
1,25$(OH)_2D_3$, pg/mL[10]	339	257	181	394	203	180	14.8	0.986	< 0.001	0.001

[1]A total of 360 pigs (initially 12.8 ± 1.18 lb) were used with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. Urine samples were collected from one pig per pen (72 pigs total) on d 45.
[2]No added 25(OH)D_3 × STTD P interactions were observed (P > 0.10) unless otherwise noted.
[3]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.
[4]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC requirement estimate treatment diet in phases 1, 2, and 3 respectively.
[5]Fecal samples were collected from two pigs per pen (144 samples total) on d 45.
[6]Creatinine was detectable for all samples and represents the total samples for each treatment.
[7]The limit of detection (LOD) was 1 mg/dL. When the concentration was below the LOD, a value of 1 mg/dL was used.
[8]The LOD was 5.5 mg/dL. When the concentration was below the LOD, a value of 5.5 mg/dL was used.
[9]Quadratic 25(OH)D_3 × STTD P interaction, P = 0.032. Quadratic STTD P within added 25(OH)D_3, P = 0.005. Quadratic STTD P within no 25(OH)D_3, P = 0.860.
[10]Quadratic 25(OH)D_3 × STTD P interaction, P = 0.002. Quadratic STTD P within added 25(OH)D_3, P < 0.001. Quadratic STTD P within no 25(OH)D_3, P = 0.877. Linear STTD P within added 25(OH)D_3 and no 25(OH)D_3, P < 0.001.

Table 4. Main effect of added 25(OH)D_3 and STTD P on nursery pig performance[1]

	Added 25(OH)D_3[2]				STTD P, % of NRC[3]				*P* =	
	No	Yes	SEM	*P* =	70%	100%	130%	SEM	Linear	Quadratic
BW, lb										
d 0	12.8	12.8	1.18	0.984	12.8	12.8	12.8	1.18	0.872	0.710
d 10	16.5	16.7	1.29	0.232	16.4	16.7	16.8	1.30	0.029	0.671
d 22	26.5	26.8	1.80	0.471	25.8	27.5	26.6	1.80	0.051	< 0.001
d 45	57.8	57.5	2.97	0.682	51.3	60.7	60.8	2.99	< 0.001	< 0.001
d 0 to 10 (phase 1)										
ADG, lb	0.38	0.40	0.014	0.233	0.36	0.39	0.41	0.016	0.031	0.707
ADFI, lb	0.43	0.44	0.018	0.413	0.43	0.43	0.44	0.020	0.803	0.930
F/G	1.15	1.13	0.015	0.324	1.21	1.12	1.08	0.017	< 0.001	0.297
d 10 to 22 (phase 2)										
ADG, lb	0.82	0.83	0.044	0.841	0.78	0.89	0.81	0.046	0.300	0.001
ADFI, lb	1.11	1.11	0.058	0.952	1.10	1.15	1.07	0.059	0.452	0.015
F/G	1.35	1.35	0.008	0.723	1.42	1.30	1.34	0.009	0.002	0.001
d 22 to 45 (phase 3)										
ADG, lb	1.35	1.33	0.057	0.455	1.11	1.44	1.46	0.059	< 0.001	< 0.001
ADFI, lb	2.09	2.12	0.111	0.522	1.88	2.23	2.21	0.113	< 0.001	< 0.001
F/G	1.57	1.61	0.008	0.036	1.70	1.55	1.52	0.009	< 0.001	0.022
d 0 to 45										
ADG, lb	0.99	0.98	0.043	0.879	0.85	1.06	1.05	0.044	< 0.001	< 0.001
ADFI, lb	1.45	1.47	0.076	0.422	1.34	1.54	1.51	0.077	< 0.001	< 0.001
F/G	1.48	1.50	0.007	0.111	1.58	1.45	1.44	0.008	< 0.001	0.003

continued

Table 4. Main effect of added 25(OH)D_3 and STTD P on nursery pig performance[1]

	Added 25(OH)D_3[2]				STTD P, % of NRC[3]				*P* =	
	No	Yes	SEM	*P* =	70%	100%	130%	SEM	Linear	Quadratic
Fecal DM, %[4]	22.5	23.6	0.48	0.133	24.0	22.5	22.7	0.59	0.123	0.255
Urine										
Creatinine										
Count of samples > LOD[5]	34	36	---	---	23	24	22	---	---	---
Concentration, mg/dL	78.2	77.4	5.67	0.922	69.7	80.5	83.3	7.04	0.175	0.635
Calcium										
Count of samples > LOD[6]	28	31	---	---	22	23	14	---	---	---
Concentration, mg/dL	49.7	61.9	7.40	0.242	114.9	42.8	9.7	9.19	< 0.001	0.078
Phosphorus										
Count of samples > LOD[7]	15	13	---	---	0	7	21	---	---	---
Concentration, mg/dL	30.0	32.2	5.22	0.770	< 5.5	7.0	80.8	6.49	< 0.001	< 0.001
Serum										
25(OH)D_3, ng/mL[8]	7.5	24.3	1.19	< 0.001	12.9	18.2	16.6	1.46	0.082	0.057
24,25$(OH)_2D_3$, ng/mL	1.6	9.5	0.30	< 0.001	5.7	5.6	5.3	0.36	0.455	0.777
1,25$(OH)_2D_3$, pg/mL[9]	259	259	8.8	0.986	367	230	180	10.6	< 0.001	0.001

[1]A total of 360 pigs (initially 12.8 ± 1.18 lb) were used with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. No 25(OH)D_3 × STTD P interactions were observed (*P* > 0.10).
[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.
[3]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC treatment diet in phases 1, 2, and 3 respectively.
[4]Fecal samples were collected from two pigs per pen (144 samples total) on d 45.
[5]Creatinine was detectable for all samples and represents the total samples for each treatment.
[6]The limit of detection (LOD) was 1 mg/dL. When the concentration was below the LOD, a value of 1 mg/dL was used.
[7]The LOD was 5.5 mg/dL. When the concentration was below the LOD, a value of 5.5 mg/dL was used.
[8]Quadratic 25(OH)D_3 × STTD P interaction, *P* = 0.032. Quadratic STTD P within added 25(OH)D_3, *P* = 0.005. Quadratic STTD P within no 25(OH)D_3, *P* = 0.860.
[9]Quadratic 25(OH)D_3 × STTD P interaction, *P* = 0.002. Quadratic STTD P within added 25(OH)D_3, *P* < 0.001. Quadratic STTD P within no 25(OH)D_3, *P* = 0.877. Linear STTD P within added 25(OH)D_3 and no 25(OH)D_3, *P* < 0.001.

Table 5. Effects of added 25(OH)D_3 and STTD P on nursery pig bone characteristics[1]

							P =		
Added 25(OH)D_3[2]:	No			Yes				STTD P	
STTD P, % of NRC[3]:	70%	100%	130%	70%	100%	130%	25(OH)D_3	Linear	Quadratic
Bone density, g/mL[4]									
Fibula	1.21	1.32	1.35	1.23	1.33	1.29	0.656	< 0.001	< 0.001
Second rib	1.13	1.20	1.25	1.11	1.23	1.25	0.789	< 0.001	0.055
Tenth rib	1.15	1.22	1.27	1.14	1.24	1.27	0.944	< 0.001	0.039
Metacarpal	1.13	1.17	1.19	1.13	1.17	1.19	0.918	0.001	0.290
Bone ash, %[5]									
Fibula	52.6	56.9	59.9	53.2	58.8	59.3	0.250	< 0.001	0.006
Second rib	50.2	57.1	60.3	52.1	58.5	59.6	0.103	< 0.001	0.001
Tenth rib	49.2	54.9	58.6	49.1	56.8	57.6	0.620	< 0.001	0.001
Metacarpal	51.9	57.2	59.5	53.4	58.2	58.6	0.337	< 0.001	0.001
Bone ash, g[6]									
Fibula	0.63	1.12	1.32	0.63	1.12	1.32	0.931	< 0.001	0.009
Second rib	0.41	0.81	1.06	0.37	0.82	0.98	0.501	< 0.001	0.065
Tenth rib	0.73	1.54	1.84	0.75	1.51	1.80	0.715	< 0.001	< 0.001
Metacarpal	1.11	1.66	1.84	1.08	1.64	1.84	0.720	< 0.001	0.001

[1]A total of 360 pigs (initially 12.8 ± 1.18 lb) were used with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 3 factorial with main effects of added 25(OH)D_3 and STTD P. Per treatment, 12 pigs were euthanized (72 pigs total) and the right metacarpal, fibula, second rib, and tenth rib were collected to determine bone density, bone ash weight, and percentage bone ash utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 221°F for 7 d in a drying oven and then ashed in a muffle furnace at 1,112°F for 24 h. Bone density was measured on each bone based on the Archimedes principle.

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC requirement estimate treatment diet in phases 1, 2, and 3 respectively.

[4]Bone density was measured on each bone based on the Archimedes principle. Linear added 25(OH)D_3 × STTD P × bone interaction, $P = 0.058$. Added 25(OH)D_3 effect for all bones, $P = 0.938$. Linear STTD P effect across all bones, $P < 0.001$. Quadratic STTD P effect across all bones, $P = 0.001$. Main effect of bone, $P < 0.001$. SEM = 0.016.

[5]Bone ash percentage was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, $P > 0.10$. Added 25(OH)D_3 effect for all bones, $P = 0.183$. Linear and quadratic STTD P effect for all bones, $P < 0.001$. Main effect of bone, $P < 0.001$. SEM = 0.67.

[6]Bone ash weight was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, $P > 0.10$. Added 25(OH)D_3 effect for all bones, $P = 0.675$. Linear STTD P effect for all bones, $P < 0.001$. Quadratic STTD P effect for all bones, $P = 0.001$. Main effect of bone, $P < 0.001$. SEM = 0.081.

Table 6. Effects of added 25(OH)D_3 and STTD P on nursery pig bone characteristics[1]

								P =				
								25(OH)D_3 × STTD P		STTD P		
Added 25(OH)D_3[2]:	**No**			**Yes**								
STTD P, % of NRC[3]:	**70**	**100**	**130**	**70**	**100**	**130**	**SEM**	**Linear**	**Quad**	**Linear**	**Quad**	**25(OH)D_3**
Bone density, g/mL[4]	1.15	1.23	1.27	1.15	1.24	1.25	0.011	0.406	0.163	< 0.001	0.001	0.938
Bone ash, %[5]	51.0	56.5	59.6	52.0	58.1	58.8	0.54	0.098	0.114	< 0.001	< 0.001	0.183
Bone ash, g[6]	0.72	1.28	1.52	0.71	1.27	1.48	0.075	0.876	0.887	< 0.001	0.001	0.675

[1]A total of 360 pigs (initially 12.8 ± 1.18 lb) were used with five pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × three factorial with main effects of added 25(OH)D_3 and STTD P. Per treatment, 12 pigs were euthanized (72 pigs total) and the right metacarpal, fibula, second rib, and tenth rib were collected to determine bone ash weight and percentage bone ash utilizing the de-fatted processing method. Bones were cleaned of tissue and then placed in Soxhlet extractors containing petroleum ether for 7 d to remove water and fat. Bones were dried at 221°F for 7 d in a drying oven and then ashed in a muffle furnace at 1,112°F for 24 h.

[2]Rovimix Hy-D (dsm-firmenich, Plainsboro, NJ) included at 50 μg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3.

[3]STTD P levels were 0.45, 0.40, and 0.33% for the 100% of NRC treatment diet in phases 1, 2, and 3 respectively.

[4]Bone density was measured on each bone based on the Archimedes principle. Linear added 25(OH)D_3 × STTD P × bone interaction, $P = 0.058$. Main effect of bone, $P < 0.001$.

[5]Bone ash percentage was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, $P > 0.10$. Main effect of bone, $P < 0.001$.

[6]Bone ash weight was measured on each bone utilizing the de-fatted processing method. Linear and quadratic added 25(OH)D_3 × STTD P × bone interaction, $P > 0.10$. Main effect of bone, $P < 0.001$.

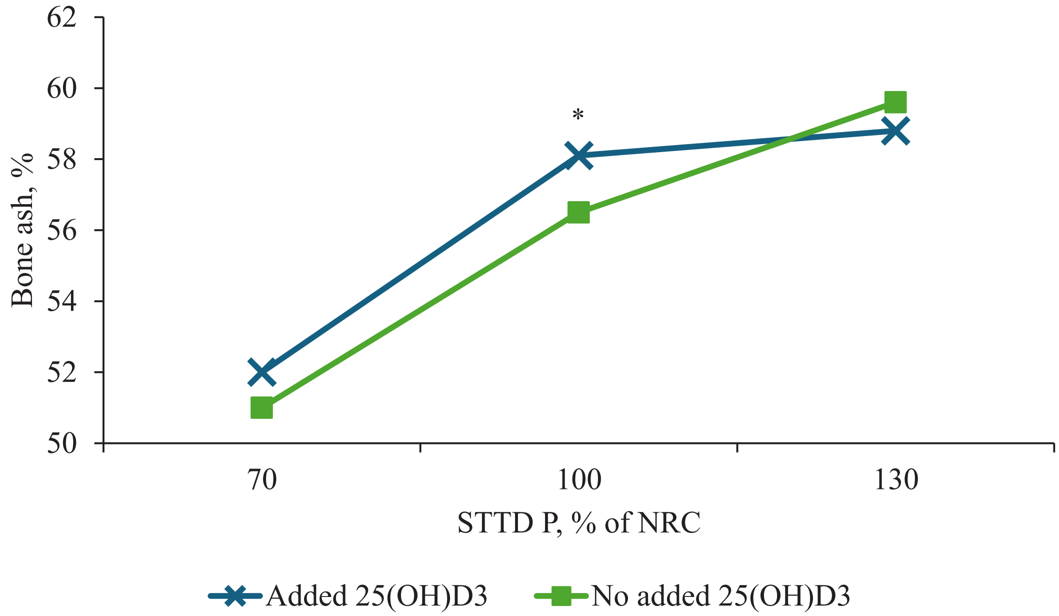

Figure 1. Effects of added 25(OH)D_3 and STTD P on bone ash percentage (25(OH)D_3 × STTD P, linear, *P* = 0.098). Added 25(OH)D_3 (Rovimix Hy-D; dsm-firmenich, Plainsboro, NJ) at 50 µg/kg estimated to provide an additional 2,000 IU/kg of vitamin D_3. *, effect of 25(OH)D_3 within STTD P level, *P* < 0.05. Error bars represent ± 1 standard error of the mean.