An evaluation of the effects of added vitamin D_3 in maternal diets on sow and pig performance[1,2]

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ABSTRACT: A total of 84 sows (PIC 1050) and their litters were used to determine the effects of supplementing maternal diet with vitamin D_3 on sow and pig performance, serum 25-hydroxyvitamin D_3 (25(OH) D_3), milk vitamin D_3, neonatal bone mineralization, and neonatal tissue vitamin D_3. After breeding, sows were randomly assigned to 1 of 3 dietary vitamin D_3 treatments (1,500, 3,000, or 6,000 IU/kg of complete diets). Sows were bled on d 0 and 100 of gestation and at farrowing and weaning (d 21). Pig BW was recorded at birth and weaning, and serum was collected from 2 pigs/litter at birth, on d 10 and at weaning. A total of 54 pigs (18/treatment) were euthanized at birth and necropsied to sample bones and tissues. Sow and suckling pig performance and neonatal bone ash and bone density did not differ among maternal vitamin D_3 treatments; however, sow 25(OH)D_3 and milk vitamin D_3 increased (linear, $P < 0.01$) with increasing maternal vitamin D_3 supplementation. Piglet serum 25(OH) D_3 increased (quadratic, $P < 0.03$) with increased maternal vitamin D_3. Neonatal kidney vitamin D_3 tended (quadratic, $P = 0.08$) to decrease with increasing maternal vitamin D_3, but liver vitamin D_3 tended (linear, $P = 0.09$) to increase with increasing maternal vitamin D_3. At weaning, a subsample of 180 pigs (PIC 327 × 1050) were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 and 2 levels of dietary vitamin D_3 (1,800 or 18,000 IU/kg) from d 0 to 10 postweaning on nursery growth and serum 25(OH)D_3. Overall (d 0 to 35), nursery ADG and G:F were not affected by either concentration of vitamin D_3, but ADFI tended (quadratic, $P < 0.06$) to decrease with increasing maternal vitamin D_3 as pigs from sows fed 3,000 IU had lower ADFI compared with pigs from sows fed 1,500 or 6,000 IU/kg. Nursery pig serum 25(OH)D_3 increased with increasing maternal vitamin D_3 (weaning) on d 0 (linear, $P < 0.01$), and maternal × diet interactions ($P < 0.01$) were observed on d 10 and 21 because pigs from sows fed 1,500 IU had greater increases in serum 25(OH)D_3 when fed 18,000 IU compared with pigs from sows fed 3,000 IU. In conclusion, sow and pig serum 25(OH)D_3, milk vitamin D_3, and neonatal tissue vitamin D_3 can be increased by increasing maternal vitamin D_3, and nursery pig 25(OH)D_3 can be increased by increasing dietary vitamin D_3; however, sow and pig performance and neonatal bone mineralization was not influenced by increasing vitamin D_3 dietary levels.

Key words: nursery pigs, sow nutrition, vitamin D, vitamin D_3, 25-hydroxyvitamin D_3

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INTRODUCTION

Speculation has surfaced recently about serum 25-hydroxyvitamin D_3 (**25(OH)D_3**) concentrations of nursery pigs reared in modern swine production facilities. This is mainly due to documented cases, in which vitamin D has been absent from premixes fed to pigs (Feedstuffs, 2010; Salas, 2011), leading to metabolic bone disease and rickets. In an attempt to prevent deficiency, veterinarians have advocated guidelines on serum 25-hydroxyvitamin D (**25(OH)D**)concentra-

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tions that are associated with deficiency and adequacy of the vitamin from similar values used in human health (IOM, 1997; Specker et al., 1992). A survey of swine indicated that a substantial proportion of pigs have serum 25(OH)D_3 concentrations below guidelines, especially weaned pigs (Madson, 2011). Additionally, recent research describing the presence of the vitamin D receptor in numerous soft tissues not associated with Ca and P homeostasis (Norman and Bouillon, 2010) indicates that vitamin D may play a role in other biologically important functions of the body.

Vitamin D can be supplemented to the suckling pig in synthetic forms; however, this increases production and labor cost. Goff et al. (1984) reported increased piglet serum 25(OH)D_3 concentrations in newborn pigs from sows dosed with vitamin D_3 intramuscularly, indicating that vitamin D and its metabolites are transferred transplacentally. No research has examined the influence of maternal dietary vitamin D_3 on vitamin D status of young pigs (measured by serum 25(OH)D_3), which would represent a less expensive and less labor intensive form of supplement for the young pig. The objectives of this experiment were to evaluate whether increasing vitamin D status through maternal vitamin D_3 supplementation (2, 4, and 8 times requirement; NRC, 2012) affects maternal performance, milk vitamin D_3 concentrations, sow and piglet serum 25(OH)D_3 concentrations, subsequent pig growth, neonatal pig liver and kidney vitamin D_3 concentrations, and neonatal bone mineralization.

MATERIALS AND METHODS

Experimental procedures and animal care were approved by the Kansas State University Institutional Animal Care and Use Committee. This experiment was conducted at the Kansas State University Swine Teaching and Research Facility (Manhattan, KS) from the months of January through August of 2012. Gestation and lactation sow diets and phase 2 and phase 3 nursery diets were prepared at the Kansas State University Animal Science Feed Mill (Manhattan, KS). Phase 1 nursery diets were prepared at the Kansas State University Grain Science Feed Mill (Manhattan, KS). All diets were formulated to meet or exceed nutrient requirement estimates (NRC, 2012).

A total of 84 sows (PIC 1050; PIC, Hendersonville, TN) and their litters were used to determine the effects of supplementing varying levels (2, 4, and 8 times requirement; NRC, 2012) of dietary vitamin D_3 on maternal performance, subsequent pig performance, sow and piglet serum 25(OH)D_3, Ca, and P, milk vitamin D, neonatal bone mineralization, and piglet tissue vitamin D_3 concentrations. Following breeding, sows were randomly assigned to 1 of 3 dietary vitamin D_3 treatments (1,500, 3,000, or 6,000 IU/kg) within 3 consecutive farrowing groups. There were 28 sows per treatment and 7 to 11 replications per farrowing group. During d 0 to 110 of gestation, sows were housed in gestation stalls (2.13 by 0.61 m) and were fed 2.0 kg/d of the gestation diets. On d 110, sows were transported to the farrowing house and were housed in farrowing crates. Both the gestation and farrowing barns were totally enclosed, environmentally controlled, and mechanically ventilated buildings. The farrowing barn contained 29 farrowing crates (2.13 by 0.61 m for the sow and 2.13 by 0.96 m for the pigs) that were each equipped with a single feeder and nipple waterer. After farrowing, sows were switched to lactation diets. Gestation and lactation diets were formulated to contain 0.56 and 0.94% standardized ileal digestible Lys, respectively (Table 1). Gestation and lactation diets contained 40 and 20% dried distillers grains with solubles, respectively. For the first 3 d after farrowing, sows were gradually provided increased feed according to appetite. After d 3, all sows were allowed ad libitum access to the lactation diet. Temperature in the farrowing house was maintained at a minimum of 20°C, and supplemental heat was provided to piglets with heat lamps.

Lactation feed intake was determine by measuring feed disappearance on d 0, 7, 14, and 21 (weaning). Sow BW was measured at breeding, d 110 of gestation, within 24 h after farrowing, and at weaning to determine gestation weight gain and lactation weight loss. Sows were bled on d 0 and 100 of gestation, within 12 h after farrowing, and on d 10 and 21 (weaning) in lactation to determine serum 25(OH)D_3, Ca, and P concentrations. Milk samples were collected within 12 h after farrowing and on d 10 and 21 (weaning) to determine milk vitamin D_3 concentrations. Milk samples were obtained after an intravenous injection of oxytocin (1 mL; Agrilabs, St. Joseph, MO), and milk was collected from all functional glands. At birth, all piglets were weighed individually and ear tagged for identification. The second and fifth pigs born within each litter were bled before suckling on d 0 and 10 and at weaning to determine piglet serum 25(OH)D_3, Ca, and P. The seventh pig born from 54 litters (18 pigs per treatment and 6 replications per farrowing group) was euthanized before suckling and necropsied for bone and tissue sample analysis to determine neonatal pig bone ash content, bone density, and tissue vitamin D_3 concentrations. Mummified and stillborn pigs were recorded to calculate total born and live born piglets. Although minimal, cross-fostering was conducted within 24 h postfarrowing to help standardize litter size within vitamin D_3 dietary treatments. Pigs were weighed after fostering to measure fostered litter weight. At weaning, piglet weights and piglet counts were recorded to determine individual and litter weight gains along with survivability.

Table 1. Composition of sow diets (as-fed basis)[1,2]

Item	Gestation	Lactation
Ingredient, %		
Corn	52.95	52.19
Soybean meal (46.5% CP)	2.99	23.88
Dried distillers grains with solubles	40.00	20.00
Monocalcium P (21% P)	0.65	0.90
Limestone	1.90	1.60
Salt	0.50	0.50
Vitamin premix[3]	0.50	0.50
Trace mineral premix[4]	0.15	0.15
L-Lys HCl	0.23	0.15
Phytase[5]	0.13	0.13
Total	100	100
Calculated analysis		
ME, kcal/kg	3,289	3,281
CP, %	17.0	21.1
Total Lys, %	0.72	1.13
Standardized ileal digestible Lys, %	0.56	0.97
Ca, %	0.88	0.88
P, %	0.59	0.64
Available P,[6] %	0.50	0.48

[1]A total of 84 sows and litters were used to determine the effects of supplemental vitamin D_3 on maternal performance, subsequent pig performance, sow and piglet serum 25-hydroxyvitamin D_3, Ca, and P, milk vitamin D, neonatal bone mineralization, and piglet tissue vitamin D concentrations.

[2]Vitamin D_3 premixes were mixed to contain 2,024,620 IU vitamin D_3/kg of premix by blending vitamin D_3 with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.

[3]Vitamin premix provided 11,023 IU vitamin A, 1,378 IU vitamin D_3, 44 IU vitamin E, 4.41mg menadione, 8.27 mg riboflavin, 27.56 mg pantothenic acid, 49.60 mg niacin, 38.5 μg vitamin B_{12}, 551 mg choline, 0.22 mg biotin, 1.65 mg folic acid, and 4.96 mg pyridoxine per kilogram of the complete diet.

[4]Trace mineral premix provided 39.68 mg Mn as manganous oxide, 151.84 mg Fe ferrous sulfate, 151.84 mg Zn as zinc oxide, 15.18 mg Cu copper sulfate, 0.30 mg I as potassium iodide, and 0.30 mg Se as sodium selenite per kilogram of the complete diet.

[5]Natuphos 600 (BASF, Florham Park, NJ). Provided 752 phytase units/kg of diet.

[6]Phytase provided 0.12% available P to gestation and lactation diets.

At weaning, a subsample of 180 multisex pigs (PIC 327 × 1050; PIC) from the first sow group were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 concentration and 2 levels of dietary vitamin D_3 (1,800 or 18,000 IU/kg from d 0 to 10 postweaning) on growth performance and serum 25(OH)D_3, Ca, and P. At weaning, pigs were allotted to pens based on their previously administered maternal vitamin D_3 treatments to maintain the integrity of weaning weights consistent with maternal vitamin D_3 effects. Pens were then randomly assigned to dietary vitamin D_3 treatments. There were 6 pigs/pen and 5 pens/treatment. Dietary vitamin D_3 treatments were provided from d 0 to 10 in the nursery and were fed in a pellet form (Table 2). Common phase 2 and 3 diets were provided to pigs from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1,800 IU vitamin D_3/kg. All pens (1.2 by 1.5 m) had woven wire flooring, one 3-hole, dry self-feeder, and a nipple waterer to allow for ad libi-

Table 2. Composition of nursery diets (as-fed basis)[1,2]

Item	Phase 1[3]	Phase 2[4]	Phase 3[5]
Ingredient, %			
Corn	39.56	44.72	65.76
Soybean meal (46.5% CP)	17.33	23.41	30.67
Dried distillers grains with solubles	5.00	15.00	–
Spray-dried porcine plasma	5.00	–	–
Spray-dried blood cells	1.25	–	–
Spray-dried whey	25.00	10.00	–
Select menhaden fish meal	–	4.50	–
Soybean oil	3.00	–	–
Monocalcium P (21% P)	0.85	0.15	1.03
Limestone	1.00	0.70	0.98
Salt	0.30	0.30	0.35
Zinc oxide	0.39	0.25	–
Trace mineral premix[6]	0.15	0.15	0.15
Vitamin premix[7]	0.25	0.25	0.25
L-Lys HCl	0.20	0.28	0.36
DL-Met	0.13	0.05	0.13
L-Thr	0.05	0.05	0.13
L-Ile	0.10	–	–
Phytase[8]	0.13	0.17	0.17
Acidifier[9]	0.20	–	–
Vitamin E, 20,000 IU	0.05	–	–
Choline chloride 60%	0.04	–	–
Vitamin D premix[10]	0.02	0.02	0.02
Total	100	100	100
Calculated analysis			
ME, kcal/kg	3,415	3,320	3,314
CP, %	21.2	23.1	20.4
Standardized ileal digestible Lys, %	1.35	1.30	1.25
Ca, %	0.80	0.70	0.68
P, %	0.71	0.63	0.61
Available P,[11] %	0.63	0.50	0.42

[1]A total of 180 pigs (initially 21 d of age; PIC 327 × 1050; PIC, Hendersonville, TN) were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 and early nursery dietary vitamin D_3 on nursery growth performance and serum 25-hydroxyvitamin D_3 concentrations.

[2]Vitamin D_3 premixes were mixed to contain 2,024,620 IU vitamin D_3/kg of premix by blending vitamin D_3 with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.

[3]Phase 1 diets were fed from d 0 to 10 and were formulated to contain either 1,800 or 18,000 IU vitamin D_3/kg of the complete diet.

[4]Common phase 2 diets were fed from d 10 to 24 and were formulated to contain 1,800 IU vitamin D_3/kg of the complete diet.

[5]Common phase 3 diets were fed from d 24 to 35 and were formulated to contain 1,800 IU vitamin D_3/kg of the complete diet.

[6]Trace mineral premix provided 39.68 mg Mn as manganous oxide, 151.84 mg Fe as ferrous sulfate, 151.84 mg Zn as zinc oxide, 15.18 mg Cu as copper sulfate, 0.30 mg I as potassium iodide, and 0.30 mg Se as sodium selenite per kilogram of the complete diet.

[7]Vitamin premix provided 11,023 IU vitamin A, 1,378 IU vitamin D_3, 44 IU vitamin E, 4.41mg menadione, 8.27 mg riboflavin, 27.56 mg pantothenic acid, 49.60 mg niacin, and 38.5 μg of vitamin B_{12} per kilogram of the complete diet.

[8]Natuphos 600 (BASF, Florham Park, NJ). Provided 780, 1,021, and 1,021 phytase units/kg of the complete diet for phase 1, 2, and 3 diets, respectively.

[9]Kem-Gest: phosphoric, fumaric, lactic, and citric acid (Kemin Industries Inc., Des Moines, IA).

[10]Vitamin D_3 premixes were mixed to contain 2,204,620 IU/kg of premix by blending vitamin D_3 (Rovimix D, DSM Nutritional Products, Parsippany, NJ) with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.

[11]Phytase provided 0.12, 0.13, and 0.12% available P for phase 1, 2, and 3 diets, respectively.

tum access to feed and water. All pigs and feeders were weighed on d 0, 5, 10, 17, 21, 28, and 35 after weaning to determine ADG, ADFI, and G:F. Blood samples were collected from 10 pigs/treatment on d 0, 10, 24, and 35 to determine serum 25(OH)D_3, Ca, and P.

Feed and Premix Vitamin D_3 Analysis

Vitamin D_3 supplement (Rovimix D_3, 500,000 IU/g; DSM Nutritional Products, Parsippany, NJ) was mixed with rice hulls to achieve a premix formulated to contain 2,204,620 IU vitamin D_3/kg. Premix was then added to the control diet (1,378 IU vitamin D_3/kg) by replacing corn to achieve desired dietary treatments. Vitamin premixes and complete diet samples were collected during feed manufacturing. These samples were pooled by specific diet type or premix and were subsampled. Subsamples were analyzed for vitamin D_3 by using a combination of HPLC and mass spectrometry (DSM Nutritional Products; Schadt et al., 2012).

Serum 25-Hydroxyvitamin D_3, Milk and Tissue Vitamin D, and Serum Ca and P Analysis

All blood, milk, and tissue sample analyses were conducted by a commercial laboratory (Heartland Assays, Ames, IA). Blood samples were collected via jugular venipuncture using 25-mm (neonatal and nursery pigs) and 38-mm (sows) × 20-gauge needles and 10-mL blood collection tubes containing a gel separator to determine circulating serum 25(OH)D_3, Ca, and P concentrations. Six hours after collection, blood was centrifuged (1,600 × *g* for 25 min at 2°C) and serum was harvested and stored at –20°C until analysis. Serum 25(OH)D_3 concentrations were determined by using a previously described RIA (Hollis et al., 1993), and serum Ca concentrations were determined by spectrophotometry with a commercial kit (Pointe Scientific, Canton, MI) using a method described by Pointe Scientific (2009a). Serum P was determined by spectrophotometry with a commercial kit (Pointe Scientific) using a method described by Pointe Scientific (2009b). Milk and whole tissue samples were frozen at –20°C until analysis. Analysis was conducted using a combination of HPLC and mass spectrometry previously described by Schadt et al. (2012).

Necropsies, Bone and Tissue Sampling, and Analysis

Necropsies were performed onsite and in compliance with the college's standard operating procedures. Pigs were euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI). Right femurs and second ribs were collected to determine bone ash content and left second ribs were used to determine bone density. Whole liver and kidney tissues were collected and frozen immediately at –20°C until samples were prepared for specific analysis.

Bone densities were determined at the Iowa State University College of Veterinary Medicine (Ames, IA). All left second ribs were stripped to the periosteum, submerged in water for 4 h under 83 kPa vacuum, and blotted dry before recording bone weight. Bone volume was determined using weight in air minus weight under water according to Archimedes principle (Keenan et al., 1997). Bone density values were then expressed as grams of bone per milliliter volume.

Bone ash analysis, which was performed on the right femurs and right second rib, was conducted at the Kansas State University Swine Nutrition Laboratory (Manhattan, KS). Bones were cleaned to the periosteum and were split perpendicular to the long axis of the diaphysis. Fat was extracted by placing bones in cellulose thimbles and inserting thimbles into the main chambers of soxhlet extractors. The extraction solvent was petroleum ether. At the completion of the 7-d extraction period, bone samples were dried in a forced-air oven at 100°C until a consistent dry weight was achieved. Then, bones were ashed at 600°C for 24 h. Ash weights were recorded and expressed as a percentage of dry fat-free bone.

Statistical Analysis

Data were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). Treatment means were analyzed using the LSMEANS statement, and preplanned contrasts were used to determine the linear and quadratic effects of increasing vitamin D_3. Unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS. Maternal performance data were analyzed with sow as the experimental unit, treatment as a fixed effect, and farrowing group as a random effect. Nursery performance was analyzed as a 3 × 2 split plot design and the pen was the experimental unit. Additional preplanned contrasts were used to determine the effects of early nursery vitamin D_3 treatments and the interaction of maternal vitamin D_3 and early nursery dietary vitamin D_3 treatments. Serum 25(OH)D_3, Ca, and P and milk vitamin D_3 data were analyzed using the REPEATED function of SAS to determine the effects of treatment variables over time, and the individual pig was the experimental unit. Bone ash, bone density, and tissue vitamin D concentrations were analyzed using the individual pig as the experimental unit. Differences among treatments were considered significant with $P \leq 0.05$ and trends if $P > 0.05$ and $P \leq 0.10$.

RESULTS

Analysis of vitamin D_3 concentrations in the diets were presented in Table 3. The results verified that they were within acceptable analytical error of formulated dietary values.

Maternal Performance

Supplementation of vitamin D_3 at levels used in this study did not influence sow lactation ADFI (Table 4) or sow BW throughout gestation and lactation. Additionally, the levels of maternal vitamin D_3 within the experiment did not affect litter size criteria or suckling pig performance. Four sows were removed from experimental treatments during the study. A first parity sow from the 3,000 IU vitamin D_3/kg group was removed due to later term abortion before entry into the farrowing house, and another first parity sow from the 3,000 IU vitamin D_3/kg treatment was euthanized and a caesarean section was performed due to farrowing complications. One fifth parity sow from the 6,000 IU vitamin D_3/kg treatment group was removed due to a uterine infection after parturition. A fourth parity sow from the 6,000 IU vitamin D_3/kg group was removed due to lingering structural issues that caused lameness within 3 wk after being administered onto the treatment diets. None of the removed sows showed signs associated with the onset of hypervitaminosis D, which would include weight loss, lethargic behavior, or feed refusal.

Nursery Performance

During the nursery portion of the study, no interactions of maternal vitamin D_3 and dietary vitamin D_3 on growth performance were observed (Table 5). Overall (d 0 to 35), ADFI tended (quadratic, $P = 0.06$) to decrease with increasing vitamin D_3, with pigs from sows fed 3,000 IU having lower ADFI than pigs from sows fed either 1,500 or 6,000 IU/kg.

Sow Serum 25-Hydroxyvitamin D_3, Ca, and P and Milk Vitamin D_3

A maternal × day interaction ($P < 0.01$; Table 6) was observed for sow serum 25(OH)D_3 because serum 25(OH)D_3 was not different among sows on d 0 of gestation; however, increasing maternal vitamin D_3 increased serum 25(OH)D_3 on d 100 of gestation, at farrowing, and at weaning. A day effect ($P < 0.01$; Fig. 1) was observed for serum Ca. Serum Ca tended (linear, $P = 0.07$) to be greater on d 0 of gestation for sows assigned to the 6,000 IU vitamin D_3 treatment compared with sows assigned to lower maternal vitamin D_3 treatments. On d 100 of gestation, increasing maternal dietary vitamin D_3 tended to decrease ($P = 0.09$) serum Ca. Serum P concentrations were not influenced by maternal vitamin D_3 treatments or by sampling day (Fig. 1). Milk vitamin

Table 3. Analyzed dietary vitamin D_3 concentrations (as-fed)[1]

				Nursery diets, IU/kg[3]			
Item	Maternal diets, IU/kg[2]			Phase 1[4]		Phase 2[5]	Phase 3[6]
Formulated	1,500	3,000	6,000	1,800	18,000	1,800	1,800
Analyzed	–	–	–	1,870	19,300	1,855	1,911
Gestation	1,505	3,370	8,025	–	–	–	–
Lactation	1,475	3,390	6,210	–	–	–	–

[1]Vitamin D_3 analysis was performed by DSM Nutritional Products (Parsippany, NJ), and values represent the average of 2 pooled samples per diet.

[2]A total of 81 sows and litters were used to determine the effects of supplemental vitamin D_3 on maternal performance, subsequent pig performance, sow and piglet serum 25-hydroxyvitamin D_3 (25(OH)D_3), Ca, and P, milk vitamin D, neonatal bone mineralization, and piglet tissue vitamin D concentrations.

[3]A total of 180 pigs (PIC 327 × 1050; initially 21 d of age) were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 and early nursery dietary vitamin D_3 on nursery growth performance and serum 25(OH)D_3 concentrations.

[4]Phase 1 diets were fed from d 0 to 10.

[5]Common phase 2 diets were fed from d 10 to 24.

[6]Common phase 3 diets were fed from d 24 to 35.

Table 4. The effects of high maternal vitamin D_3 on sow and litter performance[1,2]

	Vitamin D_3, IU/kg				*P*-value	
Item	1,500	3,000	6,000	SEM	Linear	Quadratic
Sows, *n*	28	26	26			
ADFI, kg						
d 0 to wean (d 21)	5.65	5.88	5.98	0.34	0.27	0.63
Sow BW, kg						
Gestation						
d 0	193.1	194.1	192.1	8.8	0.91	0.89
d 110	231.4	235.2	237.1	6.0	0.52	0.80
Change	+38.3	+41.1	+45.0	5.4	0.24	0.92
Lactation						
d 0	221.9	227.6	224.1	6.0	0.89	0.50
d 21 (weaning)	212.3	220.3	217.4	7.5	0.67	0.42
Change	–9.6	–7.3	–6.7	2.8	0.24	0.43
Piglets						
Litter size, no.						
Mummies	0.3	0.2	0.3	0.1	0.88	0.86
Stillborn	0.6	0.4	0.8	0.3	0.60	0.37
Total born alive	13.0	12.5	13.2	0.9	0.74	0.57
Fostered	12.3	12.1	13.0	0.7	0.50	0.48
Weaned	11.2	10.8	11.5	0.7	0.48	0.32
Survivability,[3] %	91.2	89.2	88.5	2.0	0.88	0.58
Piglet BW, kg						
Birth	1.31	1.36	1.34	0.04	0.63	0.47
Weaning	5.31	5.55	5.52	0.17	0.43	0.42

[1]A total of 84 sows (PIC 1050; PIC, Hendersonville, TN) and their litters were used. There were 2 sows removed from the 3,000 IU/kg vitamin D_3 treatment because of lameness and illness. There were 2 sows removed from the 6,000 IU/kg vitamin D_3 treatment because of late-term abortion and farrowing complications.

[2]Sow group was used as a random effect in the statistical model.

[3]Survivability was calculated by dividing the weaned litter size by the fostered litter size.

Table 5. The effects of maternal and early nursery vitamin D_3 supplementation on nursery pig growth performance (d 0 to 35)[1]

Item	Maternal vitamin D_3, IU/kg							*P*-value			
	1,500		3,000		6,000				Maternal		
Vitamin D_3[2]:	1,800	18,000	1,800	18,000	1,800	18,000	SEM	Maternal × diet	Linear	Quadratic	Diet
ADG, g	417	419	391	399	416	396	13	0.56	0.56	0.12	0.75
ADFI, g	597	618	577	565	602	576	17	0.39	0.47	0.06	0.69
G:F	0.70	0.68	0.68	0.71	0.69	0.69	0.01	0.17	0.96	0.71	0.90

[1]A total of 180 mixed-sex pigs (initially 21 d of age; PIC 327 × 1050; PIC, Hendersonville, TN) were weaned from the first sow group and used in a 3 × 2 split plot design for 35 d to determine the effects of maternal and early nursery dietary vitamin D_3 on growth performance.

[2]Early dietary vitamin D_3 treatments (IU/kg). Phase 1 diets were fed from d 0 to 10. Common phase 2 and 3 diets were fed from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1,800 IU/kg vitamin D_3. Treatments are expressed as IU/kg of the complete diet.

D_3 concentrations were not influenced by sampling day; however, milk vitamin D_3 increased (linear, $P < 0.01$) with increasing maternal dietary vitamin D_3 at farrowing, on d 10 of lactation, and at weaning.

Table 6. Effects of high maternal vitamin D_3 on serum 25-hydroxyvitamin D_3 ($25(OH)D_3$), Ca, P, milk vitamin D, neonatal bone ash content, and bone density[1,2]

	Maternal vitamin D_3, IU/kg				*P*-value	
Item	1,500	3,000	6,000	SEM	Linear	Quadratic
Sow						
$25(OH)D_3$, ng/mL						
d 0	30.1	26.2	32.0	4.7	0.54	0.27
d 100	33.2	36.5	57.9	4.7	<0.01	0.23
Farrowing	30.1	35.4	56.9	4.7	<0.01	0.38
Weaning	39.3	52.5	66.3	4.7	<0.01	0.31
Milk vitamin D3, ng/g						
Farrowing	1.02	2.33	3.97	0.31	<0.01	0.37
d 10	0.78	2.33	3.73	0.31	<0.01	0.13
Weaning	1.02	1.98	3.53	0.31	<0.01	0.73
Piglet						
$25(OH)D_3$, ng/mL						
Birth	4.5	5.9	9.4	0.8	<0.01	0.03
d 10	4.4	6.2	10.6	0.8	<0.01	<0.01
Weaning	5.6	8.0	14.0	0.8	<0.01	<0.01
Bone ash content, %						
Second rib	43.6	43.6	43.5	0.8	0.95	0.96
Femur	44.9	44.5	44.8	0.6	0.76	0.66
Bone density, g/mL						
Second rib	1.30	1.30	1.31	0.02	0.64	0.56
Tissue vitamin D_3, ng/g						
Kidney	1.68	0.10	1.37	0.84	0.99	0.09
Liver	0.04	0.04	0.19	0.05	0.08	0.16

[1]A total of 84 sows (PIC 1050, PIC, Hendersonville, TN) and their litters were used to determine the effects of high maternal vitamin D_3 on sow and pig performance, serum $25(OH)D_3$, Ca, P, milk vitamin D_3, neonatal bone mineralization, and tissue vitamin D_3.

[2]Day effects were $P < 0.01$, $P = 0.56$, and $P < 0.01$ for sow $25(OH)D_3$, milk vitamin D_3, and piglet $25(OH)D_3$, respectively. Maternal × day interactions were $P < 0.01$, $P = 0.87$, and $P = 0.13$ for sow $25(OH)D_3$, milk vitamin D_3, and piglet $25(OH)D_3$, respectively.

Piglet Serum 25-Hydroxyvitamin D_3, Ca, and P and Neonatal Bone Ash and Density and Tissue Vitamin D

A day effect was observed ($P < 0.01$; Table 6) for piglet serum $25(OH)D_3$ as serum concentrations increased over time from birth to weaning, and serum $25(OH)D_3$ increased (quadratic, $P = 0.03$) with increasing maternal vitamin D_3 at birth, on d 10 in lactation, and at weaning. A day effect was observed ($P < 0.01$; Fig. 2) for serum Ca concentrations. Additionally, serum Ca tended to decrease ($P = 0.08$) with increasing maternal vitamin D_3 on d 10 of lactation. Serum P concentrations (Fig. 2) were not influenced by maternal vitamin D_3 treatments, but they tended to differ based on day of sampling ($P = 0.08$). No differences in bone ash values were observed for femurs or second ribs. Rib bone density was not influenced by maternal vitamin D_3 concentrations. Kidney vitamin D_3 concentrations (quadratic, $P = 0.09$) tended to decrease with increasing maternal vitamin D_3 because pigs from sows fed 3,000 IU vitamin D_3 had much lower tissue vitamin D_3 concentrations than pigs from sows fed 1,500 or 6,000 IU/kg. Liver tissue vitamin D_3 concentrations tended to increase (linear, $P = 0.08$) with increased maternal dietary vitamin D_3.

Nursery Pig Serum 25-Hydroxyvitamin D_3, Ca, and P

A day effect ($P < 0.01$; Table 7) was observed for nursery pig serum $25(OH)D_3$. At weaning (d 0), pig serum $25(OH)D_3$ was increased (linear, $P < 0.01$) with increasing maternal vitamin D_3. Maternal × diet interactions ($P < 0.01$) were observed on d 10 and 21 because pigs from sows fed 1,500 IU vitamin D_3/kg had greater increases in serum $25(OH)D_3$ when fed 18,000 IU vitamin D_3/kg compared with pigs from sows fed 3,000 IU vitamin D_3/kg. On d 35, serum $25(OH)D_3$ concentrations were not different among maternal treatments, but a diet effect ($P = 0.04$) was observed with increased nursery diet vitamin D_3 increasing serum $25(OH)D_3$. Serum Ca and P concentrations were not influenced by maternal or nursery vitamin D_3 concentrations, except for a tendency (quadratic, $P = 0.08$; Fig. 3) for P concentrations to in-

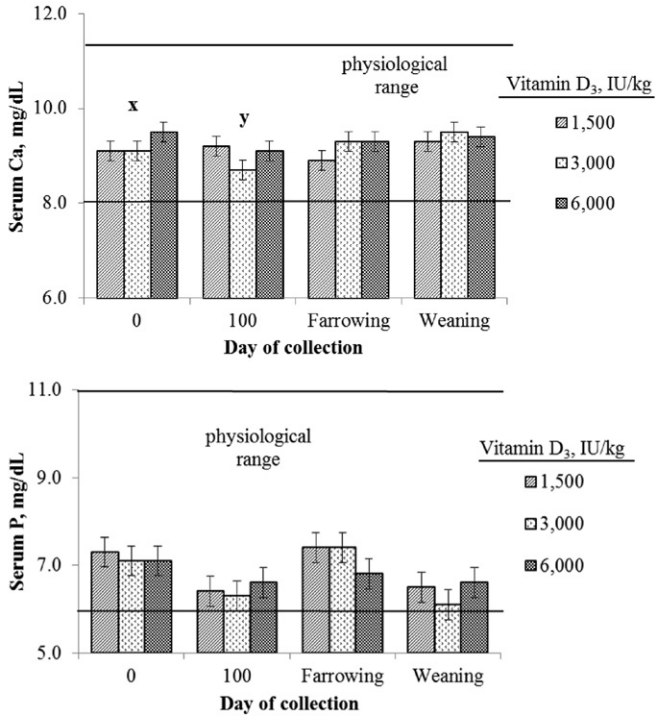

Figure 1. Serum Ca and P concentrations (mg/dL) at d 0 and 100 of gestation, farrowing, and weaning (d 21 of lactation) in sows fed diets formulated to supply 1,500, 3,000, or 6,000 IU vitamin D_3/kg of the complete diet. Superscripts denote tendencies ($0.05 < P \leq 0.10$): [x]linear dietary effects and [y]quadratic dietary effects. Physiological range based on Friendship et al. (1984).

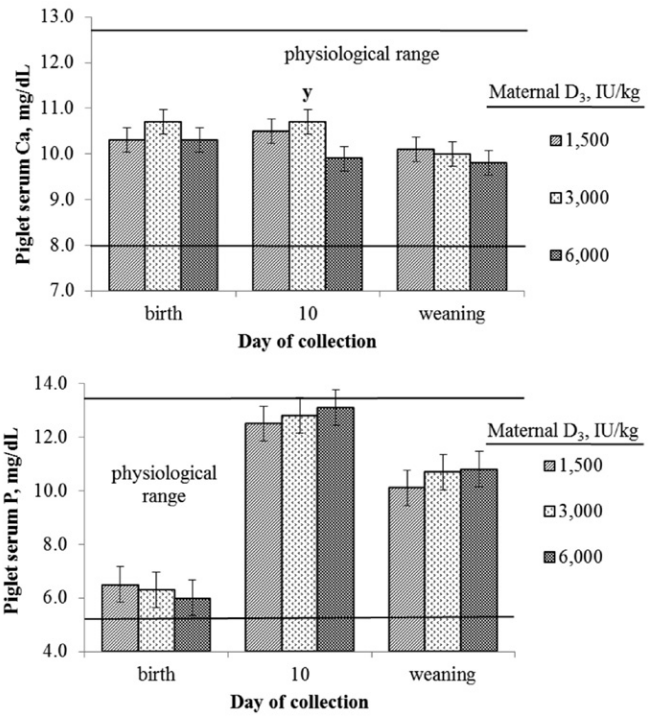

Figure 2. Serum Ca and P concentrations (mg/dL) at birth, and weaning (d 21) in pigs from sows fed diets formulated to supply 1,500, 3,000, or 6,000 IU vitamin D_3/kg of the complete diet. Superscripts denote tendencies ($0.05 < P \leq 0.10$): [y]quadratic maternal diet effect. Physiological range based on Friendship et al. (1984).

crease within increasing maternal vitamin D_3. In addition, a day effect for serum P was observed ($P < 0.01$).

DISCUSSION

The concept of supplementing levels of vitamin D in excess of the requirement is often associated with hypervitaminosis or toxicity. This is due to previous studies describing soft tissue mineralization and even death as a result of toxicity (Chineme et al., 1976; Kamio et al., 1977; Long, 1984). However, in these observed toxicity incidents, supplemented levels were more than 1,000 times the animal's requirement (NRC, 2012). The NRC (1987) presumed the upper safe levels in swine to be 2,200 IU and 33,000 IU per kilogram of the diet when exposure time is greater than 60 or less than 60 d, respectively. These values are 10 to 150 times the growing pig's requirement and 3 to 40 times the sow's requirement (NRC, 2012). Despite this, most diets in the swine industry are formulated to contain 6 to 9 times the growing pig's requirement and 2 times the sow's requirement for vitamin D_3 (Reese and Hill, 2010). To maintain industry applicability, this study was developed to examine the effects of supplementing vitamin D_3 through maternal diets at concentrations above typically formulated levels rather than established requirement levels. To our

Table 7. The effects of maternal and early nursery dietary vitamin D_3 on nursery pig serum 25-hydroxyvitamin D_3 ($25(OH)D_3$; ng/mL)[1,2]

Item	Maternal vitamin D_3, IU/kg							*P*-value			
	1,500		3,000		6,000			Maternal ×	Maternal		
Vitamin D_3[3]:	1,800	18,000	1,800	18,000	1,800	18,000	SEM	diet	Linear	Quadratic	Diet
d 0	6.3		10.5		17.6		3.1		<0.01	0.91	
d 10	20.0	53.5	21.9	49.6	24.0	60.9	2.2	0.01	<0.01	0.04	<0.01
d 21	13.2	26.7	13.6	23.9	14.4	31.6	2.2	0.01	0.16	0.15	<0.01
d 35	16.7	18.0	14.5	19.3	14.9	19.5	2.2	0.42	0.94	0.83	0.04

[1]A total of 180 mixed-sex pigs (initially 21 d of age; PIC 327 × 1050; PIC, Hendersonville, TN) were weaned from the first sow group and used in a 3 × 2 split plot design for 35 d to determine the effects of maternal and early nursery dietary vitamin D_3 on growth performance. Ten pigs per treatment were bled to determine serum $25(OH)D_3$.

[2]Ten pigs per treatment were bled to determine serum $25(OH)D_3$. Day effect, $P < 0.01$, and maternal × diet × day interaction, $P = 0.32$.

[3]Early vitamin D_3 treatments (IU/kg). Phase 1 diets were fed from d 0 to 10. Common phase 2 and 3 diets were fed from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1,800 IU/kg vitamin D_3.

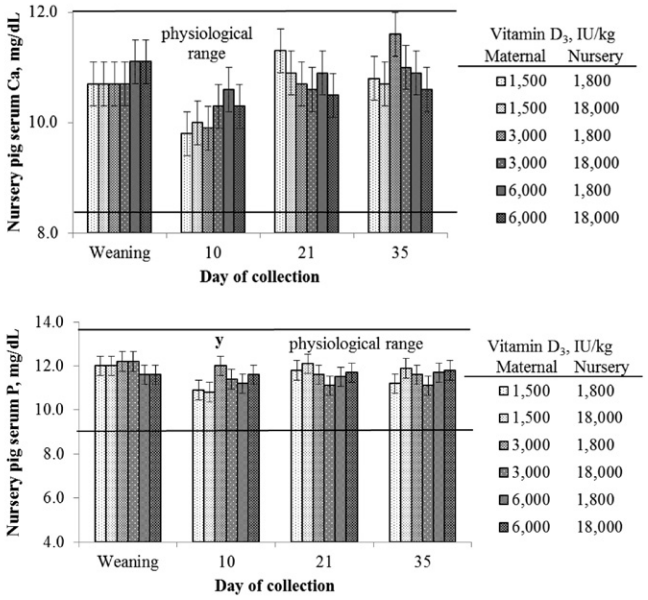

Figure 3. Serum Ca and P concentrations (mg/dL) at weaning, d 10, 21, and 35 in nursery pigs from sows fed diets formulated to supply either 1,500, 3,000, or 6,000 IU vitamin D_3/kg of the complete diet, and fed diets formulated to either 1,800 or 18,000 IU vitamin D_3/kg from weaning until d 10. Superscripts denote tendencies ($0.05 < P \leq 0.10$): ^{y}quadratic maternal diet effect. Physiological range based on Friendship et al. (1984).

knowledge, this is the first study to evaluate vitamin D_3 supplementation up to 6,000 IU vitamin D_3/kg of the complete diet.

No differences were observed in the current study with regards to sow BW change or lactation ADFI from supplementing vitamin D_3 at concentrations between 1,500 and 6,000 IU/kg of the complete diet. Similarly, in a study conducted by Lauridsen et al. (2009), no influence of vitamin D on gilt BW change was observed during the first 28 d of gestation when gilts were fed 4 levels (200, 800, 1,400, or 2,000 IU/kg) of vitamin D from 2 sources (vitamin D_3 or 25(OH)D_3). The authors also reported no influence of dose or form of vitamin D supplementation on BW changes of multiparous sows between 2 and 5 parities throughout gestation or lactation. Interestingly, the investigators discussed an interaction for total feed intake as feed intake decreased with increasing doses of vitamin D_3, which was observed mainly in parity 4 and 5 sows, but for sows fed 25(OH)D_3, the largest decrease in feed intake was observed with increased vitamin D supplementation from 200 to 800 IU. However, the authors pointed out the limitations of the results because of the complexity of the interaction.

Viganò et al. (2003) described the potential role of vitamin D in implantation because of its ability to increase expression of calbindin, an intracellular protein involved in Ca metabolism, and *HoxA* genes, which are shown to affect the viability of pre-implantation embryos. In the current study, no differences in the number of stillborns or live born pigs were observed as a result of supplementing vitamin D_3 between 1,500 and 6,000 IU/kg of the complete diet. Additionally, vitamin D_3 supplementation in maternal diets did not influence birth weight or suckling pig performance to weaning. Lauridsen et al. (2009) reported no effect of dietary form or dose of vitamin D on early reproduction in terms of the number of implanted fetuses in gilts or litter size of sows, but the authors reported reductions in the number of stillborns with increased vitamin D doses of 1,400 and 2,000 IU compared with 200 and 800 IU. Coffey et al. (2012) reported an increased number of developed fetuses in reproductive tracts harvested from first-service gilts when supplemented with 25(OH)D_3 compared with vitamin D_3 at the same supplementation level of 2,500 IU/kg of the complete diet. The authors speculated that this increase in the number of developed fetuses may have been due to the increased efficiency of absorption of 25(OH)D_3 compared with vitamin D_3 in the upper portion of the intestine, which has been observed in poultry (Bar et al., 1980). Research determining the efficiency of absorption of vitamin D_3 and 25(OH)D_3, specifically in swine, has not been conducted, and commercial use of 25(OH)D_3 has not been approved for use in swine in the United States. Ultimately, to evaluate the economic incentive of increasing supplemental vitamin D_3 on the basis of sow productivity, large-scale commercial studies with increased sample sizes will be needed to increase sensitivity and reduce the experimental error that is associated with sow reproduction measurements.

Compared with presumed upper safety guidelines established by NRC (1987), sows in the current experiment were supplemented vitamin D_3 at rates 2 to 3 times the recommended level (2,200 IU/kg of the complete diet) for exposure times greater than 60 d with no adverse effects on feed intake, sow BW, or sow productivity. This may indicate that supplementation rates up to 6,000 IU/kg of complete feeds are safe to use for sows; however, sows in this study were not followed through subsequent parities to determine potential long-term effects. In addition, because of the absence of improvement in maternal performance within this study, increasing vitamin D_3 supplementation above 1,500 IU/kg of complete diet appears to carry no benefit.

Nursery pig performance within the current experiment was not adversely influenced by vitamin D_3 supplementation in maternal diets or in early nursery diets when fed up to 18,000 IU vitamin D_3/kg of the complete diet. Flohr et al. (2013) observed decreased intake of diets supplemented with 44,100 IU/kg vitamin D_3. Also, serum 25(OH)D_3 concentrations in pigs supplemented 18,000 IU of vitamin D_3 ranged from approximately 50 to 60 ng/mL on d 10, which is below previously described concentrations experienced in periods of vitamin D intoxication (Littledike and Horst, 1982). The minimal impact of vitamin D_3 supplementation on nursery pig performance may

be interpreted similarly to previous studies conducted by Wahlstrom and Stolte (1958) and Combs et al. (1966) although vitamin D_3 was supplemented at much greater levels in the current study. Previous research observing improvements in growth performance, as a result of vitamin D_3 supplementation, have been reported consistently when pigs were fed marginal Ca and P, and vitamin D deficiency also was established (Johnson and Palmer, 1939; Bethke et al., 1946; Rortvedt and Crenshaw, 2012). A depletion period was not performed before the initiation of the current study to maintain industry applicability.

Results from this study show that increasing supplementation of vitamin D_3, either through maternal or nursery diets, resulted in increased serum 25(OH)D_3 in sows, neonatal pigs, and nursery pigs. This agrees with previous research conducted by Goff et al. (1984), Lauridsen et al. (2009), Witschi et al. (2011), and Coffey et al. (2012). The observed increase in sow and pig 25(OH)D_3 at parturition supports previous conclusions reported by Goff et al. (1984) who described a strong correlation of sow 25(OH)D_3 and piglet 25(OH)D_3 at birth because of transplacental transfer of 25(OH)D_3. Additionally, the increases in maternal vitamin D_3 supplementation resulted in increases in milk vitamin D_3 concentrations, which agrees with work conducted in dairy cows (Hollis et al., 1983). Additionally, this increase in milk vitamin D_3 concentration contributed to increased piglet serum 25(OH)D_3 from birth (before suckling) to weaning because of milk intake. Nursery pig serum 25(OH)D_3 increased as a result of an interaction between maternal and phase 1 dietary vitamin D_3 supplementation; however, by d 35, nursery pigs were similar in serum 25(OH)D_3 concentrations, confirming the half-life of 25(OH)D_3 is between 10 d and 3 wk, which has been described previously in human research (IOM, 1997).

Liver vitamin D_3 concentrations in the current study tended to increase with increased maternal vitamin D_3, and kidney vitamin D_3 concentrations decreased with increasing maternal vitamin D_3. In general, however, tissue concentrations were low, which agrees with previous research that describes 25(OH)D as the circulating and stored metabolite of the vitamin. Research in rats by Clements and Fraser (1988) showed that vitamin D was transferred transplacentally, especially in the last trimester, and it was stored in fetal muscle, most notably as 25(OH)D_3. Interestingly, research by Schröder et al. (1993) concluded that newborn piglets do not rely on vitamin D-dependent Ca transport until the fourth week postpartum. This may indicate that although newborn pigs have stored vitamin D in the form of 25(OH)D_3, it is not needed in early life Ca absorption. However, more research quantifying 25(OH)D_3 presence in a variety of tissues along with hydroxylating enzyme levels in the newborn pig will help to better understand if vitamin D is useful for preweaning Ca and P absorption during the suckling period.

To our knowledge, previous research quantifying bone mineralization in newborn pigs has not been conducted. Vitamin D supplementation has been shown to influence fetal bone development in the human fetus when mothers are vitamin D deficient (Morley et al., 2006). In the current experiment, bone ash content of ribs and femurs of pigs euthanized at birth was not influenced by maternal vitamin D_3 treatment. Percentage bone ash of ribs and femurs were 15% lower than previously referenced bone ash values of nursery pigs 2 mo of age (Crenshaw et al., 1981). This is probably a function of age, which has been described as a predominant factor in bone mineral content (Crenshaw et al., 1981). In addition, as discussed by Reinhard et al. (1976), differences in ash content of bones depend on skeletal function, and the current study agrees with these results, as rib bone ash percentage was 1% lower than femur bone ash percentage. Bone densities were not different among sampled ribs, which is similar to the result described by Witschi et al. (2011) who reported similar bone mineral content and bone mineral density for pigs (35 d postpartum) from sows fed 200 IU/kg vitamin D or 2,000 IU/kg vitamin D from supplemented 25(OH)D_3. Rortvedt and Crenshaw (2012) reported decreased mineral content and density of femurs in pigs at 9 wk of age, which were fed diets with no supplemental vitamin D and marginal Ca and P concentrations. The decreases in mineral content and density were exacerbated to a greater degree when pigs were from sows fed vitamin D_3–depleted diets. This indicates that dietary Ca and P influences bone mineralization responses of the nursery pig to maternal vitamin D.

Serum Ca and P were not adversely influenced by supplemental vitamin D_3 in maternal diets or in phase 1 nursery diets. All reported concentrations were within normally described physiological ranges (Friendship et al., 1984). In the current study, Ca and P were supplied in excess of the animal's requirements (NRC, 2012), which indicates that vitamin D_3 supplementation above 1,500 IU in maternal diets or 1,800 IU in the nursery did not influence circulating Ca and P. This conclusion is similar to results described by Witschi et al. (2011) who supplemented the complete diet with 200 or 2,000 IU vitamin D/kg using 25(OH)D_3; however, decreases in serum Ca and P have been associated with deficient supplementation rates of vitamin D_3 in growing pigs (Hagemoser et al., 2000).

Overall, the results of this study indicate that supplementing high concentrations of vitamin D_3 to sows can increase sow and piglet serum 25(OH)D_3, milk vitamin D_3 concentrations, and neonatal tissue vitamin D_3 concentrations. Maternal vitamin D_3 and dietary vitamin D_3 supplementation can increase nursery pig serum 25(OH)D_3; however, vitamin D_3 supplementation to either maternal or nursery diets beyond concentrations typically used in commercial diets failed to affect sow or pig performance, neonatal bone mineralization, or serum Ca

and P. This result indicates that supplementing vitamin D_3 levels above 1,500 IU for sows and 1,800 IU to nursery pigs has no beneficial effect.

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