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Evaluating the impact of maternal vitamin D supplementation on sow performance: II. Subsequent growth performance and carcass characteristics of growing pigs[1,2]

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ABSTRACT: A of subsample of 448 growing pigs (PIC 327 × 1050) weaned from 52 sows fed varying dietary vitamin D regimens were used in a split-plot design to determine the effects of maternal and nursery dietary vitamin D on growth performance. Sows were previously administered diets containing vitamin D as vitamin D_3 (800, 2,000, or 9,600 IU/kg) or as 25(OH)D_3 (50 µg [or 2,000 IU vitamin D equivalent]/kg from HyD; DSM Nutritional Products, Parsippany, NJ). Once weaned, pigs were allotted to pens on the basis of previous maternal vitamin D treatment, and then pens were randomly assigned to 1 of 2 nursery vitamin D dietary regimens (2,000 IU of vitamin D_3/kg or 50 µg 25(OH)D_3/kg). Pigs remained on nursery vitamin D treatments for 35 d, and then they were provided common finishing diets until market (135 kg). Growing pig serum 25(OH)D_3 suggested that maternal dietary vitamin D influenced ($P < 0.001$ at weaning) serum concentrations early after weaning, but nursery vitamin D regimen had a larger impact ($P < 0.001$) on d 17 and 35 postweaning. Overall growth performance was not influenced by nursery vitamin D dietary treatments. From d 0 to 35 in the nursery, pigs from sows fed increasing vitamin D_3 had increased (quadratic, $P < 0.003$) ADG and ADFI, but G:F was similar regardless of maternal vitamin D regimen. Also, pigs from sows fed 50 µg/kg of 25(OH)D_3 had increased ($P = 0.002$) ADG compared with pigs weaned from sows fed 800 IU of vitamin D_3. Throughout finishing (d 35 postweaning until 135 kg), ADG was increased (quadratic, $P = 0.005$) and G:F was improved (quadratic, $P = 0.049$) with increasing maternal dietary vitamin D_3. Also, pigs from sows fed 50 µg/kg of 25(OH)D_3 had increased ($P = 0.002$) ADG compared with pigs weaned from sows fed 800 IU of vitamin D_3. Carcass data were collected from a subsample population separate from that used for the growth performance portion of the study, and a total of 642 carcasses from progeny of sows fed the varying dietary vitamin D treatments were used. Live BW of pigs at marketing and HCW were heavier ($P < 0.030$) for pigs from sows previously fed 25(OH)D_3 compared with pigs from sows fed 9,600 IU of vitamin D_3. Overall, pigs from sows fed 2,000 IU of vitamin D_3 grew faster after weaning compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3. Pigs from sows fed 25(OH)D_3 hag greater ADG compared with pigs from sows fed 800 IU of vitamin D_3, and they had increased final BW and HCW compared with pigs from sows fed 9,600 IU of vitamin D_3.

Key words: 25(OH)D_3, finishing pig, growth, nursery pig, vitamin D

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INTRODUCTION

Studies evaluating maternal dietary manipulation have determined that fetal muscle development in swine can be altered on the basis of nutritional strategies (Dwyer et al., 1994; Musser et al., 2004). Dwyer et al. (1993) concluded that differences in the total number of muscle fibers at birth, resulting from

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fetal muscle development, were positively correlated with postnatal growth potential. Additionally, previous research in mice has demonstrated that vitamin D plays a role in fetal muscle development. Endo et al. (2003) concluded that skeletal muscle in knockout mice without the vitamin D receptor (**VDR**) gene had approximately 20% smaller muscle fiber diameters at 3 wk of age compared to wild-type mice.

Hines et al. (2013) evaluated feeding 25(OH)D_3 or vitamin D_3 to bred gilts and observed alterations in fetal muscle characteristics for fetuses from gilts fed the 25(OH)D_3 compared to fetuses from gilts fed vitamin D_3 when fed at concentrations above the basal requirement estimate (NRC, 2012). There was an increase in the number of muscle fibers and an increase in the number of Pax7 (satellite cells) + myoblasts within the LM. These alterations would suggest the potential for increased postnatal growth performance. Weber et al. (2014) observed increases in piglet BW at birth and weaning in piglets from dams supplemented 50 μg of 25(OH)D_3/kg compared with piglets from dams supplemented 2,000 IU of vitamin D_3/kg. However, no previous research has evaluated whether pigs from dams supplemented varying forms or concentrations of vitamin D have improved postnatal growth after weaning or the impacts of maternal vitamin D on carcass characteristics.

Therefore, the objectives of the experiments herein were 1) to determine the vitamin D status of pigs within a subsample population from dams fed varying vitamin D regimens and 2) to evaluate the influence of maternal vitamin D status and nursery dietary vitamin D regimen on growth performance and carcass characteristics.

MATERIALS AND METHODS

Experimental procedures and animal care were approved by the Kansas State University Institutional Animal Care and Use Committee. These experiments were conducted at the K-State Swine Teaching and Research Facility in Manhattan, KS, from September of 2014 to May of 2015. Nursery and finishing diets were prepared at the Kansas State University O. H. Kruse Feed Mill (Manhattan, KS). All diets were formulated to meet or exceed nutrient requirement estimates (NRC, 2012). The pigs used in this study were derived from sows fed different concentrations and sources of vitamin D as described in a companion paper (Flohr et al., 2016).

All nursery and finishing facilities were totally enclosed, environmentally controlled, and mechanically ventilated buildings. Pigs in the first weaning group were housed in nursery pens that were 1.22 × 1.52 m with a 4-hole dry self-feeder and a single nipple waterer to provide ad libitum access to feed and water. Pens had wire mesh flooring and allowed 0.28 m^2/pig. On d 55 after weaning, pigs were moved to the finishing barn into pens that were 1.52 × 3.05 m with totally slatted concrete flooring (providing 0.77 m2 space/pig). Each pen was equipped with a 2-hole dry self-feeder and 2 nipple waterers to provide ad libitum access to feed and water. Pigs in the second weaning group were housed in nursery pens that were 1.52 × 1.52 m with tri-bar flooring (providing 0.60 m2 space/pig). Each pen was equipped with a 3-hole dry self-feeder and a nipple waterer to allow for ad libitum access to feed and water. These pigs were moved to the finishing pens (2.44 × 3.05 m) with totally slatted flooring (providing 0.93 m2 space/pig). Each pen was equipped with a 2-hole dry self-feeder and bowl waterer to allow ad libitum access to feed and water. Feed was delivered to each pen individually by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

In total 448 pigs (PIC 327 × 1050, Hendersonville, TN) from 52 litters from 2 consecutive weaned pig groups (approximately 50% of pigs weaned from the maternal trial discussed by Flohr et al., 2016) were used as a subsample of the weaned pig population in a 4 × 2 split-plot design to determine the effects of maternal vitamin D treatment and nursery dietary vitamin D regimen on growth performance. Sows were previously administered 1 of 4 maternal dietary vitamin D treatments, receiving either vitamin D_3 (800, 2,000, or 9,600 IU/kg of diet) or 25(OH)D_3 (50 μg [2,000 IU vitamin D equivalent]/kg of diet; Hy-D, DSM Nutritional Products North America, Parsippany, NJ) throughout gestation and lactation as discussed by Flohr et al. (2016). Prior to weaning, all pigs were weighed to determine the average weaning weight and SD of weaning weight among maternal treatments. Because all pigs were not used, a subsample of litters was selected to represent the mean and variance among maternal treatments differences at weaning within each of the 2 weaning groups used in the study. At weaning, pigs were allotted to pens on the basis of their previously administered maternal vitamin D regimen. Pens were then randomly assigned to the nursery regimen of feeding diets containing either 2,000 IU vitamin D_3 or 50 μg 25(OH)D_3/kg. There were 7 pigs per pen and 4 pens per treatment in the first wean group and 4 pigs per pen and 8 or 9 pens per treatment in the second wean group. Dietary vitamin D regimens remained consistent in 3 consecutive nursery diets that were fed from d 0 to 10, d 10 to 21, and d 21 to 35 for phases 1, 2, and 3, respectively. The nursery diets were formulated to contain 1.40%, 1.34%, and 1.22% standardized ileal digestible (**SID**) lysine (Table 1) for phases 1, 2, and 3, respectively. Phase 1 nursery diets were pelleted, and all other diets were in meal form. Pigs and feeders were weighed on d 0, 10, 21, and 35 to determine ADG, ADFI, and G:F.

After d 35 postweaning, pigs were switched to a common growing pig diet (phase 4) and then were

Table 1. Nursery and finishing diet composition (as-fed basis)[1]

	Nursery diets[2]			Finishing diets[3]		
Item	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Ingredient, %						
Corn	35.68	46.01	56.39	71.50	78.44	82.86
Soybean meal, 46.5% CP	22.09	20.37	24.27	25.71	19.20	14.93
Corn DDGS[4]	5.00	15.00	15.00	—	—	—
Fish meal	5.00	5.00	—	—	—	—
Spray-dried whey	25.00	10.00	—	—	—	—
Choice white grease	3.00	—	—	—	—	—
Monocalcium phosphate, 21.5% P	0.15	0.23	0.88	0.55	0.33	0.30
Calcium carbonate	1.05	1.13	1.35	1.13	1.10	1.08
Sodium chloride	0.30	0.30	0.35	0.35	0.35	0.35
L-Lys HCl	0.40	0.45	0.50	0.31	0.25	0.22
DL-Met	0.20	0.14	0.13	0.06	0.02	—
L-Thr	0.17	0.16	0.17	0.09	0.05	0.05
L-Trp	0.04	0.05	0.04	—	—	—
L-Val	0.09	0.03	0.03	—	—	—
Choline chloride, 60%	0.04	—	—	—	—	—
Zinc oxide	0.39	0.25	—	—	—	—
Medication[5]	1.00	0.50	0.50	—	—	—
Phytase[6]	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral premix[7]	0.15	0.15	0.15	0.15	0.13	0.10
Vitamin premix[8]	0.25	0.25	0.25	0.15	0.13	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
SID AA,[9] %						
Lys	1.40	1.34	1.22	1.05	0.85	0.72
Met and Cys:Lys	57	57	57	55	56	59
Thr:Lys	63	63	63	61	61	64
Trp:Lys	19	19	19	18	18	18
Val:Lys	68	68	68	69	73	76
NE, Mcal/kg	2.58	2.43	2.40	2.47	2.51	2.54
SID Lys:NE, g/Mcal	5.43	5.51	5.08	4.25	3.39	2.83
CP,%	21.6	22.6	21.0	18.5	15.9	14.2
Ca,%	0.86	0.81	0.74	0.62	0.55	0.52
P, %	0.63	0.62	0.60	0.49	0.41	0.39
Available P, %	0.51	0.47	0.42	0.29	0.23	0.22
STTD P, %	0.43	0.41	0.36	0.34	0.28	0.27
Ca:P	1.36	1.30	1.23	1.28	1.34	1.35

[1]A total of 448 pigs from 52 litters in 2 farrowing groups were used in a 35-d nursery trial. There were a total of 7 pigs per pen and 4 pens per treatment in the first weaning group, and there were 4 pigs per pen and either 8 or 9 pens per treatment in the second weaning group.

[2]Phase 1 diets were fed from d 0 (weaning) until d 10, phase 2 diets were fed from d 10 to 21, and phase 3 diets were fed from d 21 to 35. Experimental treatments were made by adding either a vitamin D_3 premix (4,409,240 IU/kg of premix) in the diet replacing corn or 0.33 kg/t of 25(OH)D_3 (Hy-D; DSM Nutritional Products North America, Parsippany, NJ) replacing corn.

[3]Common finishing diets were fed from approximately 23 to 55 kg, 55 to 93 kg, and 93 kg until market for phases 4, 5, and 6, respectively. Common finishing diets were formulated to contain 827, 690, and 551 IU of vitamin D_3 per kg of complete diet for phases 4, 5, and 6, respectively.

[4]Dried distillers grains with solubles.

[5]Mecadox 2.5 (Phibro Animal Health, Ridgefiled Park, NJ). Provided 44 mg/kg of carbadox in phase 1 nursery diets and 22 mg/kg of carbadox in phase 2 and 3 diets.

[6]Ronozyme Hi-Phos (DSM Nutritional Products North America). Provided 476 phytase units (FTU/kg) of diet with an expected release of 0.10% phytate P.

[7]Provided 11,000 mg/kg Cu, 198 mg/kg I, 73,413 mg/kg Fe, 22,046 mg/kg Mn, 198 mg/kg Se, and 74,413 mg/kg Zn per kilogram of premix.

[8]Provided 3,527,392 IU vitamin A, 17,637 IU vitamin E, 1,764 mg vitamin K, 15 mg vitamin B_{12}, 33,069 mg niacin, 11,023 mg pantothenic acid, and 3,307 mg riboflavin per kilogram of premix.

[9]SID = standardized ileal digestible.

transported to the finishing facility approximately 55 d after weaning. Pigs remained penned by maternal and dietary nursery treatments in the finisher; however, because the pen sizes changed from the nursery to the finisher, pigs were remixed within treatments and were randomly allotted to finishing pens. In finishing, all pigs received common diets formulated to contain 827, 690, and 551 IU of vitamin D_3/kg for phases 4, 5, and 6, respectively. Pigs were weighed, and feed disappearance was calculated every 28 d until marketing (135 kg).

Feed Preparation and Vitamin D Analysis

To achieve the dietary vitamin D_3 concentrations, a premix was made containing a vitamin D_3 supplement (Rovimix D_3, 500,000 IU/g; DSM Nutritional Products North America). This supplement was mixed with a rice hull carrier to form the premix and was added to the control diet by replacing corn. The vitamin D premix was the only source of added vitamin D within the diets, as other vitamin premixes did not contain vitamin D. For diets formulated to contain 50 μg of 25(OH)D_3/kg, 390 g of 25(OH)D_3 (Hy-D, DSM Nutritional Products North America; 125 μg/g) were added per ton of the diet to provide 50 μg of 25(OH)D_3/kg. Complete nursery diet samples were analyzed for vitamin D_3 and 25(OH) D_3 concentrations by DSM Nutritional Products using a combination HPLC and mass spectrometry analytical technique (Schadt et al., 2012).

Serum 25-Hydroxycholecalciferol, Vitamin D_3, α-Tocopherol, and Retinol

One pig per pen (randomly selected) was bled via jugular venipuncture at weaning (d 21) and d 17, 35, and 70 postweaning to determine serum vitamin metabolites. All blood samples were collected via jugular venipuncture using 25-mm 20-gauge needles and 10-mL blood collection tubes containing a gel separator. 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. All vitamin metabolite testing (25(OH)D_3, vitamin D_3, α-tocopherol, and retinol) was conducted by DSM Nutritional Product's laboratory (Kaiseraugst, Switzerland). The analyses were performed using a liquid chromatography/electrospray ionization tandem mass spectrometry technique with multiple reaction monitoring similar to the methods described by Priego Capote et al. (2007). The lowest detectable limit for 25(OH)D_3 was 5.00 ng/mL; for vitamin D_3 it was 1.00 ng/mL, for α-tocopherol it was 250 ng/mL, and for retinol it was 25 ng/mL. Over half of the serum samples were below the detectable limit for serum vitamin D_3 concentration (n = 130 out of 256 total samples); therefore, the percentage of animals with serum concentrations above the detectable limit is reported herein along with the mean concentration of serum vitamin D_3 associated with those animals.

Carcass Characteristics

Carcass data were collected from 642 pigs, or approximately 75% (3 of the 4 weaned pig groups), of the weaned progeny from the maternal portion of the study (Flohr et al., 201). Pigs were individually weighed and tattooed for slaughter at a commercial abattoir (Triumph Foods, St. Joseph, MO). Hot carcass weights were measured immediately after evisceration, and each carcass was evaluated for percentage carcass yield, back fat, and loin depth. Percentage carcass yield was calculated by dividing HCW by live weight obtained at the farm before transport to the abattoir. Fat depth and loin depth were measured with an optical probe (SFK, Herlev, Denmark) inserted between the third and fourth ribs located anterior to the last rib at a distance approximately 7 cm from the dorsal midline.

Statistical Analysis

All growth data were analyzed as a split-plot design using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Maternal vitamin D regimen acted as the whole-plot unit, and nursery vitamin D regimen acted as the split-plot unit. Pen was the experimental unit, and weaning group was included in the model as a random effect. Contrast statements tested for maternal vitamin D treatments included 1) increasing maternal vitamin D_3 linear and quadratic polynomials and 2) 800 IU vitamin D_3 vs. 50 μg 25(OH)D_3, 3) 2,000 IU vitamin D_3 vs. 50 μg 25(OH)D_3, and 4) 9,600 IU vitamin D_3 vs. 50 μg 25(OH)D_3. The IML procedure of SAS was used to generate unequally spaced contrast coefficients for maternal dietary vitamin D_3 treatments. Because of unbalanced sample sizes for maternal treatments, a Tukey-Kramer multiple comparison adjustment was used for the maternal vitamin D pairwise comparison tests. Repeated measures analysis was performed on the serum vitamin metabolite responses, and day of collection was included as a fixed effect to determine serum changes to dietary treatments over time. For carcass data, maternal vitamin D treatment served as the fixed effect, and weaning group acted as a random effect in the model. The percentage carcass yield was analyzed using a β distribution. Results were considered significant at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

Table 2. Analyzed nursery diet composition (as-fed basis)[1]

	Nursery diets					
	Phase 1		Phase 2		Phase 3	
Item	D_3	25(OH)D_3	D_3	25(OH)D_3	D_3	25(OH)D_3
Formulated						
CP, %	21.6	21.6	22.6	22.6	21	21
Ca, %	0.86	0.86	0.81	0.81	0.74	0.74
P, %	0.63	0.63	0.62	0.62	0.60	0.60
Vitamin D_3, IU/kg	2,000	—	2,000	—	2,000	—
25(OH)D_3, IU/kg	—	2,000	—	2,000	—	2,000
Analyzed[2]						
CP, %	21.8	22.4	24.2	23.2	23.1	22.4
Ca, %	1.04	1.04	1.03	1.02	0.80	0.9
P, %	0.65	0.64	0.71	0.70	0.61	0.61
Vitamin D_3, IU/kg	2,240	—	1,700	—	2,110	—
25(OH)D_3, IU/kg	—	1,580	—	1,500	—	1,540
Percentage of formulated	112	79	85	75	106	77

[1]Means represent the average of 2 pooled samples.

[2]Crude protein, Ca, and P were determined at Ward laboratories (Kearney, NE). Vitamin D_3 and 25(OH)D_3 analysis was performed by DSM Nutrition Products (Parsippany, NJ).

RESULTS

Chemical analysis of experimental nursery diets confirmed that diets contained CP and P concentrations similar to those for which they were formulated (Table 2). The Ca concentrations analyzed higher than formulated, but all diets were above the animals' requirements. Although there is no published accepted standard for vitamin D recovery in animal feeds, analysis showed nursery diets were within 25% of their formulated targets, which would be consistent with the acceptable analytical variation and recovery of other vitamins previously discussed by the Association of American Feed Control Officials (2015).

Growth Performance

At weaning, BW of pigs subsampled for the nursery portion of the study increased (quadratic, $P = 0.001$; Table 3) with increasing maternal vitamin D_3. This occurred because pigs subsampled from sows fed the 2,000 IU of vitamin D_3/kg were heavier (6.8 kg) than pigs from sows fed either 800 (6.5 kg) or 9,600 (6.6 kg) IU of vitamin D_3/kg. In addition, pigs weaned from sows fed 800 IU of vitamin D_3/kg tended ($P = 0.088$) to have lighter BW at weaning than pigs weaned from sows fed 50 μg of 25(OH)D_3/kg. For the maternal portion of the study, these numeric differences were not statistically significant, but changing the experimental unit from sow to pen led to a significant difference in initial BW among vitamin D_3 treatments and a statistical tendency when comparing BW of pigs weaned from sows fed 800 IU of vitamin D_3/kg and pigs weaned from sows fed 50 μg of 25(OH)D_3/kg.

No nursery × maternal vitamin D interactions were observed for growth performance in the nursery or finishing portion of the growth study. Thus, only the main effects of maternal vitamin D treatment and nursery vitamin D treatments are reported herein.

Nursery dietary vitamin D regimen had no influence (Table 4) on pig growth throughout the nursery or finishing portion of the study. From d 0 to 35 in the nursery, increasing maternal vitamin D_3 increased (quadratic, $P < 0.003$) ADG and ADFI, but G:F was similar regardless of maternal vitamin D regimen. Pigs weaned from sows fed 800 IU of vitamin D_3/kg had lower ($P = 0.002$) ADG and tended ($P = 0.066$) to have lower ADFI than pigs weaned from sows fed 50 μg of 25(OH)D_3/kg. Final BW at the end of the nursery period (d 35) was increased (quadratic, $P = 0.001$) with increased maternal vitamin D_3 because pigs from sows fed 2,000 IU of vitamin D_3/kg had heavier BW at the end of the nursery compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3/kg. In addition, pigs from sows fed 800 IU of vitamin D_3/kg had lighter ($P = 0.001$) final BW at the end of the nursery period compared with pigs fed 50 μg of 25(OH)D_3/kg. Overall finisher ADG increased (quadratic, $P = 0.005$) with increased maternal vitamin D_3, which also led to increased (quadratic, $P = 0.006$) final BW. Similar to nursery growth, this was because pigs from sows fed 2,000 IU of vitamin D_3/kg had increased ADG and improved G:F compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3/kg. Also, pigs from sows fed 800 IU of vitamin D_3/kg had lower ($P = 0.004$) ADG and lighter ($P = 0.003$) final BW com-

Table 3. Main effects of maternal vitamin D regimen on the performance of growing pigs[1]

	Maternal vitamin D, IU/kg					Probability P				
	Vitamin D_3			50 µg 25(OH)D_3		Vitamin D_3				
Item	800	2,000	9,600	2,000	SEM	Linear	Quadratic	800 IU D_3 vs. 50 µg 25(OH)D_3	2,000 IU D_3 vs. 50 µg 25(OH)D_3	9,600 IU D_3 vs. 50 µg 25(OH)D_3
Nursery growth (d 0 to 35)[2]										
ADG, kg	0.42	0.44	0.43	0.45	0.02	<0.729	<0.003	<0.002	<0.917	<0.105
ADFI, kg	0.65	0.70	0.67	0.69	0.02	<0.853	<0.002	<0.066	<0.929	<0.437
G:F	0.638	0.632	0.639	0.647	0.006	<0.708	<0.407	<0.709	<0.236	<0.709
Finishing growth (d 35 to market)[3]										
ADG, kg	0.93	0.96	0.94	0.96	0.01	<0.602	<0.005	<0.004	<0.916	<0.220
ADFI, kg	2.56	2.59	2.57	2.63	0.02	<0.981	<0.492	<0.216	<0.558	<0.327
G:F	0.368	0.377	0.374	0.373	0.006	<0.610	<0.049	<0.701	<0.740	<0.997
Average BW, kg										
d 0	6.5	6.8	6.6	6.6	0.1	<0.566	<0.001	<0.088	<0.371	<0.985
d 35	21.1	22.3	21.8	22.3	0.5	<0.555	<0.001	<0.001	<0.997	<0.141
Market	132.6	136.5	134.9	137.5	3.0	<0.480	<0.006	<0.003	<0.866	<0.240

[1]A total of 448 pigs from 52 litters in 2 farrowing groups were used in a 35-d nursery trial. The treatment structure was a split-plot design with maternal treatment as the whole-plot unit and nursery treatment as the split-plot unit.

[2]For nursery performance pen was the experimental unit. Random effect of group was used in the statistical model. There were 7 pigs per pen and 8 pens per treatment in group 1, and there were 4 pigs per pen and either 16 or 17 pens per treatment in group 2.

[3]For finishing performance pen was the experimental unit. Random effect of group was used in the statistical model. There were a total of 5 to 8 pigs per pen and 19 finishing pens per treatment.

pared with pigs from sows fed 50 µg of 25(OH)D_3/kg. Feed efficiency was improved (quadratic, $P = 0.049$) with increasing maternal vitamin D_3.

Growing Pig Serum 25(OH)D_3, Vitamin D_3, α-Tocopherol, and Retinol

No 3-way maternal × nursery × day interactions were observed for serum vitamin metabolite responses. Thus, only the main effects of maternal and nursery vitamin D regimens are reported herein.

A maternal treatment × day ($P < 0.001$; Table 5) interaction was observed for growing pig serum 25(OH)D_3 because changes in serum concentrations over time were dependent on the maternal dietary treatments. At weaning, increasing maternal dietary vitamin D_3 increased (linear, $P = 0.001$) serum 25(OH)D_3 and (quadratic, $P = 0.037$) serum retinol, but it decreased (linear, $P = 0.037$) serum α-tocopherol. In addition, pigs from sows fed 9,600 IU of vitamin D_3/kg had increased ($P < 0.001$) serum 25(OH)D_3 compared with pigs from sows fed 50 µg of 25(OH)D_3/kg. Pigs from sows fed 800 IU of vitamin D_3/kg had increased ($P = 0.001$) serum α-tocopherol compared with pigs from sows fed 50 µg of 25(OH)D_3/kg. Also, increasing maternal dietary vitamin D_3 supplementation led to an increased (quadratic, $P = 0.023$) percentage of pigs exhibiting serum vitamin D_3 concentrations above the detectable limit with a decreasing (quadratic, $P = 0.001$) mean vitamin D_3 concentration. On d 17 after weaning, increasing maternal vitamin D_3 increased (quadratic; $P = 0.023$) serum retinol and tended (quadratic, $P = 0.063$) to increase serum 25(OH)D_3. Additionally, increasing maternal vitamin D_3 supplementation tended (linear, $P = 0.082$) to decrease piglet serum vitamin D_3 concentrations, although the percentage of pigs exhibiting serum concentrations above the detectable limit was not affected by maternal vitamin D dietary treatment. By d 35 postweaning, increasing maternal vitamin D_3 supplementation increased (quadratic, $P = 0.006$) serum 25(OH)D_3 and tended (quadratic, $P = 0.063$) to increase serum retinol. Also, pigs from sows fed 2,000 IU of vitamin D_3/kg had increased ($P < 0.002$) serum 25(OH)D_3 compared with pigs from sows fed 50 µg of 25(OH)D_3/kg. By d 70 after weaning, maternal dietary vitamin D treatment had no influence on growing pig serum vitamin metabolites.

A nursery × day ($P < 0.001$; Table 6) interaction was observed for growing pig serum 25(OH)D_3 because changes over time were different on the basis of nursery vitamin D regimen. At weaning, pigs moved to pens fed vitamin D_3 had lower ($P = 0.015$) serum α-tocopherol concentrations than pigs moved to pens fed 25(OH)D_3. Also, pigs moved to pens fed vitamin D_3 tended ($P = 0.099$) to have greater mean serum vitamin D_3 concentrations, although the percentage of pigs exhibiting concentrations above the detectable limit was not influenced by nursery treatment. On d 17 and 35 in the nursery, pigs fed vitamin D_3 had greater ($P < 0.001$) percentages of pigs exhibiting serum vitamin D_3 concentrations above the detectable limit; however, they also had decreased serum 25(OH)D_3 ($P = 0.001$) concentrations compared with pigs fed 25(OH)D_3. By d 70 (35 d postnursery vitamin D treatments), serum vitamin metabolites were not influenced by nursery dietary vitamin D regimens.

Table 4. Main effects of nursery dietary vitamin D regimen on the performance of growing pigs[1]

Item	Nursery source[2]		SEM	Probability *P*, nursery
	Vitamin D_3	25(OH)D_3		
Nursery growth (d 0 to 35)[3]				
ADG, kg	0.44	0.43	0.02	<0.482
ADFI, kg	0.68	0.67	0.02	<0.137
G:F	0.635	0.643	0.004	<0.224
Finishing growth (d 35 to market)[4]				
ADG, kg	0.95	0.95	0.01	<0.577
ADFI, kg	2.57	2.61	0.02	<0.126
G:F	0.374	0.369	0.006	<0.453
Average BW, kg				
d 0	6.6	6.6	0.1	<0.922
d 35	21.9	21.8	0.5	<0.537
Market	135.3	135.4	2.9	<0.911

[1]A total of 448 pigs from 52 litters in 2 farrowing groups were used in a 35-d nursery trial. The treatment structure was a split-plot design with maternal treatment as the whole-plot unit and nursery treatment as the split-plot unit.

[2]Subsequent nursery treatments consisted of supplementing vitamin D in phase 1, 2, and 3 diets from either vitamin D_3 (2,000 IU/kg) or 25(OH)D_3 (50 µg/kg).

[3]For nursery performance pen was the experimental unit. Random effect of group was used in the statistical model. There were a total of 7 pigs per pen and 16 pens per treatment in group 1, and there were 4 pigs per pen and 33 pens per treatment in group 2.

[4]For finishing performance pen was the experimental unit. Random effect of group was used in the statistical model. There were a total of 5 to 8 pigs per pen and 38 finishing pens per treatment.

Carcass Characteristics

Pigs from sows fed 50 µg of 25(OH)D_3/kg had heavier ($P < 0.047$; Table 7) final live BW and HCW than pigs from sows fed 9,600 IU of vitamin D_3/kg. Carcass yield percentage increased (quadratic, $P = 0.003$) with increasing maternal dietary vitamin D_3 supplementation. Loin depth (linear, $P = 0.047$) and back fat thickness (quadratic, $P = 0.031$) decreased with increasing maternal dietary vitamin D_3 supplementation.

DISCUSSION

The impact of maternal imprinting on postnatal performance of progeny has led to an increased interest in understanding how maternal nutrition can impact subsequent progeny growth. Mahan and Vallet (1997) concluded that the understanding of vitamin and mineral transport in utero was still very much in its infancy almost 2 decades ago. Research specifically focused on vitamin D's transport and function in utero has been more prolific than research on some other vitamins and trace minerals.

Haddad et al. (1971) illustrated, using pregnant rats, that both vitamin D_3 and 25(OH)D_3 are capable of being transported transplacentally to the fetus and concluded that maternal and fetal ratios of vitamin D_3 and 25(OH)D_3 were similar as soon as 1 h after administration. Clements and Fraser (1988) determined that supplementing vitamin D deficient pregnant rats resulted in increased in utero presence of vitamin D metabolites, predominately 25(OH)D_3 and 24,25-OH2-D_3. The active form of the vitamin (1,25-OH2-D_3) must be derived from fetal sources, but little to no data are available about how the active form is metabolized in the fetus. However, Johnson et al. (1996) and Endo et al. (2003) both illustrated the presence of vitamin D nuclear receptors (VDR) within fetal bone and muscle tissues. This suggests that the active 1,25-OH2-D_3 metabolite plays a role in the fetal development of these tissues. In fact, Endo et al. (2003) demonstrated that the absence of the VDR in mice led to aberrant expression of myogenic transcription factors (myogenic factor 5 positive cells [Myf5], myogenin, and early lymphocyte cells in development [E2A]) in hind leg muscle. High expression of these factors in utero could lead to precocious cell differentiation and impaired cell proliferation, leaving a smaller myoblast cell pool for postnatal muscle development and hypertrophic growth. Most of this research has been conducted with deficient animals; however, previous work in swine by Hines et al. (2013) concluded that differences in fetal muscle fiber number and Pax7+ cells existed within the LM of fetuses from bred gilts fed 2,500 IU of vitamin D/kg of diet as 100% vitamin D_3 or as 80% 25(OH)D_3 and 20% vitamin D_3. Their conclusion was that the increases in maternal 25(OH)D_3 concentrations (vitamin D status) were the reason for the improvements in fetal muscle development. Other researchers have observed similar increases in the serum 25(OH)D_3 response of growing pigs and sows fed 25(OH)D_3 compared with those fed similar IU equivalency concentrations of vitamin D_3. The aforementioned conclusions from previous research led to our hypothesis that altering the maternal vitamin D status of the sow could lead to alterations in fetal muscle development and, subsequently, changes in postnatal growth. The aim of the study herein was to evaluate the postnatal growth of pigs from sows fed the varying dietary vitamin D supplementation treatments and to determine whether growth was impacted by maternal dietary vitamin D treatment and/or by subsequent nursery dietary vitamin D treatments.

Nursery and finishing growth herein was not influenced by nursery vitamin D supplementation, which is consistent with conclusions reported by Wahlstrom and Stolte (1958), Combs et al. (1966), and Flohr et al. (2014b), who evaluated supplementing dietary vitamin D_3 when all other nutrient concentrations were adequate. Rortvedt and Crenshaw (2012) demonstrated a 10% reduction in the growth of nursery pigs weaned from sows fed diets deficient (45 IU vitamin D_3/kg of diet) in vita-

Table 5. Main effects of maternal dietary vitamin D regimen on growing pig serum metabolites[1]

Growing pig serum vitamin metabolites	Maternal vitamin D, IU/kg					Probability *P*				
	Vitamin D_3			50 µg 25(OH)D_3		Vitamin D_3				
	800	2,000	9,600	2,000	SEM	Linear	Quadratic	800 IU D_3 vs. 50 µg 25(OH)D_3	2,000 IU D_3 vs. 50 µg 25(OH)D_3	9,600 IU D_3 vs. 50 µg 25(OH)D_3
25(OH)D_3,[2] ng/mL										
Weaning	5.4	7.1	16.6	5.5	1.2	<0.001	<0.871	<0.925	<0.300	<0.001
d 17	22.7	25.9	25.0	23.6	1.2	<0.466	<0.063	<0.581	<0.163	<0.398
d 35	26.4	30.8	26.8	25.5	1.3	<0.366	<0.006	<0.556	<0.002	<0.452
d 70	18.3	15.7	16.1	16.5	1.5	<0.497	<0.257	<0.403	<0.686	<0.816
Vitamin D_3[3]										
Weaning										
Detectable samples, %	6.3	32.4	83.3	0.0	5.2	<0.001	<0.023	<0.395	<0.001	<0.001
Mean, ng/mL	7.3	1.2	5.6	—	0.2	<0.369	<0.001	—	—	—
d 17										
Detectable samples, %	43.8	43.8	50.0	50.0	5.7	<0.367	<0.907	<0.420	<0.420	<0.999
Mean, ng/mL	3.3	3.8	2.7	3.0	0.4	<0.082	<0.266	<0.505	<0.114	<0.614
d 35										
Detectable samples, %	43.8	50.0	50.0	50.0	5.9	<0.593	<0.459	<0.420	<0.999	<0.999
Mean, ng/mL	3.5	3.5	3.6	3.8	0.4	<0.888	<0.920	<0.590	<0.521	<0.641
d 70										
Detectable samples, %	100.0	100.0	100.0	100.0	7.1	<0.999	<0.999	<0.999	<0.999	<0.999
Mean, ng/mL	3.2	3.1	3.1	2.6	0.3	<0.855	<0.784	<0.191	<0.312	<0.277
α-tocopherol,[4] mg/L										
Weaning	5,304	4,769	4,591	4,331	198	<0.037	<0.086	<0.001	<0.101	<0.340
d 17	982	829	804	924	207	<0.641	<0.629	<0.837	<0.738	<0.679
d 35	1,521	1,401	1,242	1,291	216	<0.374	<0.758	<0.417	<0.698	<0.869
d 70	1,799	1,566	1,784	1,631	259	<0.796	<0.498	<0.632	<0.856	<0.646
Retinol,[5] ng/mL										
Weaning	254	301	286	283	20	<0.464	<0.037	<0.176	<0.427	<0.907
d 17	366	419	397	413	21	<0.599	<0.023	<0.038	<0.795	<0.491
d 35	389	435	431	421	22	<0.242	<0.063	<0.158	<0.553	<0.667
d 70	379	393	373	360	25	<0.635	<0.585	<0.507	<0.250	<0.631

[1]A total of 448 pigs from 52 litters in 2 farrowing groups were used in a 35-d nursery trial and followed through finishing. The treatment structure was a split-plot design with maternal treatment as the whole-plot unit and nursery treatment as the split-plot unit.

[2]A maternal × day ($P < 0.001$) interaction was observed for growing pig serum 25(OH)D_3 concentrations.

[3]The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit ($n = 130$ out of 256) were not used in the statistical analysis. Detectable sample represents the percentage of samples above the detectable limit, and the mean serum vitamin D_3 was calculated using only samples above the detectable limit.

[4]A day effect ($P < 0.001$) was observed for growing pig serum **α**-tocopherol concentrations.

[5]A day effect ($P < 0.001$) was observed for growing pig serum retinol concentrations.

min D compared with pigs weaned from sows fed diets formulated to 325 IU vitamin D_3/kg of feed. The authors concluded that pigs born from sows fed diets deficient in vitamin D were unable to achieve performance comparable to that of pigs born from sows fed diets replete with vitamin D, regardless of Ca and P supplementation after weaning (the researchers evaluated feeding 80% or 120% of the animal's estimated requirement [NRC, 1998]). This suggests a role of vitamin D in utero and the potential for deficiency to impact postnatal development.

Interestingly, in the study herein, maternal vitamin D influenced postweaning growth but not in the way that we had hypothesized on the basis of previous conclusions drawn from Hines et al. (2013) and Weber et al. (2014). In the current study, it appeared that the only consistent impact on growth performance was that pigs from sows fed 2,000 IU of vitamin D_3/kg had increased ADG and ADFI in the nursery and improved ADG and G:F in finishing. Considering that performance of pigs from sows fed 50 µg of 25(OH)D_3 was similar to that of pigs from sows fed 2,000 IU of vitamin D_3/kg, the conclusion is that the form of maternal vitamin D (vitamin D_3 or 25(OH)D_3) does not influence postweaning growth; however, it appeared that the level of the vitamin supplemented did result in growth differences. The data herein suggest that 2,000 IU of vitamin D/kg of diet were useful in achieving the highest growth rates compared to feeding 800 or 9,600 IU of vitamin D_3/kg. Also, pigs weaned from sows fed 2,000 IU of vitamin D_3/kg had numerically heavier weaning BW (although not

statistically significant in the sow portion of the study; Flohr et al., 2016) than pigs from sows fed 800 or 9,600 IU of vitamin D_3/kg. Pluske and Dong (1998) showed that the growth of suckling pig is predominately limited by the amount of milk produced by the sow. In addition, the amount of feed intake during lactation can impact total milk production and subsequent litter weaning weight (Eissen et al., 2003). Because of the increase in lactation ADFI observed for sows fed diets with 2,000 IU of vitamin D_3/kg discussed by Flohr et al. (2016), it is plausible to think that lactation feed intake may have been a larger reason for the numeric increase in weaning weights of pigs rather than maternal vitamin D treatment. There is no previous evidence to support that maternal vitamin D treatment would have impacted lactation feed intake except for the case of toxicity, which has been described to cause lethargy and anorexia (NRC, 1988); however, signs of these symptoms were not observed during the lactation portion of the study. Ultimately, the results herein suggest that maternal dietary vitamin D treatment impacted nursery performance, which disagrees with the results of Flohr et al. (2014a), who observed no impact of maternal vitamin D_3 treatment or nursery vitamin D_3 treatment on nursery performance of pigs weaned from sows supplemented between 1,500 and 6,000 IU of vitamin D_3/kg of diet.

The maternal and nursery vitamin D treatment impacts on growing pig serum 25(OH)D_3 in this study were largely expected. Most previous reports (Lauridsen et al., 2010; Witschi et al., 2011; Coffey et al., 2012; Weber et al., 2014) have all shown that supplementation of 25(OH)D_3 at the same IU equivalency of vitamin D_3 will result in an increased serum 25(OH)D_3 response. Also, increasing maternal vitamin D_3 supplementation has been shown to lead to an increase in subsequent pig serum 25(OH)D_3 (Flohr et al., 2014a), which is consistent with results from the current study. However, pigs weaned from sows fed 50 μg of 25(OH)D_3/kg had serum 25(OH)D_3 concentrations similar to those of pigs weaned from sows fed 2,000 IU of vitamin D_3/kg (which were formulated to be at the same IU equivalency of the vitamin), but levels were less than that of pigs from sows fed 9,600 IU of vitamin D_3/kg. This shows that for milk transfer of the vitamin (which was the lone source of the nutrient prior to weaning) the level of maternal dietary vitamin D was more impactful than the form of dietary vitamin D. Additionally, Flohr et al. (2014a) concluded that serum 25(OH)D_3 of weaned pigs was no longer impacted by maternal vitamin D_3 supplementation as soon as 21 d postweaning. However, maternal vitamin D treatment impacted serum 25(OH)D_3 of growing pigs up to 35 d postweaning in the current study. This may be largely due to, in part, the increase in ADFI of pigs weaned from sows fed the medium level of vitamin D_3, which would have increased total vitamin D intake.

Serum vitamin D_3 concentrations responded as expected in growing pigs on the basis of maternal and nursery vitamin D treatments. Particularly, supplementing only 25(OH)D_3, maternally or in the nursery diet, led to decreased serum vitamin D_3 concentra-

Table 6. Main effects of nursery dietary vitamin D regimen on growing pig serum vitamin metabolites[1]

Growing pig serum vitamin metabolites	Nursery source[2]		SEM	Probability *P*, nursery
	Vitamin D_3	25(OH)D_3		
25(OH)D_3,[3] ng/mL				
Weaning	9.3	8.0	0.8	<0.229
d 17	11.3	37.3	0.9	<0.001
d 35	16.1	38.7	0.9	<0.001
d 70	16.8	16.6	1.1	<0.889
Vitamin D_3,[4] ng/mL				
Weaning				
Detectable samples, %	33.3	27.0	4.7	<0.335
Mean, ng/mL	4.9	4.0	0.4	<0.099
d 17				
Detectable samples, %	93.8	0.0	5.0	<0.001
Mean, ng/mL	3.2	—	0.3	—
d 35				
Detectable samples, %	96.9	0.0	5.1	<0.001
Mean, ng/mL	3.6	—	0.3	—
d 70				
Detectable samples, %	100.0	100.0	6.0	<0.999
Mean, ng/mL	3.0	3.1	0.3	<0.823
α-Tocopherol,[5] mg/L				
Weaning	4,512	4,984	138	<0.015
d 17	902	868	145	<0.868
d 35	1,404	1,324	148	<0.695
d 70	1,680	1,710	178	<0.901
Retinol,[6] ng/mL				
Weaning	284	278	17	<0.663
d 17	408	390	17	<0.260
d 35	423	415	17	<0.660
d 70	373	379	20	<0.800

[1]A total of 448 pigs from 52 litters in 2 farrowing groups were used in a 35-d nursery trial and followed through finishing. The treatment structure was a split-plot design with maternal treatment as the whole-plot unit and nursery treatment as the split-plot unit.

[2]Subsequent nursery treatments consisted of supplementing vitamin D in phase 1, 2, and 3 diets from either vitamin D_3 (2,000 IU/kg) or 25(OH)D_3 (50 μg/kg).

[3]A nursery × day ($P < 0.001$) interaction was observed for growing pig serum 25(OH)D_3 concentrations.

[4]The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit ($n = 130$ out of 256) were not used in the statistical analysis. Positive sample represents the percentage of samples above the detectable limit, and the mean serum vitamin D_3 was calculated using only samples above the detectable limit.

[5]A day effect ($P < 0.001$) was observed for growing pig serum α-tocopherol concentrations.

[6]A day effect ($P < 0.001$) was observed for growing pig serum retinol concentrations.

Table 7. The effect of maternal dietary vitamin D regimen on subsequent pig carcass characteristics[1]

	Maternal vitamin D,[2] IU/kg					Probability *P*				
	Vitamin D_3			50 µg 25(OH)D_3		Vitamin D_3		800 IU D_3 vs. 50 µg 25(OH)D_3	2,000 IU D_3 vs. 50 µg 25(OH)D_3	9,600 IU D_3 vs. 50 µg 25(OH)D_3
Item	800	2,000	9,600	2,000	SEM	Linear	Quadratic			
Live BW, kg	134.8	135.5	133.8	137.1	3.2	<0.264	<0.534	<0.266	<0.574	<0.047
HCW,[3] kg	99.8	100.7	98.9	101.6	3.4	<0.155	<0.288	<0.276	<0.830	<0.037
Yield, %	73.9	74.3	73.8	74.0	0.8	<0.077	<0.002	<0.521	<0.339	<0.298
Loin depth, mm[3]	60.2	60.6	58.9	59.4	4.1	<0.037	<0.470	<0.743	<0.457	<0.905
Back fat, mm[3]	20.8	19.7	20.3	20.0	0.9	<0.923	<0.031	<0.407	<0.898	<0.941

[1]Means represent data collected from 642 finishing pigs within 3 consecutive finishing groups. Group and finishing treatment within group were used as random effects.

[2]Maternal vitamin D_3 concentrations of 800, 2,000, and 9,600 IU vitamin D_3 per kilogram of complete diet were fed for low, medium, and treatments, respectively, and 50 µg of 25(OH)D_3/kg of complete diet was fed for the maternal 25(OH)D_3 treatment.

[3]Hot carcass weight was used as a covariate in the statistical model.

tions in the growing pig. This result is expected because the demand for transport of vitamin D_3 to tissue for storage or to the liver for metabolism is lessened if the animal is not exposed to that specific metabolite. However, it is difficult to infer much about the animal's vitamin D status from serum vitamin D_3 concentrations since circulating levels will increase quickly after a meal and then clear circulation within hours after absorption (Clinton, 2013).

Little research has examined metabolic interactions of vitamin D with vitamin A and vitamin E. It was hypothesized that differences among serum retinol and α-tocopherol based on maternal or nursery vitamin D treatment would be minimal and that was largely true. Interestingly, increased growing pig serum retinol after weaning was observed for pigs from sows fed 2,000 IU of vitamin D_3/kg compared with pigs from sows fed either 800 or 9,600 IU of vitamin D_3/kg. This may be the result of the increased lactation ADFI of sows fed diets containing 2,000 IU of vitamin D_3/kg compared with sows fed diets containing 800 or 9,600 IU of vitamin D_3/kg. Daily vitamin A intake would have been approximately 650 IU/d greater for sows fed diets containing 2,000 IU of vitamin D_3/kg compared with sows fed diets containing 800 or 9,600 IU of vitamin D_3/kg.

The carcass data herein showed that pigs from sows fed 50 µg of 25(OH)D_3/kg had increased final BW and HCW compared to pigs from sows fed 9,600 IU of vitamin D_3/kg. Ultimately, this result was unexpected and, to our knowledge, is the first data associating subsequent pig carcass data with maternal dietary vitamin D supplementation. Increases in carcass yield and decreases in back fat of pigs from sows fed 2,000 IU of vitamin D_3/kg compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3/kg compliment the growth data herein, suggesting pigs from sows fed 2,000 IU of vitamin D_3 had both improved postweaning growth and improved carcass characteristics. However, it is still unclear whether these responses were the result of maternal vitamin D treatments or numeric differences in weaning weight of pigs weaned from sows fed the medium level of vitamin D.

Conclusion

Serum 25(OH)D_3 of growing pigs is influenced by maternal dietary vitamin D treatment early after weaning, but afterward, it is largely dependent on nursery dietary vitamin D supplementation. Growing pigs fed 25(OH)D_3 in the nursery had increased serum 25(OH)D_3 compared with pigs fed vitamin D_3 at the same IU equivalency, but by 35 d after nursery treatment serum levels were similar regardless of nursery vitamin D source. Also in this study, pigs from sows fed 2,000 IU of vitamin D_3/kg had increased ADG and ADFI in the nursery, increased ADG and G:F in finishing, and increased percentage carcass yield and decreased back fat compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3/kg. These results show a benefit to supplementing maternal vitamin D_3 at 2,000 IU/kg of diet compared with 800 or 9,600 IU/kg of diet. In addition, ADG was improved for pigs weaned from sows fed 50 µg of 25(OH)D_3/kg compared with pigs weaned from sows fed 800 IU of vitamin D_3/kg, and carcass data suggested that pigs weaned from sows fed 50 µg of 25(OH)D_3/kg had increased final BW and HCW compared with pigs from sows fed 9,600 IU/kg. More research examining the potential relationships of maternal vitamin D supplementation with subsequent pig growth and carcass characteristics is needed to elucidate if there are potential benefits of maternal vitamin D supplementation strategies different from those currently employed in commercial sow diets.

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