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Evaluating the impact of maternal vitamin D supplementation: I. Sow performance, serum vitamin metabolites, and neonatal muscle characteristics[1,2]

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ABSTRACT: In Exp. 1, 56 gestating sows (PIC 1050; 35 d postinsemination) were used in a 30-d trial to determine serum 25(OH)D_3 response to increasing concentrations of dietary vitamin D_3. Sows were randomly allotted to 1 of 7 dietary D_3 treatments (200, 800, 1,600, 3,200, 6,400, 12,800, or 25,600 IU of added D_3 per kilogram of complete diet) with 8 sows per treatment. Increasing D_3 increased (quadratic; $P < 0.001$) serum 25(OH)D_3 with the response depicted by the prediction equation: serum 25(OH)D_3, ng/mL = 35.1746 + (0.002353 × dietary D_3, IU/d) − (0.0000000156 × dietary D_3, IU/d^2). In Exp. 2, 112 sows and their litters were used to determine the effects of dietary vitamin D regimen on sow performance, subsequent preweaning pig performance, neonatal bone and muscle characteristics, and serum vitamin metabolites. Sows were allotted to 1 of 4 dietary treatments 3 to 5 d following breeding: 800, 2,000, or 9,600 IU of D_3 per kilogram of the diet or 50 µg of 25(OH)D_3 (2,000 IU of D_3 equivalent from Hy-D, DSM Nutritional Products, Parsippany, NJ) per kilogram of diet. There were 25 to 27 sows per treatment. Increasing dietary D_3 increased (linear, $P = 0.001$) serum 25(OH)D_3 of sows on d 100 of gestation, at farrowing, and at weaning. Increasing D_3 in sow diets increased piglet serum 25(OH)D_3 at birth (linear, $P = 0.001$) and weaning (quadratic, $P = 0.033$). Sows fed 50 µg of 25(OH)D_3/kg had intermediate ($P < 0.004$) serum 25(OH)D_3 concentrations on d 100 of gestation, at farrowing, and at weaning compared with sows fed 2,000 IU of D_3/kg and sows fed 9,600 IU of D_3/kg. Pigs from sows fed 50 µg of 25(OH)D_3/kg had greater serum 25(OH)D_3 compared with pigs from sows fed 2,000 IU of D_3/kg, but at weaning, serum 25(OH)D_3 concentrations were similar. Also, pigs from sows fed 9,600 IU of D_3/kg had greater ($P = 0.011$) serum 25(OH)D_3 at birth and weaning compared with pigs from sows fed 50 µg of 25(OH)D_3/kg. Maternal performance, litter characteristics, neonatal bone ash content, and neonatal muscle fiber characteristics were largely unaffected by the dietary vitamin D treatments. Overall, D_3 and 25(OH)D_3 are both useful at increasing serum 25(OH)D_3 concentrations, but more D_3 (on an equivalent IU basis) is needed to achieve similar serum 25(OH)D_3 responses compared with feeding 25(OH)D_3. Concentration of maternal vitamin D supplementation in lactation impacted milk transfer of the vitamin more so than the form of the vitamin, as evidence by the weaned pig serum 25(OH)D_3 concentrations.

Key words: 25(OH)D_3, sow nutrition, vitamin D, muscle characteristics

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INTRODUCTION

The most common form of dietary vitamin D supplemented in livestock diets is cholecalciferol (vitamin D_3). Research examining a synthetically produced 25(OH)D_3 (Hy-D, DSM Nutritional Products North America, Parsippany, NJ) has shown increased serum 25(OH)D_3 compared with vitamin D_3 when

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both vitamin D_3 and 25(OH)D_3 were added in diets at 2,000 IU of vitamin D (Lauridsen et al., 2010). This is because the vitamin D binding protein (chaperone protein for vitamin D metabolites in circulation) has a higher affinity for 25(OH)D_3 compared with vitamin D_3 (Bouillon et al., 1980).

Research examining the role of vitamin D in skeletal muscle development concluded that vitamin D is involved in myogenic signaling pathways, and the in utero alterations were evident in postnatal skeletal muscle growth (Endo et al., 2003). A study in gestating gilts concluded that when adding either 2,500 IU of vitamin D_3 or 500 IU of vitamin D_3 and 50 μg of 25(OH)D_3 in the diet (both treatments having similar IU equivalency), maternal and fetal serum 25(OH)D_3 were increased with added dietary 25(OH)D_3 (Coffey et al., 2012). Additionally, the authors concluded that reproductive performance of gilts was improved with 25(OH)D_3 supplementation. Interestingly, Hines et al. (2013) found alterations in fetal muscle characteristics in fetuses from gilts fed 25(OH)D_3 when compared with fetuses from gilts fed vitamin D_3. If these changes in fetal muscle development lead to improvements in postnatal performance, they will increase profitability of swine producers.

Therefore, the objectives were 1) to determine a feeding level of vitamin D_3 that would result in a serum 25(OH)D_3 response similar to that observed from feeding 50 μg/kg of 25(OH)D_3 in gestating sows and 2) to evaluate the effects of vitamin D_3 (above the basal requirement level) or the supplementation with 50 μg/kg of 25(OH)D_3 on sow performance, serum vitamin metabolites, subsequent pig performance, and neonatal muscle and bone characteristics.

MATERIALS AND METHODS

General Description

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. These experiments were conducted at the K-State Swine Teaching and Research Facility in Manhattan, KS, and were conducted from January through December of 2014. Both the gestation and farrowing barns were totally enclosed, environmentally controlled, and mechanically ventilated buildings. In gestation, sows were housed in gestation stalls (2.1 × 0.6 m). The farrowing barn contained 29 farrowing crates (2.1 × 0.6 m for the sow and 2.1 × 1.0 m for the pigs) that were each equipped with a single feeder and nipple waterer. Temperature in the farrowing house was maintained at a minimum of 21°C, and supplemental heat was provided to piglets with heat lamps. Gestation and lactation sow 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).

Animals, Diets, and Treatment Design

In Exp. 1, a total of 56 sows (PIC 1050) from 2 consecutive breeding groups were used in a 30-d study to determine the serum 25(OH)D_3 response to varying concentrations of dietary vitamin D_3. The study began 35 d postinsemination and after sows were confirmed pregnant. At initiation, the sows were randomly allotted to 1 of 7 dietary treatments receiving 200, 800, 1,600, 3,200, 6,400, 12,800, or 25,600 IU vitamin D_3/kg of complete diet. There were 8 sows per treatment. The gestation diets were common corn–soybean meal–based diets formulated to contain 0.56% standardized ileal digestible (**SID**) Lys and 0.82% Ca (Table 1). All sows were fed once daily (at 0800 h) and received 2.5 kg of feed. Prior to receiving their daily meal, sows were bled on d 0 and 30 of the trial via jugular venipuncture to collect serum for 25(OH)D_3 analysis. Results from this study were then used to develop a prediction equation to determine the dietary vitamin D_3 concentration needed to achieve a serum 25(OH)D_3 response in gestating sows similar to levels previously reported in the literature (Weber et al., 2014) for females fed 50 μg of 25(OH)D_3/kg of complete diet as their sole source of vitamin D.

In Exp. 2, a total of 112 sows (PIC 1050) from 4 consecutive farrowing groups and their litters were used in the study. Following breeding, sows were randomly assigned to 1 of 4 dietary vitamin D treatments receiving 800 IU, 2,000 IU, or 9,600 IU of vitamin D_3/kg of complete diet or 50 μg of 25(OH)D_3/kg of complete diet. The treatment of 800 IU of vitamin D_3/kg was selected since it represents the basal requirement of the sow (NRC, 2012). The treatment of 2,000 IU of vitamin D_3/kg was used to directly compare to feeding 50 μg of 25(OH)D_3, representing the same IU equivalency. The treatment of 9,600 vitamin D_3/kg was determined following the results found in Exp. 1 and was predicted to provide mean serum 25(OH)D_3 values that would be similar to the treatment fed 50 μg of 25(OH)D_3/kg. There were 28 sows per treatment and 6 to 8 replications per farrowing group. During d 0 through 110 of gestation, sows were fed once daily at 0800 h and received 2.5 kg/d of the gestation diets. On d 110, sows were moved to the farrowing house and were housed in farrowing stalls. After farrowing, sows were fed lactation diets. Gestation and lactation diets were formulated to contain 0.56% and 1.07% SID Lys, respectively. Farrowing crate feeders were equipped with an electronic feeding system (Gestal Solo; JYGA Technologies, Quebec, Canada) that used a built-in

feeding curve based on parity to feed individual sows. The feeding curves were monitored and adjusted daily for individual sows to allow for ad libitum feed intake while reducing feed wastage. Lactation feed intake was confirmed by measuring feed disappearance on d 7, 14, and 21 (weaning). Sow BW was measured at breeding, d 110 of gestation, within 24 h of farrowing, and at weaning to determine gestation BW gain and lactation weight loss. Back fat measurements were collected when sows arrived in the farrowing house and at weaning to determine back fat loss. Sows were bled on d 0 and 100 of gestation, within 24 h after farrowing, and at weaning (d 21) to determine serum 25(OH)D_3, vitamin D_3, vitamin A (retinol), and vitamin E (α-tocopherol).

Within 24 h of parturition, all piglets were weighed and ear notched for identification. The male pig closest to the average BW of the litter was euthanized to collect bone and muscle samples for neonatal bone ash content and neonatal muscle immunohistochemistry measurements. The male and female piglets next closest to the average BW of the litter were bled via jugular venipuncture within 24 h of birth and again at weaning to determine preweaned piglet serum 25(OH)D_3, vitamin D_3, vitamin A (retinol), and vitamin E (α-tocopherol). Mummified and stillborn pigs were recorded to calculate total born. Although minimal, cross-fostering was conducted within vitamin D dietary treatments within 48 h after farrowing to help standardize litter size. 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.

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 into 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 25(OH)D_3/kg, 370 g of 25(OH)D_3 (Hy-D, DSM Nutritional Products North America; 125 µg/g of product) were added per ton of the diet to reach desired finished feed concentrations. Complete diet samples from Exp. 1 and 2 were analyzed for vitamin D_3 and 25(OH)D_3 concentrations by DSM Nutritional Products North America using a combination HPLC and mass spectrometry analytical technique (Schadt et al., 2012).

Table 1. Sow diet composition (as-fed basis)[1]

Item	Gestation[2]	Lactation
Ingredient, %		
Corn	80.28	62.99
Soybean meal, 46.5% CP	15.62	30.21
Choice white grease	—	2.50
Monocalcium phosphate	1.48	1.48
Calcium carbonate	1.15	1.05
Sodium chloride	0.50	0.50
L-Lysine HCl	—	0.20
DL-Methionine	—	0.05
L-Threonine	0.03	0.08
Phytase[3]	0.02	0.02
Trace mineral premix[4]	0.15	0.15
Vitamin premix[5]	0.75	0.75
Vitamin D premix[6]	0.02	0.02
Total	100.00	100.00
Calculated analysis		
SID AA,[7] %		
Lys	0.56	1.07
Met and Cys:Lys	76	56
Thr:Lys	80	64
Trp:Lys	24	20
NE, Mcal/kg	2.47	2.51
SID Lys:NE, g/Mcal	2.27	4.26
CP, %	14.1	19.9
Ca,%	0.82	0.83
P, %	0.64	0.70
Available P, %	0.47	0.49
STTD P,[8] %	0.49	0.53
Ca:P	1.28	1.19
Vitamin A, IU/kg	1,102	1,102
Vitamin E, IU/kg	66.1	66.1

[1]In Exp. 1, 56 gestating sows were used to determine the serum 25(OH)D_3 response from feeding titrated concentrations of vitamin D_3. In Exp. 2, 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D_3 or from synthetic 25(OH)D_3 on maternal performance, subsequent pig performance, sow and piglet 25(OH)D_3, neonatal bone mineralization, and piglet muscle development.

[2]Gestation diets for Exp. 1 and 2 were similar in composition.

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

[4]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.

[5]Provided 3,527,392 IU vitamin A, 26,455 IU vitamin E, 1,764 mg vitamin K, 15 mg vitamin B_{12}, 33,069 mg niacin, 11,023 mg pantothenic acid, 3,307 mg riboflavin, 661 mg folic acid, 882 mg pyridoxine, 220,460 mg choline, 19,842 mg carnitine, and 79 mg chromium per kilogram of premix.

[6]Vitamin D premix was mixed to contain 4,409,240 IU of vitamin D_3/kg of premix by blending vitamin D_3 (Rovimix D, DSM Nutritional Products) with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations in Exp. 1 and 2. For diets containing 25(OH)D_3, the vitamin D premix was not included, and Hy-D (DSM Nutritional Products; 0.0123% 25(OH)D_3) was added into the diet, replacing a percentage of corn, at 0.37 kg/t to achieve the desired concentration of 50 µg of 25(OH)D_3/kg of diet.

[7]SID = standardized ileal digestible.

[8]Standardized total tract digestible

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

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 serum 25(OH)D_3 testing for Exp. 1 was performed by Heartland Assays Inc. (Ames, IA) using a previously described RIA (Hollis et al., 1993). All vitamin metabolite testing (25(OH)D_3, vitamin D_3, α-tocopherol, and retinol) from Exp. 2 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 was 5.00 ng/mL for 25(OH)D_3, 1.00 ng/mL for vitamin D_3, 250 ng/mL for α-tocopherol, and 25 ng/mL for retinol. Some samples were below the detectable limit for serum vitamin D_3 concentration; 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.

Necropsies, Bone and Tissue Sampling, and Bone Ash Procedure

Necropsies were performed on site and in compliance with the university's standard operating procedures. Pigs were euthanized using CO_2 gas administered via a Euthanex AgPro system (Nutriquest, Mason City, IA). Right femurs and second ribs were collected to determine percentage bone ash, and whole-muscle cross sections of the longissimus thoracis (2-cm section over the fifth and sixth ribs caudal to the trapezius) and the semitendinosus (2-cm section medial to the insertion and origin) were collected for immunohistochemistry. Bones were boiled for 60 min, and adhering tissue was removed. Then the bones were dried at 100°C for 7 d. After drying, the bones were ashed in a muffle furnace at 600°C for 24 h.

Immunohistochemistry

After dissecting the whole-muscle cross sections, the cross sections were blotted using blotting paper to measure whole-muscle cross-sectional area. Then the cross sections were embedded in optimal cutting temperature tissue-embedding media (Fisher Scientific, Pittsburgh, PA), frozen by submersion in supercooled isopentane, and stored at −80°C until analysis. For each muscle sample, two 10-µm cryosections were collected on positively charged slides (MidSci), and muscle fibers were immunostained with antibodies validated by Town et al. (2004) for the detection of primary and secondary muscle fibers and merged with the methods of Paulk et al. (2014) to simultaneously identify muscle fiber cross-sectional area. Briefly, nonspecific antigen-binding sites were inhibited by incubating cryosections in 5% horse serum and 0.2% TritonX-100 (Fisher Scientific) in PBS for 30 min. All sections were incubated with the following primary antibodies in blocking solution for 60 min: 1:500 α-dystrophin (Thermos Scientific, Waltham, MA), 1:10 supernatant myosin heavy-chain, slow IgG2b (BA-D5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and 1:10 supernatant myosin heavy-chain type 2A, IgG1 (SC-71, Developmental Studies Hybridoma Bank). After incubation, sections were washed with PBS 3 times for 5 min, followed by incubation in the following secondary antibodies (1:1,000) in blocking solution for 30 min: Alexa-Fluor 488 goat anti-mouse IgG1 for SC-71 (Invitrogen, San Diego, CA), Alexa-Fluor 633 goat anti-mouse IgG2b for BA-D5 (Invitrogen), and Alexa-Fluor 594 goat anti-rabbit H&L for α-dystrophin (Invitrogen). In addition, 1:1,000 Hoechst Dye 33342 (Invitrogen) was utilized to identify all fiber-associated nuclei. Finally, sections were washed for three 5-min periods in PBS and then covered with 5 µL of 9:1 glycerol in PBS and cover slipped for imaging.

Cryosections were imaged using a Nikon Eclipse T1-U inverted microscope with 20× working distance magnification (Nikon Instruments Inc., Melville, NY). Four representative photomicrographs per section were captured using a Nikon DS-QiMc digital camera (Nikon Instruments Inc.) calibrated to the 20 × objective. For myosin heavy-chain fiber-type data collection, a minimum of 2 photomicrographs per section (minimum of 500 fibers per animal) were analyzed for isoform distribution with NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.). Fibers that were positively stained for the BA-D5 antibody were counted as primary muscle fibers, and the fibers that were positively stained for SC-71 were labeled as secondary fibers. Total muscle fiber number was calculated by dividing the whole muscle cross-sectional area by the average cross-sectional area of all muscle fibers. To calculate the total number of primary muscle fibers per muscle section, the percentage of primary muscle fibers was multiplied by the total number of muscle fibers. Similarly, the total number of secondary muscle fibers was calculated by multiplying the percentage of secondary fibers by the total number of muscle fibers.

Statistical Analysis

All data were analyzed as a generalized randomized complete block design using the GLIMMIX procedure

Table 2. Analyzed dietary vitamin D_3 in the complete diets, Exp. 1[1]

	Vitamin D_3, IU/kg						
Item	200	800	1,600	3,200	6,400	12,800	25,600
Formulated	200	800	1,600	3,200	6,400	12,800	25,600
Analyzed	194	714	1,600	2,440	6,116	13,049	24,540
Percentage of claim	96.7	89.3	100.0	76.3	95.6	101.9	95.9

[1]Samples were collected and pooled together, then shipped to a DSM Nutritional Products laboratory (Parsippany, NJ) for analysis. Means represent the average analyzed value of 2 samples.

of SAS (SAS Inst. Inc., Cary, NC). For Exp. 1, a mixed effect model was used to regress the sow serum 25(OH)D_3 concentrations against dietary vitamin D_3 concentrations per day. In the model, sow group was used as a random effect, and dietary treatment was used as the predictor variable. Fit of prediction models was evaluated by using the Bayesian information criterion (**BIC**). A model with a reduced BIC value of 2 or greater was considered an improved model (Kass and Raftery, 1995). Residuals during the model development process were evaluated to distinguish any potential biases in the prediction equations. Maternal performance data were analyzed with sow as the experimental unit, maternal treatment as a fixed effect, and farrowing group as a random effect. Responses not normally distributed were analyzed with a negative binomial distribution (total born and number after cross fostering), a binomial distribution (stillborns, mummies, and number born alive), or a β distribution (bone ash). Preplanned comparisons consisted of 1) linear and quadratic polynomials for increasing vitamin D_3 (Exp. 1 and 2), 2) 800 IU vitamin D_3 vs. 50 µg 25(OH)D_3 (Exp. 2), 3) 2,000 IU vitamin D_3 vs. 50 µg 25(OH)D_3 (Exp. 2), and 4) 9,600 IU vitamin D_3 vs. 50 µg 25(OH)D_3 (Exp. 2). The IML procedure of SAS was used to generate unequally spaced linear and quadratic contrast coefficients for dietary vitamin D_3 treatments in Exp. 1 and 2. Additionally, the repeated measures analysis was performed on serum vitamin metabolite responses, and day of collection was included as a fixed effect to determine serum changes to dietary treatments over time. Results were considered significant at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

Supplementation of dietary vitamin D is required for swine reared in environmentally controlled production facilities because of the lack of exposure to direct sunlight needed for the endogenous conversion of 7-dehydrocholesterol to vitamin D_3 in the skin. Previously documented cases of vitamin D being absent from premixes fed to pigs (Feedstuffs, 2010) has led to a resurgence of interest in the animal's requirement for vitamin D to safeguard from potential deficiency. Additionally, recent genomic data, which have shown the presence of the vitamin D receptor in many soft tissues not associated with normal Ca and P homeostasis (Norman and Bouillon, 2010), have led to increased efforts to understand vitamin D's role in other normal bodily processes. The aim of the current study was to evaluate maternal vitamin D supplementation as either vitamin D_3 (at varying levels) or 25(OH)D_3 on sow and subsequent pig response criteria.

Experiment 1

Although there is no published accepted standard for vitamin D recovery in animal feeds, analysis showed diets were within 25% of their formulated targets (Table 2). which would be consistent with the acceptable analytical variation and recovery of other vitamins previously discussed by the Association of American Feed Control Officials (**AAFCO**, 2015).

Gestating sows fed increasing vitamin D_3 had increased (quadratic, $P = 0.001$; Table 3) serum 25(OH)D_3 concentrations. The serum results were used to develop an equation to predict the serum 25(OH)D_3 response to increasing vitamin D_3 supplementation in gestating females. The equation was as follows: serum 25(OH)D_3, ng/mL (±SE) = 35.1746 ± 5.56 + (0.002353 ± 0.00024 × dietary vitamin D_3, IU/d) − (0.0000000156 ± 0.0000000036 × dietary vitamin D_3, IU/d^2; Fig. 1). Fitting a quadratic term model improved the BIC value compared with a single linear model, suggesting a better model fit to the observed data. To our knowledge, this

Table 3. Effects of increasing dietary vitamin D_3 on serum 25(OH)D_3 in gestating sows, Exp. 1[1]

Serum 25(OH)D^3,	Vitamin D_3, IU/kg								Probability *P*, Vitamin D_3	
ng/mL	200	800	1,600	3,200	6,400	12,800	25,600	SEM	Linear	Quadratic
d 0	46.1	40.3	46.0	43.8	46.3	48.2	43.9	6.47	<0.826	<0.318
d 30	37.2	35.9	46.1	51.9	73.8	91.1	122.4	6.62	<0.001	<0.001

[1]A total of 56 gestating sows were used in a 30-d trial to determine the serum 25(OH)D_3 response from feeding titrated concentrations of vitamin D_3. There were 8 sows per treatment, and sows were fed 2.5 kg/d.

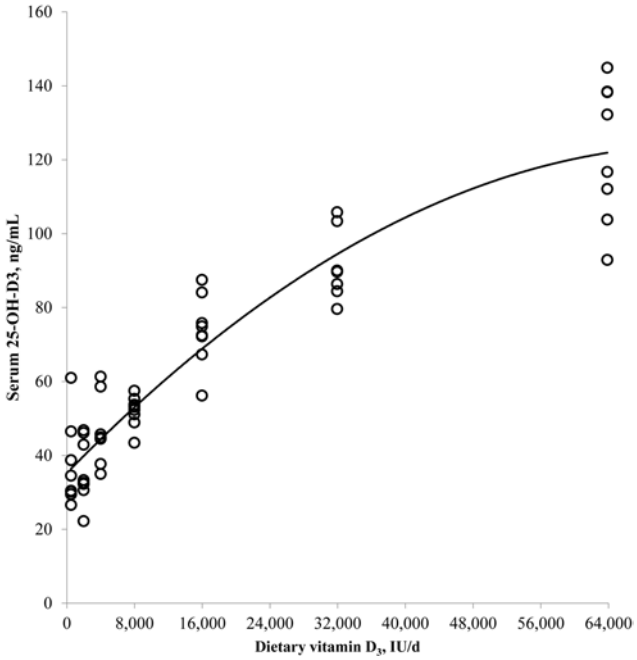

Figure 1. Plot of predicted serum 25(OH)D_3 response to daily vitamin D_3 intake of gestating sows (Exp. 1) based on the observed serum 25(OH)D_3. The equation used for predicted values was serum 25(OH)D_3, ng/mL (±SE) = 35.1746 ± 5.56 + (0.002353 ± 0.00024 × dietary vitamin D_3, IU/d) − (0.0000000156 ± 0.0000000034 × dietary vitamin D_3, IU/d^2).

is the first study to develop a prediction equation based on dietary vitamin D intake in swine. This information was used to predict the vitamin D_3 supplementation rate needed to achieve serum 25(OH)D_3 results similar to that of sows fed a known amount of 25(OH)D_3. Previous literature examining the serum 25(OH)D_3 response of sows fed 50 µg/kg of 25(OH)D_3 (Weber et al., 2014) in gestation concluded that the range of serum 25(OH)D_3 response appeared to be between 50 and 90 ng/mL depending on time of sampling (gestation or lactation) and parity of the female. This range was supported by Lauridsen et al. (2010), who reported a mean serum 25(OH)D_3 concentration of approximately 85 ng/mL for sows fed 50 µg of 25(OH)D_3/kg of diet. Additionally, Coffey et al. (2012) observed serum 25(OH)D_3 concentrations of approximately 80 to 90 ng/mL for first-parity gestating gilts fed diets containing 50 µg of 25(OH)D_3/kg along with 500 IU of vitamin D_3/kg. In this preliminary experiment, we did not examine serum 25(OH)D_3 concentrations from sows fed 50 µg of 25(OH)D_3/kg because of the breadth of data supporting a response at approximately 70 to 80 ng/mL in the sow. Using the prediction equation developed herein, serum 25(OH)D_3 concentrations between 50 and 90 ng/mL could be achieved by supplementing between 17,000 and 29,000 IU of vitamin D_3/d. To ensure the vitamin D_3 supplementation rate was high enough to elicit a serum response similar to that reported in the literature when feeding 50 µg/kg of 25(OH)D_3, a targeted feeding level of 9,600 IU of vitamin D_3 per kilogram of complete feed (12 to 14 times the NRC [2012] vitamin D requirement, approximately 24,000 IU/d) was selected as the highest level of vitamin D_3 supplementation for Exp. 2.

Table 4. Analyzed sow diet composition, Exp. 2[1]

	Maternal vitamin D supplementation, IU/kg			
	Vitamin D_3			50 µg 25(OH)D_3
Item	800	2,000	9,600	2,000
Formulated gestation diets				
CP, %	14.1	14.1	14.1	14.1
Ca, %	0.82	0.82	0.82	0.82
P, %	0.64	0.64	0.64	0.64
Vitamin D_3, IU/kg	800	2,000	9,600	—
25(OH)D_3, µg/kg	—	—	—	50
Analyzed gestation diets				
CP, %	15.0	15.2	14.8	14.8
Ca, %	1.01	0.86	0.87	1.06
P, %	0.62	0.62	0.64	0.63
Vitamin D_3, IU/kg	730	2,000	9,057	—
25(OH)D_3, µg/kg	—	—	—	46.4
Vitamin D, % of formulated	91.2	100.0	94.3	92.7
Formulated lactation diets				
CP, %	19.9	19.9	19.9	19.9
Ca, %	0.83	0.83	0.83	0.83
P, %	0.70	0.70	0.70	0.70
Vitamin D_3, IU/kg	800	2,000	9,600	—
25(OH)D_3, µg/kg	—	—	—	50
Analyzed lactation diets				
CP, %	19.3	20.1	19.5	19.5
Ca, %	1.05	1.10	0.94	0.94
P, %	0.65	0.66	0.67	0.70
Vitamin D_3, IU/kg	906	1,986	9,310	—
25(OH)D_3, µg/kg	—	—	—	45.4
Vitamin D, % of formulated	113.1	99.3	97.0	90.7

[1]Samples were collected and pooled together, then shipped to DSM Nutritional Products laboratory (Parsippany, NJ) for vitamin D analysis and to a commercial laboratory (Ward laboratories, Kearney, NE) for proximate analysis. Means represent the average analyzed value of 2 samples.

Experiment 2

Proximate analysis of gestation and lactation diets fed in Exp. 2 (Table 4) showed CP and P concentrations similar to formulated levels. Analyzed Ca concentrations were more variable, but all values were above the requirements of the sow. Analysis showed diets were within 10% of their formulated targets, which is within the acceptable analytical variation and recovery of other vitamins (AAFCO, 2015).

Sow Performance and Litter Characteristics

Vitamin D treatment did not affect gestation BW gain (Table 5). Increasing vitamin D_3 increased (quadratic, $P = 0.011$) lactation ADFI and decreased

Table 5. The effects of maternal dietary vitamin D supplementation on sow and preweaned pig performance, Exp. 2[1]

	Maternal vitamin D, 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			
Sows, *n*	27	28	25	28	—	—	—	—	—	—
Parity	2.2	2.2	2.1	2.2	0.30	<0.807	<0.822	<0.914	<0.963	<0.775
Lactation ADFI, kg	5.36	5.88	5.27	5.65	0.199	<0.137	<0.011	<0.184	<0.294	<0.088
Sow BW, kg										
Gestation										
d 0	193.2	190.8	190.4	192.0	9.55	<0.835	<0.835	<0.905	<0.908	<0.876
d 110	234.7	226.1	233.7	233.9	7.85	<0.721	<0.232	<0.923	<0.293	<0.980
BW gain, kg	41.4	35.4	43.9	42.0	3.74	<0.330	<0.190	<0.901	<0.191	<0.771
Lactation										
d 0	229.3	222.3	226.6	231.2	7.31	<0.909	<0.348	<0.800	<0.231	<0.547
d 21	221.2	221.8	220.5	227.0	6.94	<0.889	<0.926	<0.452	<0.494	<0.406
BW loss, kg	−8.1	−0.6	−6.1	−4.2	2.44	<0.677	<0.003	<0.129	<0.153	<0.464
Sow back fat, mm										
Farrowing	14.3	13.5	14.9	14.1	0.72	<0.245	<0.305	<0.796	<0.539	<0.343
Weaning	12.7	12.5	13.3	12.6	0.63	<0.303	<0.661	<0.868	<0.892	<0.339
Lactation back fat loss	−1.6	−1.1	−1.6	−1.5	0.58	<0.734	<0.395	<0.876	<0.516	<0.883
Litter characteristics										
Total born, *n*	13.93	12.96	12.96	13.57	0.718	<0.584	<0.573	<0.783	<0.645	<0.652
Born alive, %	91.0	94.5	93.2	93.4	1.48	<0.763	<0.329	<0.428	<0.651	<0.929
Stillborn, %	7.7	4.1	6.2	6.1	1.38	<0.956	<0.294	<0.534	<0.447	<0.958
Mummies, %	1.3	1.4	0.6	0.5	0.61	<0.497	<0.854	<0.466	<0.454	<0.899
Total after foster, *n*	12.00	11.29	11.53	11.76	0.706	<0.824	<0.457	<0.797	<0.606	<0.810
Number weaned	10.70	10.21	10.20	10.54	0.639	<0.761	<0.698	<0.880	<0.773	<0.770
Survivability, %	89.5	90.8	88.8	88.9	2.27	<0.573	<0.524	<0.809	<0.426	<0.972
Piglet BW, kg										
Birth	1.43	1.41	1.44	1.42	0.052	<0.816	<0.842	<0.989	<0.989	<0.770
Weaning	6.48	6.76	6.55	6.40	0.237	<0.882	<0.349	<0.231	<0.231	<0.622

[1]A total of 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D_3 or from synthetic 25(OH)D_3 on maternal performance, subsequent pig performance, sow and piglet serum vitamin metabolites, neonatal bone mineralization, and piglet muscle development. Three sows (1 from the 800 IU/kg treatment and 2 from the 9,600 IU/kg treatment) were removed because of farrowing complications. One sow from the treatment fed 9,600 IU/kg was removed from the data set due to a late-term abortion.

(quadratic, $P = 0.003$) BW loss during lactation. This was because sows fed diets with 2,000 IU of vitamin D_3/kg had greater lactation ADFI compared with sows fed diets with 800 or 9,600 IU of vitamin D_3/kg. Also, sows consuming diets with 9,600 IU vitamin D_3/kg tended ($P = 0.088$) to have lower lactation feed intake compared with sows fed diets with 50 µg/kg of 25(OH)D_3. Total daily vitamin D intake during lactation was approximately 4,300, 11,800, and 50,600 IU/d for sows fed diets containing 800, 2,000, and 9,600 IU of vitamin D_3/kg, respectively, and approximately 11,300 IU/d for sows fed diets containing 50 µg/kg IU of 25(OH)D_3/kg. The current study observed no impact of vitamin D treatment on litter characteristics or piglet BW at birth or weaning. The results herein suggest little to no influence of maternal vitamin D treatment above basal requirement on sow performance. Flohr et al. (2014) also concluded that varying vitamin D_3 supplementation rates (1,500 to 6,000 IU/kg of diet) had no influence on sow performance or litter characteristics. However, Lauridsen et al. (2010) observed reductions in stillborns from sows fed 1,400 or 2,000 IU of vitamin D/kg of diet compared with sows fed 200 or 800 IU of vitamin D/kg of diet. Weber et al. (2014) observed increases in the birth and weaning weight of pigs from sows fed 50 µg/kg of 25(OH)D_3 compared with pigs from sows fed 2,000 IU of vitamin D_3. They hypothesized that this was the result of improvements in the intrauterine development of the embryos. Coffey et al. (2012) observed an increase in the number of developed fetuses in the reproductive tracts of first-service gilts when supplemented with 25(OH)D_3 rather than vitamin D_3 at the same IU equivalency. Although some significant differences have been observed with different vitamin D supplementation strategies, the lack of consistency in measured responses across studies makes it difficult to determine whether vitamin D supplementation (above basal NRC, 2012, requirement) truly impacts maternal performance. Ultimately, commercial-scale studies with large sample sizes will be needed to increase sensitivity

Table 6. The effects of maternal dietary vitamin D supplementation on sow serum metabolites, Exp. 2[1]

Sow serum vitamin metabolites	Maternal vitamin D, 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
	800	2,000	9,600	2,000	SEM[2]	Linear	Quadratic			
25(OH)D_3,[3] ng/mL										
d 0 of gestation	44.6	43.9	41.1	45.9	3.54	<0.405	<0.957	<0.768	<0.650	<0.278
d 100 of gestation	27.6	29.2	82.5	59.5		<0.001	<0.157	<0.001	<0.001	<0.001
Farrowing	25.1	26.1	68.2	55.4		<0.001	<0.241	<0.001	<0.001	<0.004
Weaning	34.6	50.9	110.6	94.6		<0.001	<0.153	<0.001	<0.001	<0.001
Vitamin D_3,[4] ng/mL										
d 0 of gestation										
Detectable samples, %	100.0	100.0	100.0	100.0	4.74	<0.999	<0.999	<0.999	<0.999	<0.999
Serum D_3, ng/mL	7.6	7.5	7.1	7.6	0.926	<0.677	<0.965	<0.954	<0.877	<0.646
d 100 of gestation										
Detectable samples, %	100.0	100.0	100.0	100.0		<0.999	<0.999	<0.999	<0.999	<0.999
Serum D_3, ng/mL	3.5	5.2	26.6	1.9	0.926	0.001	0.217	<0.188	<0.006	<0.001
Farrowing										
Detectable samples, %	100.0	100.0	100.0	91.7		<0.999	<0.999	<0.216	<0.216	<0.216
Serum D_3, ng/mL	3.0	4.7	19.5	1.8	0.961	<0.001	<0.645	<0.357	<0.020	<0.001
Weaning										
Detectable samples, %	91.7	100.0	100.0	58.3		<0.387	<0.255	<0.001	<0.001	<0.001
Serum D_3, ng/mL	4.5	10.9	33.7	1.8	1.17	<0.001	<0.035	<0.063	<0.001	0.001
α-tocopherol,[5] mg/L										
d 0 of gestation	2,187	2,063	1,979	2,099	131.1	<0.275	<0.545	<0.601	<0.830	<0.473
d 100 of gestation	2,096	1,668	2,112	1,803		<0.211	<0.007	<0.081	<0.420	<0.066
Farrowing	1,247	1,054	1,219	1,329		<0.748	<0.231	<0.622	<0.102	<0.508
Weaning	2,338	2,611	2,295	2,358		<0.305	<0.077	<0.905	<0.132	<0.705
Retinol,[6] ng/mL										
d 0 of gestation	285	294	254	279	17.6	<0.113	<0.569	<0.833	<0.565	<0.301
d 100 of gestation	231	210	237	225		<0.492	<0.353	<0.807	<0.554	<0.604
Farrowing	128	165	149	192		<0.593	<0.713	<0.177	<0.291	<0.089
Weaning	299	393	337	325		<0.957	<0.001	<0.299	<0.006	<0.625

[1]In total 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D_3 or from synthetic 25(OH)D_3 on maternal performance, subsequent pig performance, sow and piglet serum vitamin metabolites, neonatal bone mineralization, and piglet muscle development. Means represent the average serum metabolite from 12 randomly selected sows within treatment and day combinations.

[2]Standard error of the mean representing the within–sampling day variation. Because the same number of treatments was analyzed for each day, the variance estimates were the same.

[3]A treatment × day interaction ($P = 0.001$) was observed for serum 25(OH)D_3.

[4]The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit ($n = 144$ out of 192) were not used in the statistical analysis. Detectable samples represent 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 tendency ($P = 0.052$) for a treatment × day interaction was observed for serum **α**-tocopherol.

[6]A treatment × day interaction ($P = 0.035$) was observed for serum retinol.

and reduce the experimental error associated with sow reproduction measurements to evaluate dietary supplementation of vitamin D above the current requirement.

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

A treatment × day interaction ($P = 0.001$; Table 6) for serum 25(OH)D_3 of sows was observed because sow serum 25(OH)D_3 was similar on d 0 of gestation regardless of dietary vitamin D treatment, but increasing vitamin D_3 increased (linear, $P < 0.001$) serum 25(OH)D_3 on d 100 of gestation, after farrowing, and at weaning. Also, sows fed diets with 800 or 2,000 IU of vitamin D_3/kg had less serum 25(OH)D_3 on d 100 of gestation ($P = 0.001$), after farrowing ($P = 0.001$), and at weaning ($P = 0.001$) compared with sows fed 50 µg of 25(OH)D_3/kg. Sows fed the diets with 9,600 IU of vitamin D_3/kg had greater serum 25(OH)D_3 concentrations on d 100 of gestation ($P = 0.001$), after farrowing ($P = 0.004$), and at weaning ($P = 0.001$) compared with sows fed 25(OH)D_3. Lauridsen et al. (2010), Coffey et al. (2012), and Weber et al. (2014) have all discussed similar responses when comparing the supplementation of 25(OH)D_3 and vitamin D_3 at the same IU equivalency. It is clear that 25(OH)D_3 provides a greater serum

Table 7. The effect of maternal dietary vitamin D supplementation on preweaned pig serum vitamin metabolites and neonatal bone ash, Exp. 2[1,2]

	Maternal vitamin D, 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[2]	Linear	Quadratic			
Preweaned pig serum vitamin metabolites										
25(OH)D_3,[3] ng/mL										
Birth	2.0	2.2	5.5	3.5	0.43	<0.001	<0.548	<0.004	<0.011	<0.001
Weaning	4.3	7.0	16.3	6.1	0.43	<0.001	<0.033	<0.001	<0.101	<0.001
Vitamin D_3[4]										
Birth										
Detectable samples, %	0.0	0.0	54.2	0.0	5.61	<0.001	<0.299	<0.999	<0.999	<0.001
Serum vitamin D_3, ng/mL	—	—	1.7	—	0.45	—	—	—	—	—
Weaning										
Detectable samples, %	0.0	41.7	100	4.2	5.61	<0.001	<0.001	<0.582	<0.001	<0.001
Serum vitamin D_3, ng/mL	—	1.4	5.7	2.1	1.24	—	—	—	—	—
α-tocopherol, mg/L										
Birth	2,718	2,494	2,190	2,662	395.9	<0.319	<0.757	<0.912	<0.741	<0.342
Weaning	5,331	4,584	5,379	4,844	380.2	<0.439	<0.107	<0.326	<0.601	<0.286
Retinol,[5] ng/mL										
Birth	108	80	93	106	9.6	<0.714	<0.031	<0.909	<0.038	<0.288
Weaning	254	266	268	255	9.6	<0.395	<0.384	<0.924	<0.381	<0.305
Bone ash content, %										
Second rib	53.7	55.7	54.0	54.0	3.11	<0.753	<0.265	<0.863	<0.358	<0.973
Femur	46.1	45.6	45.5	46.4	0.53	<0.519	<0.566	<0.681	<0.285	<0.246

[1]In total 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D_3 or from synthetic 25(OH)D_3 on maternal performance, subsequent pig performance, sow and piglet serum vitamin metabolites, neonatal bone mineralization, and piglet muscle development. Means represent the average serum metabolite from 48 randomly selected litters (2 pigs per litter were bled for serum analysis) within treatments, and the same litters within each day were analyzed. One pig per litter (n = 104) was euthanized for bone ash percentage determination.

[2]Standard error of the mean representing the within–sampling day variation. Because the same number of treatments was analyzed for each day, the variance estimates were the same.

[3]A treatment × day interaction (P = 0.001) was observed for serum 25(OH)D_3.

[4]The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit (n = 144 out of 192) 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.

[5]A tendency (P = 0.065) for a treatment × day interaction was observed for serum retinol.

25(OH)D_3 response in sows. Although the exact reason for this improved response is not completely clear, Bar et al. (1980) demonstrated that 25(OH)D_3 is absorbed more efficiently than vitamin D_3 in the upper portion of the intestine of young broiler chicks. Another potential reason may be due to the postabsorptive transport of the different forms. Because 25(OH)D_3 is the circulating form of the vitamin that binds with the vitamin D binding protein in the bloodstream, it does not require the hydroxylation step of metabolism in the liver. On the other hand, vitamin D_3 must enter the bloodstream as a part of a chylomicron (Clinton, 2013). Lipoprotein lipases in adipose tissue can interact with circulating chylomicrons to store a portion of their lipids and, consequently, the vitamin D_3 transported within them. This suggests that a portion of the vitamin D_3 that is absorbed may be stored in adipose tissue rather than being transported to the liver for hydroxylation. The serum 25(OH)D_3 concentrations achieved in gestation from supplementing 25(OH)D_3 were less than those in the reports of previous researchers (Lauridsen et al., 2010; Coffey et al., 2012; Weber et al., 2014); this may be due to the time of sampling and duration of feeding, which Weber et al. (2014) discussed as potential influencers of the serum response. Also, Lauridsen et al. (2010) summarized results using only the main effect of dietary treatment on serum 25(OH)D_3 concentrations from sows fed 50 µg of 25(OH)D_3/kg rather than reporting the interactive means of time × dietary treatment, which may have led to an inflated mean serum concentration due to increased vitamin intake during the lactation period. The increases in serum 25(OH)D_3 with increasing vitamin D_3 agree with previous data from Flohr et al. (2014). To our knowledge, this is the first study that has shown a level of vitamin D_3 supplementation that has elicited a serum response above feeding 50 µg of 25(OH)D_3/kg.

For serum vitamin D_3, maternal vitamin D treatment did not affect the percentage of sows exhibiting serum

Table 8. The effect of maternal dietary vitamin D supplementation on neonatal muscle immunohistochemistry, Exp. 2[1]

	Maternal vitamin D, 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			
Litters sampled, *n*	25	27	25	27						
Longissimus thoracis										
Whole muscle area,[2] mm^2	117.3	113.7	113.5	111	13.98	<0.795	<0.749	<0.543	<0.792	<0.810
Average fiber CSA,[3] μm^2	101.1	106.4	96.8	109.8	9.56	<0.291	<0.362	<0.200	<0.609	<0.057
Average primary fiber CSA,[4] μm^2	191.5	209.7	197.7	213.4	11.47	<0.946	<0.254	<0.173	<0.813	<0.325
Average secondary fiber CSA,[5] μm^2	95.8	99.8	91.0	102.9	9.52	<0.272	<0.450	<0.276	<0.632	<0.070
Total fiber number[6] (1×10^6)	1.2	1.1	1.3	1.1	0.18	<0.540	<0.296	<0.235	<0.823	<0.177
Total primary fibers[7] (1×10^4)	6.8	6.9	6.5	8.5	1.06	<0.776	<0.924	<0.234	<0.254	<0.158
Total secondary fibers[8] (1×10^6)	1.8	1.1	1.2	1.0	0.17	<0.502	<0.270	<0.169	<0.716	<0.117
Secondary:primary[9]	18.0	16.5	18.8	15.7	1.63	<0.289	<0.238	<0.112	<0.577	<0.035
Semitendinosus										
Whole muscle area,[2] mm^2	60.0	64.3	61.6	62.0	7.30	<0.985	<0.460	<0.730	<0.695	<0.939
Average fiber CSA,[3] μm^2	135.4	139.7	128.8	140.4	10.89	<0.409	<0.633	<0.671	<0.954	<0.303
Average primary fiber CSA,[4] μm^2	185.4	198.7	171.8	202.9	12.47	<0.142	<0.279	<0.243	<0.767	<0.031
Average secondary fiber CSA,[5] μm^2	131.7	135.8	125.7	136.2	10.59	<0.449	<0.656	<0.700	<0.968	<0.349
Total fiber number[6] (1×10^5)	4.7	4.6	4.8	4.7	0.54	<0.771	<0.799	<0.949	<0.875	<0.810
Total primary fibers[7] (1×10^4)	3.5	3.5	3.4	3.6	0.54	<0.822	<0.923	<0.905	<0.957	<0.766
Total secondary fibers[8] (1×10^5)	4.4	4.3	4.5	4.4	0.51	<0.739	<0.775	<0.932	<0.871	<0.773
Secondary:primary[9]	15.5	19.7	16.9	18.1	3.83	<0.943	<0.312	<0.544	<0.688	<0.769

[1]In total 112 sows and their subsequent litters were used to evaluate the effects of maternal vitamin D supplementation on fetal muscle development. For all litters larger than 6 pigs, 1 pig per litter (the male piglet closest to the mean BW within 24 h of birth) was euthanized for muscle fiber identification.

[2]Cross-sectional area of the whole muscle.

[3]Average cross-sectional area of all muscle fibers.

[4]Average cross-sectional area of a representative sample of primary muscle fibers.

[5]Average cross-sectional area of a representative sample of secondary muscle fibers.

[6]Total muscle fiber number is calculated as the whole muscle area divided by the average muscle fiber cross-sectional area of all muscle fibers.

[7]Total primary muscle fiber number was calculated as the percentage of primary fibers × total fiber number.

[8]Total secondary muscle fiber number was calculated as the percentage of secondary fibers × total fiber number.

[9]The average number of secondary muscle fibers per primary muscle fiber.

concentrations above the detectable limit on d 0 or 100 of gestation or at farrowing. However, at weaning, greater percentages of sows fed vitamin D_3 ($P < 0.001$) had serum vitamin D_3 concentrations above the detectable limit. Increasing vitamin D_3 increased serum vitamin D_3 on d 100 of gestation (linear, $P = 0.001$), after farrowing (linear, $P = 0.001$), and at weaning (quadratic, $P = 0.035$). Also, sows fed the diets with 2,000 or 9,600 IU of vitamin D_3/kg had greater serum vitamin D_3 concentrations on d 100 of gestation ($P < 0.006$), after farrowing ($P < 0.020$), and at weaning ($P = 0.001$) compared with sows fed 25(OH)D_3. Sows fed diets containing 800 IU of vitamin D_3/kg tended to have greater ($P = 0.063$) serum vitamin D_3 concentrations at weaning compared with sows fed diets with 50 µg/kg of 25(OH)D_3. Serum vitamin D_3 is typically much more variable than 25(OH)D_3 since it will increase rapidly after exposure (either in the diet or through the skin) and will be cleared from circulation by the liver or storage tissue within hours. Also, the vitamin D binding protein, which accompanies vitamin D metabolites in circulation, has a much lower affinity for vitamin D_3 compared with 25(OH)D_3 (Institute of Medicine, 2011). In the current study, it is understandable that increasing dietary vitamin D_3 led to increased serum concentrations of the nutrient. Additionally, because of less vitamin D_3 exposure of sows fed 25(OH)D_3, it is justified that their serum vitamin D_3 was lower than that of sows fed vitamin D_3.

There was a tendency ($P = 0.052$) for a treatment × day interaction for sow serum α-tocopherol concentrations because serum α-tocopherol was similar across maternal treatments on d 0 of gestation and after farrowing, but on d 100 of gestation increasing vitamin D_3 supplementation decreased (quadratic, $P = 0.007$) serum α-tocopherol concentrations. Additionally, on d 100 of gestation, serum α-tocopherol tended ($P < 0.081$) to be greater for sows fed 800 or 9,600 IU of vitamin D_3/kg compared with sows fed 50 µg of 25(OH)D_3/kg. These differences observed in serum α-tocopherol were unexpected since all diets were formulated to contain similar concentrations of vitamin E (66 IU/kg of diet), resulting in a daily intake of 165 IU of vitamin E. Additionally,

there are no previous data that have evaluated a vitamin E and vitamin D interaction in livestock diets. However, Goncalves et al. (2015) concluded that there is the potential for common absorption pathways for vitamin D and E since increasing vitamin D uptake resulted in decreased vitamin E uptake in human epithelial colorectal adenocarcinoma cells (Caco-2) in vitro cells. At weaning, there was a tendency (quadratic, $P = 0.077$) for sows fed increasing vitamin D_3 to have increasing serum α-tocopherol. This tendency for increased serum α-tocopherol may be the result of increased lactation feed intake observed for sows fed 2,000 IU of vitamin D_3/kg. On the basis of lactation feed intake, sows consuming diets with 2,000 IU of vitamin D_3/kg had vitamin E intakes of approximately 390 IU/d, compared with sows fed either 800 or 9,600 IU of vitamin D_3/kg, which had vitamin E intakes of approximately 350 IU/d.

A treatment × day interaction ($P = 0.001$) for sow serum retinol was observed because serum retinol was similar regardless of maternal vitamin D treatment on d 0 and 100 of gestation; however, after farrowing, sows fed 9,600 IU of vitamin D_3/kg tended ($P = 0.089$) to have less serum retinol compared with sows fed 50 μg of 25(OH)D_3/kg. In addition, sows fed increasing levels of vitamin D_3 had increased (quadratic, $P = 0.001$) serum retinol concentrations at weaning. Sows fed diets with 2,000 IU of vitamin D_3/kg had greater ($P = 0.006$) serum retinol compared with sows fed 50 μg of 25(OH)D_3/kg at weaning. Again, this increase in serum retinol at weaning was likely the result of increased vitamin A intake for sows fed the diets with 2,000 IU of vitamin D_3/kg because of the increase in lactation feed intake. Sows consuming diets with 2,000 IU of vitamin D_3 consumed approximately 6,500 IU of vitamin A/D compared with sows fed 800 IU of vitamin D_3/kg (approximate vitamin A intake of 5,900 IU/d), sows fed 9,600 IU of vitamin D_3/kg (approximate vitamin A intake of 5,800 IU/d), and sows fed 50 μg of 25(OH)D_3/kg (approximate vitamin A intake of 6,225 IU/d). Little information has been reported on the interactions of vitamin A and vitamin D. Abawi and Sullivan (1989) concluded that supplying higher supplemental levels of vitamin D helped improve performance in broilers supplemented high levels of vitamin A and E. Also, Payne and Manston (1967) concluded that increasing the supplementation of vitamin A with high supplementation of vitamin D may reduce the chance of vitamin D toxicity.

Piglet Serum 25(OH)D_3, Vitamin D_3, α-Tocopherol, Retinol, and Neonatal Percentage Bone Ash

For piglet serum 25(OH)D_3, there was a treatment × day interaction ($P = 0.001$; Table 7) because increasing maternal dietary vitamin D_3 increased (linear, $P < 0.001$) piglet serum 25(OH)D_3 at birth and at weaning (quadratic, $P = 0.033$), with a greater magnitude of increase occurring at weaning. This observation agrees with reports from Flohr et al. (2014), who found that increasing maternal vitamin D_3 supplementation from 1,500 to 6,000 IU/kg of diet increased subsequent piglet serum 25(OH)D_3 throughout lactation. Also, in the current study, piglets from sows fed 25(OH)D_3 had greater ($P < 0.011$) serum 25(OH)D_3 compared with piglets from sows fed 800 or 2,000 IU of vitamin D_3/kg at birth; however, at weaning, piglets from sows fed 50 μg of 25(OH)D_3/kg had similar serum 25(OH)D_3 compared with piglets from sows fed the 2,000 IU of vitamin D_3/kg and greater ($P = 0.001$) serum 25(OH)D_3 concentrations compared with piglets from sows fed 800 IU of vitamin D_3/kg. Additionally, piglets from sows fed 9,600 IU of vitamin D_3/kg had increased ($P = 0.001$) serum 25(OH)D_3 at birth and weaning compared with piglets from sows fed 50 μg/kg of 25(OH)D_3/kg.

Previous reports from Coffey et al. (2012) and Witschi et al. (2011) have discussed increases in serum 25(OH)D_3 concentrations in fetuses (d 90 of gestation) and piglets from sows supplemented dietary 25(OH)D_3 compared with those from sows supplemented with dietary vitamin D_3 at the same IU equivalency. Goff et al. (1984) demonstrated that the 25(OH)D_3 of the neonate is largely correlated to the 25(OH)D_3 status of the sow at birth, and 25(OH)D_3 has clearly been demonstrated as the vitamin D metabolite associated with transplacental transfer (Haddad et al., 1971). The current data agree with previous reports and are the first to show a maternal dietary vitamin D_3 supplementation rate that provided a larger serum 25(OH)D_3 response in piglets compared with piglets from sows supplemented 50 μg of 25(OH)D_3. Human research has shown that the transfer of vitamin D metabolites into breast milk is limited (Hollis and Wagner, 2004). Flohr et al. (2014) concluded that increasing supplementation of vitamin D_3 led to increasing milk vitamin D_3 concentrations throughout a 21-d lactation period when milk samples were taken immediately after parturition (colostrum), on d 10, and at weaning. Clements and Fraser (1988) reported that vitamin D_3 was the predominant vitamin D constituent in colostrum of rats, but vitamin D_3 concentrations declined after a few days, and 25(OH)D_3 became the predominant metabolite in milk. The current study suggests that the form of dietary vitamin D supplementation (25(OH)D_3 or vitamin D_3) did not impact milk vitamin D concentrations since feeding either 2,000 IU of vitamin D_3 or 25(OH)D_3 resulted in similar piglet serum 25(OH)D_3 concentrations at weaning. Witschi et al. (2011) observed increased serum 25(OH)D_3 of piglets from sows fed 25(OH)D_3 compared with piglets from sows fed vitamin D_3 at the same IU equivalency,

but their results were confounded with creep feed diets that were provided to suckling pigs starting on the third week of lactation, with pigs being weaned at 5 wk of age. The data herein suggest that the level of maternal dietary vitamin D supplementation is more impactful on milk transfer of the vitamin rather than form (either vitamin D_3 or 25(OH)D_3) of the vitamin when pigs are weaned at approximately 21 d of age and creep feed is not provided prior to weaning.

The majority of piglet serum vitamin D_3 samples were below the laboratory detectable limit of 1.00 ng/mL, which was expected because of the quick clearance of vitamin D_3 from circulation. Samples below that threshold (144 out of 192) were not included in the statistical analysis; therefore, the results were summarized as the percentage of samples that were above the lowest detectable limit, and then the average serum concentration of the detectable samples was calculated. Only 54.2% of pigs from sows fed 9,600 IU of vitamin D_3/kg exhibited serum vitamin D_3 concentrations above the detectable limit, with mean serum concentrations of 1.7 ng/mL. Increasing maternal dietary vitamin D_3 increased (quadratic, $P = 0.001$) the percentage of pigs with serum vitamin D_3 concentrations above the detectable limit, and greater percentages of pigs from sows fed 2,000 or 9,600 IU of vitamin D_3/kg had serum vitamin D_3 concentrations ($P < 0.001$) above the detectable limit compared with pigs from sows fed 50 μg of 25(OH)D_3/kg.

Piglet serum α-tocopherol was similar after birth and at weaning regardless of vitamin D maternal treatment. A tendency ($P = 0.065$) for a treatment × day interaction for piglet serum retinol was observed because at birth piglet serum retinol was reduced (quadratic, $P = 0.031$) with increasing maternal vitamin D_3, and piglets from sows fed diets with a medium level of vitamin D_3 had lower ($P = 0.038$) serum retinol compared with piglets from sows fed 25(OH)D_3; however, by weaning, serum retinol was similar regardless of maternal vitamin D treatment. These differences in serum retinol in piglets at birth were unexpected and may be due to piglets from sows fed the medium level of vitamin D_3 having lower serum retinol in later gestation, although it is unclear why this would have occurred.

Percentage bone ash for second ribs and femurs from pigs euthanized after birth was similar regardless of vitamin D treatment. Similarly, Flohr et al. (2014) observed no impact of increasing maternal vitamin D_3 concentration (1,500 to 6,000 IU/kg of diet) on the bone ash percentage of neonates when maternal vitamin D_3 was above the animal's requirement. Alternatively, Rortvedt and Crenshaw (2012) clearly demonstrated the impact of maternal vitamin D deficiency on subsequent pig kyphosis; however, visual impacts of maternal deficiency were not observed until after weaning. A previous study with rat (Johnson et al., 1996) fetuses detected vitamin D receptor within fetal tissues prior to ossification, alluding to the functional role of vitamin D in the proliferation and differentiation of chondrocytes in skeletal tissue. In the current study, the maternal vitamin D supplementation concentrations were well above those needed to induce a vitamin D deficiency in sows.

Neonatal Muscle Characteristics

Previous research by Hines et al. (2013) concluded that replacing 80% (2,000 IU of the total 2,500 IU/kg of diet) of the vitamin D_3 supplemented to gestating gilts with 25(OH)D_3 increased the maternal vitamin D status and in turn altered fetal muscle development. The authors observed an increase in the number of skeletal muscle fibers and Pax7 (satellite cells) + myoblasts in the LM of fetuses collected on d 90 of gestation. Additionally, after isolating and culturing myoblasts from the semitendinosus muscle, the satellite cells from fetuses of gilts supplemented 25(OH)D_3 had a higher proportion of cells in the proliferation stage 96 h postplating, which suggests increased hyperplasia of myoblasts. These conclusions suggest that vitamin D status of the dam can alter fetal skeletal muscle development with positive changes resulting from the use of 25(OH)D_3 compared with vitamin D_3 itself. Previous work in poultry has elicited similar results (Giuliani and Boland, 1984) and has shown that exogenous addition of 1,25-OH2-D_3 to primary cultures of embryonic chick myoblasts stimulated proliferation and differentiation. In the current study, a subsample of pigs was euthanized to obtain longissimus thoracis (**LT**) and semitendinosus (**ST**) whole-muscle cross sections for immunohistochemistry to characterize potential development differences among maternal vitamin D treatments. Although Pax7+ myoblasts within muscles were not quantified in the current study, we hypothesized that neonatal muscle samples of pigs born from sows fed the 25(OH)D_3 compared with those from sows fed 800 or 2,000 IU of vitamin D_3/kg would have an increased number of muscle fibers. Additionally, if vitamin D status were the reason for the change in fetal muscle fiber numbers, then muscle samples from pigs born from sows fed 9,600 IU of vitamin D_3/kg should be similar to the fiber numbers from muscles of pigs born from sows fed 50 μg of 25(OH)D_3/kg of diet.

Results from the current study showed that whole-muscle areas of the LT and ST were similar regardless of maternal vitamin D treatment (Table 8). Maternal vitamin D treatment did not influence ST average muscle fiber cross-sectional area (**CSA**), but LT average muscle fiber CSA tended ($P = 0.057$) to be greater for piglets from sows fed 25(OH)D_3 compared with piglets

from sows fed 9,600 IU of vitamin D_3/kg. Average primary muscle fiber CSA was similar for the LT regardless of maternal vitamin D treatment; however, primary muscle fiber CSA for the ST was greater ($P = 0.031$) for piglets from sows fed 25(OH)D_3 compared with piglets from sows fed 9,600 IU of vitamin D_3/kg. Secondary muscle fiber CSA for the ST was not influenced by maternal vitamin D treatments, but LT secondary muscle fiber CSA tended to be greater ($P = 0.070$) for piglets from sows fed 25(OH)D_3 compared with piglets from sows fed 9,600 IU of vitamin D_3/kg. Total fiber number, primary fiber number, and secondary fiber number for LT and ST muscles were not influenced by maternal dietary vitamin D treatment. The LT secondary to primary fiber ratio was lower ($P = 0.035$) for piglets from sows fed 25(OH)D_3 compared with piglets from sows fed 9,600 IU of vitamin D_3/kg; however, maternal dietary vitamin D treatment did not influence ST secondary to primary muscle fiber ratio.

The results herein contradict those previously reported by Hines et al. (2013) in the sense that total muscle fiber numbers were not different among maternal vitamin D treatments. The current data suggest little to no impact of the maternal vitamin D treatments on neonatal muscle characteristics except for increases in the hypertrophic growth of the primary muscle fibers of the ST and the secondary muscle fibers of the LT for pigs from sows fed 25(OH)D_3 compared to pigs from sows fed 9,600 IU of vitamin D_3/kg. More research is needed to help elucidate whether there are distinct impacts of maternal vitamin D supplementation from vitamin D_3 or 25(OH)D_3 on fetal muscle development and what levels of the vitamin are optimal.

Conclusion

Overall, the results of this study indicate that supplementing increasing levels of maternal vitamin D_3 to sows can increase sow and piglet serum 25(OH)D_3. Additionally, when supplementing 25(OH)D_3 and vitamin D_3 at the same IU equivalency, serum 25(OH)D_3 of sows and piglets at birth will be increased for sows fed 25(OH)D_3. It appears that maternal dietary vitamin D level impacted weaned pig serum 25(OH)D_3 more so than the form (vitamin D_3 or 25(OH)D_3) of vitamin D. This is likely due to increased vitamin D in milk as a result of an increased level of the maternal dietary supplementation rather than the vitamin D form. Maternal vitamin D treatment (above the basal requirement) had minimal impact on sow performance, neonatal percentage bone ash, or neonatal muscle development characteristics.

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