

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)D₃ response to increasing concentrations of dietary vitamin D₃. Sows were randomly allotted to 1 of 7 dietary D₃ treatments (200, 800, 1,600, 3,200, 6,400, 12,800, or 25,600 IU of added D₃ per kilogram of complete diet) with 8 sows per treatment. Increasing D₃ increased (quadratic; $P < 0.001$) serum 25(OH)D₃ with the response depicted by the prediction equation: serum 25(OH)D₃, ng/mL = 35.1746 + (0.002353 × dietary D₃, IU/d) – (0.0000000156 × dietary D₃, IU/d²). 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 D₃ per kilogram of the diet or 50 µg of 25(OH)D₃ (2,000 IU of D₃ equivalent from Hy-D, DSM Nutritional Products, Parsippany, NJ) per kilogram of diet. There were 25 to 27 sows per treatment. Increasing dietary D₃ increased (linear, $P = 0.001$) serum 25(OH)D₃ of sows on d 100 of gestation, at farrowing, and at weaning. Increasing D₃ in

sow diets increased piglet serum 25(OH)D₃ at birth (linear, $P = 0.001$) and weaning (quadratic, $P = 0.033$). Sows fed 50 µg of 25(OH)D₃/kg had intermediate ($P < 0.004$) serum 25(OH)D₃ concentrations on d 100 of gestation, at farrowing, and at weaning compared with sows fed 2,000 IU of D₃/kg and sows fed 9,600 IU of D₃/kg. Pigs from sows fed 50 µg of 25(OH)D₃/kg had greater serum 25(OH)D₃ compared with pigs from sows fed 2,000 IU of D₃/kg, but at weaning, serum 25(OH)D₃ concentrations were similar. Also, pigs from sows fed 9,600 IU of D₃/kg had greater ($P = 0.011$) serum 25(OH)D₃ at birth and weaning compared with pigs from sows fed 50 µg of 25(OH)D₃/kg. Maternal performance, litter characteristics, neonatal bone ash content, and neonatal muscle fiber characteristics were largely unaffected by the dietary vitamin D treatments. Overall, D₃ and 25(OH)D₃ are both useful at increasing serum 25(OH)D₃ concentrations, but more D₃ (on an equivalent IU basis) is needed to achieve similar serum 25(OH)D₃ responses compared with feeding 25(OH)D₃. 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)D₃ concentrations.

Key words: 25(OH)D₃, 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 D₃). Research examining a synthetically produced 25(OH)D₃ (Hy-D, DSM Nutritional Products North America, Parsippany, NJ) has shown increased serum 25(OH)D₃ compared with vitamin D₃ when

both vitamin D₃ and 25(OH)D₃ 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)D₃ compared with vitamin D₃ (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 D₃ or 500 IU of vitamin D₃ and 50 µg of 25(OH)D₃ in the diet (both treatments having similar IU equivalency), maternal and fetal serum 25(OH)D₃ were increased with added dietary 25(OH)D₃ (Coffey et al., 2012). Additionally, the authors concluded that reproductive performance of gilts was improved with 25(OH)D₃ supplementation. Interestingly, Hines et al. (2013) found alterations in fetal muscle characteristics in fetuses from gilts fed 25(OH)D₃ when compared with fetuses from gilts fed vitamin D₃. 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 D₃ that would result in a serum 25(OH)D₃ response similar to that observed from feeding 50 µg/kg of 25(OH)D₃ in gestating sows and 2) to evaluate the effects of vitamin D₃ (above the basal requirement level) or the supplementation with 50 µg/kg of 25(OH)D₃ 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)D₃ response to varying concentrations of dietary vitamin D₃. 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 D₃/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)D₃ analysis. Results from this study were then used to develop a prediction equation to determine the dietary vitamin D₃ concentration needed to achieve a serum 25(OH)D₃ response in gestating sows similar to levels previously reported in the literature (Weber et al., 2014) for females fed 50 µg of 25(OH)D₃/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 D₃/kg of complete diet or 50 µg of 25(OH)D₃/kg of complete diet. The treatment of 800 IU of vitamin D₃/kg was selected since it represents the basal requirement of the sow (NRC, 2012). The treatment of 2,000 IU of vitamin D₃/kg was used to directly compare to feeding 50 µg of 25(OH)D₃, representing the same IU equivalency. The treatment of 9,600 vitamin D₃/kg was determined following the results found in Exp. 1 and was predicted to provide mean serum 25(OH)D₃ values that would be similar to the treatment fed 50 µg of 25(OH)D₃/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)D₃, vitamin D₃, 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)D₃, vitamin D₃, 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 D₃ concentrations, a premix was made containing a vitamin D₃ supplement (Rovimix D₃, 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 μ g 25(OH)D₃/kg, 370 g of 25(OH)D₃ (Hy-D, DSM Nutritional Products North America; 125 μ g/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 D₃ and 25(OH)D₃ 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

¹In Exp. 1, 56 gestating sows were used to determine the serum 25(OH)D₃ response from feeding titrated concentrations of vitamin D₃. In Exp. 2, 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D₃ or from synthetic 25(OH)D₃ on maternal performance, subsequent pig performance, sow and piglet 25(OH)D₃, neonatal bone mineralization, and piglet muscle development.

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

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

⁴Provided 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.

⁵Provided 3,527,392 IU vitamin A, 26,455 IU vitamin E, 1,764 mg vitamin K, 15 mg vitamin B₁₂, 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.

⁶Vitamin D premix was mixed to contain 4,409,240 IU of vitamin D₃/kg of premix by blending vitamin D₃ (Rovimix D, DSM Nutritional Products) with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D₃ concentrations in Exp. 1 and 2. For diets containing 25(OH)D₃, the vitamin D premix was not included, and Hy-D (DSM Nutritional Products; 0.0123% 25(OH)D₃) was added into the diet, replacing a percentage of corn, at 0.37 kg/t to achieve the desired concentration of 50 μ g of 25(OH)D₃/kg of diet.

⁷SID = standardized ileal digestible.

⁸Standardized total tract digestible

Serum 25-Hydroxycholecalciferol, Vitamin D₃, 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 ($1,600 \times g$ for 25 min at 2°C), and serum was harvested and stored at -20°C until analysis. All serum 25(OH)D₃ 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)D₃, vitamin D₃, 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)D₃, 1.00 ng/mL for vitamin D₃, 250 ng/mL for α -tocopherol, and 25 ng/mL for retinol. Some samples were below the detectable limit for serum vitamin D₃ concentration; therefore, the percentage of animals with serum concentrations above the detectable limit is reported herein along with the mean concentration of serum vitamin D₃ 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 CO₂ 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- μ m cryosections were collected on positively charged slides (MidSci), and muscle fibers were immu-

nostained 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 μ L of 9:1 glycerol in PBS and cover slipped for imaging.

Cryosections were imaged using a Nikon Eclipse T1-U inverted microscope with 20 \times 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 \times 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 D₃ in the complete diets, Exp. 1¹

Item	Vitamin D ₃ , IU/kg						
	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

¹Samples 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)D₃ concentrations against dietary vitamin D₃ 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 D₃ (Exp. 1 and 2), 2) 800 IU vitamin D₃ vs. 50 μ g 25(OH)D₃ (Exp. 2), 3) 2,000 IU vitamin D₃ vs. 50 μ g 25(OH)D₃ (Exp. 2), and 4) 9,600 IU vitamin D₃ vs. 50 μ g 25(OH)D₃ (Exp. 2). The IML procedure of SAS was used to generate unequally spaced linear and quadratic contrast coefficients for dietary vitamin D₃ 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 D₃ 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 D₃ (at varying levels) or 25(OH)D₃ 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 D₃ had increased (quadratic, $P = 0.001$; Table 3) serum 25(OH)D₃ concentrations. The serum results were used to develop an equation to predict the serum 25(OH)D₃ response to increasing vitamin D₃ supplementation in gestating females. The equation was as follows: serum 25(OH)D₃, ng/mL (\pm SE) = $35.1746 \pm 5.56 + (0.002353 \pm 0.00024 \times \text{dietary vitamin D}_3, \text{ IU/d}) - (0.0000000156 \pm 0.0000000036 \times \text{dietary vitamin D}_3, \text{ IU/d}^2$; 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 D₃ on serum 25(OH)D₃ in gestating sows, Exp. 1¹

Serum 25(OH)D ₃ , ng/mL	Vitamin D ₃ , IU/kg							SEM	Probability <i>P</i> , Vitamin D ₃	
	200	800	1,600	3,200	6,400	12,800	25,600		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

¹A total of 56 gestating sows were used in a 30-d trial to determine the serum 25(OH)D₃ response from feeding titrated concentrations of vitamin D₃. There were 8 sows per treatment, and sows were fed 2.5 kg/d.

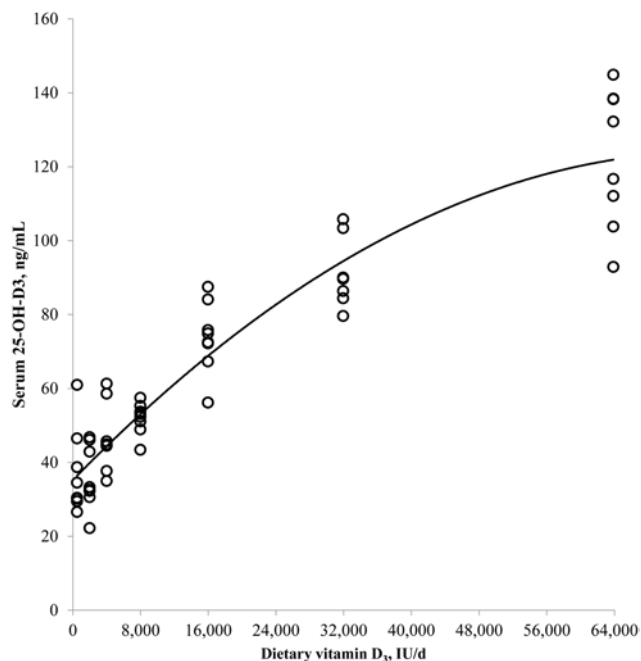


Figure 1. Plot of predicted serum 25(OH)D₃ response to daily vitamin D₃ intake of gestating sows (Exp. 1) based on the observed serum 25(OH)D₃. The equation used for predicted values was serum 25(OH)D₃, ng/mL (\pm SE) = $35.1746 \pm 5.56 + (0.002353 \pm 0.00024 \times \text{dietary vitamin D}_3, \text{ IU/d}) - (0.000000156 \pm 0.000000034 \times \text{dietary vitamin D}_3, \text{ IU/d}^2)$.

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 D₃ supplementation rate needed to achieve serum 25(OH)D₃ results similar to that of sows fed a known amount of 25(OH)D₃. Previous literature examining the serum 25(OH)D₃ response of sows fed 50 $\mu\text{g/kg}$ of 25(OH)D₃ (Weber et al., 2014) in gestation concluded that the range of serum 25(OH)D₃ 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)D₃ concentration of approximately 85 ng/mL for sows fed 50 μg of 25(OH)D₃/kg of diet. Additionally, Coffey et al. (2012) observed serum 25(OH)D₃ concentrations of approximately 80 to 90 ng/mL for first-parity gestating gilts fed diets containing 50 μg of 25(OH)D₃/kg along with 500 IU of vitamin D₃/kg. In this preliminary experiment, we did not examine serum 25(OH)D₃ concentrations from sows fed 50 μg of 25(OH)D₃/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)D₃ concentrations between 50 and 90 ng/mL could be achieved by supplementing between 17,000 and 29,000 IU of vitamin D₃/d. To ensure the vitamin D₃ supplementation rate was high enough to elicit a serum response similar to that reported in the literature when feeding 50 $\mu\text{g/kg}$ of 25(OH)D₃, a targeted feeding level of 9,600 IU

Table 4. Analyzed sow diet composition, Exp. 1¹

Item	Maternal vitamin D supplementation, IU/kg			
	Vitamin D ₃			50 μg 25(OH)D ₃
	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 , $\mu\text{g/kg}$	—	—	—	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 , $\mu\text{g/kg}$	—	—	—	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 , $\mu\text{g/kg}$	—	—	—	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 , $\mu\text{g/kg}$	—	—	—	45.4
Vitamin D, % of formulated	113.1	99.3	97.0	90.7

¹Samples 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.

of vitamin D₃ 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 D₃ supplementation for Exp. 2.

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 D₃ 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. 1¹

Item	Maternal vitamin D, IU/kg				SEM	Probability, <i>P</i>				
	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			Linear	Quadratic			
Sows, <i>n</i>	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, <i>n</i>	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, <i>n</i>	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 D₃ or from synthetic 25(OH)D₃ 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 D₃/kg had greater lactation ADFI compared with sows fed diets with 800 or 9,600 IU of vitamin D₃/kg. Also, sows consuming diets with 9,600 IU vitamin D₃/kg tended (*P* = 0.088) to have lower lactation feed intake compared with sows fed diets with 50 µg/kg of 25(OH)D₃. 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 D₃/kg, respectively, and approximately 11,300 IU/d for sows fed diets containing 50 µg/kg IU of 25(OH)D₃/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 D₃ 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)D₃ compared with pigs from sows fed 2,000 IU of vitamin D₃. 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)D₃ rather than vitamin D₃ 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. 2¹

Sow serum vitamin metabolites	Maternal vitamin D, IU/kg				SEM ²	Probability <i>P</i>				
	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			Linear	Quadratic			
25(OH)D₃,³ 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 D₃,⁴ 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

¹In total 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D₃ or from synthetic 25(OH)D₃ 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.

²Standard 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.

³A treatment × day interaction ($P = 0.001$) was observed for serum 25(OH)D₃.

⁴The assay for serum vitamin D₃ 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 D₃ was calculated using only samples above the detectable limit.

⁵A tendency ($P = 0.052$) for a treatment × day interaction was observed for serum α-tocopherol.

⁶A 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)D₃, Vitamin D₃, α-Tocopherol, and Retinol

A treatment × day interaction ($P = 0.001$; Table 6) for serum 25(OH)D₃ of sows was observed because sow serum 25(OH)D₃ was similar on d 0 of gestation regardless of dietary vitamin D treatment, but increasing vitamin D₃ increased (linear, $P < 0.001$) serum 25(OH)D₃ on d 100 of gestation, after farrowing, and

at weaning. Also, sows fed diets with 800 or 2,000 IU of vitamin D₃/kg had less serum 25(OH)D₃ 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)D₃/kg. Sows fed the diets with 9,600 IU of vitamin D₃/kg had greater serum 25(OH)D₃ 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)D₃. 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)D₃ and vitamin D₃ at the same IU equivalency. It is clear that 25(OH)D₃ 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}

Item	Maternal vitamin D, IU/kg					Probability <i>P</i>				
	Vitamin D ₃			50 µg 25(OH)D ₃	SEM ²	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		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

¹In total 112 sows and litters were used to determine the effects of supplemental vitamin D from varying levels of vitamin D₃ or from synthetic 25(OH)D₃ 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.

²Standard 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.

³A treatment × day interaction (*P* = 0.001) was observed for serum 25(OH)D₃.

⁴The assay for serum vitamin D₃ 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 D₃ was calculated using only samples above the detectable limit.

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

25(OH)D₃ response in sows. Although the exact reason for this improved response is not completely clear, Bar et al. (1980) demonstrated that 25(OH)D₃ is absorbed more efficiently than vitamin D₃ 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)D₃ 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 D₃ 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 D₃ transported within them. This suggests that a portion of the vitamin D₃ that is absorbed may be stored in adipose tissue rather than being transported to the liver for hydroxylation. The serum 25(OH)D₃ concentrations achieved in gestation from supple-

menting 25(OH)D₃ 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)D₃ concentrations from sows fed 50 µg of 25(OH)D₃/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)D₃ with increasing vitamin D₃ agree with previous data from Flohr et al. (2014). To our knowledge, this is the first study that has shown a level of vitamin D₃ supplementation that has elicited a serum response above feeding 50 µg of 25(OH)D₃/kg.

For serum vitamin D₃, 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. 2¹

Item	Maternal vitamin D, IU/kg					Probability <i>P</i>				
	Vitamin D ₃		50 µg 25(OH)D ₃		SEM	Vitamin D ₃		800 IU D ₃ vs. 50	2,000 IU D ₃ vs. 50	9,600 IU D ₃ vs. 50
	800	2,000	9,600	2,000		Linear	Quadratic	µg 25(OH)D ₃	µg 25(OH)D ₃	µg 25(OH)D ₃
Litters sampled, <i>n</i>	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

¹In 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.

²Cross-sectional area of the whole muscle.

³Average cross-sectional area of all muscle fibers.

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

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

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

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

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

⁹The 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 D₃ ($P < 0.001$) had serum vitamin D₃ concentrations above the detectable limit. Increasing vitamin D₃ increased serum vitamin D₃ 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 D₃/kg had greater serum vitamin D₃ 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)D₃. Sows fed diets containing 800 IU of vitamin D₃/kg tended to have greater ($P = 0.063$) serum vitamin D₃ concentrations at weaning compared with sows fed diets with 50 µg/kg of 25(OH)D₃. Serum vitamin D₃ is typically much more variable than 25(OH)D₃ 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 D₃ compared with 25(OH)D₃ (Institute of Medicine, 2011). In the current study, it is understandable that increasing dietary vitamin D₃ led to increased serum concentrations of the nutrient. Additionally, because of less vitamin D₃ exposure of sows fed 25(OH)D₃, it is justified that their serum vitamin D₃ was lower than that of sows fed vitamin D₃.

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 D₃ 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 D₃/kg compared with sows fed 50 µg of 25(OH)D₃/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 D₃ 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 D₃/kg. On the basis of lactation feed intake, sows consuming diets with 2,000 IU of vitamin D₃/kg had vitamin E intakes of approximately 390 IU/d, compared with sows fed either 800 or 9,600 IU of vitamin D₃/kg, which had vitamin E intakes of approximately 350 IU/d.

A treatment \times 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 D₃/kg tended ($P = 0.089$) to have less serum retinol compared with sows fed 50 μ g of 25(OH)D₃/kg. In addition, sows fed increasing levels of vitamin D₃ had increased (quadratic, $P = 0.001$) serum retinol concentrations at weaning. Sows fed diets with 2,000 IU of vitamin D₃/kg had greater ($P = 0.006$) serum retinol compared with sows fed 50 μ g of 25(OH)D₃/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 D₃/kg because of the increase in lactation feed intake. Sows consuming diets with 2,000 IU of vitamin D₃ consumed approximately 6,500 IU of vitamin A/D compared with sows fed 800 IU of vitamin D₃/kg (approximate vitamin A intake of 5,900 IU/d), sows fed 9,600 IU of vitamin D₃/kg (approximate vitamin A intake of 5,800 IU/d), and sows fed 50 μ g of 25(OH)D₃/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)D₃, Vitamin D₃, α -Tocopherol, Retinol, and Neonatal Percentage Bone Ash

For piglet serum 25(OH)D₃, there was a treatment \times day interaction ($P = 0.001$; Table 7) because increasing maternal dietary vitamin D₃ increased (linear, $P <$

0.001) piglet serum 25(OH)D₃ 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 D₃ supplementation from 1,500 to 6,000 IU/kg of diet increased subsequent piglet serum 25(OH)D₃ throughout lactation. Also, in the current study, piglets from sows fed 25(OH)D₃ had greater ($P < 0.011$) serum 25(OH)D₃ compared with piglets from sows fed 800 or 2,000 IU of vitamin D₃/kg at birth; however, at weaning, piglets from sows fed 50 μ g of 25(OH)D₃/kg had similar serum 25(OH)D₃ compared with piglets from sows fed the 2,000 IU of vitamin D₃/kg and greater ($P = 0.001$) serum 25(OH)D₃ concentrations compared with piglets from sows fed 800 IU of vitamin D₃/kg. Additionally, piglets from sows fed 9,600 IU of vitamin D₃/kg had increased ($P = 0.001$) serum 25(OH)D₃ at birth and weaning compared with piglets from sows fed 50 μ g/kg of 25(OH)D₃/kg.

Previous reports from Coffey et al. (2012) and Witschi et al. (2011) have discussed increases in serum 25(OH)D₃ concentrations in fetuses (d 90 of gestation) and piglets from sows supplemented dietary 25(OH)D₃ compared with those from sows supplemented with dietary vitamin D₃ at the same IU equivalency. Goff et al. (1984) demonstrated that the 25(OH)D₃ of the neonate is largely correlated to the 25(OH)D₃ status of the sow at birth, and 25(OH)D₃ 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 D₃ supplementation rate that provided a larger serum 25(OH)D₃ response in piglets compared with piglets from sows supplemented 50 μ g of 25(OH)D₃. 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 D₃ led to increasing milk vitamin D₃ 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 D₃ was the predominant vitamin D constituent in colostrum of rats, but vitamin D₃ concentrations declined after a few days, and 25(OH)D₃ became the predominant metabolite in milk. The current study suggests that the form of dietary vitamin D supplementation (25(OH)D₃ or vitamin D₃) did not impact milk vitamin D concentrations since feeding either 2,000 IU of vitamin D₃ or 25(OH)D₃ resulted in similar piglet serum 25(OH)D₃ concentrations at weaning. Witschi et al. (2011) observed increased serum 25(OH)D₃ of piglets from sows fed 25(OH)D₃ compared with piglets from sows fed vitamin D₃ 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 D₃ or 25(OH)D₃) 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 D₃ samples were below the laboratory detectable limit of 1.00 ng/mL, which was expected because of the quick clearance of vitamin D₃ 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 D₃/kg exhibited serum vitamin D₃ concentrations above the detectable limit, with mean serum concentrations of 1.7 ng/mL. Increasing maternal dietary vitamin D₃ increased (quadratic, $P = 0.001$) the percentage of pigs with serum vitamin D₃ concentrations above the detectable limit, and greater percentages of pigs from sows fed 2,000 or 9,600 IU of vitamin D₃/kg had serum vitamin D₃ concentrations ($P < 0.001$) above the detectable limit compared with pigs from sows fed 50 µg of 25(OH)D₃/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 \times day interaction for piglet serum retinol was observed because at birth piglet serum retinol was reduced (quadratic, $P = 0.031$) with increasing maternal vitamin D₃, and piglets from sows fed diets with a medium level of vitamin D₃ had lower ($P = 0.038$) serum retinol compared with piglets from sows fed 25(OH)D₃; 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 D₃ 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 D₃ concentration (1,500 to 6,000 IU/kg of diet) on the bone ash percentage of neonates when maternal vitamin D₃ 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 D₃ supplemented to gestating gilts with 25(OH)D₃ 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)D₃ 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)D₃ compared with vitamin D₃ itself. Previous work in poultry has elicited similar results (Giuliani and Boland, 1984) and has shown that exogenous addition of 1,25-OH₂-D₃ 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)D₃ compared with those from sows fed 800 or 2,000 IU of vitamin D₃/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 D₃/kg should be similar to the fiber numbers from muscles of pigs born from sows fed 50 µg of 25(OH)D₃/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)D₃ compared with piglets

from sows fed 9,600 IU of vitamin D₃/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)D₃ compared with piglets from sows fed 9,600 IU of vitamin D₃/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)D₃ compared with piglets from sows fed 9,600 IU of vitamin D₃/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)D₃ compared with piglets from sows fed 9,600 IU of vitamin D₃/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)D₃ compared to pigs from sows fed 9,600 IU of vitamin D₃/kg. More research is needed to help elucidate whether there are distinct impacts of maternal vitamin D supplementation from vitamin D₃ or 25(OH)D₃ 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 D₃ to sows can increase sow and piglet serum 25(OH)D₃. Additionally, when supplementing 25(OH)D₃ and vitamin D₃ at the same IU equivalency, serum 25(OH)D₃ of sows and piglets at birth will be increased for sows fed 25(OH)D₃. It appears that maternal dietary vitamin D level impacted weaned pig serum 25(OH)D₃ more so than the form (vitamin D₃ or 25(OH)D₃) 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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