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Evaluating the Impact of Maternal Vitamin D Supplementation on Sow Performance, Serum Vitamin Metabolites, Neonatal Muscle and Bone Characteristics, and Subsequent Pre-weaning Pig Performance

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Abstract

A total of 56 gestating sows (PIC 1050; 35-d post-insemination) were used in a 30-d trial to determine the serum 25(OH)D_3 response to increasing concentrations of vitamins D3. At initiation, sows were randomly allotted to 1 of 7 dietary vitamin D_3 treatments (91, 363, 726, 1,451, 2,903, 5,806, or 11,612 IU of vitamin D_3/lb of complete diet) with 8 sows per treatment. All sows were fed 5.5 lb daily at 0800. Increasing vitamin D3 increased (quadratic; $P < 0.001$) serum 25(OH)D_3 with the response depicted by the following prediction equation:

Serum 25(OH)D3, ng/mL = 35.1746 + (0.002353 × dietary vitamin D_3, IU/d) - (0.0000000156 × dietary vitamin D_3, IU/d^2)

In Exp. 2, 112 sows and their litters were used to determine the effects of supplemented vitamin D on sow performance, subsequent pre-weaning pig performance, neonatal pig bone and muscle characteristics, and serum vitamin metabolites. Sows were allotted to 1 of 4 dietary regimens: 1) low vitamin $_{D3}$ (363 IU/lb); 2) medium vitamin D_3 (907 IU/lb); 3) high vitamin D_3 (4,354 IU/lb); or 4) 23 μg 25(OH)D_3/lb (Hy-D, DSM Nutritional Products Inc, Parsippany, NJ), which were fed throughout gestation and lactation. There were 25 to 27 sows per treatment. Overall, increasing maternal vitamin D_3 increased (linear, $P = 0.001$) serum 25 (OH)D3 of sows on d 100 of gestation, at farrowing, and at weaning. Also increasing vitamin D_3 in diets fed to sows increased piglet serum 25(OH)D_3 at birth (linear, $P = 0.001$) and weaning (quadratic, $P = 0.033$). Sows fed 25(OH)D_3 had greater ($P < 0.001$) serum 25(OH)D_3 concentrations on d 100 of gestation, at farrowing, and at weaning compared to sows fed the low or medium concentration of vitamin D_3; however, they were reduced ($P < 0.004$) compared to the serum 25(OH)D_3 concentrations of sows fed the high concentration of vitamin D_3 on the same collection days. Piglets from sows fed 25(OH)D_3 had greater serum 25(OH)D_3 compared to piglets from sows fed the low and medium concentration of vitamin D_3; however, at weaning, serum 25(OH)D_3 concentrations were only greater compared to the low concentration of vitamin D_3. Also, piglets from sows fed the high concentration of vitamin D had greater ($P = 0.011$) serum 25(OH)D_3 concentration at birth and at weaning, compared to piglets from sows fed 25(OH)D_3. Maternal performance, litter characteristics, neonatal bone ash content, and neonatal muscle fiber characteristics were largely unaffected by the maternal vitamin D regimen. Overall, vitamin D_3 and 25(OH)D_3 both appear to be useful at increasing serum 25(OH) D_3 concentrations, but more vitamin D_3 (on an IU basis) is needed to achieve similar serum 25(OH)D_3 responses compared to feeding 25(OH)D3. Interestingly, sows fed 25(OH)D_3 in lactation had less vitamin D transport to the pig than sows fed medium and high concentrations of vitamin D_3 suggesting that vitamin D_3 is still a more useful metabolite for milk transfer of the vitamin. Due to the lack of impact of maternal vitamin D regimen on sow or pre-weaned pig performance, and neonatal muscle characteristics, more research examining the impact of vitamin D on immune function and novel biological processes is needed to assess the value of vitamin D supplementation strategies in pigs.

Keywords

25(OH)D3, sow nutrition, vitamin D

Cover Page Footnote

Appreciation is expressed to DSM Nutritional Products Inc, Parsippany, NJ, for funding and laboratory analysis of this project.

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K-STATE Research and Extension

SWINE DAY 2015

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2015

Evaluating the Impact of Maternal Vitamin D Supplementation on Sow Performance, Serum Vitamin Metabolites, Neonatal Muscle and Bone Characteristics, and Subsequent Pre-weaning Pig Performance[1]

J. R. Flohr, J. C. Woodworth, M. D. Tokach, S. S. Dritz[2], R. D. Goodband, J. M. DeRouchey, and J. R. Bergstrom[3]

Summary

A total of 56 gestating sows (PIC 1050; 35-d post-insemination) were used in a 30-d trial to determine the serum 25(OH)D_3 response to increasing concentrations of vitamins D_3. At initiation, sows were randomly allotted to 1 of 7 dietary vitamin D_3 treatments (91, 363, 726, 1,451, 2,903, 5,806, or 11,612 IU of vitamin D_3/lb of complete diet) with 8 sows per treatment. All sows were fed 5.5 lb daily at 0800. Increasing vitamin D_3 increased (quadratic; $P < 0.001$) serum 25(OH)D_3 with the response depicted by the following prediction equation:

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In Exp. 2, 112 sows and their litters were used to determine the effects of supplemented vitamin D on sow performance, subsequent pre-weaning pig performance, neonatal pig bone and muscle characteristics, and serum vitamin metabolites. Sows were allotted to 1 of 4 dietary regimens: 1) low vitamin D_3 (363 IU/lb); 2) medium vitamin D_3 (907 IU/lb); 3) high vitamin D_3 (4,354 IU/lb); or 4) 23 µg 25(OH)D_3/lb (Hy-D, DSM Nutritional Products Inc, Parsippany, NJ), which were fed throughout gestation and lactation. There were 25 to 27 sows per treatment. Overall, increasing maternal vitamin D_3 increased (linear, $P = 0.001$) serum 25 (OH)D_3 of sows on d 100 of gestation, at farrowing, and at weaning. Also increasing vitamin D_3 in diets fed to sows increased piglet serum 25(OH)D_3 at birth (linear, $P = 0.001$) and weaning (quadratic, $P = 0.033$). Sows fed 25(OH)D_3 had greater ($P < 0.001$) serum 25(OH)D_3 concentrations on d 100 of gestation, at farrowing, and at weaning compared to sows fed the

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low or medium concentration of vitamin D_3; however, they were reduced ($P < 0.004$) compared to the serum 25(OH)D_3 concentrations of sows fed the high concentration of vitamin D_3 on the same collection days. Piglets from sows fed 25(OH)D_3 had greater serum 25(OH)D_3 compared to piglets from sows fed the low and medium concentration of vitamin D_3; however, at weaning, serum 25(OH)D_3 concentrations were only greater compared to the low concentration of vitamin D_3. Also, piglets from sows fed the high concentration of vitamin D had greater ($P = 0.011$) serum 25(OH)D_3 concentration at birth and at weaning, compared to piglets from sows fed 25(OH)D_3. Maternal performance, litter characteristics, neonatal bone ash content, and neonatal muscle fiber characteristics were largely unaffected by the maternal vitamin D regimen. Overall, vitamin D_3 and 25(OH)D_3 both appear to be useful at increasing serum 25(OH)D_3 concentrations, but more vitamin D_3 (on an IU basis) is needed to achieve similar serum 25(OH)D_3 responses compared to feeding 25(OH)D_3. Interestingly, sows fed 25(OH)D_3 in lactation had less vitamin D transport to the pig than sows fed medium and high concentrations of vitamin D_3 suggesting that vitamin D_3 is still a more useful metabolite for milk transfer of the vitamin. Due to the lack of impact of maternal vitamin D regimen on sow or pre-weaned pig performance, and neonatal muscle characteristics, more research examining the impact of vitamin D on immune function and novel biological processes is needed to assess the value of vitamin D supplementation strategies in pigs.

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

Introduction

Within the last five years, the swine industry has seen a rise in interest and speculation associated with vitamin D supplementation. This was largely due to several documented cases in which vitamin D_3 was absent from vitamin premixes fed to pigs (Feedstuffs, 2010[4]). Deficiency of vitamin D_3 can lead to metabolic bone disease, which is categorized as a disturbance of normal bone formation and remodeling and can lead to bone fractures and clinical signs of rickets. Additionally, human nutrition has experienced a resurgence of interest in vitamin D's role due to increasing genomic data that has identified the presence of vitamin D receptors within tissue not typically associated with normal Ca and P homeostasis (Norman and Bouillon, 2010[5]). One specific tissue that has been identified with vitamin D receptors is skeletal muscle cells.

Historically, vitamin D_3 has been the most common form of vitamin D supplemented to livestock. This metabolite must undergo two steps of hydroxylation to become the active 1, 25 dihydroxycholecalciferol which is commonly involved in gut Ca and P absorption, and bone resorption pathways. The first step of hydroxylation occurs in the liver, while the second has notably occurred in the kidney. A synthetically produced 25 hydroxycholecalciferol (25(OH)D_3; Hy-D; DSM Nutritional Products, Parsippany, NJ) metabolite is also available for use in the domestic poultry industry and internationally, and is recognized as being more readily available to the animal because it has already undergone the first step in the conversion to 1, 25 dihydroxycholecalciferol.

[4] Feedstuffs. 2010. Kent feeds recalls certain swine feeds. Accessed April 4, 2011. http://www.feedstuffs.com.

[5] Norman, A. W. and R. Bouillon. 2010. Vitamin D nutritional policy needs a vision for the future. Experimental Biology and Medicine. 235: 1034-1045.

Hines et al. (2013[6]) evaluated fetal muscle development from fetuses (90 d of age) from gestating gilts fed diets containing either vitamin D_3 (1,113 IU/lb) or vitamin D_3 plus 25(OH)D_3 (227 IU/lb of D_3 and 23 µg/lb of 25(OH)D_3), with both treatments resulting in similar vitamin D on an international units equivalency in the diet. They observed an increase in the number of muscle fibers within longissimus muscles of fetuses from gilts fed 25(OH)D_3 compared to those fetuses from gilts only fed vitamin D_3. Also the authors observed increased fetal 25(OH)D_3 concentrations in fetuses from gilts fed 25(OH)D_3 compared to those fetuses from gilts only fed vitamin D_3. The study suggests that when vitamin D_3 and 25(OH)D_3 are evaluated on an international unit equivalency, 25(OH)D_3 is more available and efficiently utilized. This makes sense considering that 25(OH)D_3 can bypass the first step of hydroxylation and metabolism in the liver; however, no one has examined how much vitamin D_3 would be needed to achieve a similar serum 25(OH)D_3 response and if those greater concentrations would also influence fetal muscle characteristics similar to results found in the previously mentioned study.

The objectives of the experiments discussed herein were to: 1) determine a feeding concentration of vitamin D_3 that would result in a similar serum 25(OH)D_3 response as that observed from feeding 23 µg/lb of 25(OH)D_3, and 2) evaluate the influence of varying concentrations of vitamin D_3 or 25(OH)D_3 supplementation on sow performance, serum vitamin metabolites, subsequent pig performance, and neonatal muscle and bone characteristics.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved experimental procedures and animal care for this study. These experiments were conducted at the K-State Swine Teaching and Research Facility in Manhattan, KS, and were conducted from January through December 2014.

Both the gestation and farrowing barns were totally enclosed, environmentally controlled, and mechanically ventilated buildings. In gestation, sows were housed in gestation stalls (7.0 × 2.0 ft). The farrowing barn contained 29 farrowing crates (7.0 × 2.0 ft for the sow and 7.0 × 3.2 ft 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 68°F, and supplemental heat was provided to piglets with heat lamps.

In Exp. 1, a total of 56 sows (PIC 1050) from two consecutive breeding groups were used in a 30-d study to determine the serum 25(OH)D_3 response to varying concentrations of vitamin D_3. The study began 35-d post insemination after sows were confirmed pregnant. At initiation, the sows were randomly allotted to 1 of 7 dietary treatments receiving 91, 363, 726, 1,451, 2,903, 5,806, or 11,612 IU vitamin D_3/lb of the complete diet. There were 8 sows per treatment. The gestation diets were common corn-soybean meal-based diets and were formulated to contain 0.56% standardized ileal digestible (SID) lysine and 0.82% Ca (Table 1). Sows were bled via jugular venipuncture to collect serum for 25(OH)D_3 analysis on d 0 and 30 of the trial, prior to receiving their daily meal. All sows were fed 5.5 lb of feed once daily (0800). Results from this study were

[6] Hines, E. A., J. D. Coffey, C. W. Starkey, T. K. Chung and J. D. Starkey. 2013. Improvement of maternal vitamin D status with 25-hydroxycholecalciferol positively impacts porcine fetal skeletal muscle development and myoblast activity. J. Anim. Sci. 91:9 4116–4122.

then used to develop a prediction equation used to determine the vitamin D_3 feeding concentration needed to achieve a serum 25(OH)D_3 response in gestating sows similar to concentrations previously reported in the literature (Weber et al., 2014) for females fed 25(OH)D_3 as their source of vitamin D.

In Exp. 2, a total of 112 sows (PIC 1050) from four consecutive farrowing groups and their litters were used in the study. Following breeding, sows were randomly assigned to 1 of 4 dietary vitamin D regimens receiving: 1) low (363 IU); 2) medium (907 IU); 3) high (4,354 IU) concentrations of vitamin D_3/lb in the complete diet; or 4) 23 μg of 25(OH)D_3/lb in the complete diet. Vitamin D dietary regimens were fed throughout gestation and lactation. The high vitamin D_3 feed concentration was determined following the results found in Exp. 1 and was predicted to have mean serum 25(OH)D_3 values that would be similar to the regimen fed the synthetic 25(OH)D_3. 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 and received 5.5 lb/d of the gestation diets. On d 110, sows were transported to the farrowing house and housed in farrowing crates. After farrowing, sows were switched to lactation diets. Gestation and lactation diets were formulated to contain 0.56% and 0.94% SID lysine, respectively. Farrowing crate feeders were equipped with an electronic feeding system (Gestal Solo; JYGA Technologies, Quebec, Canada), which used a built-in feeding curve based on parity to feed individual sows. The feeding curves were monitored and adjusted daily to allow for ad libitum feed intake while reducing feed waste. 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. Backfat measurements were collected when sows arrived in the farrowing house and at weaning to determine BF 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 pre-weaned 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 and live-born piglets. Although minimal, cross-fostering was conducted within 48 h post-farrowing to help standardize litter size within vitamin D dietary regimen. 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.

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 Inc., Parsippany, NJ). This supplement was mixed into a rice hull carrier to form the premix and 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_3. For diets formulated to contain 23 µg $25(OH)D_3$/lb, 0.73 lb of $25(OH)D_3$ (Hy-D, DSM Nutritional Products Inc, Parsippany, NJ) was added per ton of the diet in order to reach desired finished feed concentration. Complete diet samples from Exp. 1 and 2 were analyzed for vitamin D_3 and $25(OH)D_3$ concentrations by a commercial laboratory (DSM Nutritional Products, Parsippany, NJ).

All blood samples were collected in serum separator tubes and refrigerated for at least 6 h after collection. Blood was centrifuged at 2,800 rpm for 25 min. Serum was extracted and stored in 2-mL vials and frozen in a freezer at -4°F. All serum $25(OH)D_3$ testing for Exp. 1 was performed by Heartland Assays Inc. (Ames, IA). All vitamin metabolite testing ($25(OH)D_3$, vitamin D_3, α-tocopherol, and retinol) from Exp. 2 was conducted by the DSM Nutritional Laboratory (Kaiseraugst, Switzerland).

Necropsies were performed onsite and in compliance with the university's standard Institutional Animal Care and Use 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 bone ash content, and whole muscle cross sections of the longissimus thoracis and the semitendinosus were collected for immunohistochemistry. Bones were boiled for 60 min and adhering tissue was removed. Then the bones were dried at 221°F for 7 d. After drying, the bones were ashed in a muffle furnace at 1,112°F for 24 h. After dissecting the whole muscle cross sections, they were embedded in Optimal Cutting Temperature (OCT) tissue-embedding media (Fisher Scientific, Pittsburgh, PA), frozen by submersion in supercooled isopentane, and stored at -112°F.

For each muscle sample, two 10-µm cryosections were collected on frost-resistant slides (Fisher Scientific). Nonspecific antigen-binding sites were inhibited by incubating cryosections in 5% horse serum and 0.2% TritonX-100 in phosphate-buffered saline (PBS) for 30 min. All sections were incubated with the following primary antibodies in blocking solution for 60 min: 1:500 α-dystrophin (Thermo 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, University of Iowa, Iowa City, IA). After incubation sections were washed with PBS 3 times for 5 min, followed by incubation in the following secondary antibodies (1:1000) 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, 1:1000 Hoechst Dye 33342 (Invitrogen) was utilized to identify all fiber-associated nuclei. Finally, sections were washed for three 5-min periods in PBS, then covered with 5 µL of 9:1 glycerol in PBS, then coverslipped 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 positively stained for SC-71 were labeled as secondary fibers.

Statistical analysis

All data were analyzed as a generalized randomized complete block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC). Maternal performance data were analyzed with sow as the experimental unit, maternal regimen 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 beta distribution (% bone ash). Contrast statements consisted of: (1) increasing vitamin D_3 linear and quadratic polynomials (Exp. 1 and 2); (2) 363 IU vitamin D_3 vs. 23 µg 25(OH)D_3 (Exp. 2); (3) 907 IU vitamin D_3 vs. 23 µg 25(OH)D_3 (Exp. 2); and (4) 4,354 IU vitamin D_3 vs. 23 µg 25(OH)D_3 (Exp. 2). 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 regimen over time. Results were considered significant at $P \leq 0.05$ and a tendency at $P \leq 0.10$.

Results and discussion

Experiment 1

Dietary vitamin D_3 analysis results (Table 2) showed that diets were within 25% of their formulated values. Although there is no reference for analytical variation of vitamin D within animal feed, a recovery rate of 75% of the formulated value is viewed as an accepted analytical recovery of other vitamins within animal feeds (AAFCO, 2015[7]); therefore, the formulated concentrations were used for statistical analyses and results. Gestating sows fed increasing vitamin D_3 had increased (quadratic, $P = 0.001$; Table 3) serum 25(OH)D_3 concentrations. This data set was 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: Serum 25(OH)D_3, ng/mL = 35.1746 + (0.002353 × dietary vitamin D_3, IU/d) - (0.0000000156 × dietary vitamin D_3, IU/d^2; Figure 1). The corresponding coefficient of variation (r^2) for this fitted prediction equation was 0.852, suggesting a high correlation of dietary vitamin D_3 supplementation to serum 25(OH) D_3 which was expected since the sole source of vitamin D for commercially reared swine is from the diet. This information was used to predict a dietary vitamin D_3 concentration 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 23 µg/lb of 25(OH)D_3 shows variation in response (Weber et al., 2014[8]) based on parity and sampling time, with the range of serum responses appearing to be between 50 and 90 ng/mL. Based on the prediction equation developed herein, similar results could be achieved by supplementing between 7,000 and 29,000 IU of vitamin D_3/d. In order to ensure the supplementation rate was high enough to elicit a serum re-

[7] AAFCO. 2015. 2015 Official publication association of American feed control officials incorporated. p. 302.

[8] Weber, G. M., A.-K. M. Witschi, C. Wenk and H. Martens. 2014. TRIENNIAL GROWTH SYMPOSIUM— Effects of dietary 25-hydroxycholecalciferol and cholecalciferol on blood vitamin D and mineral status, bone turnover, milk composition, and reproductive performance of sows. J. Anim. Sci. 92:3:899–909.

sponse, a targeted feeding concentration of 4,354 IU of vitamin D_3/lb of complete feed (12 to 14 times the NRC vitamin D requirement and approximately 24,000 IU/d) was selected as the highest concentration of vitamin D_3 supplementation for Exp. 2.

Experiment 2

Proximate analysis of gestation and lactation diets fed in Exp. 2 (Table 4) showed similar CP and P concentrations to formulated concentrations. Analyzed Ca concentrations were more variable, but all values were above the requirement for the sow. The analyzed vitamin D concentration in gestation and lactation diets were within 90% of their formulated concentrations; therefore, the formulated concentrations were used for statistical analyses and for the results.

For maternal performance, vitamin D regimen did not affect gestation BW gain (Table 5). During the lactation phase, increasing vitamin D_3 increased (quadratic, $P = 0.011$) ADFI and decreased (quadratic, $P = 0.003$) BW loss. This was due to sows having greater ADFI when fed diets with a medium concentration of vitamin D_3 compared to sows fed low or high concentrations of vitamin D_3. Also, sows consuming diets with a high concentration of vitamin D_3 tended ($P = 0.088$) to have less lactation feed intake compared to sows fed diets with 25(OH)D_3. Maternal vitamin D regimen did not affect litter performance criteria or piglet BW at birth or weaning.

A treatment × day interaction ($P = 0.001$; Table 6) for serum 25(OH)D_3 of sows was observed because on d 0 of gestation sow serum 25(OH)D_3 was similar regardless of dietary vitamin D regimen, 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 low or medium concentrations of vitamin D_3 had reduced 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 to sows fed 25(OH)D_3. Sows fed the diets with a high concentration of vitamin D_3 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 to sows fed 25(OH)D_3. These results agree with previous data suggesting that feeding the low or medium concentration of vitamin D_3 (907 IU/lb of the diet) results in a reduced serum 25(OH)D_3 response when compared to feeding 23 µg/lb 25(OH)D_3. A difference in serum bioavailability is expected considering 25(OH)D_3 does not require the first step of metabolism that vitamin D_3 requires. However, these are the first data, to our knowledge, that show a greater serum 25(OH)D_3 response from feeding a high concentration of vitamin D_3 compared to feeding 25(OH)D_3 at its recommended supplementation rate of 23 µg/lb. This suggests that supplementing vitamin D_3 or 25(OH)D_3 can effectively increase serum 25(OH)D_3 response. By definition, 1 µg of vitamin D has the same biological activity as 40 IU. Therefore, simply based on the definition of an international unit of vitamin D, sows fed 23 µg of 25(OH)D_3/lb were fed the same amount of vitamin D as sows fed 907 IU of vitamin D_3/lb. But when using serum 25(OH)D_3 as the response, more vitamin D_3 is needed to achieve as similar serum concentration as feeding 25(OH)D_3. No research has observed a potentially toxic threshold associated with too high a supplementation rate of vitamin D_3 or 25(OH)D_3 in sows. No clinical signs associated with toxic supplementation of vitamin D were observed throughout the feeding period (1 parity) of this trial.

There was a treatment × day interaction ($P = 0.001$) for serum vitamin D_3 because on d 0 of gestation sow serum vitamin D_3 was similar regardless of dietary vitamin D regimen; but increasing vitamin D_3 increased serum vitamin D_3 on d 100 of gestation (linear, $P = 0.001$), after farrowing (linear, $P = 0.005$), and at weaning (quadratic, $P = 0.017$). Sows fed diets with a low concentration of vitamin D_3 had greater serum vitamin D_3 concentrations that were higher at weaning ($P = 0.023$) compared to sows fed 25(OH)D_3. Also, sows fed the diets with a medium or high concentration of vitamin D_3 had greater serum vitamin D_3 concentrations on d 100 of gestation ($P < 0.005$), after farrowing ($P < 0.014$), and at weaning ($P = 0.001$) compared to sows fed 25(OH)D_3. In this study the only source of vitamin D provided to sows fed 25(OH)D_3 was the hydroxylated metabolite since a vitamin D_3-free premix was used. Although this metabolite enters circulation sooner, it cannot be converted back to the vitamin D_3 form that is the most common form stored in fat tissues. It appears that serum concentrations of vitamin D_3 were reduced when sows were fed 25(OH)D_3 but it is unclear whether this is due to depleting fat stores of vitamin D_3, or simply from a decrease in vitamin D_3 intake.

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 regimen on d 0 of gestation and after farrowing, but on d 100 increasing vitamin D_3 supplementation decreased (quadratic, $P = 0.007$) serum α-tocopherol concentrations. This result was unexpected, and it appears to have been driven by a low serum α-tocopherol concentration for sows fed the medium concentration of vitamin D_3. In addition, on d 100 of gestation, serum α-tocopherol tended to be greater for sows fed a low ($P = 0.081$) or high ($P = 0.066$) concentration of vitamin D_3 compared to sows fed 25(OH)D_3. At weaning, sows fed increasing vitamin D_3 tended (quadratic, $P = 0.077$) to have increasing serum α-tocopherol. This was largely due to sows fed the medium concentration of vitamin D_3 tending to have high serum α-tocopherol concentrations, likely due to greater feed intakes in lactation.

A treatment × day interaction ($P = 0.001$) for sow serum retinol was observed because serum retinol was similar regardless of maternal vitamin D regimen on d 0 and 100 of gestation; however, after farrowing, sows fed a high concentration of vitamin D_3 tended to have reduced ($P = 0.089$) serum retinol concentrations compared to sows fed 25(OH)D_3. Additionally, sows fed increasing concentrations of vitamin D_3 had increased (quadratic, $P = 0.001$) serum retinol concentrations at weaning. Sows fed diets with a medium concentration of vitamin D_3 had greater ($P = 0.006$) serum retinol compared to sows fed 25(OH)D_3 at weaning. Both of the responses at weaning were probably the result of greater daily retinol intake due to increased lactation intake for sows fed the medium concentration of vitamin D_3.

For piglet serum 25(OH)D_3, a treatment × day interaction ($P = 0.001$; Table 7) was observed. This was because increasing maternal 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. Piglets from sows fed 25(OH)D_3 had greater ($P = 0.004$) serum 25(OH)D_3 compared to piglets from sows fed the low concentration of vitamin D_3 at birth and weaning. Piglets from sows fed 25(OH)D_3 had greater ($P = 0.011$) serum 25(OH)D_3 compared to piglets from sows fed the

medium concentration of vitamin D_3 at birth; however, at weaning, piglets from sows fed 25(OH)D_3 had similar serum 25(OH)D_3 compared to piglets from sows fed the medium concentration of vitamin D_3. The serum 25(OH)D_3 concentrations at birth were expected since 25(OH)D_3 is the common circulating form of the vitamin, which can be transferred trans-placentally. However, it was surprising that the pigs from sows fed 25(OH)D_3 did not appear to receive additional 25(OH)D_3 through the sow's milk, even though sows had greater serum 25(OH)D_3 concentrations than those sows fed the medium concentration of vitamin D_3. Piglets from sows fed a high concentration of vitamin D_3 had greater ($P = 0.001$) serum 25(OH)D_3 at birth and weaning compared to piglets from sows fed 25(OH)D_3.

A majority of piglet serum vitamin D_3 samples were below the laboratory detectable limit of 1.00 ng/mL. Samples below that threshold (144 out of 192) were assigned a value of 1.00. There was a treatment × day interaction ($P = 0.001$) for piglet serum vitamin D_3 because increasing maternal vitamin D_3 supplementation increased piglet serum vitamin D_3 at both birth (linear, $P = 0.003$) and weaning (quadratic, $P = 0.006$) with a greater magnitude of increase at weaning. Serum vitamin D_3 was greater ($P < 0.008$) at birth and weaning for piglets from sows fed the diets with the high concentration of vitamin D_3 compared to piglets from sows fed 25(OH)D_3.

Piglet serum α-tocopherol was similar after birth and at weaning regardless of vitamin D maternal regimen. 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 concentration of vitamin D_3 had reduced (P = 0.038) serum retinol compared to piglets from sows fed 25(OH)D_3; however, by weaning, serum retinol was similar regardless of maternal vitamin D regimen. Percentage bone ash for second ribs and femurs from pigs euthanized after birth were similar regardless of vitamin D regimen.

Whole muscle longissimus thoracis (LT) and semitendinosus (ST) area were similar (Table 8) regardless of maternal vitamin D regimen. Maternal vitamin D regimen 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 to piglets from sows fed the high concentration of vitamin D_3. Average primary muscle fiber CSA was similar for the LT regardless of maternal vitamin D regimen; however, primary muscle fiber CSA for the ST was greater ($P = 0.031$) for piglets from sows fed 25(OH)D_3 compared to piglets from sows fed the high concentration of vitamin D_3. Secondary muscle fiber CSA for the ST was not influenced by maternal vitamin D regimen; but LT secondary muscle fiber CSA tended to be greater ($P = 0.070$) for piglets from sows fed 25(OH)D_3 compared to piglets from sows fed the high concentration of vitamin D_3. Total fiber number, primary fiber number, and secondary fiber number for LT and ST muscles were not influenced by maternal vitamin D regimen. The LT secondary to primary fiber ratio was reduced ($P = 0.035$) for piglets from sows fed 25(OH)D_3 compared to piglets from sows fed the high concentration of vitamin D_3; however, maternal vitamin D regimen did not influence ST secondary to primary muscle fiber ratio. The results herein would contradict the results from Hines et al. (2013), who concluded that average muscle fiber CSA was smaller and there were more total muscle fibers in the LT of fetuses from gilts fed 25(OH)D_3 compared to

fetuses from gilts fed vitamin D_3 on the same international unit equivalency. In the current study, feeding 907 IU of vitamin D_3 or 23 μg of 25(OH)D_3 (treatments with the same international unit equivalency) had no impact on muscle fiber counts or CSA of neonates.

Overall, the experiments herein suggest that both vitamin D_3 and 25(OH)D_3 can be useful sources of vitamin D for sows and pre-weaned pigs to increase serum 25(OH)D_3 concentrations. It also appears that serum vitamin D_3 is decreased when 25(OH)D_3 is fed. Although 25(OH)D_3 supplementation can increase the fetal transfer of vitamin D, after parturition supplementation of 25(OH)D_3 was not as useful as the high concentration of vitamin D_3 for milk transfer of vitamin D. This may be due to the increased vitamin D_3 concentrations found in colostrum (Weber et al., 2014). Maternal performance and litter characteristics were not influenced by vitamin D_3 concentration. Additionally, maternal vitamin D regimen had little to no impact on muscle fiber characteristics or bone ash content in the neonatal pig. Further research examining potential impacts of vitamin D on the immune system or other novel biological processes in pigs may offer additional insight into possible benefits of vitamin D supplementation strategies for pigs.

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

	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, 21.5% P	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
Sow vitamin add pack[5]	0.50	0.50
Vitamin premix[6]	0.25	0.25
Vitamin D premix[7]	0.02	0.02
Total	100.00	100.00
Calculated analysis		
SID[8] amino acids, %		
Lys	0.56	1.70
Met & cys:lys	76	56
Thr:lys	80	64
Trp:lys	24	20
NE, Mcal/lb	1.12	1.14
SID Lys:NE, g/Mcal	2.27	6.76
CP, %	14.1	19.9
Ca, %	0.82	0.83
P, %	0.64	0.70
Available P, %	0.47	0.49
STTD P, %	0.49	0.53
Ca:P	1.28	1.19

[1] In Exp. 1, a total of 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, a total of 112 sows and litters were used to determine the effects of supplemental vitamin D from varying concentrations 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, Parsippany, NJ, provided 216 phytase units (FTU/lb) of diet with an expected release of 0.10% phytate P.

[4] Provided 11,000 ppm Cu, 198 ppm I, 73,413 ppm Fe, 22,046 ppm Mn, 198 ppm Se, and 74,413 ppm Zn per lb of premix.

[5] Sow add pack provided: 4,000 IU vit. E, 40 mg biotin, 300 mg folic acid, 400 mg pyridoxine, 100,000 mg choline, 9,000 mg carnitine, and 36 mg chromium per lb of premix.

[6] Provided 1,600,000 IU vit. A, 8,000 IU vit. E, 800 mg vit. K, 7 mg vit. B_{12}, 15,000 mg niacin, 5,000 mg pantothenic acid, and 1,500 mg riboflavin per lb of premix.

[7] Vitamin D premix was mixed to contain 2,000,000 IU of vitamin D_3/lb of premix by blending vitamin D_3 (Rovimix D: DSM Nutritional Products, Parsippany, NJ) with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations 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, Parsippany, NJ) was added into the diet, replacing a percentage of corn, at 0.73 lb/ton to achieve the desired concentration of 23 µg/lb 25(OH)D_3 of the diet.

[8] Standardized ileal digestible.

Table 2. Dietary vitamin D_3 (IU/lb) concentration of the complete diet (Exp. 1)[1]

	Vitamin D_3, IU/lb						
	91	363	726	1,451	2,903	5,806	11,612
Formulated	91	363	726	1,451	2,903	5,806	11,612
Analyzed	88	324	726	1,107	2,774	5,919	11,131
% 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 commercial laboratory (DSM Nutritional Products Inc., Parsippany, NJ) for analysis. Means represent the average analyzed value of two samples.

Table 3. Effects of titrated vitamin D_3 supplemental on serum 25(OH)D_3 in gestation sows (Exp. 1)[1]

									Probability, *P*<	
	Vitamin D_3, IU/lb								Vitamin D_3	
Item	91	363	726	1,451	2,903	5,806	11,612	SEM	Linear	Quadratic
Serum 25(OH)D_3, ng/mL										
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 5.5 lb/d.

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

	Maternal vitamin D supplementation			
	Vitamin D_3			
Item	Low	Medium	High	25(OH)D_3
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/lb	363	907	4,354	---
25(OH)D_3, µg/lb	---	---	---	23
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/lb	331	907	4,108	---
25(OH)D_3, µg/lb	---	---	---	21
Vitamin D, % of formulated	91.2	99.8	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/lb	363	905	4,354	---
25(OH)D_3, µg/lb	---	---	---	23
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/lb	411	901	4,223	---
25(OH)D_3, µg/lb	---	---	---	21
Vitamin D, % of formulated	113.1	99.3	97.0	90.7

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

Table 5. The effects of maternal vitamin D supplementation on sow and pre-weaned pig performance (Exp. 2)[1]

	Maternal vitamin D[2]					Probability, *P* <				
	Vitamin D_3					Vitamin D_3		Low D_3 vs.	Medium D_3 vs.	High D_3 vs.
Item	Low	Medium	High	25(OH)D_3	SEM	Lin	Quad	25(OH)D_3	25(OH)D_3	25(OH)D_3
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, lb	11.81	12.97	11.61	12.46	0.439	0.137	0.011	0.184	0.294	0.088
Sow BW, lb										
Gestation										
d 0	425.9	420.7	419.7	423.2	21.04	0.835	0.835	0.905	0.908	0.876
d 110	517.3	498.5	515.3	515.7	17.31	0.721	0.232	0.923	0.293	0.980
BW gain, lb	91.2	78.1	95.9	92.6	8.24	0.330	0.190	0.901	0.191	0.771
Lactation										
d 0	505.6	490.2	499.7	509.7	16.12	0.909	0.348	0.800	0.231	0.547
d 21	487.7	488.9	486.0	500.4	15.29	0.889	0.926	0.452	0.494	0.406
BW loss, lb	-17.9	-1.3	-13.5	-9.3	5.37	0.677	0.003	0.129	0.153	0.464
Sow BF, 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
BF 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
Cross-foster, n	12.00	11.29	11.53	11.76	0.706	0.824	0.457	0.797	0.606	0.810
Total weaned, n	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, lb										
Birth	3.1	3.1	3.2	3.1	0.114	0.816	0.842	0.878	0.989	0.770
Weaning	14.3	14.9	14.4	14.1	0.523	0.882	0.349	0.779	0.231	0.622

[1] A total of 112 sows and litters were used to determine the effects of supplemental vitamin D from varying concentrations 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 (one from the 363 IU/lb treatment and 2 from the 4,354 IU/lb treatment) were removed due to farrowing complications. One sow from the treatment fed 4,354 IU/lb was removed from the dataset due to a late-term abortion.

[2] Vitamin D_3 concentrations of 363, 907, and 4,354 IU vitamin D_3 per lb of complete diet were fed for low, medium, and high treatments, respectively, and 23 µg of 25(OH)D_3/lb of the complete diet for the 25(OH)D_3 treatment.

Table 6. The effect of maternal vitamin D supplementation on sow serum vitamin metabolites (Exp. 2)[1,2]

	Maternal vitamin D[3]					Probability, $P <$				
	Vitamin D_3					Vitamin D_3				
Item	Low	Medium	High	25(OH)D_3	SEM	Lin	Quad	Low D_3 vs. 25(OH)D_3	Medium D_3 vs. 25(OH)D_3	High D_3 vs. 25(OH)D_3
Sow serum vitamin metabolites										
25(OH)D_3, ng/mL[4]										
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	3.54	0.001	0.157	0.001	0.001	0.001
Farrowing	25.1	26.1	68.2	55.4	3.54	0.001	0.241	0.001	0.001	0.004
Weaning	34.6	50.9	110.6	94.6	3.54	0.001	0.153	0.001	0.001	0.001
Vitamin D_3, ng/mL[5,6]										
d 0 of gestation	7.6	7.5	7.1	7.6	0.914	0.672	0.964	0.953	0.875	0.640
d 100 of gestation	3.5	5.2	26.6	1.9	0.914	0.001	0.209	0.181	0.005	0.001
Farrowing	3.0	4.7	19.5	1.8	0.914	0.001	0.640	0.319	0.014	0.001
Weaning	4.2	10.9	33.7	1.5	0.914	0.001	0.017	0.023	0.001	0.001
α-tocopherol, mg/L[7]										
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	131.1	0.211	0.007	0.081	0.420	0.066
Farrowing	1,247	1,054	1,219	1,329	131.1	0.748	0.231	0.622	0.102	0.508
Weaning	2,338	2,611	2,295	2,358	131.1	0.305	0.077	0.905	0.132	0.705
Retinol, ng/mL[8]										
d 0 of gestation	285	294	254	279	17.59	0.113	0.569	0.833	0.565	0.301
d 100 of gestation	231	210	237	225	17.59	0.492	0.353	0.807	0.554	0.604
Farrowing	128	165	149	192	17.59	0.593	0.713	0.177	0.291	0.089
Weaning	299	393	337	325	17.59	0.957	0.001	0.299	0.006	0.625

[1] A total of 112 sows and litters were used to determine the effects of supplemental vitamin D from varying concentrations 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.

[2] Means represent the average serum metabolite from 12 randomly selected sows within treatment and day combinations.

[3] Vitamin D_3 concentrations of 363, 907, and 4,354 IU vitamin D_3/lb of complete diet were fed for low, medium, and high treatments, respectively; and 23 μg of 25(OH)D_3 /lb of the complete diet for the 25(OH)D_3 treatment.

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

[5] The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit (n=8) were assigned concentrations of 1.00 ng/mL.

[6] A treatment × day interaction ($P = 0.001$) was observed for serum vitamin D_3.

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

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

Table 7. The effect of maternal vitamin D supplementation on pre-weaned pig serum vitamin metabolites and neonatal bone ash (Exp. 2)[1,2]

	Maternal vitamin D[3]					Probability, $P <$				
	Vitamin D_3					Vitamin D_3				
Item	Low	Medium	High	25(OH)D_3	SEM	Lin	Quad	Low D_3 vs. 25(OH)D_3	Medium D_3 vs. 25(OH)D_3	High D_3 vs. 25(OH)D_3
Pre-weaned pig serum vitamin metabolites										
25(OH)D_3, ng/mL[4]										
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, ng/mL[5,6]										
Birth	1.0	1.0	1.5	1.0	0.15	0.003	0.698	0.999	0.999	0.008
Weaning	1.0	1.2	5.7	1.0	0.15	0.001	0.006	0.929	0.441	0.001
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, ng/mL[7]										
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, %										
2nd 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] A total of 112 sows and litters were used to determine the effects of supplemental vitamin D from varying concentrations 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.

[2] Means represent the average serum metabolite from 48 randomly selected litters (two pigs per litter were bled for serum analysis) within treatment and day combinations.

[3] Vitamin D_3 concentrations of 363, 907, and 4,354 IU vitamin D_3/lb of complete diet were fed for low, medium, and high treatments, respectively; and 23 µg of 25(OH)D_3 /lb of the complete diet for the 25(OH)D_3 treatment.

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

[5] The assay for serum vitamin D_3 had a lower detectable limit of 1.00 ng/mL. Samples below the detectable limit (n=144) were assigned concentrations of 1.00 ng/mL.

[6] A treatment × day interaction ($P = 0.001$) was observed for serum vitamin D_3.

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

Table 8. The effect of maternal vitamin D supplementation on fetal muscle development (Exp. 2)[1]

	Maternal vitamin D[2]					Probability, *P*<				
	Vitamin D_3					Vitamin D_3		Low D_3 vs.	Med. D_3 vs.	High D_3 vs.
Item	Low	Medium	High	25(OH)D_3	SEM	Lin	Quad	25(OH)D_3	25(OH)D_3	25(OH)D_3
Litters sampled, n	25	27	25	27						
Longissimus Thoracis										
Whole muscle area (mm^2)[3]	117.3	113.7	113.5	111.0	14.0	0.795	0.749	0.543	0.792	0.810
Average fiber CSA, (μm^2)[4]	101.1	106.4	96.8	109.8	9.6	0.291	0.362	0.200	0.609	0.057
Average 1° fiber CSA, (μm^2)[5]	191.5	209.7	197.7	213.4	11.5	0.946	0.254	0.173	0.813	0.325
Average 2° fiber CSA, (μm^2)[6]	95.8	99.8	91.0	102.9	9.5	0.272	0.450	0.276	0.632	0.070
Total fiber number (1×10^6)[7]	1.2	1.1	1.3	1.1	0.2	0.540	0.296	0.235	0.823	0.177
Total 1° fibers (1×10^4)[8]	6.8	6.9	6.5	8.5	1.1	0.776	0.924	0.234	0.254	0.158
Total 2° fibers (1×10^6)[9]	1.2	1.1	1.2	1.0	0.2	0.502	0.270	0.169	0.716	0.117
Secondary:primary[10]	18.0	16.5	18.8	15.7	1.6	0.289	0.238	0.112	0.577	0.035
Semitendinosus										
Whole muscle area (mm^2)[3]	60.0	64.3	61.6	62.0	7.3	0.985	0.460	0.730	0.695	0.939
Average fiber CSA, (μm^2)[4]	135.4	139.7	128.8	140.4	10.9	0.409	0.633	0.671	0.954	0.303
Average 1° fiber CSA, (μm^2)[5]	185.4	198.7	171.8	202.9	12.5	0.142	0.279	0.243	0.767	0.031
Average 2° fiber CSA, (μm^2)[6]	131.7	135.8	125.7	136.2	10.6	0.449	0.656	0.700	0.968	0.349
Total fiber number (1×10^5)[7]	4.7	4.6	4.8	4.7	0.5	0.771	0.799	0.949	0.875	0.810
Total 1° fibers (1×10^4)[8]	3.5	3.5	3.4	3.6	0.5	0.822	0.923	0.905	0.957	0.766
Total 2° fibers (1×10^5)[9]	4.4	4.3	4.5	4.4	0.5	0.739	0.775	0.932	0.871	0.773
Secondary:primary[10]	15.5	19.7	16.9	18.1	3.8	0.943	0.312	0.544	0.688	0.769

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

[2] Vitamin D_3 concentrations of 363, 907, and 4,354 IU vitamin D_3/lb of complete diet were fed for low, medium, and high treatments, respectively; and 23 µg of 25(OH)D_3 /lb of the complete diet for the 25(OH)D_3 treatment.

[3] Cross-sectional area (mm^2) of the whole muscle.

[4] Average cross-sectional area (μm^2) of all muscle fibers.

[5] Average cross-sectional area (μm^2) of a representative sample of primary muscle fibers.

[6] Average cross-sectional area (μm^2) of a representative sample of secondary muscle fibers.

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

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

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

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

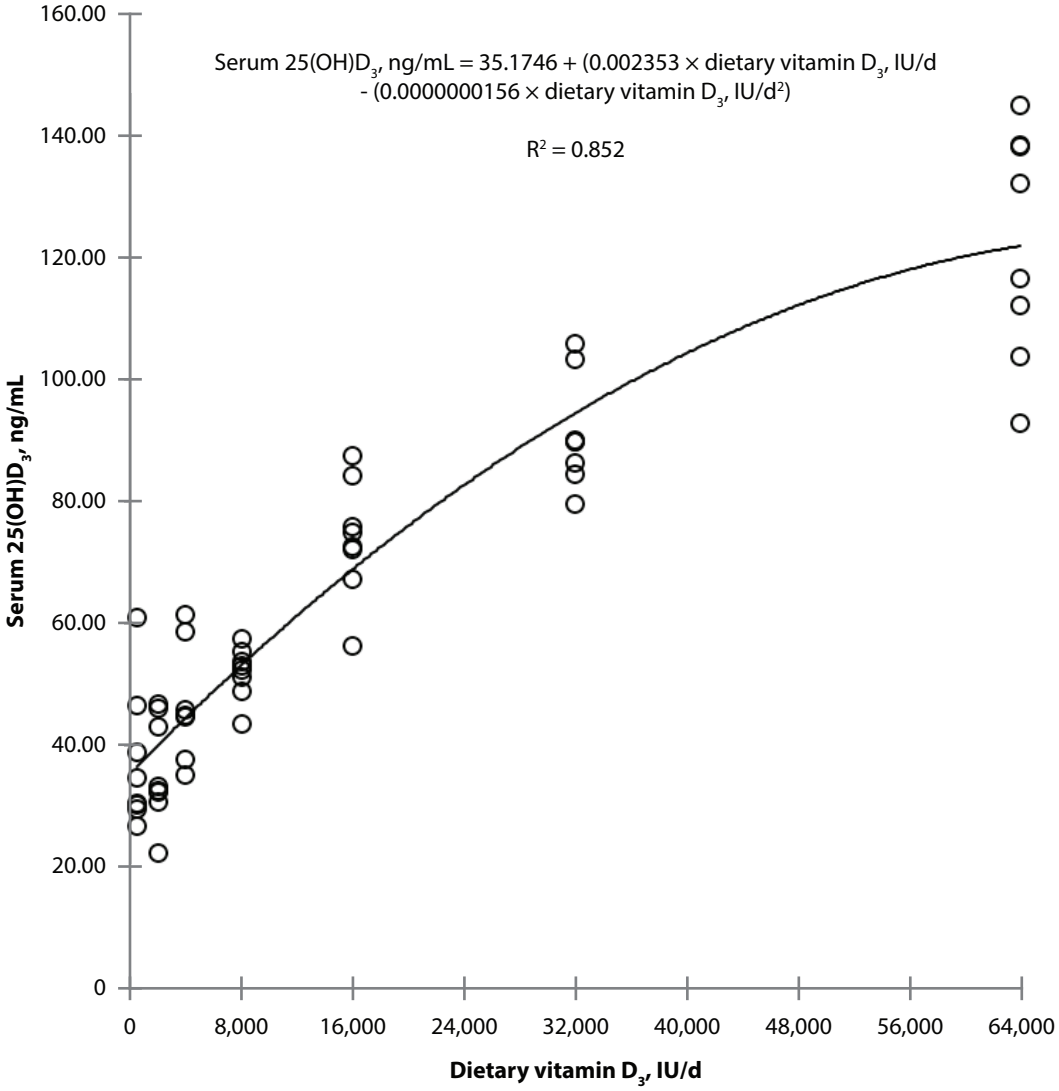

Figure 1. Influence of daily vitamin D_3 intake on serum 25(OH)D_3 of gestating sows (Exp. 1)