The effects of maternal dietary supplementation of cholecalciferol (vitamin D_3) and 25(OH)D_3 on sow and progeny performance[1]

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ABSTRACT: A total of 69 sows (DNA Line 200 × 400) and their progeny were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow vitamin D status, muscle fiber morphometrics at birth and weaning, and subsequent growth performance. Within 3 d of breeding, sows were allotted to one of three dietary treatments fortified with 1,500 IU/kg vitamin D_3 (CON), 500 IU/kg vitamin D_3 + 25 µg/kg 25(OH)D_3 (DL), or 1,500 IU/kg vitamin D_3 + 50 µg/kg 25(OH)D_3 (DH). When pigs were sacrificed at birth, there were no treatment effects for all fiber morphometric measures ($P > 0.170$), except primary fiber number and the ratio of secondary to primary muscle fibers ($P < 0.016$). Pigs from CON fed sows had fewer primary fibers than pigs from sows fed the DH treatment ($P = 0.014$), with pigs from sows fed DL treatment not differing from either ($P > 0.104$). Pigs from CON and DL fed sows had a greater secondary to primary muscle fiber ratio compared to pigs from DH sows ($P < 0.022$) but did not differ from each other ($P = 0.994$). There were treatment × time interactions for all sow and pig serum metabolites ($P < 0.001$). Therefore, treatment means were compared within the time period. At all time periods, sow serum 25(OH)D_3 concentrations differed for all treatments with the magnitude of difference largest at weaning ($P < 0.011$), where serum 25(OH)D_3 concentration was always the greatest when sows were fed the DH diet. At birth, piglets from DH fed sows had greater serum 25(OH)D_3 concentrations than piglets from sows fed the DL treatment ($P = 0.003$), with piglets from sows fed CON treatment not differing from either ($P > 0.061$). At weaning, serum concentrations of 25(OH)D_3 in piglets from all sow treatments were different ($P < 0.001$), with the greatest concentration in piglets from DH sows, followed by CON, and followed by DL. There were no treatment × time interactions for any of the metabolites measured in milk and no treatment or time main effects for 24,25$(OH)_2D_3$ concentration ($P > 0.068$). Colostrum collected within 12 h of parturition contained less ($P = 0.001$) 25(OH)D_3 than milk collected on day 21 of lactation. Regardless of time, concentrations of 25(OH)D_3 in milk were different ($P < 0.030$), with the largest 25(OH)D_3 concentration from DH fed sows, followed by DL, and then CON. In conclusion, combining vitamin D_3 and 25(OH)D_3 in the maternal diet improves the vitamin D status of the dam and progeny and it increases primary muscle fiber number at birth.

Key words: 25(OH)D_3, milk, muscle fibers, serum, sows, vitamin D_3

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INTRODUCTION

Vitamin D_3 plays a major role in regulating Ca and P absorption and metabolism in animals, especially for bone homeostasis (DeLuca, 1967). This is important for the growth and maintenance of a functional skeleton to sustain health and improve welfare and longevity. Farms in North America house pigs in environmentally controlled barns, which eliminates the animal's exposure to sunlight and ultraviolet B radiation. Vitamin D_3 is the most common form of vitamin D used to supplement swine diets. After ingestion and absorption of vitamin D_3, the 25th carbon of vitamin D_3 is hydroxylated in the liver to form 25(OH)D_3. This metabolite is then transported through the blood to the kidney where additional hydroxylation occurs to form different metabolites of vitamin D_3, including 24,25$(OH)_2D_3$ and the active hormone form of vitamin D_3, 1,25$(OH)_2D_3$, each with its own function in the body (Dittmer and Thompson, 2011). The level of 25(OH)D_3 found in blood serum is considered a good indicator of an animal's D status (i.e., deficient, adequate, or toxic) (Soares et al., 1995).

During myogenesis in a developing embryo, precursor cells proliferate to form myoblasts. When signaled, myoblasts differentiate and fuse to form multinucleated muscle fibers or differentiate into satellite cells (Rehfeldt et al., 2000). Wigmore and Stickland (1983) determined primary muscle fiber myogenesis occurs until day 50 of gestation, which is when secondary muscle fiber myogenesis begins to occur and lasts until day 90. At birth, the number of muscle fibers are fixed and there is no change in fiber number during postnatal growth (Stickland and Goldspink, 1973). Postnatal growth is due to an increase in size of the muscle fiber known as hypertrophy.

Using swine, Zhou et al. (2016) investigated the effects of maternal vitamin D_3 nutrition and status on the sow and her offspring. They evaluated muscle fiber characteristics of newborn and weanling piglets whose mothers were fed 25(OH)D_3 in addition to vitamin D_3 and observed increased total muscle fiber numbers in newborn and weanling pigs as well as an increased muscle fiber cross-sectional area in weanling pigs. The differences in skeletal muscle developments found after the piglets were born indicates there is potential for enhancing lean development and growth performance when dams are fed greater levels of dietary vitamin D from supplementing 25(OH)D_3 to an existing level of vitamin D_3. Flohr et al. (2016b) observed no effect of maternal dietary vitamin D_3 on neonatal pig muscle fiber numbers; however, the average number of secondary muscle fibers per primary muscle fiber decreased when dams were fed 25(OH)D_3 compared with 9,600 IU vitamin D_3. Also, pigs from sows fed 25(OH)D_3 had a tendency for increased hypertrophic growth of secondary muscle fibers of the longissimus muscle and primary fibers of the semitendinosus compared with pigs from sows fed 9,600 IU vitamin D_3.

A large number of muscle fibers is a prerequisite for potential to grow well. When Dwyer et al. (1993) used a pig to model from 25 kg to slaughter weight, the number of muscle fibers positively correlated with postnatal growth and feed efficiency. Miller et al. (1975) measured the longissims muscle area, ham weight, and loin weight, which positively correlated with total fiber number. Muscle mass is also determined by the size of muscle fibers increased by hypertrophy (Rehfeldt et al., 2000).

Cashman et al., (2012) observed humans orally consuming 1 μg of 25(OH)D_3 was about 5 times more effective in raising serum 25(OH)D_3 than the same amount of vitamin D_3. Therefore, the combination of vitamin D_3 and 25(OH)D_3 was fed in this study because it could possibly prove to be more available to the animal. The objective of this study was to determine if feeding a combination of vitamin D_3 (Rovimix D3, 500,000 IU/g; DSM Nutritional Products, Parsippany, NJ) and 25(OH)D_3 (Hy-D, DSM) influences sow and pig performance. Performance parameters included sow reproductive and litter performance, sow and pig vitamin D status, muscle fiber morphometrics of the progeny, and subsequent growth performance of the piglets.

MATERIALS AND METHODS

General

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). Feed samples were analyzed for vitamin D_3 and 25(OH)D_3 by DSM Nutritional Products (Parsippany, NJ) and for Ca, P, and CP by Ward Laboratories (Kearney, NE). Serum, colostrum, and milk sample testing was performed by Heartland Assays LLC (Ames, IA).

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

Ingredient, %	Gestation[1]	Lactation[2]
Corn	80.33	63.04
Soybean meal	15.60	30.20
Monocalcium phosphate, 21% P	1.48	1.48
Limestone	1.15	1.05
Salt	0.50	0.50
L-Lysine-HCL	------	0.20
DL-Methionine	------	0.05
L-Threonine	0.03	0.075
Choice white grease	------	2.50
Trace mineral premix[3]	0.15	0.15
Vitamin premix without vitamin D[4]	0.25	0.25
Sow add pack[5]	0.25	0.25
Phytase[6]	0.015	0.015
Vitamin D premix[7]	0.25	0.25
Total	100.00	100.00
Calculated analysis[8]		
Standardized ileal digestible (SID) lysine, %	0.56	1.07
Net energy, kcal/kg	2,475	2,506
Crude protein, %	14.10	19.90
Calcium, %	0.76	0.77
Available phosphorus, %	0.46	0.48
Standardized digestible phosphorous, %	0.48	0.52

[1]Diets were fed from within 3 d of breeding to parturition.

[2]Diets were fed from day 0 to 21 of lactation.

[3]Provided per kg of premix: 73 g Fe from ferrous sulfate, 73 g Zn from zinc sulfate, 22 g Mn from manganous oxide, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

[4]Provided per kg of premix: 4,409,171 IU vitamin A, 17,637 IU vitamin E, 15.4 mg vitamin B12, 1,764 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

[5]Provided per kg of premix: 4,409 IU vitamin E, 44 mg biotin, 992 mg vitamin B6, 331 mg folic acid, 110,229 mg choline, 40 mg chromium, and 9,921 mg of L-carnitine.

[6]Ronozyme Hiphos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ), with a release of 0.10% available P.

[7]Vitamin D premixes contain 1,500 or 3,500 IU of total vitamin D activity per kg of diet by adding a combination of vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ), 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ), and corn to achieve desired vitamin D concentrations for each treatment.

[8]NRC. 2012. Nutrient requirements of swine. 11th ed. Washington (DC): National Academies Press.

Animals and Diets

A total of 69 sows (DNA Line 200 × 400) and their progeny from 3 consecutive farrowing groups were used in this study. Within 3 d of breeding, each sow was weighed and assigned to one of three dietary treatments equalized for parity and body weight (BW). Treatments were gestation and lactation diets fortified with either 1,500 IU/kg vitamin D_3 (CON); 500 IU/kg vitamin D_3 + 25 μg/kg 25(OH)D_3 (DL); or 1,500 IU/kg vitamin D_3 + 50 μg/kg 25(OH)D_3 (DH). The total intended vitamin D_3 activities for the CON, DL, and DH diets were 1,500, 1,500, and 3,500 IU/kg of the diet, respectively. For all other nutrients, the experimental diets (Table 1) were equally formulated to meet or exceed the dietary requirements suggested by the Swine National Research Council (NRC, 2012). Sow diets were analyzed for total vitamin D_3 activities (Table 2).

During gestation, sows were housed in individual gestation stalls equipped with individual water nipples and a feed trough. From day 0 to 74 of gestation, sows were fed 2.0 kg of feed once per day at 0800 h. Feed allowance increased to 2.5 kg/d from day 75 to 110. After consuming gestation feed on day 110, sows were moved into farrowing crates equipped with individual water nipples and water misters. Sows were fed the gestation diet 4 times throughout the day using an electronic feeding system (Gestal Solo; JYGA Technologies, Quebec, Canada) until the sow farrowed. Once the sows gave birth, they were weighed and transitioned to lactation diets. Lactation feed intake was determined from the recordings of feed disappearance on days 7, 14, and 21. Individual sow and piglet weights were recorded within 24 h of birth and at weaning on day 21 of lactation.

The progeny from one farrowing group was double ear-tagged and monitored through the nursery and finisher until harvest. For the nursery, a total of 216 pigs weaned from one of the groups were randomly placed in 36 pens within maternal dietary treatment with six pigs per pen. Nursery pens allowed 0.304 m^2 of floor space per pig and were equipped with a four-hole feeder and a nipple waterer for ad libitum access. Nursery diets were fed in two phases and pigs maintained the same treatment as their mother. Experimental nursery diets (Table 3) were equally formulated to meet or exceed the dietary requirements suggested by the Swine National Research Council (NRC, 2012). Nursery diets were analyzed for total

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

	Gestation diets			Lactation diets		
Item	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3
Formulated						
Vitamin D_3, IU/kg	1,500	500	1,500	1,500	500	1,500
25(OH)D_3, µg/kg	---	25	50	---	25	50
Crude protein, %	14.1	14.1	14.1	19.9	19.9	19.9
Calcium, %	0.76	0.76	0.76	0.77	0.77	0.77
Phosphorus, %	0.64	0.64	0.64	0.70	0.70	0.70
Analyzed						
Vitamin D_3, IU/kg	1,300	620	2,060	1,690	540	1,350
25(OH)D_3, µg/kg	---	30	53	---	27	55
Crude protein, %	14.8	15.2	14.6	19.3	20.5	20.9
Calcium, %	0.83	0.87	0.85	1.09	0.84	0.83
Phosphorus, %	0.63	0.63	0.63	0.77	0.74	0.66

[1]Samples were collected at the feed mill, pooled by diet, subsampled, and stored at −20 °C. Samples were shipped to DSM Nutritional Products (Parsippany, NJ) for vitamin D3 and 25(OH)D3 analysis and to Ward Laboratories (Kearney, NE) for proximate analysis.

vitamin D_3 activities (Table 4). Phase 1 diets were fed in meal form from day 0 to 14 post weaning. Phase 2 diets were fed in meal form from day 14 to 59 post weaning. Individual pen weights and feeder weights were measured on days 14, 21, 28, and 59 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Pigs were transferred from the nursery to the finisher on day 59 post weaning. All pigs maintained the same pen mates after their transfer to maintain pen integrity throughout the trial. Finisher pens allowed for 0.836 m^2 of slatted floor space per pig and were equipped with a two-hole, dry self-feeder and a cup waterer for ad libitum access. The finisher dietary treatments were fed in three phases. Phase 1 and 2 diets were fed until pigs weighed approximately 61 and 100 kg, respectively. The third and final phase diets were fed from 100 kg of body weight until market. Finisher phase diets contained a decreased amount of vitamin D due to an expected increase feed intake. Finisher phase 1 and 2 diets contained the same added levels of vitamin D within each treatment and were fortified with 1,000 IU/kg vitamin D_3 (CON), 25 µg/kg 25(OH)D_3 (DL), or 50 µg/kg 25(OH)D_3 (DH). The total intended vitamin D_3 activities of the CON, DL, and DH diets were 1,000, 1,000, and 2,000 IU/kg of the diet, respectively. For finisher phase 3, diets were fortified with 800 IU/kg vitamin D_3 (CON), 20 µg/kg 25(OH)D_3 (DL), or 40 µg/kg 25(OH)D_3 (DH). The total intended vitamin D_3 activity of the CON, DL, and DH diets were 800, 800, and 1,600 IU/kg of the diet, respectively. Feed was distributed and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) four times a day. Individual pen weights and feeder weights were measured approximately every 16 d to determine ADG, ADFI, and G:F ratio. Final live weight was collected on individual pigs 1 d before marketing. Hot carcass weight (HCW) was collected on individual pigs at Triumph Foods (St. Joseph, MO).

Chemical Analyses

All diets were prepared at the K-State O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). Gestation, lactation, and nursery diets were bagged and sampled at the feed mill. Finisher diets were delivered in bulk and sampled from 80% of the feeders in the finisher facility occurring once per feed delivery. Samples were pooled, subsampled, and stored at −20 °C. Feed samples were analyzed for vitamin D_3 as well as 25(OH)D_3 (Hy-D; DSM Nutritional Products, Parsippany, NJ) using a combination of high performance liquid chromatography (HPLC) and mass spectrometry (Schadt et al., 2012). Feed samples were also analyzed for Ca (Campbell and Plank, 1992; Kovar, 2003), P (Campbell and Plank, 1992; Kovar, 2003; Wolf et al., 2003), and CP (AOAC 990.03, 2006) by Ward Laboratories (Kearney, NE).

Longissimus Muscle Sample Collection and Immunohistochemistry

One average body weight male from each of the 36 litters were euthanized within 24 h of birth and at weaning. Selected pigs were euthanized by exposure to CO_2 gas administered via a Euthanex® AgProTM system (Nutriquest, Mason City, IA) for 10 min. A 2.54 cm section of the longissimus

Table 3. Nursery diet composition (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2
Corn	41.04	47.14
Soybean meal	30.30	32.00
Blood meal	1.25	------
Corn DDGS, >6 and <9% oil	10.00	15.00
Fish meal combined	1.25	------
Milk, whey powder	10.00	------
Monocalcium phosphate, 21% P	0.80	1.00
Limestone	1.10	1.03
Salt	0.30	0.35
L-Lysine-HCL	0.30	0.30
DL-Methionine	0.18	0.12
L-Threonine	0.15	0.06
Choice white grease	2.00	2.00
Trace mineral premix[2]	0.15	0.15
Vitamin premix without vitamin D[3]	0.25	0.25
Zinc oxide	0.42	0.28
Copper sulfate	0.05	0.05
Acidifier[4]	0.20	------
Phytase[5]	0.02	0.02
Vitamin D premix[6]	0.25	0.25
Total	100.00	100.00
Calculated analysis[7]		
Standardized ileal digestible (SID) lysine, %	1.40	1.24
Net energy, kcal/kg	2,457	2,440
Crude protein, %	24.10	23.70
Calcium, %	0.79	0.69
Available phosphorus, %	0.53	0.49
Standardized digestible phosphorous, %	0.55	0.52

DDGS, dried distillers grains with solubles.

[1]Phase 1 diets were fed from day 0 to 14 and phase 2 diets were fed from day 14 to 59 in the nursery.

[2]Provided per kg of premix: 73 g Fe from ferrous sulfate, 73 g Zn from zinc sulfate, 22 g Mn from manganous oxide, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

[3]Provided per kg of premix: 4,409,171 IU vitamin A, 17,637 IU vitamin E, 15.4 mg vitamin B12, 1,764 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

[4]Kem-gest (Kemin Industries, Inc., Des Moines, IA).

[5]Ronozyme Hiphos (GT) 2700 (DSM Nutritional Products, Parsippany, NJ), with a release of 0.10% available P.

[6]Vitamin D Premixes contain 1,500 or 3,500 IU of total vitamin D activity per kg of diet by adding a combination of vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ), 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ), and corn to achieve desired vitamin D concentrations for each treatment.

[7]NRC. 2012. Nutrient requirements of swine. 11th ed.Washington (DC): National Academies Press.

muscle was removed between the last rib and fourth vertebrae. Whole muscle cross-sectional area (CSA) was collected by gently placing the LM muscle on blotting paper and tracing the outline of the blot. Blots, including a reference scale, were imaged using a scanner (Hewlett-Packard, Palo Alto, CA) and CSA was determined using NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc., Melville, NY) by calibrating the images and measuring the area within the outlined blot.

A 1.27 cm portion of the LM was collected from the anterior portion of the 2.54 cm section and embedded in Optimal Cutting Temperature tissue embedding media (Fisher Scientific, Pittsburgh, PA). Tissue samples were frozen by submersion in dry ice supercooled isopentane and were stored at −80 °C until analysis. Two cryosections, 10 μm thick, per slide were collected on 1 (birth sections) or 2 (wean sections) frost-resistant slides (Fisher Scientific). The methods of Noel et al. (2016) were followed for fiber type immunohistochemistry with modifications.

Cryosections stained for pigs harvested at birth were incubated in blocking solution, which contained 5% horse serum and 0.2% TritonX-100 (Fisher Scientific) in phosphate-buffered saline (PBS) for 30 min to inhibit nonspecific antigen-binding sites. Cryosections were incubated in blocking solution with the following primary antibodies: undiluted supernatant α-Pax7 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), 1:500 α-dystrophin (Thermo Scientific, Waltham, MA), and 1:10 supernatant myosin heavy chain, slow, IgG2b (BA-D5; Developmental Studies Hybridoma Bank) for 18 h at 4 °C in a humidified environment. Following incubation, cryosections were rinsed with PBS three times for 5 min each and incubated with the following secondary antibodies in blocking solution for 30 min: 1:1,000 Alexa-Flour 488 goat-anti-mouse IgG1 heavy and light chains (Life Technologies) for Pax7, 1:1,000 Alexa-Flour 594 goat-anti-rabbit heavy and light chains (Life Technologies) for α-dystrophin, 1:1,000 Alexa-Flour 633 goat anti-mouse IgG2b (Life Technologies) for BA-D5, and 1:1,000 Hoechst 33342 dye (Thermo Scientific) for nuclei.

The first slide for pigs harvested at weaning was used for muscle fiber type and CSA analyses and the second slide was used for satellite cell analysis. All slides were incubated in blocking solution as previously described. Muscle fiber type slides were incubated in blocking solution with the following primary antibodies: 1:500 α-dystrophin (Thermo Scientific), 1:10 supernatant myosin heavy chain, slow, type I, IgG2b (BA-D5; Developmental Studies Hybridoma Bank), 1:10 supernatant myosin heavy chain, type IIA, IgGl (SC-71; Developmental Studies Hybridoma Bank), and 1:10 supernatant myosin heavy chain, type IIB, IgM (BF-F3; Developmental Studies Hybridoma Bank). Cryosections were rinsed and the following secondary antibodies and dilutions used were

Table 4. Analyzed nursery diet composition (as-fed basis)[1]

	Phase 1			Phase 2		
Item	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3
Formulated						
Vitamin D_3, IU/kg	1,500	500	1,500	1,500	500	1,500
25(OH)D_3, µg/kg	---	25	50	---	25	50
Crude protein, %	24.1	24.1	24.1	23.7	23.7	23.7
Calcium, %	0.79	0.79	0.79	0.69	0.69	0.69
Phosphorus, %	0.66	0.66	0.66	0.66	0.66	0.66
Analyzed						
Vitamin D_3, IU/kg	2,190	562	1,620	1,540	830	1,480
25(OH)D_3, µg/kg	---	23	47	---	32	59
Crude protein, %	24.8	24.5	24.2	23.7	23.9	24.4
Calcium, %	0.90	1.02	0.96	0.69	0.71	0.70
Phosphorus, %	0.67	0.69	0.68	0.67	0.63	0.68

[1]Samples were collected at the feed mill, pooled by diet, subsampled, and stored at −20 °C. Samples were shipped to DSM Nutritional Products (Parsippany, NJ) for vitamin D_3 and 25(OH)D_3 analysis and to Ward Laboratories (Kearney, NE) for proximate analysis.

1:1,000 Alexa-Flour 594 goat-anti-rabbit heavy and light chains (Life Technologies) for α-dystrophin, 1:1,000 Alexa-Flour 633 goat anti-mouse IgG2b (Life Technologies) for BA-D5, 1:1,000 Alexa-Flour 594 goat anti-mouse IgG1 (Life Technologies) for SC-71, 1:1,000 Alexa-Flour 488 Goat anti-mouse IgM (Life Technologies, Carlsbad, CA) for BF-F3, and 1:1,000 Hoechst 33342 dye (Thermo Scientific) for fiber-associated nuclei.

Satellite cell cryosections were incubated in undiluted α-Pax7 supernatant (Developmental Studies Hybridoma Bank) with 1:500 α-dystrophin (Thermo Scientific) primary antibodies, rinsed and incubated in secondary antibodies, including 1:1,000 Alexa-Flour 488 goat-anti-mouse IgG1 heavy and light chains (Life Technologies) for Pax7, and 1:1,000 Alexa-Flour 594 goat-anti-rabbit heavy and light chains (Life Technologies) for α-dystrophin, and 1:1,000 Hoechst 33342 dye (Thermo Scientific) for fiber-associated nuclei.

After secondary antibody incubation, cryosections were washed three times for 5 min with PBS, covered with 5 µL of 9:1 glycerol in PBS and coverslipped for imaging. All cryosections were imaged at 200× magnification with a Nikon Eclipse TI-U inverted microscope (Nikon Instruments Inc., Melville, NY). A Nikon DS-QiMC digital camera (Nikon Instruments, Inc.) was used to take five photomicrographs per section.

For muscle fiber morphometric data collection, a minimum of 1,000 fibers per animal (minimum of 2 photomicrographs per section) were analyzed with NIS-Elements Imaging Software (Nikon Instruments Inc.). When analyzing muscle fibers of pigs harvested at birth, primary muscle fibers stained positively for BA-D5 and secondary muscle fibers stained negative for BA-D5. When analyzing muscle fibers of pigs harvested at weaning, fibers that stained exclusively positive for BA-D5, SC-71, and BF-F3 were labeled type I, type IIA, and type IIB, respectively. Fibers that stained positive for both SC-71 and BF-F3 were labeled as type IIX fibers (Noel et al., 2016). The periphery of all muscle fibers was identified with α-dystrophin, Hoechst 33342 dye identified all nuclei and Pax7 identified satellite cells for all cryosections. The total number of muscle fibers within the LM was calculated by determining the number of photomicrograph frames in the whole muscle CSA multiplied by the average number of fibers in a frame.

Serum Collection and Analyses

Blood samples were collected via jugular venipuncture from sows within 3 d of breeding, except for the first group of sows. All sows were bled on day 100 of gestation within 24 h after farrowing and at weaning (lactation day 21) for analysis of serum 25(OH)D_3. Within 24 h of birth and at weaning, blood samples were collected via the mammary vein from one average BW male and female piglet per litter for serum vitamin D_3, 25(OH)D_3, and 24,25$(OH)_2D_3$ analysis. On day 59 of the nursery phase, a blood sample was collected via jugular venipuncture from one average weight gilt per pen for serum 25(OH)D_3 analysis. One day before market, a blood sample was collected via jugular venipuncture from one average weight gilt per pen, preferably the same gilt bled in the nursery, for serum 25(OH)D_3 analysis. Whole blood samples

were stored at 4 °C for 24 h after collection, centrifuged (1,800 × *g* for 30 min at 4 °C), and serum was collected for analysis. Serum samples were stored at −80 °C in polypropylene tubes before being sent to Heartland Assays LLC (Ames, IA) for analysis.

Serum samples and standard curve and controls were protein precipitated with 0.2 M zinc sulfate solution (Polson et al., 2003) and vortexed, followed by methanol addition and vortexing. Then, d3-vitamin D_3/d3-25(OH)D_2/d3-25(OH)D_3/d6-24,25$(OH)_2D_3$ internal standards were added to appropriate samples and controls followed by vortexing. Hexane was added to all samples and controls, then tubes were capped and vortexed, followed by centrifugation. The organic layer was then transferred followed by drying. All standards, controls, and samples were then reconstituted with liquid chromatography/mass spectrometry (LC/MS) grade methanol and water with both containing 0.1% formic acid, then loaded onto the auto-sampler for analysis. The LC/MS/MS system used was an Agilent 1290 infinity HPLC coupled to an Agilent 6460 MS/MS with electrospray ionization source. Assay accuracy was determined to be >95% based on National Institute of Standards and Technology-certified standard assessment (Verone-Boyle et al., 2016; Makowski et al., 2017; Weidner et al., 2017) for 25(OH)D and 24,25$(OH)_2$D. Controls for vitamin D_2/D_3 in serum were also found to be >90% accurate. Reagents, solvents, and supplies were purchased through Sigma-Aldrich/Cerilliant (St. Louis, MO), Fischer Scientific (Fairlawn, NJ), Isosciences (King of Prussia, PA), Agilent Technologies (Santa Clara, CA), and Medical Isotopes(Pelham, NH).

Colostrum and Milk Collection and Analyses

Sow colostrum was collected within 12 h of farrowing and milk samples were collected at weaning to be analyzed for 25(OH)D_3 and 24,25$(OH)_2D_3$. Colostrum and milk samples were stored at −80°C in 50 mL conical tubes before being sent to Heartland Assays LLC (Ames, IA) for analysis using the following method.

Milk samples were weighed out along with assay controls containing 25(OH)D_2/D_3 and 24,25$(OH)_2D_2$/D_3 samples and controls were then spiked with d3-vitamin D_3/d3-25(OH)D_2/d3-25(OH)D_3/d6-24,25$(OH)_2D_3$ internal standards. Methanolic potassium hydroxide was then added to all samples and controls and saphonified (Roseland et al., 2016; Larson-Meyer et al., 2017) in a water bath at 60 °C. After 2.0 h, samples and controls were vortexed and then liquid-liquid extracted with hexanes: methylene chloride (80:20) solution. The organic layer was dried and then reconstituted with hexanes and methylene chloride (90:10) and then applied to 1.0 g silica solid phase extraction (SPE) columns for further purification and isolation. Elution was then dried and derivatized with 0.75 mg/mL 4-phenyl-1,2,4-triazole-3,5-dione (Aronov et al., 2008) in acetonitrile for 2.0 h at room temperature. Samples and controls were then dried and reconstituted with LC/MS/MS mobile phase containing acetonitrile, methanol, water, and 0.1% formic acid and then loaded onto the auto-sampler for analysis. The LC/MS/MS system used was an Agilent 1290 infinity HPLC coupled to an Agilent 6460 MS/MS with ESI source. All controls were found to be >94% accurate with %CV for inter-assay <10.0% and intra-assay of <5.0%. All analytes had R^2 values of >0.99 with assay range from 0.062 to 8.000 ng/g. Reagents, solvents, and supplies were purchased through Sigma-Aldrich/Cerilliant (St. Louis, MO), Fischer Scientific (Fairlawn, NJ), Isosciences (King of Prussia, PA), Agilent Technologies (Santa Clara, CA), and Medical Isotopes(Pelham, NH).

Statistical Analyses

Data were analyzed as a completely randomized design using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) with sow or pen as the experimental unit. Dietary treatment was the fixed effect. For sow and litter performance, muscle fiber morphometrics, nursery and finisher performance, and HCW, data were analyzed as a completely randomized design. Normal distribution was used for symmetrically distributed numeric responses, whereas Beta or Gamma distributions were used to model percentage responses such as stillborn percentage with logit or log link function, respectively. Count responses were analyzed under Negative Binomial distribution and log link. Serum metabolite and milk analyses were analyzed as a completely randomized design with repeated measures on time controlled for baseline measures at gestation day 0. Fixed effects included treatment, time, and their interaction. Time served as the repeated measure, sow/piglet as the subject, and ANTE(1) as the covariance structure as the best fit based on Bayesian Information Criterion. Differences were considered significant at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$.

RESULTS

It should be noted that analyzed values of vitamin D_3 and 25(OH)D_3 in the feed were relatively

Table 5. Effects of feeding vitamin D_3 alone or in combination with 25(OH)D_3 on sow and preweaned piglet performance[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Sows, *n*	23	23	23	---	---
Parity	2.35	2.35	2.52	---	---
Lactation ADFI, kg	5.61	5.76	5.67	0.198	0.865
Sow BW, kg					
Gestation					
Day 0	184.6	184.0	191.1	6.226	0.672
Day 110	229.4	231.1	231.3	4.672	0.950
BW gain, kg	44.8	47.1	40.2	3.894	0.444
Lactation					
Day 0	212.2	215.0	216.5	4.633	0.801
Day 21	208.0	210.6	210.6	4.996	0.912
BW loss, kg	−4.2	−4.3	−5.9	1.993	0.807
Litter characteristics					
Total born, *n*	17.28	16.73	17.86	0.881	0.652
Born alive, %	87.80	92.13	89.67	1.960	0.283
Stillborn, %	9.53	6.93	9.42	5.048	0.891
Mummies, %	3.90	2.27	2.86	2.771	0.864
Standardized litter size,[3] *n*	14.00	13.83	13.96	0.780	0.987
Weaning litter size, *n*	13.00	13.09	13.00	0.754	0.996
Survivability, %	93.08	95.07	93.57	1.766	0.706
Piglet BW, kg					
Birth	1.37	1.42	1.33	0.041	0.307
Weaning	5.62	5.45	5.33	0.155	0.409

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets.

[2]Three maternal dietary treatments were fed. Vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) were used to achieve desired vitamin D_3 concentrations for each treatment.

[3]Cross fostering occurred within treatment and within 48 h to equalize litter size.

close to target concentrations; however, there is variability (Tables 2 and 4). This variation could be due to the analytical methods used or collection of a very small feed sample in relation to the large volume manufactured.

There were no treatment effects on sow and preweaned pig performance ($P > 0.283$; Table 5). For pigs sacrificed at birth, there were no treatment effects for fiber morphometric measures ($P > 0.170$; Table 6), except primary fiber number and the ratio of secondary to primary muscle fibers ($P < 0.016$). Pigs from CON-fed sows had fewer primary fibers than pigs from sows fed the DH treatment ($P = 0.014$), with pigs from sows fed DL treatment not differing from either ($P > 0.104$). Pigs from the CON- and DL-fed sows had greater secondary to primary muscle fiber ratios compared with pigs from sows fed the DH treatment ($P < 0.022$) but did not differ from each other ($P = 0.994$). When pigs were sacrificed at weaning, there were no treatment effects for fiber morphometric measures ($P > 0.129$; Table 7).

All pig serum samples analyzed for vitamin D_3 had concentrations that were not greater than the detectable limit of 1.5 ng/mL. There were treatment × time interactions for all other sow and pig serum metabolites ($P < 0.001$; Table 8). Therefore, treatment means were compared within time period. At all time periods, sow serum 25(OH)D_3 concentrations differed for all treatments with the magnitude of difference largest at weaning ($P < 0.011$), and serum 25(OH)D_3 concentration was always the greatest when sows were fed the DH diet. At birth, piglets from DH fed sows had greater serum 25(OH)D_3 concentrations than piglets from sows fed the DL treatment ($P = 0.003$), with piglets from sows fed CON treatment intermediate ($P > 0.061$). At weaning, serum concentrations of 25(OH)D_3 in piglets from all sow treatments were different ($P < 0.001$), with the greatest concentration in piglets from

Table 6. Whole Longissimus lumborum and muscle fiber characteristics of pigs at birth from sows fed vitamin D_3 alone or in combination with 25(OH)D_3[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Pigs, *n*	12	12	12	---	---
Live birth weight, kg	1.43	1.44	1.35	0.061	0.517
Whole muscle CSA, mm^2	192	195	186	11.7	0.838
All fiber characteristics[3]					
Number[4]	720,711	829,512	711,181	73,763	0.409
CSA, µm^2	108	106	103	6.7	0.875
Myonuclei[5]	1.21	1.22	1.36	0.336	0.939
Satellite cells[5]	0.06	0.06	0.07	0.078	0.988
Fiber type characteristics[6]					
Primary					
Number[7]	37,501[b]	44,111[a,b]	66,139[a]	8,914	0.016
CSA, µm^2	222	240	215	20.3	0.661
Secondary					
Number[7]	683,210	785,401	647,715	70,119	0.302
CSA, µm^2	101	99	93	6.4	0.614
Secondary fibers per primary fiber[8]	20.4[a]	20.0[a]	11.9[b]	2.05	0.009

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets. Thirty-six piglets were sacrificed within 24 h of birth.

[2]Three maternal dietary treatments were fed from artificial insemination until weaning on day 21 of lactation. Vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) were used to achieve desired vitamin D_3 concentrations for each treatment.

[3]Overall fiber characteristics independent of fiber type.

[4]Total number of muscle fibers was determined by the number of photomicrograph frames in the whole muscle CSA multiplied by the average number of fibers in a frame.

[5]Myonuclei and satellite cells are expressed as number per fiber.

[6]Fibers that stained exclusively positive for BA-D5 were labeled as primary muscle fibers and fibers that stained negative for BA-D5 were labeled as secondary muscle fibers.

[7]Total number of a specific fiber isoform was determined by the number of photomicrograph frames in the whole muscle CSA multiplied by the average number of the specific fiber isoform in a frame.

[8]Ratio of secondary muscle fibers present per primary muscle fiber.

DH sows, followed by CON, and then by DL. At birth, serum concentrations of 24,25$(OH)_2D_3$ in piglets from all sow treatments were different ($P < 0.001$), with the greatest concentration in pigs from DH sows, followed by DL, and then by CON. At weaning, pigs from the CON and DL fed sows had serum 24,25$(OH)_2D_3$ concentrations that were less than that of pigs from sows fed the DH treatment ($P < 0.001$) but did not differ from each other ($P = 0.944$). During grower and finisher phases, pig serum 25(OH)D_3 concentrations for all treatments differed from each other with the magnitude of difference greatest in grower pigs ($P < 0.001$).

There were no treatment × time interactions for any of the metabolites measured in milk and no treatment or time main effects for 24,25$(OH)_2D_3$ concentration ($P > 0.068$; Table 9). Colostrum collected within 12 h of parturition contained less ($P = 0.001$) 25(OH)D_3 than milk collected on day 21 of lactation. Regardless of time, concentrations of 25(OH)D_3 in milk were different ($P < 0.030$), with the largest 25(OH)D_3 concentration from DH fed sows, followed by DL, and then by CON.

When pigs were in the nursery, there were no treatment effects for any of the growth performance measures ($P > 0.132$), except for feed efficiency from day 28 to 59 and day 0 to 59 ($P < 0.015$; Table 10). From day 28 to 59, DL pigs had a poorer feed efficiency than DH pigs ($P = 0.002$), with CON pigs not intermediate ($P > 0.107$). From day 0 to 59, DL pigs had a poorer feed efficiency than DH pigs ($P = 0.018$), with CON pigs intermediate ($P > 0.191$). When pigs were in the finishing barn, there were no treatment effects for any of the growth performance measures ($P > 0.171$; Table 11). Also, there were no treatment effects for live weight,

Table 7. Whole Longissimus lumborum and muscle fiber characteristics of pigs at weaning from sows fed vitamin D_3 alone or in combination with 25(OH)D_3[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Pigs, *n*	11	12	12	---	---
Live birth weight, kg	1.46	1.43	1.28	0.091	0.323
Live weaning weight, kg	5.82	5.64	5.24	0.276	0.334
Whole muscle CSA, mm^2	656	604	541	57.3	0.360
All fiber characteristics[3]					
Number[4]	754,550	753,211	684,633	66,665	0.657
CSA, μm^2	563	565	505	45.6	0.553
Myonuclei[5]	1.59	1.48	1.38	0.380	0.916
Satellite cells[5]	0.12	0.11	0.12	0.105	0.999
Fiber type characteristics[6]					
Type I					
Number[7]	91,248	93,984	79,139	9,894	0.468
Distribution,[8] %	12.2	12.7	11.6	0.83	0.646
CSA, μm^2	434	393	416	21.5	0.384
Type IIA					
Number[7]	117,943	132,715	119,140	12,253	0.616
Distribution,[8] %	15.7	17.7	17.9	0.81	0.129
CSA, μm^2	401	360	345	26.1	0.291
Type IIX					
Number[7]	200,114	206,098	175,679	23,920	0.584
Distribution,[8] %	26.8	26.3	24.6	1.42	0.498
CSA, μm^2	539	532	469	37.2	0.329
Type IIB					
Number[7]	345,264	320,416	310,674	29,229	0.658
Distribution,[8] %	45.7	42.9	45.8	1.44	0.256
CSA, μm^2	671	727	613	74.4	0.537

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets. Thirty-five piglets were sacrificed at weaning.

[2]Three maternal dietary treatments were fed from artificial insemination until weaning on day 21 of lactation. Vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) were used to achieve desired vitamin D_3 concentrations for each treatment.

[3]Overall fiber characteristics independent of fiber type.

[4]Total number of muscle fibers was determined by the number of photomicrograph frames in the whole muscle CSA multiplied by the average number of fibers in a frame.

[5]Myonuclei and satellite cells are expressed as number per fiber.

[6]Fibers that stained exclusively positive for BA-D5, SC-71, and BF-F3 were labeled type I, type IIA, and type IIB, respectively. Fibers that stained positive for both SC-71 and B-FF3 were labeled as type IIX fibers.

[7]Total number of a specific fiber isoform was determined by the number of photomicrograph frames in the whole muscle CSA multiplied by the average number of the specific fiber isoform in a frame.

[8]Distribution was calculated by the number of the specific fiber divided by the overall total fibers multiplied by 100%.

HCW, or dressing percentage in the pigs marketed ($P > 0.826$; Table 12).

DISCUSSION

According to the most recent survey of current vitamin and trace minerals fed in the U.S. swine industry, Flohr et al. (2016a) found that the median concentration of vitamin D_3 fed to gestating and lactating sows that represented about 40% of the industry was 1,762 IU/kg. The maximum level of dietary vitamin D_3 allowed for pigs in Canada is 1,500 IU/kg. Therefore, we chose to use 1,500 IU vitamin D_3/kg diet for the CON treatment in this study. Interestingly, in humans, orally consuming 1 µg of 25(OH)D_3 was about five times more effective in raising serum 25(OH)D_3 than the same amount of vitamin D_3 (Cashman et al., 2012). Therefore, although the units of total vitamin D_3 activity are equivalent in CON and DL diets, 25(OH)D_3 in

Table 8. Effects of feeding 25(OH)D_3 on serum concentrations of vitamin D_3 metabolites[1]

	Maternal diet[2]				Probability, $P <$		
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Treatment	Time	Treatment × Time
Sow serum[3]							
25(OH)D_3, ng/mL					<0.001	<0.001	<0.001
Gestation, day 100	21.2[c]	31.4[b]	52.1[a]	1.90			
Farrowing	17.8[c]	25.3[b]	43.3[a]	1.46			
Weaning	27.6[c]	48.8[b]	82.3[a]	2.82			
Piglet serum[4]							
25(OH)D_3,[5] ng/mL					<0.001	<0.001	<0.001
Birth	2.1[a,b]	2.0[b]	3.0[a]	0.27			
Weaning	4.7[b]	3.6[c]	7.6[a]	0.19			
24,25$(OH)_2D_3$,[6] ng/mL					<0.001	<0.001	<0.001
Birth	1.9[c]	2.8[b]	4.8[a]	0.15			
Weaning	0.9[b]	1.1[b]	2.4[a]	0.09			
Pig serum							
25(OH)D_3, ng/mL					<0.001	<0.001	<0.001
Grower[7]	16.6[c]	36.4[b]	61.3[a]	1.63			
Finisher[8]	17.8[c]	30.0[b]	53.4[a]	1.76			

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$) within the row's respective time.

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets.

[2]Three dietary treatments were fed using vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) to achieve desired vitamin D_3 concentrations for each maternal treatment.

[3]Sow serum 25(OH)D_3 was analyzed using gestation day 0 as a covariate.

[4]Piglet serum vitamin D_3 was analyzed but none of the samples contained > 1.5 ng/mL at birth or weaning.

[5]Means were calculated using only samples greater than the detectable limit for 25(OH)D_3 (1.5 ng/mL). Birth means were derived from 31.3% of submitted samples for 1,500 IU D_3, 60.9% for 500 IU D_3 and 25 µg 25(OH)D_3, and 97.9% for 1,500 IU D_3 and 50 µg 25(OH)D_3. Weaning means were derived from 100% of submitted samples for all treatments.

[6]Means were calculated using only samples greater than the detectable limit for 24,25$(OH)_2D_3$ (0.3 ng/mL). Birth means were derived from 100% of submitted samples for all treatments. Weaning means were derived from 95.7% of submitted samples for 1,500 IU D_3, 97.8% for 500 IU D_3 and 25 µg 25(OH)D_3, and 100% for 1,500 IU D_3 and 50 µg 25(OH)D_3.

[7]Grower serum was collected immediately after being transferred to the finisher, 59 d post weaning.

[8]Finisher serum was collected the day before marketing, 156 d post weaning.

Table 9. Effects of feeding 25(OH)D_3 on colostrum and milk concentrations of vitamin D_3 metabolites[1,2]

	Maternal diet[3]				Probability, $P <$		
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Treatment	Time	Treatment × Time
25(OH)D_3, ng/g					<0.001	0.001	0.518
Colostrum, day 0	0.333	0.537	0.852	0.091			
Milk, day 21	0.487	0.728	1.180	0.070			
24,25$(OH)_2D_3$,[4] ng/g					0.619	0.166	0.068
Colostrum, day 0	0.118	0.262	0.382	0.081			
Milk, day 21	0.242	0.211	0.114	0.048			

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$) within the row's respective time.

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets.

[2]Colostrum means represent the average metabolite from a total 36 sows. Milk means represent the average metabolite from a total 34 sows.

[3]Three dietary treatments were fed using vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) to achieve desired vitamin D_3 concentrations for each maternal treatment.

[4]Means were calculated using only samples greater than the detectable limit for 24,25$(OH)_2D_3$ (0.062 ng/g). Colostrum means were derived from 25.0% of submitted samples for 1,500 IU D_3, 58.3% for 500 IU D_3 and 25 µg 25(OH)D_3, and 75.0% for 1,500 IU D_3 and 50 µg 25(OH)D_3. Milk means were derived from 18.2% of submitted samples for 1,500 IU D_3, 16.7% for 500 IU D_3 and 25 µg 25(OH)D_3, and 18.2% for 1,500 IU D_3 and 50 µg 25(OH)D_3.

Table 10. Effects of feeding 25(OH)D_3 on nursery pig growth performance[1,2]

	Maternal and nursery diet[3]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Days 0–14					
ADG, kg	0.143	0.161	0.156	0.009	0.385
ADFI, kg	0.215	0.223	0.215	0.009	0.762
G:F	0.665	0.712	0.729	0.022	0.132
Day 0–21					
ADG, kg	0.230	0.251	0.233	0.009	0.242
ADFI, kg	0.383	0.397	0.377	0.009	0.287
G:F	0.601	0.630	0.619	0.017	0.496
Days 21–28					
ADG, kg	0.502	0.521	0.523	0.014	0.513
ADFI, kg	0.723	0.750	0.725	0.016	0.421
G:F	0.694	0.694	0.721	0.013	0.277
Days 28–59					
ADG, kg	0.702	0.675	0.713	0.014	0.149
ADFI, kg	1.217	1.211	1.196	0.021	0.773
G:F	0.577[a,b]	0.557[b]	0.596[a]	0.007	0.002
Days 0–59					
ADG, kg	0.508	0.504	0.517	0.010	0.643
ADFI, kg	0.858	0.863	0.845	0.014	0.649
G:F	0.593[a,b]	0.583[b]	0.613[a]	0.007	0.015
BW, kg					
Day 0	5.71	5.67	5.71	0.058	0.827
Day 14	7.72	7.98	7.90	0.156	0.472
Day 21	10.70	11.03	10.61	0.225	0.398
Day 28	14.22	14.75	14.35	0.262	0.341
Day 59	35.98	35.68	36.59	0.575	0.529

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets. A total of 216 weaned pigs were used in a 59 d nursery growth trial with 6 pigs per pen and 12 pens per treatment.

[2]Experimental diets were fed from day 0 to 59 in two phases.

[3]Three dietary treatments were fed using vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) to achieve desired vitamin D_3 concentrations for each treatment.

combination with vitamin D_3 in the DL diet could prove to be more available to the animal.

Varying results have been detected in studies investigating sow reproductive performance and preweaned piglet growth performance in response to differing levels of dietary vitamin D_3. Based on results from this experiment, there was no evidence for differences in sow performance or preweaned piglet performance, which is consistent with Flohr et al. (2014a, 2016b). In contrast, Weber et al. (2014) detected total litter birth weight, and birth weight per piglet increased when dams were fed diets replacing vitamin D_3 with 25(OH)D_3 at the same level. When vitamin D_3 sources were fed to gestating gilts in a combination similar to that in the current study, Zhou et al. (2016) found that adding 50 µg/kg 25(OH)D_3 to a diet already containing 50 µg/kg vitamin D_3 (4,000 vs. 2,000 IU total vitamin D_3 activity) resulted in an increased number of piglets born alive by one piglet. Zhou et al. (2016) suggested that the born alive increase was due to increased vitamin D_3 affecting the maternal-conceptus interaction to improve implantation. In a separate report from the same study, Zhou et al. (2017) reported that feeding the combined addition of the two vitamin D_3 sources during gestation and lactation increased piglet growth during the first 2 weeks of lactation. The authors reported that the improved performance in the first 2 weeks was likely because the maternal intake of 25(OH)D_3 improved the fat and protein contents in the milk. However, there were no differences in piglet growth performance detected at weaning on day 28 of lactation.

Research in humans and other animal species suggest that there is a role of vitamin D_3 in the

Table 11. Effects of feeding 25(OH)D_3 on finishing pig growth performance[1,2]

	Maternal diet[3]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Day 0–35					
ADG, kg	0.97	0.97	0.98	0.014	0.802
ADFI, kg	2.16	2.10	2.15	0.035	0.479
G:F	0.45	0.46	0.46	0.004	0.171
Day 35–67					
ADG, kg	1.04	1.04	1.00	0.018	0.249
ADFI, kg	2.91	2.90	2.85	0.045	0.647
G:F	0.36	0.36	0.35	0.004	0.332
Day 67–97					
ADG, kg	1.02	1.03	1.03	0.013	0.809
ADFI, kg	3.08	3.20	3.14	0.047	0.171
G:F	0.33	0.32	0.33	0.004	0.233
Day 0–97					
ADG, kg	1.01	1.01	1.00	0.011	0.894
ADFI, kg	2.68	2.70	2.69	0.036	0.878
G:F	0.38	0.37	0.37	0.003	0.832
BW, kg					
Day 0	35.98	35.68	36.59	0.575	0.529
Day 35	69.95	71.40	69.80	1.102	0.533
Day 67	103.05	103.40	102.98	1.153	0.963
Day 97	133.73	134.41	133.95	1.268	0.929

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets. A total of 216 weaned pigs were used to continue the nursery growth trial into the finisher with consistent pen integrity of 6 pigs per pen and 12 pens per treatment.

[2]Experimental diets were fed from finisher day 0 to 97 in three phases.

[3]Three dietary treatments were fed using vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) to achieve desired vitamin D_3 concentrations for each treatment. Columns are divided into maternal dietary treatments from which the progeny inherited their treatment. Finishing pigs were fed three phases of diets with different concentrations of vitamin D_3 and 25(OH)D_3 than the dams.

Table 12. Effects of feeding 25(OH)D_3 on HCW[1]

	Maternal diet[2]				
	1,500 IU D_3	500 IU D_3 and 25 µg 25(OH)D_3	1,500 IU D_3 and 50 µg 25(OH)D_3	SEM	Probability, $P <$
Live weight, kg	134.0	134.0	134.4	1.39	0.967
HCW, kg	101.3	101.1	101.4	1.25	0.987
Dressing,[3] %	75.6	75.4	75.4	0.29	0.826

[1]A total of 69 sows (DNA Line 200 × 400) and their progeny over three consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D status, muscle fiber morphometrics, and subsequent growth performance of the piglets. A total of 168 market pigs were used for these calculations out of the 202 pigs that made it to the plant. The remaining pigs either could not be identified or were skinned, causing incorrect recording of HCW.

[2]Three dietary treatments were fed using vitamin D_3 (Rovimix D3-500, DSM Nutrition Products, Parsippany, NJ) and/or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products, Parsippany, NJ) to achieve desired vitamin D_3 concentrations for each treatment.

[3]Dressing percentage was calculated by taking the HCW divided by the live weight of that animal times 100%.

formation of skeletal muscle. In swine, investigators have observed an increase in total muscle fiber number of the LM of day 90 fetuses (Hines et al., 2013) and pigs at birth and weaning (Zhou et al., 2016) when the maternal diet contained a combination of added vitamin D_3 and 25(OH)D_3. Wigmore and Stickland (1983) determined secondary muscle fiber myogenesis begins to occur around day 50 and lasts until day 90 of gestation, which is why Hines et al. (2013) chose to evaluate day 90 fetuses. In the current study, although there was no difference in total muscle fiber number at birth or weaning, which agrees with Flohr et al. (2016b), we did observe an increase in primary muscle fiber numbers

of piglets at birth when born to dams fed the DH diet. However, there were no differences in the individual fiber type numbers at weaning. For the DH treatment fed to the dams since mating, results suggest that their progeny went through a longer prenatal period of primary myogenesis that may have delayed the onset of secondary myogenesis. Interestingly, Wigmore and Stickland (1983) concluded that secondary fibers are more susceptible to external influences (i.e., nutritional environment of the embryo) than primary fibers. In the present experiment, it appears that primary myogenesis was influenced by maternal vitamin D nutrition but not secondary.

Flohr et al. (2016b) observed that the average number of secondary muscle fibers per primary muscle fiber ratio of the longissimus thoracis muscle was smaller when dams were fed 50 μg/kg 25(OH)D_3 (2,000 IU/kg vitamin D_3 activity) compared with 9,600 IU/kg of vitamin D_3, with a mean ratio of 15.7 and 18.8, respectively. Nevertheless, Flohr et al. (2016b) observed no difference in the mean ratios of pigs born to dams fed 2,000 IU/kg vitamin D_3 compared with 50 μg/kg 25(OH)D_3.

The current study provided evidence for a smaller secondary muscle fibers per primary muscle fiber ratio when dams were fed the DH diet (3,500 IU/kg total vitamin D_3 activity) compared with CON (1,500 IU/kg D_3), with a mean ratio of 11.8 and 20.4, respectively. In the experiment completed by Flohr et al. (2016b), it appeared that increasing the total vitamin D_3 activity in the diet fed to sows increased the mean ratio of secondary fibers per primary, which is different from the current study where an increased number of primary muscle fibers was observed. Although we cannot fully explain why results from the two experiments are conflicting, there were differences in the amount of total vitamin D_3 tested in each experiment. Other differences include whether a single or combined source of vitamin D_3 was fed, the muscle fibers evaluated, and the genetics of the pigs. Flohr et al. (2016b) fed a single source of vitamin D_3 up to a relatively large quantity, whereas a combination of vitamin D sources were used in this study to provide a high vitamin D treatment that was 64% less total vitamin D_3. Also, Flohr et al. (2016b) analyzed muscle fibers from the longissimus thoracis muscle of Pig Improvement Company pigs, whereas the current study analyzed muscle fibers from the longissimus lumborum of pigs from DNA Genetics.

The vitamin D status of the animal is typically assessed by measuring 25(OH)D_3 concentration in circulating serum or plasma. In research evaluating the replacement of vitamin D_3 with 25(OH)D_3 or their combination in the diet, sow blood levels of 25(OH)D_3 were greater during gestation, at farrowing, and at weaning when the sow's diet included 25(OH)D_3 (Lauridsen et al., 2010; Coffey et al., 2012; Weber et al., 2014; Zhou et al., 2016), which is in agreement with the current study. At all days of collection, serum 25(OH)D_3 concentration was the greatest when sows were fed the DH diet, with the DL diet being intermediate and the sows fed the CON diet having the least amount of 25(OH)D_3 in the serum. The time × treatment interaction that was observed was the result of the greater differences between treatments at weaning compared with gestation day 100 and farrowing. Lauridsen et al. (2010) and Weber et al. (2014) also observed a general decrease in serum levels around farrowing and an increase as lactation days accumulate. This could simply be a reflection of the sow's feed intake pattern. Generally, the gilt's or sow's feed intake is less around the time of parturition and increases as the demands for lactation increase.

Coffey et al. (2012) euthanized gilts on day 90 of gestation and observed increased fetal plasma 25(OH)D_3 when dams were fed a combination of vitamin D_3 and 25(OH)D_3 when compared with an equivalent amount of vitamin D_3 alone, reflecting that the proposed improvement in maternal vitamin D status was shared with the fetuses. In the present experiment, the DH diet fed to dams caused a 153% increase in progeny 25(OH)D_3 from birth to weaning, where only an 80% and 124% increase was observed in DL and CON progeny, respectively. At both sampling days, the greatest concentration of 25(OH)D_3 was observed in DH progeny. In agreement, Zhou et al. (2017) measured the vitamin D status of newborn piglets and those at weaning and observed greater serum 25(OH)D_3 in piglets at birth when the maternal diet contained a combination of vitamin D_3 and 25(OH)D_3. In contrast, however, Zhou et al. (2017) observed no difference in serum levels of piglets at weaning, which Zhou et al. (2017) suggested was due to the short 21 d half-life of serum 25(OH)D_3. Flohr et al. (2016b) also observed no differences in the piglet 25(OH)D_3 status at weaning. Lastly, in the current study, the piglet 25(OH)D_3 concentrations across maternal treatments were similar, ranging between 2.0 and 7.0 ng/mL, to those presented by Flohr et al. (2016b) during the suckling period. However, suckling pigs in the experiment by Zhou et al. (2017) had a much greater concentration of 25(OH)D_3 in the blood ranging between 33 and 82 nmol/L or 13 and 33 ng/mL.

Seo et al. (1997) conducted research feeding $24,25(OH)_2D_3$ to chickens and concluded that when this metabolite is present at physiological levels, it is essential for normal bone integrity and healing of bone fractures in chicks. Zhou et al. (2017) found an increase in bone strength, density, and ash content of newborn piglets when dams were fed 50 μg $25(OH)D_3$ in combination with 50 μg vitamin D_3. These differences were not found in the piglets at weaning, and serum $24,25(OH)_2D_3$ was not evaluated in the study by Zhou et al. (2017). In the current study, pigs born to dams fed the CON diet had concentrations of $24,25(OH)_2D_3$ that decreased 47% from birth to weaning; however, pigs from sows fed the DL and DH diets had larger decreases at 61% and 50%, respectively. At both sampling days, the greatest concentration of $24,25(OH)_2D_3$ was observed in the DH progeny. To our knowledge, this is the first study investigating the effect of improving maternal vitamin D status on serum $24,25(OH)_2D_3$ of the piglets.

The progeny from one farrowing group of 22 sows was followed through the nursery and finisher to market. At the conclusion of the nursery period (day 59 post weaning) and the day before marketing (day 156 post weaning), blood was collected and analyzed for serum $25(OH)D_3$ concentration. No evidence of differences over time were observed in pigs fed the CON diets; however, the DL and DH fed pigs produced a 18% and 12% decrease in serum $25(OH)D_3$ concentration over time, respectively. The decrease in serum $25(OH)D_3$ concentration in the finisher might be due to the decreased amount of $25(OH)D_3$ added to the finisher phase diets. Although, this might not be the case when considering how the increase in ADFI of pigs at these two ages would increase the daily consumption of total vitamin D_3 activity. Flohr et al. (2016c) observed a quadratic effect of serum $25(OH)D_3$ of nursery pigs on day 35 and a tendency on day 17 post weaning due to increasing maternal dietary supplementation of vitamin D_3 but noted these differences might be due to the increase in ADFI and total vitamin D_3 intake of the nursery pigs.

Vitamin D_3 is thought to be transferred from the sow to the piglets via the placenta and/or milk. In previous research, milk concentrations of $25(OH)D_3$ increased when sows were fed $25(OH)D_3$ replacing vitamin D_3 (Weber et al., 2014) and when sows were fed $25(OH)D_3$ in combination with vitamin D_3 (Zhou et al., 2017). Flohr et al. (2014a) observed milk vitamin D_3 concentration increasing linearly with increasing concentrations of vitamin D_3 supplementation to the sow. Although the present experiment did not analyze vitamin D_3 in the milk due to the limited quantity of milk collected, $25(OH)D_3$ was analyzed and we observed that DH sows produced colostrum and milk with the greatest concentration of $25(OH)D_3$. Milk concentrations of $25(OH)D_3$ increased above the colostrum $25(OH)D_3$ means by 48.5%, 35.2%, and 38.8% for the CON, DL, and DH fed sows, respectively. We believe the increase in milk $25(OH)D_3$ concentrations consumed by the piglets contributed to the increase in progeny vitamin D status, measured by $25(OH)D_3$ concentrations in progeny serum, from birth to weaning.

Analyses of $1,25(OH)_2D_3$ in colostrum and milk was of interest to us in this study because it is known to be the active form of vitamin D_3. However, Hollis et al. (1983) investigated bovine milk vitamin D_3 metabolites and reported relative concentrations of each based on the percentage of serum levels in the cow. Their conclusions suggest that $1,25(OH)_2D_3$ levels would be very low and have little significance to the piglet at such low levels. During our attempt to analyze for $1,25(OH)_2D_3$ in colostrum and milk, we found the matrix noise and low endogenous levels at or around the limit of detection difficult to overcome for quantitation and was not reported. Although novel, the application for $1,25(OH)_2D_3$ levels at such low concentrations in both milk and colostrum are questionable and difficult to justify.

Progeny feed efficiency was improved in the nursery from day 0 to 59 when the dams and nursery pigs were fed the DH diet compared with pigs from dams fed the DL diet, with feed efficiency from the CON diet similar to both DH and DL. In contrast, Flohr et al. (2014b) reported that there were no differences in feed efficiency due to vitamin D_3 supplementation in the nursery. In another study, Flohr et al. (2016c) observed that pigs from sows fed 2,000 IU of vitamin D_3 had increased ADG and ADFI in the nursery but not feed efficiency when compared with pigs from sows fed 800 or 9,600 IU of vitamin D_3 per kg of the diet.

There were no differences in the growth performance of the progeny in the finisher phases of this study. In contrast, Flohr et al. (2016c) placed finishing pigs on a common diet and did observe improved ADG and ADFI for pigs from sows fed 2,000 IU vitamin D_3 per kg of feed compared with pigs from sows fed 800 or 9,600 IU vitamin D_3. Also, Flohr et al. (2016c) observed that pigs from sows fed 50 μg $25(OH)D_3$ per kg of feed achieved higher ADG than those pigs from sows fed 800 IU vitamin D_3 per kg of feed.

There were no differences in HCW due to combining vitamin D_3 with 25(OH)D_3 in the feed of the pigs or the maternal diet. In contrast, Flohr et al. (2016c) observed that pigs from sows fed 50 μg 25(OH)D_3/kg had a heavier final live BW and HCW compared with pigs from sows fed 9,600 IU vitamin D_3. Also, as maternal vitamin D_3 increased to 2,000 IU/kg, marketed pigs from those sows had increased dressing percentage and decreased loin depth and back fat thickness. Flohr et al. (2016c) discussed these responses may truly be due to maternal treatments or possibly due to numeric differences in weaning weight of pigs whose dams were fed 2,000 IU vitamin D_3.

In conclusion, combining vitamin D_3 and 25(OH)D_3 in the maternal diet did not affect sow or preweaning pig performance in the farrowing house. The combination did, however, improve the vitamin D status of the dam and progeny and increased primary muscle fibers at birth. Although improvements were observed in primary muscle fibers at birth, the total number of muscle fibers was not improved at birth or weaning, which may explain why there were no differences in progeny growth performance to market.

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