Effects of supplemental vitamin D_3 on serum 25-hydroxycholecalciferol and growth of preweaning and nursery pigs[1,2]

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ABSTRACT: Four experiments were conducted to investigate the effects of varying concentrations of supplemental vitamin D_3 on pig growth, feed preference, serum 25-hydroxycholecalciferol [25(OH)D_3] , and bone mineralization of nursing and weanling pigs. In Exp. 1, 270 pigs (1.71 ± 0.01 kg BW) were administered 1 of 3 oral vitamin D_3 dosages (none, 40,000, or 80,000 IU vitamin D_3) on d 1 or 2 of age. Increasing oral vitamin D_3 increased serum 25(OH)D_3 on d 10 and 20 (quadratic, $P < 0.01$) and d 30 (linear, $P < 0.01$). No differences were observed in ADG before weaning or for nursery ADG, ADFI, or G:F. Vitamin D_3 concentration had no effect on bone ash concentration or bone histological traits evaluated on d 19 or 35. In Exp. 2, 398 barrows (initially 7 d of age) were used in a 2 × 2 split plot design to determine the influence of vitamin D_3 before (none or 40,000 IU vitamin D_3 in an oral dose) or after weaning (1,378 or 13,780 IU vitamin D_3/kg in nursery diets from d 21 to 31 of age) in a 45-d trial. Before weaning (7 to 21 d of age), oral vitamin D_3 dose did not influence growth but increased ($P < 0.01$) serum 25(OH)D_3 at weaning (d 21) and tended ($P = 0.08$) to increase 25(OH)D_3 on d 31. Increasing dietary vitamin D_3 concentration from d 21 to 31 increased ($P < 0.01$) serum 25(OH)D_3 on d 31. Neither the oral vitamin D_3 dose nor nursery vitamin D_3 supplements influenced nursery ADG, ADFI, or G:F. In Exp. 3, 864 pigs (initially 21 d of age) were allotted to 1 of 2 water solubilized vitamin D_3 treatments (none or 16,516 IU/L vitamin D_3 provided in the drinking water from d 0 to 10) in a 30-d study. Providing vitamin D_3 increased serum 25(OH)D_3 concentrations on d 10, 20, and 30; however, vitamin D_3 supplementation did not affect overall (d 0 to 30) ADG, ADFI, or G:F. In Exp. 4, 72 pigs were used in a feed preference study consisting of 2 feed preference comparisons. Pigs did not differentiate diets containing either 1,378 or 13,780 IU vitamin D_3/kg but consumed less ($P < 0.01$) of a diet containing 44,100 IU vitamin D_3/kg compared with the diet containing 1,378 IU vitamin D_3/kg. Overall, these studies demonstrate that supplementing vitamin D_3 above basal concentrations used in these studies is effective at increasing circulating 25(OH)D_3, but the supplement did not influence growth or bone mineralization. Also, concentrations of vitamin D_3 of 44,100 IU/kg of the diet may negatively affect feed preference of nursery pigs.

Key words: bone traits, nursery pigs, vitamin D, vitamin D_3, 25-hydroxycholecalciferol

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INTRODUCTION

Vitamin D is a group of fat-soluble secosteriods. The 2 major dietary sources of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Pigs discriminate in their metabolism and more readily utilize vitamin D_3 (Horst et al., 1981). Vitamin D_3 is produced in the photochemical conversion of 7-dehydrocholesterol within the skin of animals when exposed to sunlight or a synthetic UVb light source (De Luca, 1979). Both vita-

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min D_2 and vitamin D_3 are hydroxylated in the liver to the 25-hydroxy forms {25-hydroxyergocalciferol [**25(OH) D_2**] and 25-hydroxycholecalciferol [**25(OH)D_3**]}. This metabolite of vitamin D is the main circulating form in the blood and is commonly used as a clinical marker for vitamin D status. Then 25(OH)D_3 is hydroxylated again in the renal tubules of the kidney to 1,25-dihydroxycholecalciferol [**1,25$(OH)_2D_3$**] by the 25-hydroxyvitamin D [**25(OH) D**] 1α-hydroxylase enzyme or to 24,25-dihydroxycholecalciferol [**24,25$(OH)_2D_3$**] by the 24α-hydroxylase enzyme. The 1,25 dihydroxycholecalciferol [**1,25$(OH)_2$D**] metabolite is the physiologically active form that with parathyroid hormone and calcitonin maintains Ca and P homeostasis in the body (Dittmer and Thompson, 2010).

Recently, the presence of the vitamin D receptor in many soft tissues not associated with normal Ca and P homeostasis (Norman and Bouillon, 2010) has been found. This has raised interest in its involvement in other normal bodily functions. Since serum 25(OH)D concentrations are the best biomarker of vitamin D exposure (IOM, 2011) human health researchers have used serum 25(OH) D concentrations of 5 ng/mL or less to be a diagnostic tool for vitamin D deficiency. In the swine industry, commercially reared pigs at weaning exhibited lower than expected serum 25(OH)D concentrations (Madson, 2011), which suggest additional supplementation may be beneficial. Therefore, our objectives were to determine if supplementation of vitamin D_3 above amounts typically supplied in diets by the U.S. swine industry affects 25(OH) D_3 concentrations, growth, bone mineralization, and feed preference of preweaned and nursery pigs.

MATERIALS AND METHODS

All experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 2012).

Experiments were conducted in totally enclosed, environmentally controlled, mechanically ventilated facilities. Experiments 1 and 4 were conducted at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). The preweaning portion of Exp. 2 was performed at a commercial farrowing facility (Innovative Swine Solutions, Carthage, IL), and the nursery portion was conducted at the Kansas State University Segregated Early Weaning Facility (Manhattan, KS). Experiment 3 was conducted in a commercial nursery facility (New Fashion Pork Inc., Buffalo Center, IA).

Experiment 1

A total of 270 pigs from 29 litters (327 × 1050; PIC, Hendersonville, TN; initially 1 to 2 d of age) were used in a 52-d study to determine the effects of oral vitamin D_3 supplementation on growth, serum 25(OH)D_3 concentrations, and bone mineralization of pre- and postweaning pigs.

Shortly after farrowing, pigs were allotted to 1 of 3 oral dosage vitamin D_3 treatments. Glycomyr Inc. (Ames, IA) prepared the oral vitamin D_3 dosage treatments by solubilizing varying concentrations of vitamin D_3 in ethanol and this solution was mixed with peanut oil. The solution also contained antioxidant ingredients (tertiary butylhydroquinone and citric acid) and an antifoaming ingredient (dimethylpolysiloxane). Confirmation of vitamin D_3 concentrations were performed using an HPLC technique (Hofsass et al., 1976). Dosage treatments were 1) a control 1 mL oral dosage with no supplemental vitamin D_3, 2) 1 mL of treatment 1 but containing 40,000 IU vitamin D_3, or 3) 1 mL of treatment 1 but containing 80,000 IU vitamin D_3. Pigs were allotted to treatments on 2 different days (d 0 or 2 of the trial) during the week of farrowing. This allowed pigs to be placed on test at either 1 or 2 d after farrowing. Pigs were allotted to treatments in a randomized complete block design and matched sets within litter were considered blocks. To perform the allotment, pigs were weighed on their respective allotment days and 3 pigs closest in weight within a litter were considered a matched set. The number of matched sets per litter was variable depending on number of pigs born and weight variation; however, gender was balanced across treatments. Each pig was ear tagged for identification, and pigs within each matched set were randomly allotted and dosed with 1 of the 3 oral treatments. No cross-fostering was performed on treatment pigs; however, extra pigs were cross-fostered to balance the number of suckling pigs across litters. Pigs were weighed again on d 10, 18, and 20 to determine preweaning growth. During the lactation period, neither creep feed nor other supplements were provided except the respective oral vitamin D_3 dosage. Management of all pigs, including processing methods, was similar throughout the trial and consistent with standard farm procedures. Sow gestation and lactation diets were corn–soybean meal based with 40% dried distillers grains with solubles (**DDGS**) in gestation and 20% DDGS in lactation and contained added vitamin D_3 at 1,378 IU/kg of complete diet. The diets were formulated to 0.55 and 0.94% standardized ileal digestible Lys in gestation and lactation diets, respectively. The farrowing barn contained 29 farrowing crates (2.13 by 0.61 m for the sow and 2.13 by 0.91 m for the pigs) that were each equipped with a single feeder and nipple waterer. Necropsies were performed on pigs that died during the lactation period to verify that there were no lesions associated with vitamin D toxicity (metastatic calcification of soft tissues). In total, 18 pigs (5–7 per treatment excluding pigs euthanized for tissue collection) died throughout the preweaning portion of the study.

On d 20, the remaining 234 pigs (pigs that survived to weaning) were weaned into the nursery facility and

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Table 1. Composition of nursery diets (as-fed basis) used in Exp. 1[1], 2[2], and 4[3]

Ingredient, %	Exp. 1[4] Phase 1 A	Exp. 1[4] Phase 1 B	Exp. 2[5] Phase 1	Phase 2[6]	Phase 3
Corn	36.10	38.23	39.61	57.06	65.80
Soybean meal (46.5% CP)	12.44	19.98	17.34	25.90	30.67
Spray-dried whey	25.00	25.00	25.00	10.00	–
Dried distillers grains with solubles	–	–	5.00	–	–
Select menhaden fish meal	6.00	5.00	–	4.50	–
Spray-dried animal plasma	6.70	2.50	5.00	–	–
Spray-dried blood cells	1.65	1.25	1.25	–	–
Lactose	5.00	–	–	–	–
Choice white grease	5.00	5.00	–	–	–
Soybean oil	–	–	3.00	–	–
Monocalcium P (21% P)	–	0.70	0.85	0.38	1.02
Limestone	0.45	0.45	1.00	0.58	0.98
Salt	0.25	0.30	0.30	0.30	0.35
Zinc oxide	0.38	0.38	0.39	0.25	–
Vitamin premix[7]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[8]	0.15	0.15	0.15	0.15	0.15
Vitamin E (44,092 IU/kg)[9]	0.05	0.05	0.03	–	–
Choline chloride (60%)[10]	–	–	0.02	–	–
Amino acids	0.38	0.57	0.48	0.49	0.62
Phytase[11]	–	–	0.13	0.17	0.17
Acidifier[12]	0.20	0.20	0.20	–	–
Total	100	100	100	100	100
Calculated analysis					
ME, kcal/kg	3,544	3,498	3,406	3,311	3,309
Standardized ileal digestible, amino acids, %					
Lys	1.56	1.51	1.35	1.30	1.25
Met:Lys	30	33	29	35	33
Thr:Lys	64	63	64	63	62
Trp:Lys	17	17	18	17	17
Val:Lys	69	66	72	68	68
Ca, %	0.79	0.83	0.80	0.70	0.68
P, %	0.73	0.77	0.71	0.63	0.61
Available P,[13] %	0.68	0.68	0.63	0.47	0.42

[1]A total of 270 mixed-sex pigs from 29 litters (327 × 1050; PIC, Hendersonville, TN; initially 1 to 2 d of age) were used in a 52-d nursery study to determine the effects of oral vitamin D_3 supplementation on growth performance, serum 25-hydroxycholecalciferol [25(OH)D_3] concentrations, and bone mineralization of pre- and postweaning pigs.

[2]A 38-d study was conducted using a total of 398 barrows (1050; PIC, Hendersonville, TN; initially 7 d of age) in a 2 × 2 split plot design to determine the effects of supplementing vitamin D_3 from either a single oral dose or from varying vitamin D_3 concentrations in early nursery diets on pig growth performance and serum 25(OH)D_3.

[3]Two 14-d feed preference comparisons were conducted using 72 mixed-sex pigs (PIC 327 × 1050; initially 6.6 ± 0.1 kg BW and 28 d of age) to evaluate if pigs differentiate between feeds containing different concentrations of vitamin D_3.

[4]In Exp. 1, phase 1 diets were supplied from d 20 to 25 of the study (weaning to d 5 postweaning). A and B diets were allotted at 0.45 and 1.36 kg/pig, respectively (1.81 kg/pig total). Pigs were fed common phase 2 and 3 diets from d 25 to d 39 and d 39 to 52, respectively.

[5]In Exp. 2, at weaning (d 21) a subsample of 300 barrows were allotted to 1 of 2 phase 1 vitamin D_3 treatments (1,378 or 13,780 IU/kg). To achieve formulated vitamin D_3 concentrations a premix of vitamin D_3 (2,204,620 IU/kg) was added to the common phase 1 diet by replacing corn. Phase 1 diets were fed from d 21 to 31 of the study, and common phase 2 diets were fed from d 31 to d 45 of the study. Dietary vitamin D_3 analysis was performed by DSM Nutritional Products (Parsippany, NJ), and phase 1 diets formulated to contain 1,378 and 13,780 IU vitamin D_3/kg analyzed with vitamin D_3 concentrations of 1,267, and 10,347 IU/kg of complete diets, respectively.

[6]Pigs used in Exp. 4 were fed a common phase 1 diet for 7 d before initiation of the experimental treatments, which were phase 2 diets formulated to varying vitamin D_3 concentrations for 14 d. To achieve formulated vitamin D_3 concentrations a premix of vitamin D_3 (2,204,620 IU/kg) was added to the common Phase 2 diets by replacing corn. Dietary vitamin D_3 analysis was performed by DSM Nutritional Products (Parsippany, NJ). Phase 2 diets from Exp. 4 were formulated to contain 1,378, 13,780, and 44,100 IU vitamin D_3/kg analyzed with vitamin D_3 concentrations of 1,711, 15,554, and 49,604 IU/kg of the complete diets, respectively.

[7]Vitamin premix provided 11,023 IU vitamin A, 1,378 IU vitamin D_3, 44 IU vitamin E, 4.41 mg vitamin K, 38.5 μg vitamin B_{12}, 49.6 mg niacin, 27.56 mg pantothenic acid, and 8.27 mg riboflavin per kilogram of the complete diet.

[8]Trace mineral premix provided 39.68 mg Mn, 151.84 mg Fe, 151.84 mg Zn, 15.18 mg Cu, 0.30 mg I, and 0.30 mg Se per kilogram of the complete diet.

[9]Provided an additional 22 and 13 IU/kg of complete diets for Phase 1 diets used in Exp. 1 and 2, respectively.

[10]Provided 100 mg choline/kg of the complete diets for phase 1 diets used in Exp. 2.

[11]Natuphos 600; BASF, Florham Park, NJ. Provided 780, 1,021, and 1,021 phytase units/kg of the complete diet for phase 1, 2, and 3 diets, respectively. Phase 1 diets used in Exp. 1 diet not contain phytase.

[12]Kem-Gest: phosphoric, fumaric, lactic, and citric acid. Kemin Industries Inc., Des Moines, IA.

[13]Phytase provided 0.12, 0.13, and 0.12% available P for phase 1, 2, and 3, diets, respectively. Phase 1 diets used in Exp. 1 did not contain phytase.

penned by treatment. The nursery barn had 34 pens (1.22 by 1.52 m) with wire mesh flooring; each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Sets of pens were blocked to minimize the effect of location. Pigs were assigned to a set of pens, maintaining the integrity of the initial matched sets within a pen set. There were 6 to 7 pigs per pen and a total of 11 or 12 replications per treatment (due to preweaning death, replications were uneven during the nursery portion of the study). Nursery diets were fed in a common 3-phase dietary program (Table 1). The phase 1 diets (A and B diets) were fed from d 20 to 25 in a pelleted form. The phase 2 and 3 diets were fed from d 25 to 39 and d 39 to 52, respectively, in meal form. All pigs and feeders were weighed on d 20, 25, 32, 39, 46, and 52 to determine ADG, ADFI, and G:F.

Within each litter, 1 matched set that was closest to the mean pig weight at time of allotment was bled via jugular venipuncture to determine initial serum 25(OH)D_3 concentrations. The same pigs were bled again on d 10, 20 (weaning), 30, and 52 to determine serum 25(OH)D_3 concentrations.

Tissue and Bone Sampling

On d 18, 6 matched sets (6 pigs/treatment and 1 matched set/litter) were selected for necropsy, which was conducted on d 19. Matched sets selected for necropsy

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were chosen to balance for gender and litter among experimental treatments. Mean BW of pigs selected for necropsy were consistent with the mean BW of treatment populations. Necropsies were conducted at the Kansas State University College of Veterinary Medicine. All necropsies performed were in compliance with the college's standard operating procedures. All pigs selected for necropsy were euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI). On d 19, both femurs and second ribs were collected to determine bone ash content, and the fourth ribs and tibias were sampled for histopathology examination. Mesenteric lymph nodes were collected from control pigs and pigs that received 80,000 IU vitamin D_3. Mesenteric lymph nodes were isolated, rinsed in cold saline, and homogenized in approximately 10 volumes of TRIzol reagent (Invitrogen, Carlsbad, CA). Samples were then kept frozen at –86°C before analysis for abundance of tumor necrosis factor (**TNFα**) mRNA. On d 35, 12 pigs (6 from the control treatment and 6 from the 80,000 IU vitamin D_3 treatment) balanced for BW, litter, and gender were selected for necropsy, and bones were sampled with the same procedures performed on d 19.

Collection and Preparation of Bones for Histological Examination

Muscles and connective tissues were cleaned from bone surfaces, and bones were split longitudinally with a hacksaw. Blood was washed from the cut surfaces, and gross evaluations were performed. Bones were then placed in 10% neutral buffered formalin and allowed to fix at room temperature for 24 h, after which they were decalcified in commercial decalcification solution (Cal-Ex Decalcifier; Fisher Scientific, Fair Lawn, NJ) according to the manufacturer's directions. The bones were then washed for 30 min in running water, and the proximal portion of each tibia, including the proximal growth plate, and both ribs, including the costochondral junction and 2 to 3 cm of adjacent bone, were routinely embedded in paraffin wax, sectioned at 4 µm, mounted on glass slides, and stained with hematoxylin and eosin. All bone samples were collected and examined by a board-certified pathologist who was blinded to vitamin D_3 treatments.

Bone Ash Analysis

After collection, femurs and ribs were stored at –20°C until they were thawed and placed in petroleum ether for fat extraction. Bones were soaked in the ether for 7 d at room temperature (25°C) and then removed from the ether and dried at 100°C until a consistent dry weight was achieved. Upon completion of drying, all bones were ashed at 600°C for 24 h. Final ash contents were collected and expressed as a percentage of dry fat-free bone.

Ribonucleic Acid Sample Analysis

Each homogenate of lymphoidal tissue was thawed at room temperature; 500 µL was placed in a clean microfuge tube and then the sample was mixed thoroughly with 100 µL chloroform for 15 sec and centrifuged at 12,000 × *g* for 15 min at 4°C. The upper aqueous phase was removed (250 µL) and mixed with 0.93 volumes of 75% ethanol. The mixture was then applied to an RNeasy spin column (Qiagen Inc., Germantown, MD) and processed as described by the manufacturer with the exception that an additional wash with 2 *M* NaCl and 2 m*M* EDTA (pH 4.0) was included. Ribonucleic acid was eluted in 50 µL of water, and the concentration was obtained by UV spectrometry. One microgram of RNA was then used as a template for production of cDNA in a 20-µL reaction volume using random hexamers and Superscript III (Invitrogen, Carlsbad, CA) as described by the manufacturer. Afterward, samples were diluted to 100 µL final volume with Tris-EDTA buffer and stored at –20°C before PCR analysis.

Quantitative real-time PCR was performed using a Stratagene Mx3005p cycler (Stratagene, La Jolla, CA) and PerfeCTa SYBR Green Fast mix reagent (Quanta Biosciences, Gaithersburg, MD). Amplification of porcine target cDNAs was accomplished with the following primers (synthesized by Integrated DNA Technologies, Coralville, IA): pGAPDH-For, 5′-TGTCCCCACCCCCAACGTGT; pGAPDH-Rev, 5′-GAGGGCAATGCCAGCCCCAG; pTNFα-For, 5′-GCAGGAGCCACCACGCTCTT; and pTNFα-Rev, 5′-CGTGGGCGACGGGCTTATCT. Aliquots (8.3 ng) of cDNA were amplified under the following conditions: 95°C for 30 s followed by 45 cycles of 95°C for 1 s and 57°C for 30 s. All reactions were performed in duplicate with 6 pigs/treatment, and relative mRNA abundance was quantified by the delta cycles to threshold (**C_t**) method with glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) to normalize values as previously described by Giulietti et al. (2001) and Das et al. (2009).

Experiment 2

A 38-d study was conducted using a total of 398 barrows (1050; PIC, Hendersonville, TN; initially 7 d of age) in a commercial farrowing facility in a 2 × 2 split plot design to determine the effects of supplementing vitamin D_3 from either a single oral dose or from dietary concentrations up to 60 times the requirement (220 IU) for nursery pigs (NRC, 2012) on pig growth and serum $25(OH)D_3$. The hypothesis was that vitamin D_3 supplementation would increase preweaning growth of pigs suckled in a commercial production facility.

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On d 7 after birth, weight-matched pairs of barrows within litters were allotted to 1 of 2 oral dosage treatments (none or 40,000 IU vitamin D_3) in a randomized complete block design. Barrows were weighed on d 7 and at weaning (d 21) to determine preweaning growth. Matched pair barrows were not cross-fostered; however, extra pigs were cross-fostered to balance the number of suckling pigs across litters. The study used barrows from 80 litters in 3 farrowing rooms that contained 39 stalls (2.13 by 0.61 m for the sow and 2.13 by 0.91 m for the pigs), 1 self-feeder, and a nipple waterer.

At weaning (d 21), pigs were transported approximately 7 h (623 km) to the nursery facility, which contained 80 pens (1.52 by 1.52 m) with metal slatted floors. Each pen contained one 5-hole dry self-feeder and a nipple waterer to allow for ad libitum access to feed and water. A subset of 300 barrows were used from d 21 to 45 to determine the effects of the previously administered vitamin D_3 dose and 2 concentrations of dietary vitamin D_3 (1,378 or 13,780 IU vitamin D_3/kg of complete diet) in early nursery diets (d 21 to 31) on pig performance and serum 25(OH)D_3. Barrows were subsampled to reduce the number of lightweight, nonviable pigs in the nursery portion of the study and to maintain the integrity of matched pairs originally established on d 7 after birth. Barrows were allotted to pens based on their previously administered vitamin D_3 dose, and pens were randomly assigned to dietary treatments. There were 5 pigs per pen and 15 pens per treatment. The only difference between the diets fed from d 21 to 31 was vitamin D_3 level (Table 1). The diets contained 0.80% Ca and 0.63% available P. A common diet (1,378 IU/kg, 0.70% Ca, and 0.47% available P) was fed from d 31 to 45. Pigs and feeders were weighed on d 21, 26, 31, 38, and 45 to determine ADG, ADFI, and G:F.

Serum was collected from 12 pigs per treatment at weaning (d 21), d 31, and d 45 to determine serum 25(OH)D_3. Pigs selected for serum sampling were from 12 randomly selected pens per treatment; selected pigs were closest to the average pen weight at allotment to dietary treatments.

Barrows were vaccinated for porcine circovirus type 2 (**PCV2**) and *Mycoplasma hyopnuemoniae* (**M. hyo**) on d 29. A 1-dose product (Ingelvac CircoFLEX, CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was given for PCV2. A 2-dose product was used for the M. hyo vaccine (Respisure; Pfizer Animal Health, New York, NY). Serum samples collected at weaning (d 21) and d 64 were analyzed for PCV2 antibody titers to distinguish potential effects of supplemental vitamin D_3 on acquired immunity.

Porcine Circovirus Type 2 Antibody Titer Analysis

Serum was analyzed for antibodies against PCV2 using an indirect fluorescent assay (**IFA**) at the Kansas State University Veterinary Diagnostic Laboratory. Titration endpoints were calculated as the reciprocal of the last serum dilution that gave a positive fluorescence result. Before analysis, all IFA titers were log 2 transformed to approximate a normal distribution of titers. Log 2 transformed antibody titers were used to quantify the change in antibody titer from weaning (d 21) to d 64 based on supplemental vitamin D_3 treatments.

Experiment 3

A total of 864 pigs (PIC TR4 × FAST ADN; initially 21 d of age) were used in a 30-d nursery study to determine the effects of vitamin D_3 supplementation via the water supply on nursery growth performance and serum 25(OH)D_3 concentrations.

Upon arrival to the facility, pigs were placed in pens and pens were randomly assigned to 1 of 2 water solubilized vitamin D_3 treatments (none [control] or 16,516 IU vitamin D_3/L of drinking water; Hi-D 2X; Alpharma LLC, Eagle Grove, IA). The experimental treatment was achieved by adding 15.63 mL of Hi-D 2X (135,256 IU vitamin D_3/mL) into a 1 L stock solution, which was added to the water supply at a rate of 1:128. All pens (1.75 by 4.06 m) contained a 5-hole dry self-feeder and a nipple waterer to allow for ad libitum access to feed and water. There were 24 pigs per pen and 18 pens per treatment. Vitamin D_3 treatments in the drinking water were provided from d 0 to 10. From d 10 to 30, all pens were provided the control water source with no supplemental vitamin D_3. Nursery diets were fed in a common 3-phase dietary program. The phase 1 diet (2,200 IU vitamin D_3/kg, 0.96% Ca, and 0.59% available P) was fed from d 0 to 10 and was in pelleted form. Phase 2 (2,200 vitamin D_3/kg, 0.98% Ca, and 0.59% available P) and phase 3 (2,200 IU vitamin D_3/kg, 0.68% Ca, and 0.49% available P) diets were fed from d 10 to 20 and d 20 to 30, respectively, and were in pelleted form. Pigs and feeders were weighed on d 0, 10, 20, and 30 to determine ADG, ADFI, and G:F. A subsample of 12 pigs per treatment was bled on weighing days to determine serum 25(OH)D_3 concentrations.

Experiment 4

A 14-d feed preference study consisting of 2 feed preference comparisons were conducted using 72 mixed-sex pigs (327 × 1050; PIC, Hendersonville, TN; initially 6.6 ± 0.1 kg BW and 28 d of age) to evaluate if pigs differentiate between feeds containing different concentrations of vitamin D_3. All pigs received a common phase 1 diet (1,378 IU/kg vitamin D_3) for 7 d before the start of the study. On d 0, pigs were weighed and allotted to pens based on BW. There were 6 pigs per pen and 6 pens per treatment. Pens were randomly assigned to 1 of 2 feed comparisons in

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corn–soybean meal–based diets containing 10% whey and 4.5% fish meal (Table 1). The first preference comparison was between diets containing 1,378 (control) or 13,780 IU vitamin D_3/kg of the complete diet, and the second comparison was between diets containing 1,378 (control) and 44,100 IU vitamin D_3/kg of the complete diet. The levels were selected to represent feeding concentrations approximately 6x, 60x, and 200x the requirement (NRC, 2012). All pens (1.22 by 1.52 m) contained two 3-hole dry self-feeders and a nipple waterer to allow for ad libitum access to feed and water. Diets were placed in separate feeders, and feeders were positioned adjacent to each other. Every morning, feeders were weighed and relocated in the pen to discourage location bias. Total pen feed intake was calculated, and intake of each diet for both comparisons was expressed as a percentage of total intake.

Serum 25-Hydroxycholecalciferol, Calcium, and Phosphorus Analysis

All blood samples were collected via jugular venipuncture using 25-mm × 20-gauge needles and 10-mL blood collection tubes containing a gel separator to determine circulating 25(OH)D_3 serum concentrations. Six hours after collection, blood was centrifuged (1,600 × *g* for 25 min at 2°C) and serum was harvested and stored at –20°C until analysis. Serum 25(OH)D_3 concentrations were determined by Heartland Assays (Ames, IA) using a previously described RIA (Hollis et al., 1993). Assays conducted by this laboratory have a lower detectable limit for 25(OH)D_3 of 2.5 ng/mL. Individual samples that were below the detectable limit ($n = 3$) were assigned concentrations of 2.5 ng/mL. Calcium and P analyses for Exp. 1 were conducted at the Iowa State University College of Veterinary Medicine (Ames, IA) by using spectrophotometry with commercial kits (Pointe Scientific Inc., Canton, MI) and methods described by Pointe Scientific (2009a,b).

Dietary Vitamin D_3 Analysis

Feed samples were collected from Exp. 2 and 4 to validate vitamin D_3 concentrations. Samples were collected at the conclusion of the experimental diet feeding period, pooled by treatment, and subsampled for analysis. Premixes containing vitamin D_3 from Exp. 2 and 4 were also sampled for analysis. All diet and premix samples were analyzed by DSM Nutritional Products Inc. (Parsippany, NJ) for vitamin D_3 analysis using a combination of HPLC and mass spectrometry (Schadt et al., 2012).

Statistical Analysis

All data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and treatment means were analyzed using the LSMEANS statement. All serum 25(OH)D_3 analyses and PCV2 antibody titer analyses from Exp. 2 were conducted using the REPEATED function of SAS to determine treatment main effects over time and treatment × time interactions. Data from Exp. 1 were analyzed as a randomized complete block design with litter and matched set within litter as the blocking factors. Individual pig was the experimental unit for preweaning growth performance, serum 25(OH)D_3, bone ash determination, and TNFα mRNA relative abundance. Pen was the experimental unit for nursery growth performance. Preplanned CONTRAST statements were used to determine linear and quadratic contrasts based on oral vitamin D_3 treatment. For Exp. 2, data were analyzed as a randomized complete block design with the main effects of oral dosage and diet treatment and dosage × diet interactions. Individual pig was the experimental unit for preweaning growth performance, serum 25(OH)D_3, and PCV2 antibody titers. For preweaning growth performance, initial weight was used as a covariate and sow was a random effect in the statistical model. Pen was the experimental unit for nursery growth performance. Data from Exp. 3 were analyzed as a randomized complete block design with barn location as a blocking factor and water vitamin D_3 level as the main effect. Pen was the experimental unit and initial BW on d 0 was used as a covariate. Serum 25(OH)D_3 was analyzed using individual pig as the experimental unit. Finally, data for Exp. 4 were analyzed as a completely randomized design, and differences associated with the main effect of diet on the percentage of total feed intake were determined in both comparisons. All results were considered significant at $P \leq 0.05$ and considered trends at $P \leq 0.10$.

RESULTS

Experiment 1

Before weaning (d 0 to 20), no significant differences were observed for ADG (Table 2). During the nursery phase (d 20 to 52), oral vitamin D_3 dosage did not affect ADG, ADFI, or G:F. Before vitamin D_3 supplementation, initial serum 25(OH)D_3 concentrations were similar among all pigs (Table 3; Fig. 1). A vitamin D_3 dose × day interaction ($P < 0.01$) was observed for serum 25(OH)D_3. The interaction was a result of serum 25(OH)D_3 increasing (quadratic, $P < 0.01$) over time with the greatest values observed on d 10 for pigs dosed with vitamin D_3. Pigs orally dosed with vitamin D_3 had greater serum 25(OH)D_3 on d 10 (quadratic, $P < 0.01$), d 20 (quadratic, $P < 0.01$), and d 30 (linear, $P < 0.01$) than control pigs. On d 52, serum 25(OH)D_3 concentrations were similar regardless of oral vitamin D_3 supplementation. Supple-

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Table 2. Effects of oral vitamin D_3 dose on preweaning and nursery growth performance, Exp. 1[1]

		Vitamin D_3, IU			Probability, P <	
	Control	40,000	80,000	SEM	Linear	Quadratic
Preweaning[2]						
Initial[3] BW, kg	1.71	1.70	1.71			
ADG, g	216	222	223	28	0.69	0.42
Weaning BW, kg	5.91	6.04	6.05	0.18	0.69	0.44
Nursery, d 20 to 52[4]						
ADG, g	360	371	369	8	0.45	0.49
ADFI, g	530	551	541	12	0.52	0.30
G:F	0.68	0.67	0.68	0.01	0.96	0.58
Final BW, kg	17.80	18.02	18.03	0.35	0.58	0.76

[1]A total of 270 pigs from 29 litters (PIC 327 × 1050) were used in a 52 d study to determine the effects of oral vitamin D_3 dose at 1 or 2 d of age on growth performance, 25-hydroxycholecalciferol [25(OH)D_3] , and bone mineralization of pigs pre- and postweaning. Pig was the experimental unit for preweaning growth, and pen was the experimental unit for nursery growth.

[2]Data were analyzed using performance records from pigs that survived (234 total; 79, 78, and 77 for the control; 40,000, and 80,000 IU vitamin D_3 treatments, respectively) through weaning (d 20).

[3]Initial refers to pigs placed on test on both d 0 and d 2 of the trial. Pigs were placed on test 1 or 2 d postfarrowing. Pig days were adjusted to account for differences in trial starting d for calculating preweaning ADG.

[4]There were 6 or 7 pigs per pen with 12 replications for the control and 40,000 IU vitamin D_3 treatments and 11 replications for the 80,000 IU vitamin D_3 treatment.

Table 3. Effects of oral vitamin D_3 dose on serum 25-hydroxycholecalciferol [25(OH)D_3] and bone ash, Exp. 1[1]

		Vitamin D_3, IU			Probability, P <	
	Control	40,000	80,000	SEM	Linear	Quadratic
Serum 25(OH)D_3, ng/mL						
Initial	3.6	3.5	3.6	1.2	0.99	0.99
d 10	14.7	57.3	68.5	1.2	0.01	0.01
d 20	8.0	28.1	35.8	1.2	0.01	0.01
d 30	10.4	17.8	22.5	1.2	0.01	0.36
d 52	13.9	15.0	15.4	1.2	0.36	0.82
Bone ash,[2] %						
d 19						
Femur	42.0	42.7	40.5	1.6	0.54	0.46
Rib	35.5	32.6	30.8	1.8	0.09	0.82
d 35						
Femur	39.0		39.7	0.6	0.47[3]	
Rib	31.5		33.0	1.7	0.55[3]	

[1]A total of 87 pigs or 29 pigs per treatment (1 matched set per litter) were bled before dosing (initial: includes pigs placed on test on both d 0 and 2) and on d 10 of lactation, and d 20, 30, and 52 in the nursery to determine serum 25(OH)D_3, Ca, and P concentrations. Individual pig was the experimental unit.

[2]A total of 18 pigs, 6/treatment (6 matched sets), were necropsied and bone samples were collected on d 19; 12 pigs (6 control pig and 6 pigs from the 80,000 IU treatment) were necropsied and bone samples were collected on d 35.

[3]P-values represent main effect of oral dosage.

mentation of vitamin D_3 did not influence (Fig. 2) serum Ca concentrations on the initial day of collection, d 10, or d 30. However, differences for serum Ca were observed on d 20 (linear, $P = 0.05$) and d 52 (quadratic, $P = 0.02$), with serum Ca increasing with increasing supplementation of vitamin D_3. Serum P concentrations were not influenced (Fig. 3) by supplementation of vitamin D_3 in an oral dose. Correlation analysis showed that serum 25(OH) D_3 was a poor indicator of serum Ca ($r^2 \leq 0.03$) or serum P ($r^2 \leq 0.05$) on any sampling day.

Bone ash from femurs of pigs euthanized on d 19 showed no effect of vitamin D_3 dosage (Table 3), but second-rib ash content tended (linear, $P = 0.09$) to decrease as oral vitamin D_3 dosage increased. No differences were found in bone ash content of femurs or second ribs collected on d 35.

No macroscopically visible differences were observed in the growth plates of either the tibias or the ribs. Histologically, all ribs from both collection days (d 19 and 35) were similar in their progression of chondrocytes through the normal maturation zones. The zones had a normal, even, and abrupt transition to primary spongiosa, which undergoes remodeling to form secondary spongiosa and trabecular bone. The growth plates were uniform in width across their length. The growth plates of all tibias were uniform and were undergoing the normal progression from cartilage to bone formation and mineralization. Finally, mesenteric lymph node abundance of TNFα mRNA was ($P < 0.01$; Fig. 4) lower for pigs dosed with 80,000 IU of vitamin D_3 than for control pigs.

Experiment 2

Analysis of vitamin D_3 concentrations in the diets verified that they were within acceptable analytical error of formulated dietary values. Analysis of experimental diets showed that vitamin D_3 mean concentrations were 1,267 and 10,347 IU/kg for diets formulated to contain added vitamin D_3 at 1,378 and 13,780 IU/kg of the complete diet, respectively. Vitamin D_3 oral dose did not influence preweaning growth (Table 4). During the nursery phase (d 21 to 45), neither previously administered oral vitamin D_3 dose nor dietary concentration of vitamin D_3 in early nursery diets affected ADG, ADFI, or G:F. No dose × diet interactions were observed for any response criteria.

At weaning (d 21), serum concentrations increased ($P < 0.01$) in pigs that received an oral dose of 40,000 IU vitamin D_3. On d 31, a tendency ($P = 0.08$) for an increase in serum 25(OH)D_3 was observed for pigs dosed with vitamin D_3 before weaning. Also on d 31, increased serum 25(OH)D_3 concentrations were observed ($P < 0.01$) in pigs fed increased concentrations of vitamin D_3, but serum 25(OH)D_3 concentrations were similar by d 45 regardless of oral vitamin D_3 dosage before weaning or early nursery dietary vitamin D_3 concentration. Also, PCV2 antibody titer results showed no influence

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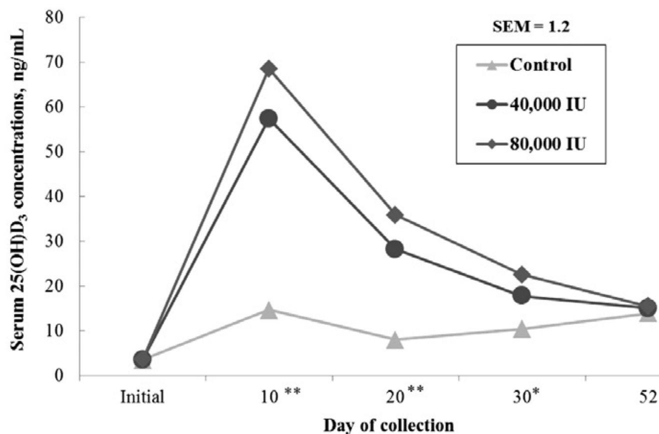

Figure 1. Mean serum 25-hydroxycholecalciferol [25(OH)D_3] concentrations (ng/mL) at initial (d 0 or 2), d 10, 20, 30, and 52 in pigs dosed with either 0, 40,000, or 80,000 IU of vitamin D_3 (29 pigs per treatment). Super scripts denote differences ($P < 0.01$) due to *, a linear oral dosage effect, and **, a quadratic oral dosage effect.

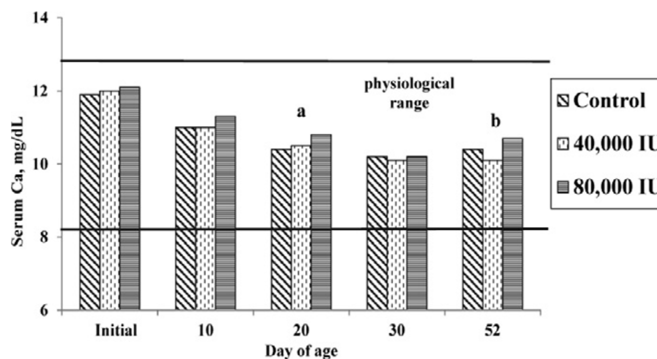

Figure 2. Mean serum Ca concentrations (mg/dL) from Exp. 1 at initial (d 0 or 2), d 10, 20, 30, and d 52 in pigs dosed with either 0, 40,000, or 80,000 IU of vitamin D_3 (29 pigs per treatment). Superscripts denote differences ($P < 0.05$) due to a, linear oral dosage effect, and b, a quadratic oral dosage effect, or tendencies ($0.05 < P \leq 0.10$) due to x, linear oral dosage effect, and y, quadratic oral dosage effect. Physiological range: based on Friendship et al. (1984).

of either vitamin D_3 oral dosage or early nursery dietary vitamin D_3 concentration associated with the change in log 2 reciprocal dilutions from d 21 to 64.

Experiment 3

Supplementation of vitamin D_3 in the water supply did not affect (Table 5) overall ADG, ADFI, or G:F from d 0 to 30. For serum 25(OH)D_3 concentrations, supplementing 16,516 IU vitamin D_3/L from d 0 to 10 increased ($P < 0.01$) serum 25(OH)D_3 concentrations in pigs on d 10, 20, and 30.

Experiment 4

Analysis of vitamin D_3 concentrations in the diets verified that they were within the acceptable analytical error of formulated dietary values. Analysis of experimental diets showed that vitamin D_3 mean concentrations were 1,711, 15,554, and 49,604 IU/kg for diets formulated to contain added vitamin D_3 at 1,378, 13,780, and 44,100 IU/kg of the complete diet, respectively. No preference differences, expressed as a percentage of total feed intake, were observed between diets formulated to contain 1,378 or 13,780 IU of vitamin D_3/kg (49 vs. 51% of 0.54 kg ADFI) throughout the length of the study (Fig. 5). Conversely, when pigs were offered a choice between diets formulated to contain either 1,378 IU or 44,100 IU vitamin D_3/kg (67 vs. 33% of 0.52 kg ADFI), pigs consumed a greater portion ($P = 0.03$) of the diet containing 1,378 IU of vitamin D_3/kg.

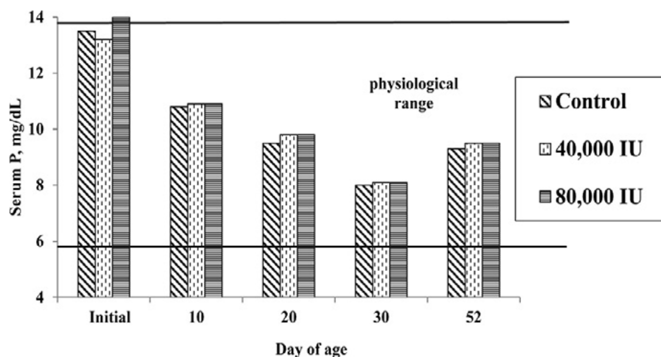

Figure 3. Mean serum P concentrations (mg/dL) from Exp. 1 at initial (d 0 or 2), d 10, 20, 30, and d 52 in pigs dosed with either 0, 40,000, or 80,000 IU of vitamin D_3(29 pigs per treatment). Superscripts denote differences ($P < 0.05$) due to a, linear oral dosage effect, and b, a quadratic oral dosage effect, or tendencies ($0.05 < P \leq 0.10$) due to x, linear oral dosage effect, and y, quadratic oral dosage effect. Physiological range: based on Friendship et al. (1984).

DISCUSSION

Vitamin D_3 requirements set by the NRC (2012) for the nursery pig are 220 IU/kg of the complete diet for pigs ranging from 5 to 11 kg and 200 IU/kg of the complete diet for pigs from 11 to 25 kg. On the other hand, vitamin D_3 levels in commercial diets often contain 5 to 7 times these concentrations (Reese and Hill, 2010). Previous research has extensively evaluated the supplementation of

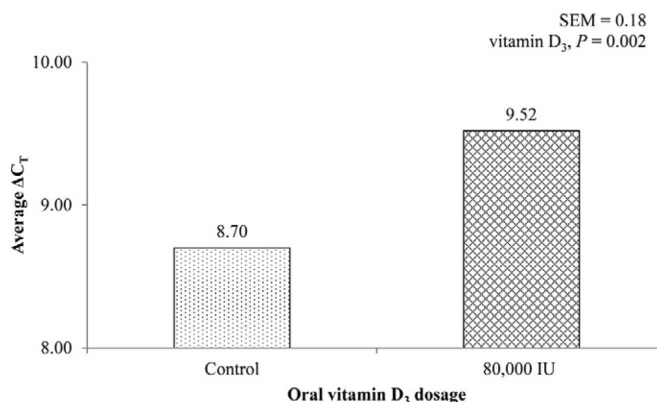

Figure 4. Effects of oral vitamin D_3 (either 0 or 80,000 IU; $n = 6$) on relative abundance of tumor necrosis factor (TNFα) in lymphatic tissue sampled on d 19, Exp. 1. ΔC_T = cycles to threshold.

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Table 4. Effects of supplemental vitamin D_3 by an oral dose or in early nursery diets on preweaning and nursery growth performance, serum 25-hydroxycholecalciferol [25(OH)D_3], and porcine circo virus antibody titers, Exp. 2[1]

Oral dosage[2]	None		40,000 IU D_3			Probability, $P <$		
Dietary D_3,[3] IU/kg:	1,378	13,780	1,378	13,780	SEM	Dose × diet interaction	Dosage	Diet
Preweaning[4]								
Weight gain, kg	3.21		3.30		0.07		0.17	
Weaning BW, kg	5.18		5.26		0.07		0.17	
Nursery[5]								
d 21 to 45								
ADG, g	311	306	305	308	8	0.59	0.83	0.92
ADFI, g	386	378	380	388	9	0.28	0.83	0.99
G:F	0.80	0.81	0.80	0.79	0.01	0.65	0.57	0.84
25(OH)D_3,[6,7] ng/mL								
d 21	7.8	7.9	26.8	21.6	2.6	0.30	0.01	0.32
d 31	21.3	33.5	28.6	35.6	2.6	0.33	0.08	0.01
d 45	10.1	14.3	15.6	13.7	2.6	0.25	0.35	0.66
PCV2 antibody titer,[8,9] log								
d 21 (weaning)	6.6	7.6	6.6	6.6	0.4	0.16	0.14	0.21
d 64 (5 wk postvaccination)	8.4	9.4	7.5	8.2	1.0	0.84	0.23	0.35
Change	1.8	1.8	0.9	1.6	1.1	0.74	0.59	0.70

[1]A total of 400 barrows from 80 litters (PIC 1050; initially 7 d of age) were used in a 45-d study to determine the effects of supplementing vitamin D_3 in a single oral dose before weaning or in early nursery diets on preweaning and nursery growth and 25(OH)D_3. Pig was the experimental unit for preweaning growth, serum 25(OH)D_3 concentrations and PCV2 antibody titers. Pen was the experimental unit for nursery growth.

[2]Oral dosage treatments were administered at 7 d of age.

[3]Dietary vitamin D_3 concentrations were fed in phase 1 diets (d 21 to 31) and then pigs were fed common diets containing 1,378 IU/kg vitamin D_3 from d 31 to 45.

[4]Initial BW (d 7) was used as a covariate and sow was included as a random effect in the statistical model for preweaning growth. Data were analyzed using performance records from pigs that survived until weaning (398 total; 200 and 198 for the none and 40,000 IU vitamin D_3 oral dosage treatments, respectively.

[5]At weaning (d 21), a subsample of 300 barrows were used in the 24-d nursery portion of the exp. There were 5 pigs per pen and 15 replications per treatment.

[6]Twelve pigs/treatment were bled on d 21 (weaning), 31, and 45 to determine serum 25(OH)D_3 concentrations.

[7]Dose × diet × day interaction, $P = 0.99$; day main effect, $P < 0.01$.

[8]PCV2 = porcine circovirus type 2. Serum collected on d 21 (weaning) and 5 wk postvaccination was sent to the K-State Veterinary Diagnostic Laboratory for indirect fluorescent antibody assay (IFA).

[9]Endpoint antibody titers determined by IFA were log 2 transformed.

dietary vitamin D_3 at concentrations similar to the dietary requirement of nursery pigs (100 to 250 IU/kg of the diet), but no research has looked at supplementing vitamin D_3 in alternative forms as discussed in the current studies or at levels above those typically supplemented in commercial diets. Comparing results in the present experiments to those previously discussed is challenging due to the difference in concentration of vitamin D_3 supplementation.

In the present experiments, preweaning and nursery growth performance were not influenced by supplementing vitamin D_3 above the normal industry inclusion rates. Although numerical differences were observed in weaning weights of pigs dosed with vitamin D_3, no statistical differences were found; therefore, it appears additional supplementation may not be a significant factor in preweaning growth. Rortvedt and Crenshaw (2012) found that growth performance was decreased in nursery pigs that were fed diets with no supplemental vitamin D and marginal Ca and P concentrations after sows were fed maternal diets without supplemental vitamin D_3. The authors did not report any observed decreases in preweaning performance, which may suggest that vitamin D is not a significant factor in Ca and P homeostasis in the neonatal pig; however, when the authors fed marginal Ca and P levels in the nursery diets (80% of NRC [1998] requirement) of the same pigs, they observed decreased performance. When Ca and P were supplemented above the animal's requirement (120% of NRC [1998] requirement), they were able to retain normal growth performance. Wahlstrom and Stolte (1958) supplemented pigs with 90 IU of vitamin D_2/kg of the diet with adequate dietary Ca and P and observed no improvement in growth performance. Combs et al. (1966) also observed that supplementation of vitamin D_2 at 220 or 880 IU/kg of the diet did not influence growth performance. Johnson and Palmer (1939) and Bethke et al. (1946) observed increased growth performance of pigs supplemented with vitamin D_2. Both of these studies, however, were preceded with vitamin D depletion periods before the vitamin supplementation, and Ca and P concentrations were marginal in the diets, which caused clinical symptoms of rickets and Ca tetany that were

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Table 5. Effects of water supplemented vitamin D_3 on nursery growth performance and 25-hydroxycholecalciferol [25(OH)D_3], Exp. 3[1,2]

	Water supplemented D_3,[3,4,5] IU/L			
	None	16,516	SEM	Probability, P <
d 0 to 30				
ADG, kg	470	460	5	0.15
ADFI, kg	586	577	7	0.31
G:F	0.80	0.80	0.003	0.28
Serum 25(OH)D_3,[6,7] ng/mL				
d 0	11.6	16.0	2.8	0.27
d 10	27.4	90.2	2.8	0.01
d 20	17.8	47.7	2.8	0.01
d 30	21.0	32.6	2.8	0.01

[1]A total of 864 pigs (PIC TR4 × FAST ADN; initially 21 d of age) were used in a 30-d nursery study to determine the effects of water supplementation of vitamin D_3 on growth performance. Pen was the experimental unit for nursery growth, and pig was the experimental unit for serum 25(OH)D_3 concentrations.

[2]Common diets formulated to contain 2,200 IU/kg of vitamin D_3 were provided throughout the trial.

[3]Hi-D 2X (Alpharma LLC, Eagle Grove, IA) was included in water source to achieve the desired experimental vitamin D_3 supplementation concentrations.

[4]Experimental water treatments were administered from d 0 to 10; from d 10 to 30, pigs were provided a control water source with no supplemental vitamin D_3.

[5]There were 24 pigs per pen and 18 replications per treatment.

[6]A total of 12 pigs/treatment were bled via jugular venipuncture to determine serum 25(OH)D_3 concentrations.

[7]Day × treatment interaction, $P < 0.01$; day main effect, $P < 0.01$.

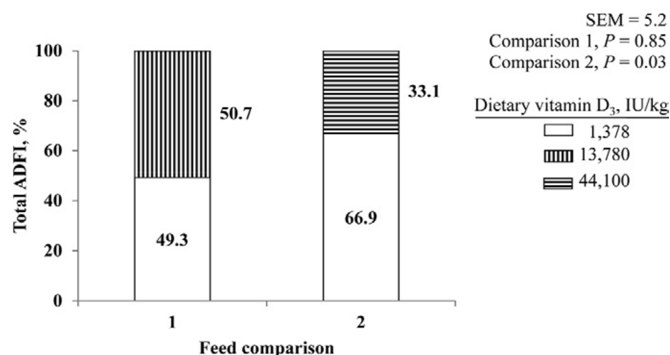

Figure 5. Effects of formulated vitamin D_3 concentration on feed preference Exp. 4. There were 6 mixed sex pigs per pen and 6 replications per feed comparison.

avoided due to the vitamin D supplementation treatments. Ultimately, conclusions from previous research have suggested that dietary supplementation of vitamin D above the animal's requirement will not affect growth performance unless the animal is deficient in the vitamin or in Ca and P. The present studies suggest that supplementation of vitamin D_3 at levels above commercially formulated dietary concentrations does not affect growth performance regardless of the form of supplementation.

Bone ash data collected from second ribs and femurs of pigs in Exp. 1 showed no change in bone mineralization of pigs dosed with vitamin D_3 compared with control pigs. Overall, bone ash percentages determined in Exp. 1 as a percentage of dry fat-free bone were lower than typical reference values (58 to 62%; Salas, 2011). Our finding of the lower-than-normal bone ash percentages determined in this study may be a function of pig age when euthanized (19 d and 35 d of age). Previous work conducted by Crenshaw et al. (1981) found increased bone ash content with increased age from pigs at 2, 4, 6, and 8 mo of age. Additionally, the bones collected in Exp. 1 were placed in petroleum ether as whole bones and were not split, which may have not allowed for complete fat extraction of internal organic matter associated with the medullary cavity. As a result, the bone ash percentages were intermediate compared with those typically referenced for wet bone and dry fat-free bone. A statistical tendency was observed for bone ash percentage to decrease in second ribs of pigs dosed with increasing vitamin D_3. This may coincide with increased 1,25(OH)$_2D_3$ activity increasing osteoclastic mobilization of Ca to resupply blood calcium concentrations; however, this process is tightly regulated, and because 1,25(OH)$_2D_3$ activity within bones or mature osteoclast cell numbers was not determined, this is not a definitive conclusion. More research quantifying the impact of vitamin D_3 supplementation on 1,25(OH)$_2D_3$ concentrations and interactions with bone Ca mobilization may help clarify this relationship. Similar to growth performance, bone ash as an indicator of bone mineralization has proven to be affected only when dietary Ca and P are limiting or when vitamin D has been deficient in the animal (Rortvedt and Crenshaw, 2012).

Bone histology conducted on the tibias and fourth ribs collected from pigs euthanized in Exp. 1 were considered consistent with normal bone development because microscopic evaluation showed a normal progression of chondrocytes through their maturation zones and abrupt transitions from cartilage tissue to mineralized bone tissue. Dittmer and Thompson (2010) reviewed the role of vitamin D and rickets in domestic animals and described distinct histological differences in animals with clinical signs of rickets. These differences include cartilage plugs extending in the metaphysis and thickening of the physis. Overall, the bone histological examinations conducted in Exp. 1 concluded that no significant differences were observed due to the supplementation of vitamin D_3 in an oral dose.

The role of vitamin D in immunity is a topic of growing interest, especially in human health research. For innate immunity, human research conducted by Liu et al. (2006), showed that toll-like receptors (**TLR**) can be stimulated by an antimicrobial peptide in macrophages resulting in an increased expression of the cytochrome P450 enzyme (CYP27B1) responsible for conversion of 25(OH)D to 1,25(OH)$_2$D. If enough 25(OH)D substrate is available when TLR stimulate CYP27B1, then 1,25(OH)$_2$D can stimulate the expression of cathelicidin,

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a potent antimicrobial peptide, within the macrophage. In a review on acquired immunity, Bikle (2009) discussed evidence that vitamin D inhibited maturation of dendritic cells and promoted the TH2 type immune responses with a shift from TH1 type responses. These qualities may be beneficial for those suffering from autoimmune diseases such as inflammatory arthritis, inflammatory bowel disease, and experimental allergic encephalitis (**EAE**; a model for multiple sclerosis) but may be detrimental to immune defense against specific infectious agents.

In Exp. 1, a reduction in TNFα mRNA abundance was observed in lymphatic tissue of pigs dosed with 80,000 IU of vitamin D_3 compared with control pigs. These results agree with the work of Cantorna et al. (1998), who suggested that vitamin D_3 promotes anti-inflammatory cytokine gene expression, but more research is needed to verify this initial finding and whether these differences would have any biological significance. In Exp. 2, acquired immunity was assessed by measurements of PCV2 antibody titers after vaccination with a single 1 mL dose. No differences were detected among vitamin D_3 supplemented groups. A majority of the research conducted in an attempt to quantify the role of vitamin D in immunity has been conducted in mice. The initial data from the current studies suggest similar results may be true for swine; however, the work done in the current studies was performed in an attempt to quantify the cytokine mRNA abundance and PCV2 antibody titer. More research using controlled disease and infectious challenge models need to be conducted to draw truly valid conclusions.

For vitamin D, the most widely used biomarker of exposure in humans is circulating 25(OH)D concentrations (IOM, 2011). This is because circulating 25(OH)D has the longest serum half-life of 10 d to 3 wk. However, serum 25(OH)D has yet to be clearly established as a biomarker of effect, this is due to the tight metabolic regulation of 1,25$(OH)_2$D (calcitriol), the active form of vitamin D that elicits effects within the body.

In the current experiments, half-life of circulating 25$(OH)D_3$ concentrations appeared to be approximately 10 d for pigs dosed with supplemental vitamin D_3, which agrees with previous research conducted in humans. The determination of “adequate” circulating concentrations of 25(OH)D in humans has been debated due to a lack of information available on the level needed for optimal calcium metabolism and peak bone mass. Health has been introduced into this discussion due to observational studies that describe the relationship of low serum 25(OH)D concentrations in individuals who have tuberculosis (Nnoaham and Clarke, 2008), but no work has defined whether vitamin D plays a distinct role in reducing the risk of the disease. Similar debates have occurred in swine research due to increased incidence of metabolic bone disease in production systems.

The normal range of circulating 25(OH)D concentrations defined in human nutrition is the mean serum 25(OH) D concentration ± 2 SD from a group of healthy individuals in human recommendations (IOM, 1997). Specker et al. (1992) concluded that circulating 25(OH)D concentrations below 11 ng/mL are consistent with vitamin D deficiency in human infants and neonates. Additionally, Salas (2011) described normal serum 25$(OH)D_3$ concentrations in neonatal swine to range from 5 to 15 ng/mL. Circulating 25(OH)D concentrations appear to be similar for swine and humans, but an adequate level may be more closely defined in human research due to studies that have observed elevations in alkaline phosphatase and parathyroid hormone (**PTH**) concentrations to be associated with low serum 25(OH)D (Demay, 1995); to our knowledge, this type of comparison has not been performed in swine. In the current experiments, serum 25$(OH)D_3$ concentrations of control pigs were slightly lower than the range previously described for neonatal pigs (3.6 ± 1.15 ng/mL for Exp. 1). Based on the definition of normal range to be the mean ± 2 SD, the value would fall into the previously described range for young swine, but the previously mentioned reference values did not describe the recommended range of 25$(OH)D_3$ for nursery pigs. Because no pigs in the current set of studies exhibited clinical symptoms associated with metabolic bone disease or rickets, our results suggest that circulating 25$(OH)D_3$ concentrations in pigs were adequate to maintain calcium homeostasis and ideal bone development. Serum Ca and P concentrations determined in Exp. 1 were within previously described normal ranges (Friendship et al., 1984) of weanling pigs. Based on this information, even though vitamin D_3 supplementation level significantly influenced serum Ca on d 20 and 52, all serum Ca and P values obtained throughout the study were at elevated concentrations well above reference values associated with deficiency. Correlations of circulating 25$(OH)D_3$ concentrations to serum Ca and P suggest that no correlations exist when vitamin D_3 is supplemented at levels above typically formulated concentrations.

Interestingly, in Exp. 4, the inclusion of vitamin D_3 at 44,100 IU/kg of the diet reduced the intake preference of the particular diet. Previous recommendations by NRC (1987) established maximum vitamin D_3 concentrations for pigs at 33,000 IU/kg of diet if they were fed for less than 60 d and at 2,200 IU/kg if fed for more than 60 d. The concentration fed in Exp. 4 was 44,100 IU/kg of the complete diet, thus possibly explaining the reduced percentage of overall feed intake for that diet. A study conducted by Quarterman et al. (1964) concluded that daily supplementation of 250,000 IU vitamin D_3 for 4 wk reduced feed intake and growth rate, and calcification was observed in several soft tissues after necropsy. This phenomenon of soft organ calcification was previously described by Holmes and Kummerow (1983) as a result of increased Ca retention in the body.

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In conclusion, the supplementation of vitamin D_3 at levels above those typically used in commercial diets did not influence growth performance, bone mineralization, serum Ca and P, or bone histology. Supplementation of vitamin D_3 increased the circulating concentration of 25(OH)D_3. Additionally, at concentrations greater than 200 times the requirement (200 IU/kg; NRC, 2012) vitamin D_3 affects feed preference. Therefore, future research is needed to determine optimal serum 25(OH)D_3 levels for proper bone development and ideal Ca and P absorption in pigs. Research to examine the potential effects of vitamin D on the immune system or other novel biological processes in pigs may offer additional insights into the potential benefits of vitamin D supplements for pigs.

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