THE EFFECTS OF VARIOUS SOURCES AND LEVELS OF SUPPLEMENTAL VITAMIN D_3 ON GROWTH PERFORMANCE AND SERUM 25(OH)D_3 OF YOUNG PIGS

by

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B.A., Texas Tech University, 2010

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

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Abstract

Seven experiments using a total of 3,251 preweaned pigs, nursery pigs, and sows were used to determine the effects of: 1) supplemental vitamin D_3 on suckling and nursery pig growth, and maternal performance, and 2) high sulfate water, dietary zeolite and humic substance on nursery pig performance. Also, a web-based survey was developed to question pork producers and advisors of the swine industry on their knowledge of feed efficiency. Experiment 1 tested an oral dose of either; none, 40,000 or 80,000 IU vitamin D_3 given to pigs 24 to 48 h after farrowing. No differences in growth performance or bone mineralization were observed, but vitamin D_3 supplementation increased serum 25(OH)D_3 on d 10, 20, and 30, but returned to control values by d 52. Experiments 2 and 3 evaluated an oral dose of vitamin D_3 to pigs just before weaning, as well as added D_3 in nursery diets and in drinking water. There were no effects on growth performance; however, serum 25(OH)D_3 increased with all sources of vitamin D_3 supplementation. Experiment 4 evaluated if pigs had a preference to 1 of 3 dietary concentrations of vitamin D_3. Pigs ate less feed from diets containing very high levels of vitamin D_3 compared to commonly supplemented levels. Experiment 5 evaluated 3 levels of vitamin D_3 in sow diets. There were no effects on sow productivity, subsequent pig performance, or piglet bone ash content. However, increasing vitamin D_3 increased sow serum 25(OH)D_3, milk vitamin D, and pig serum 25(OH)D_3. Experiment 6 and 7 evaluated the effects of dietary zeolite and humic substances in nursery pigs drinking high sulfate water. Ultimately, pigs drinking high sulfate water had increased fecal moisture content and decreased growth performance, and feed additives evaluated were ineffective in ameliorating these negative effects. Finally, data collected from the feed efficiency survey suggest that there are knowledge gaps about practices that effect feed efficiency. Results from this survey will help extension educators better target specific industry segments with current information and provide more specific areas of future research where lack of information has been identified.

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Chapter 1 - The effects of supplemental vitamin D_3 from various sources on serum 25(OH)D_3 and growth performance of pre-weaning and nursery pigs

Abstract

Four experiments were conducted to investigate the effects of varying concentrations and sources of vitamin D_3 on pig performance, feed preference, serum 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 ($P > 0.10$) were observed in ADG prior to weaning, or for nursery ADG, ADFI, or G:F. Vitamin D_3 level had no effect on bone ash concentration or bone histological evaluation on d 19 or 35. In Exp. 2, 400 barrows (initially 7 d of age) were used in a 2 × 2 factorial 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/kg vitamin D_3 in nursery diets from d 21 to 31 of age) in a 45 d trial. Prior to weaning (7 to 21 d of age), oral vitamin D_3 dose did not influence ($P > 0.10$) 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 level 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 early nursery vitamin D_3 influenced ($P > 0.10$) nursery ADG, ADFI, or G:F. In Exp. 3, 864 pigs (initially 21 d of age) were allotted to 1 of 2 water vitamin D_3 treatments (none or 1,056,700 IU/liter vitamin D_3 from d 0 to 10) in a 30 d study. Providing vitamin D_3 in the water increased serum 25(OH)D_3 concentrations on d 10, 20, and 30; however, water vitamin D_3 did not influence ($P > 0.10$) overall (d 0 to 30) ADG, ADFI, or G:F. In Exp. 4, 72 pigs were used in 2 feed preference studies. Pigs did not differentiate diets containing either 1,378 or 13,780 IU/kg vitamin D_3, but consumed less ($P < 0.01$) of a diet containing 44,100 IU vitamin D_3 compared to the diet containing 1,378 IU vitamin D_3. 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 it did not influence growth performance or bone

mineralization. Also, feeding extremely high levels of vitamin D_3 may reduce feed intake of nursery pigs.

Keywords: nursery pigs, vitamin D, vitamin D_3, 25(OH)D_3

Introduction

Vitamin D is a group of fat-soluble secosteriods. The two major physiologically relevant forms 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, 1982). 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 vitamin D_2 and vitamin D_3 are hydroxylated in the liver to the 25-hydroxy forms (25(OH)D_2 and 25(OH)D_3). This metabolite of vitamin D is the main circulating form in the blood and acts as a clinically useful marker for vitamin D status. 25(OH)D_3 is then hydroxylated again in the renal tubules of the kidney to 1,25$(OH)_2D_3$ by the 25(OH)D 1α-hydroxylase enzyme or to 24,25$(OH)_2D_3$ by the 24α-hydroxylase enzyme. The 1,25$(OH)_2D_3$ metabolite is important in the regulation of Ca and P absorption across the intestinal wall by acting on mucosal cells of the small intestine to form calcium-binding proteins. These proteins facilitate Ca and Mg absorption and influence P absorption. Together with a parathyroid hormone and calcitonin, they maintain a Ca and P homeostasis in the body (Dittmer and Thompson, 2011).

Recently, anecdotal reports of vitamin D being excluded from diet premixes (Feedstuffs, 2010), and more speculation of the role of vitamin D in metabolic bone disease (Madsen, 2011) have sparked more research needs. There is currently a lack of research examining different methods of delivering vitamin D_3 to the nursery pig and almost no research has been conducted with supplementation of vitamin D_3 to the pre-weaned pig. Therefore, our objectives were to determine if supplementation of vitamin D_3, above typically used dietary levels in the U.S. swine industry, impacts growth performance, 25(OH)D_3 concentrations, bone mineralization, feed preference, and immune function of pre-weaned 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, 1998).

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 pre-weaning 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 performance, serum 25(OH)D_3 concentrations, and bone mineralization of pre- and post-weaning pigs.

Shortly after farrowing, pigs were allotted to 1 of 3 oral vitamin D_3 treatments which included: 1) a control treatment of 1 mL of a peanut-oil and ethanol based liquid carrier without 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 RCBD with litter, and matched set within litter, functioning as the 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 numbers of matched sets per litter were 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. Pigs were weighed again on d 10, 18, and 20 to determine pre-weaning growth performance. 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 lysine in gestation and lactation diets, respectively. The farrowing barn contained 29 farrowing crates (2.13 × 0.46 m for the sow and 2.13 × 0.48 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 toxicity symptoms associated with vitamin D and no clinical signs were observed.

On d 20, the remaining 234 pigs (pigs who survived to weaning) were weaned into the nursery facility and penned by treatment. The nursery barn had 34 pens (1.22 × 1.52 m) with woven wired flooring and 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 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 1-1). The phase 1 diets (SEW and transition 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, one matched set which was closest to the mean pig weight at time of allottment 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 were chosen to reduce random effects of litter, or sex and 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 were euthanized with an intravenous

overdose of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). On d 19, both femurs and 2nd ribs were collected to determine bone ash content, and the 4th 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 ca. 10 volumes of TRIzol® reagent. Samples were then kept frozen at -86° C prior to being analyzed for relative abundance of the specific mRNA sequence associated with the inflammatory cytokine tumor necrosis factor (TNFα) (Guilietti, 2001). On d 35, 12 pigs (6 from the control treatment and 6 from the 80,000 IU vitamin D_3 treatment) were selected for necropsy and bones were sampled similarly to procedures performed on d 19.

Collection and preparation of bones for histological examination

After euthanasia both fourth ribs and a tibia were collected from each pig. The muscles and connective tissues were cleaned from bones' surfaces and the tibias were split longitudinally with a hacksaw. The blood was washed from the cut surfaces and the tibias examined. All bones were 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 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 class slides, and stained with hematoxylin and eosin. All bone samples were collected and examined by a board certified pathologist who was unaware of treatment vitamin D status.

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 left in the ether for 7 d at room temperature (25°C), and then were 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.

RNA processing

Each TRIzol® homogenate was thawed at room temperature and 500 ul was placed in a clean microfuge tube, mixed thoroughly with 100 ul chloroform for 15 sec and then 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 2M NaCl/2 mM EDTA (pH 4.0) was included. Ribonucleic acid was eluted in 50 μL of water and the concentration 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. Afterwards, samples were diluted to 100 μL final volume with TE buffer and stored at -20°C prior to PCR analysis.

Quantitative real-time-PCR (qRT-PCR)

Quantitative real-time-PCR was performed using a Stratagene Mx3005p cycler (Stratagene, La Jolla, CA) and PerfeCTa SYBR Green FastMix, low ROX 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; pTNF□-For, 5'-GCAGGAGCCACCACGCTCTT; pTNF□-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 target gene expression was estimated using the ΔC_t method normalized relative to GAPDH expression 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 high dietary concentrations on pig growth performance and serum 25(OH)D_3. The hypothesis was that vitamin D_3 supplementation may have a larger impact on pre-weaning performance of pigs suckled in a commercial production facility.

On d 7 after birth, 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 RCBD. Barrows were weighed on d 7 and at weaning (d 21) to determine pre-weaning growth. The study used litters from 3 farrowing rooms which contained 39 stalls (1.69 × 0.49 m for the sow and 1.69 × 1.25 m for the pigs), and 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 × 1.52 m) with metal slatted floors, 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 levels of dietary vitamin D_3 (1,378 or 13,780 IU/kg vitamin D_3) in early nursery diets (d 21 to 31) on pig performance and serum 25(OH)D_3. Barrows were subsampled in order to reduce the number of light weight non-viable 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 then 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 were the vitamin D_3 levels (Table 1-1). 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 with the pigs within those pens being selected that 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. For the Mycoplasma hyopneumoniae (M. Hyo) vaccine, Respisure (Pfizer Animal Health, New York, NY), a 2 –dose product was used. Serum samples collected at weaning (d 21) and d 64 were analyzed for PCV antibody titers to distinguish potential effects of supplemental vitamin D_3 on acquired immunity.

PCV antibody titer analysis

Serum was analyzed at the Kansas State University Veterinary Diagnostic Laboratory using an indirect fluorescent assay (IFA). Titration endpoints were calculated as the reciprocal of the last serum dilution that gave a positive fluorescence result. Prior to 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 water supplementation of vitamin D_3 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 vitamin D_3 treatments (none [control] or 1,056,700 IU vitamin D_3/L; Hi-D 2X, Alpharma LLC., Eagle Grove, IA). All pens (1.75 × 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. Water treatments 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 a pelleted form. The phase 2 (2,200 vitamin D_3/kg, 0.98% Ca and 0.59% available P) and 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 a 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 were bled on weigh days to determine serum 25(OH)D_3 concentrations.

Experiment 4

Two 14-d 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 levels 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 corn-soybean meal based

diets containing 10% whey and 4.5% fish meal (Table 1-1). The first preference comparison was between diets containing 1,378 (control) or 13,780 IU/kg vitamin D_3, and the second comparison was between diets containing 1,378 (control) and 44,100 IU/kg vitamin D_3, the levels selected were to represent feeding concentrations approximately 6, 60, and 200 × requirement (NRC, 2012). All pens (1.22 × 1.52 m) contained two 3-hole dry self-feeder 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 switched in pen location to discourage any location bias by the pig. Total pen feed intake was calculated, and intake of each diet for both comparisons was expressed as a percentage of total intake.

Serum 25(OH)D_3, Ca, and P 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 for use in determining circulating 25(OH)D_3 serum concentrations. Six h after collection, blood was centrifuged (1,600 × g, 25 min at 2°C), serum was harvested and stored at -20°C until analysis. Serum 25(OH)D_3 concentrations were determine 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. Calcium and P analyses for Exp. 1 was conducted at Iowa State University College of Veterinary Medicine (Ames, IA) by using spectrophotometry with commercial kits (Pointe Scientific Inc., Canton, MI) using methods described by Pointe Scientific (2009a; 2009b).

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 were also 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 Institute, Inc., Cary, NC) and treatment means were analyzed using the LSMEANS statement. All serum 25(OH)D_3 analysis and PCV antibody titer analysis from Exp. 2 were conducted using the REPEATED function of SAS to determine treatment main effects over time and the treatment × time interactions. Data from Exp. 1 were analyzed as a RCBD with litter and matched set within litter acting as the blocking factors. Individual pig was the experimental unit for pre-weaning growth performance, serum 25(OH)D_3, bone ash determination, and TNFα relative abundance. Pen was the experimental unit for nursery growth performance. Pre-planned 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 RCBD with the main effects of oral dosage and diet treatment and dosage × diet interactions. Individual pig was the experimental unit for pre-weaning growth performance, serum 25(OH)D_3, and PCV antibody titers. For pre-weaning 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 RCBD 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 CRD 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 a trend at $P \leq 0.10$.

Results

Experiment 1

Prior to weaning (d 0 to 20), no significant differences were observed ($P > 0.10$) for ADG (Table 1-2). During the nursery phase (d 20 to 52), oral vitamin D_3 dosage did not affect ($P > 0.10$) ADG, ADFI, or G:F (Table 1-3). Prior to vitamin D_3 supplementation, initial serum 25(OH)D_3 concentrations were similar ($P = 0.99$) among all pigs (Table 1-4; Figure 1-1). 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, $P < 0.01$), 20 (quadratic, $P < 0.01$) and 30 (linear, $P < 0.01$) compared to control pigs. On d 52, serum 25(OH)D_3 concentrations were similar ($P = 0.36$) regardless of oral vitamin D_3 supplementation. Supplementation of vitamin D_3 did not influence ($P > 0.10$) serum Ca concentrations on the initial d of collection, d 10, or d 30. However, significant 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. Circulating P was not influenced ($P > 0.10$) by supplementation of vitamin D_3 in an oral dose. Correlation analysis showed that serum 25(OH)D_3 is 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 ($P > 0.10$) of vitamin D_3 dosage (Table 1-4), but 2nd rib ash content tended (linear, $P < 0.09$) to decrease as oral vitamin D_3 dosage increased. No differences ($P > 0.10$) were found in bone ash content of femurs or 2nd ribs collected on d 35.

No differences could be discerned in the hardness of ribs or tibias between individual pigs. There were no macroscopically visible differences in the growth plates of either the tibias (Figure 1-2) 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 (Figure 1-3). 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 normal progression from cartilage to bone formation and mineralization. Finally, mesenteric lymph node relative abundance of the inflammatory cytokine TNF-α, was ($P < 0.01$; Figure 1-4) lower for pigs dosed with 80,000 IU of vitamin D_3 compared to 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. Experimental diets analyzed with vitamin D_3 mean concentrations of 1,267 and 10,347 IU/kg for those 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 ($P > 0.10$) pre-weaning growth performance or BW at weaning (d 21), but BW at weaning was numerically heavier for pigs dosed with 40,000 IU of vitamin D_3 compared

to control pigs (5.26 vs. 5.18 kg; Table 1-5). During the nursery phase (d 21 to 45), neither previously administered oral vitamin D_3 dose nor dietary level of vitamin D_3 in early nursery diets affected ($P > 0.10$) ADG, ADFI, or G:F. No dose × diet interactions were observed for any response criteria, except for a tendency ($P < 0.09$) for G:F from d 21 to 31. Here, G:F was worsened with increasing dietary vitamin D_3 for pigs initially dosed on d 7 with 40,000 IU vitamin D_3, but for pigs not orally dosed with vitamin D_3, G:F was improved with increasing dietary vitamin D_3.

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 prior to weaning. Also on d 31, increased serum 25(OH)D_3 concentrations were observed ($P < 0.01$) in pigs fed increased levels of vitamin D_3. But, by d 45 serum 25(OH)D_3 concentrations were similar ($P > 0.10$) regardless of oral vitamin D_3 dosage prior to weaning or early nursery dietary vitamin D_3 concentration. Also, PCV antibody titer results showed no influence ($P > 0.10$) 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 ($P > 0.10$) overall ADG, ADFI, or G:F, but during the first phase (d 0 to 10) G:F improved ($P < 0.05$) in pigs supplemented with 1,056,700 IU vitamin D_3/L (Table 1-6). From d 10 to 30, ADG decreased ($P < 0.03$) and G:F worsened ($P < 0.05$) for pigs supplemented 1,056,700 IU vitamin D_3/L during the first phase.

For serum 25(OH)D_3 concentrations, supplementing 1,056,700 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 acceptable analytical error of formulated dietary values. Experimental diets analyzed with mean vitamin D_3 concentrations of 1,711, 15,554, and 49,604 IU/kg for those 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 ($P > 0.10$) were observed between diets containing 1,378 or 13,780

IU/kg of vitamin D_3 throughout the length of the study (Table 1-7). Conversely, when pigs were offered a choice between diets containing either 1,378 IU or 44,100 IU/kg vitamin D_3, pigs consumed a greater portion ($P < 0.03$) of the diet containing 1,378 IU of vitamin D_3.

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 6 to 9 times these levels. Previous research has extensively evaluated the supplementation of dietary vitamin D_3 at levels similar to the dietary requirement of nursery pigs, 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. Therefore it's difficult to compare results in the present experiments to those previously discussed due to the difference in level of vitamin D_3 supplementation.

In the present experiments pre-weaning 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 and thus it appears additional supplementation may not be a significant factor in pre-weaning growth. Rohrvedt and Crenshaw (2012) found that growth performance was decreased in nursery pigs that were deficient in vitamin D due to the absence of vitamin D_3 in maternal diets. The authors did not report any observed decreases in pre-weaning 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 requirement) of the same pigs, they observed decreased performance. But when Ca and P were supplemented above the animal's requirement (120% of NRC 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 and adequate Ca and P, and observed no improvement in growth performance. Also, Combs et al. (1966), 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 (1946) observed increased growth performance of pigs supplemented with vitamin D_2. However, both of these studies were

preceded with vitamin D depletion periods prior to the vitamin supplementation and Ca and P levels were marginal in the test diets, which caused clinical symptoms of rickets and Ca tetany that were 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 impact 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 impact growth performance regardless of the form of supplementation.

Interestingly, in Exp. 4 the inclusion of extremely high levels of vitamin D_3 (44,100 IU/kg) 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 longer periods of time. The level fed in Exp. 4 was 44,100 IU/kg of the complete diet, thus possibly explaining the reduced feed intake of the 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, growth rate, and after necropsy, calcification was observed in the heart, lungs and aorta. This phenomenon of soft organ calcification has been previously described by Holmes and Kummerow (1983) as a result of increased Ca retention in the body. An experiment conducted by Long (1984) resulted in the death of pigs after supplementation of vitamin D_3 at a dietary level of 473,000 IU/d for 4 d.

Bone ash data collected from 2nd 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. There was actually a statistical tendency for bone ash percentage to decrease in 2nd ribs of pigs dosed with increasing vitamin D_3. Overall, bone ash percentages determined in Exp. 1, as a percentage of dry fat-free bone, were lower (53 to 60% of reference value for ribs and 67 to 72% of reference value for femurs) 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 lipids associated with the medullary cavity. As a result, the bone ash percentages were intermediate to

those typically referenced for wet bone and dry fat-free bone. As far as the statistical tendency for a decrease in bone ash of ribs sampled on d 19 with increased vitamin D_3 supplementation, it 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 were not determined this is not a definitive conclusion. More research quantifying the impact of vitamin D_3 supplementation on $1,25(OH)D_3$ concentrations and interactions with bone Ca mobilization may help clarify this relationship. Similarly to growth performance, bone ash as an indicator of bone mineralization has only proven to be affected 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 4^{th} ribs collected from pigs euthanized in Exp. 1 were considered to be consistent with normal bone development. This was because microscopic evaluation showed 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 of either 40,000 or 80,000 IU.

The role of vitamin D in immunity is a topic that has grown in interest especially in human health research. For innate immunity, human research conducted by Lui et al. (2006), showed that toll like receptors (TLRs) 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 $1,25(OH)_2D$. If enough 25(OH)D substrate is available when TLRs stimulate CYP27B1, then 1,25(OH)2D can stimulate the expression of cathelicidin within the macrophage which is a potent antimicrobial peptide.

As far as acquired immunity, research by Chen et al. (2007) has reported that $1,25(OH)_2D$ enacts an inhibitory effect by suppressing the proliferation and differentiation of B cell precursors into plasma cells. Additionally, the vitamin D active metabolite inhibits T cell proliferation, particularly the T helper (Th)-1 cell capable of producing interferon γ (INF-γ) and

interleukin-2 (IL-2) and activation of macrophages (Lemire et al., 1995). These actions prevent further antigen presentation to and recruitment of T lymphocytes (role of INF- γ) and T lymphocyte proliferation (role of IL-2). More recently, the inhibition of $1,25(OH)_2D$ on the development of Th-17 has been described similarly to the suppression of Th-1 development (Daniel et al., 2008). However, IL-4, IL-5, and IL-10 production is increased with $1,25(OH)_2D$ stimulation which demonstrates a shift to increased development of Th-2 cell phenotypes (Boonstra et al., 2001). Also, increased IL-10 production is an inhibitory factor on Th-1 cells by means of increasing Treg cell production. Overall, the shift in Th-2 and Treg cell phenotype of acquired immunity due to vitamin D would result in a suppressed acquired immunity (Bikle, 2009) which is desired for several autoimmune diseases such as inflammatory arthritis, inflammatory bowel disease, and experimental allergic encephalitis (EAE, a model for multiple sclerosis), but it may be detrimental towards immune defense against specific infectious agents.

During a disease challenge model, using EAE in mice, Cantorna et al. (1998) reported reductions in lymphocyte INF-γ and TNF-α gene expression when $1,25(OH)_2D_3$ was supplemented. The investigators concluded that the results did not differentiate between the direct effects of vitamin D on cytokine gene expression, or potential indirect effects of vitamin D as a regulator of other cells and genes that may have resulted in a net change in cytokine expression. In Exp. 1, TNF-α relative abundance was measured in the mesenteric lymph nodes of control pigs or pigs dosed with 80,000 IU vitamin D_3 on d 19. Based on qRT-PCR results, a reduction in relative abundance of TNF-α was observed in lymphatic tissue of pigs dosed with 80,000 IU of vitamin D_3, compared to control pigs. These results agree with the work and suggestion of Cantorna et al. (1998) that vitamin D_3 influences cytokine gene expression. However, more research is needed to verify this initial finding. In Exp. 2, acquired immunity was measured by way of PCV antibody titers following vaccination. The results showed no differences based on oral vitamin D_3 treatment prior to weaning or based on vitamin D_3 levels in early nursery diets. Based on previous mentioned research describing the role of $1,25(OH)_2D$ on acquired immunity, we hypothesized that a reduction in antibody titers following vaccination may result from increased vitamin D_3 supplementation due to the suppression of Th-1 and Th-17 cells. However, another experiment conducted by Cantorna et al. (2000) showed that mice and rats who received transplanted organs did not have increased chances of susceptibility to fungal or viral infection with treatment of $1,25(OH)_2D_3$. In fact, those animals also had increased bone

density compared to mice that were treated with typically used transplant antirejection drugs. 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 relative abundance of the specific gene sequence for TNFα, and PCV2 antibody titer. More research using controlled disease and infectious challenge models need to be conducted to truly draw valid conclusions.

The most widely used indicator of vitamin D status in humans is circulating 25(OH)D concentrations (IOM, 1997). This is because both vitamin D and 1,25$(OH)_2$D have short circulating half-life's. Circulating vitamin D accumulates within the liver a few hours after ingestion and can vary greatly depending on time after ingestion and sun exposure. Additionally, circulating 1,25$(OH)_2$D also has a half-life of 4 to 6 h (Kumar, 1986). Due to the tight regulation of this active metabolite, it is not believed to be a valuable marker for vitamin D deficiency, adequacy, or excess. On the other hand, 25(OH)D has a half-life of 10 d to 3 wk.

Within 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. Ultimately, the determination of "adequate" circulating concentrations of 25(OH)D has been debated greatly for human recommendations due to a lack of information available on the level needed for optimal calcium metabolism and peak bone mass. Health is another factor that has been introduced more recently in 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 has not been conducted. Similar debates in swine have also been discussed due to the increased incidences of metabolic bone disease in production systems.

The normal range of circulating 25(OH)D concentrations has been defined as the mean serum 25(OH)D concentration ± 2 SD from a group of health individuals in human recommendations (OIM, 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 PTH concentrations to be associated with low serum 25(OH)D (Demay, 1995), this type of comparison has not been assessed 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 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. However, the previously mentioned reference values did not describe a 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 it may suggest circulating 25(OH)D_3 concentrations in pigs of the current studies were adequate in maintaining calcium homeostasis and ideal bone development. Serum Ca and P were also determined from serum collected in Exp. 1. Similar to recommended circulating levels of 25(OH)D_3, Salas (2011) referenced deficient circulating Ca and P values to be < 8 and < 5 mg/dL for Ca and P, respectively. Based on this information, even though significant differences in serum Ca were observed on d 20 and 52 based on vitamin D_3 supplementation level, all serum Ca and P values obtained throughout the study were at elevated concentrations believed to be well above reference values associated with deficiency. In an attempt to correlate circulating 25(OH)D_3 concentrations to serum Ca and P. Results suggest that a correlation does not exist between 25(OH)D_3 and serum Ca and P when vitamin D_3 is supplemented at levels above those typically used in commercial diet formulation regardless of the form of supplemental vitamin D_3.

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. However supplementation of vitamin D_3 increased the circulating concentration of 25(OH)D_3. Additionally, preliminary results attempting to quantify the relative abundance of TNFα suggest that vitamin D_3 supplementation may affect immune function, but more research using established disease challenge models is needed to verify this conclusion and determine if additional supplementation above levels used in commercial diets are a beneficial practice to utilize in modern swine production systems.

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Figures and Tables

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

	Phase 1, Exp. 1[4]				
Ingredient, %	SEW	Transition	Phase 1, Exp. 2[5]	Phase 2[6]	Phase 3
Corn	36.10	38.23	39.57	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	---
DDGS[7]	---	---	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	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[8]	0.15	0.15	0.15	0.15	0.15
L-Lys HCl	0.15	0.26	0.20	0.25	0.36
DL-Met	0.15	0.18	0.13	0.13	0.13
L-Thr	0.08	0.13	0.05	0.11	0.13
L-Ile	---	---	0.10	---	---
Phytase[9]	---	---	0.13	0.17	0.17
Vit E, 20,000 IU	0.05	0.05	0.03	---	---
Choline chloride (60%)	---	---	0.02	---	---
Acidifier[10]	0.20	0.20	0.20	---	---
Vitamin D_3 premix[11]	---	---	---	---	---
Total	100	100	100	100	100
Calculated analysis					
ME, kcal/kg	3,544	3,498	3,406	3,311	3,309
CP, %	22.7	22.3	21.2	21.3	20.4
Total Lys, %	1.7	1.65	1.5	1.3	1.38
Standardized ileal digestible amino acids, %					
Lys	1.56	1.51	1.35	1.3	1.25
Ile:Lys	49	52	61	61	60

Met:Lys	30	33	29	35	33
Met & Cys:Lys	55	56	58	59	58
Thr:Lys	64	63	64	63	62
Trp:Lys	17	17	18	17	17
Ca, %	0.79	0.83	0.8	0.7	0.68
P, %	0.73	0.77	0.71	0.63	0.61
Available P, %[12]	0.68	0.68	0.63	0.47	0.42
Ca:P	1.08	1.08	1.13	1.12	1.12
Vitamins (added levels)					
Vit A, IU/kg	11,023	11,023	11,023	11,023	11,023
Vit D, IU/kg[13,14,15]	1,378	1,378	1,378	1,378	1,378
Vit E, IU/kg	44	44	44	44	44
Vit K (menadione), mg/kg	4.41	4.41	4.41	4.41	4.41
Vit B_{12}, µg/kg	38.5	38.5	38.5	38.5	38.5
Niacin, mg/kg	49.6	49.6	49.6	49.6	49.6
Pantothenic Acid, mg/kg	27.56	27.56	27.56	27.56	27.56
Riboflavin, mg/kg	8.27	8.27	8.27	8.27	8.27

[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(OH)D_3 concentrations, and bone mineralization of pre- and post-weaning 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 factorial to determine the effects of supplementing vitamin D_3 from either a single oral dose or from high 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 levels 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). SEW and transition diets were allotted at .45 and 1.36 kg/pig, respectively (1.81 kg/pig). 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), phase 1 diets were fed from d 21 to 31 of the study. Then common phase 2 diets were fed from d 31 to d 45 of the study.

[6] Pigs used in Exp. 4 were fed Common phase 2 diets, formulated to varying vitamin D_3 levels, from d0 to 14 of the study.

[7] DDGS: dried distillers grains with solubles.

[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 per kg of the complete diet.

[9] 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.

[10] KemGest, Kemin Industries Inc., Des Moines, IA.

[11] Vitamin D_3 premixes were mixed to contain 2,204,620 IU/kg of premix by blending vitamin D_3 with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.

[12] 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.
[13] Analysis of dietary vitamin D_3 was performed by DSM Nutritional Products Inc. (Parsippany, NJ). Variability associated with laboratory vitamin D_3 assays were ± 25 % for diets containing levels below 10,000 IU/kg, and ± 20% for diets formulated between 10,000 and 100,000 IU/kg.
[14]Phase 1 diet samples from Exp. 2 had analyzed vitamin D_3 concentrations of 1,267 and 10,347 for diets formulated to contain 1,378 and 13,780, respectively.
[15] Phase 2 diets from Exp. 4 had analyzed vitamin D_3 concentrations of 1,711, 15,554, and 49,604 IU/kg for diets formulated to contain 1,378, 13,780, and 44,100, respectively.

Table 1-2. Effects of oral vitamin D_3 dose on pre-weaning growth performance, Exp. 1[1,2]

		Vitamin D_3, IU			*Probability, P <*	
	Control	40,000	80,000	SEM	Linear	Quadratic
Pigs, n						
Initial[3]	90	90	90			
d 10	87	88	85			
d 18	86	86	83			
d 20[4]	79	78	77			
BW, kg						
Initial	1.71	1.70	1.71			
d 10	3.64	3.68	3.65	0.104	0.66	0.89
d 18	5.53	5.62	5.67	0.165	0.86	0.41
d 20	5.91	6.04	6.05	0.177	0.69	0.44
ADG, g						
d 0 to 10	204	208	205	8.2	0.60	0.90
d 10 to 18	236	242	251	9.5	0.86	0.14
d 18 to 20	188	205	190	11.6	0.17	0.90
d 0 to 20	216	222	223	28.1	0.69	0.42

[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(OH)D_3, and bone mineralization of pigs pre- and post-weaning.

[2] Data were analyzed using performance records from pigs which survived 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 post-farrowing. Pig days were adjusted to account for differences in trial starting d for calculating ADG from d 0 to 10.

[4] Six pigs per treatment (6 matched sets) were removed on d 19 for necropsy.

Table 1-3. Effects of oral vitamin D_3 dose on nursery growth performance, Exp. 1[1]

		Vitamin D_3, IU			*Probability, P <*	
	Control	40,000	80,000	SEM	Linear	Quadratic
d 20 to 25						
ADG, g	238	241	234	13.7	0.78	0.69
ADFI, g	232	240	233	8.3	0.96	0.29
G:F	1.02	1.00	1.00	0.037	0.61	0.78
d 25 to 39						
ADG, g	300	302	315	10.8	0.34	0.64
ADFI, g	439	443	450	13.3	0.56	0.93
G:F	0.69	0.68	0.70	0.016	0.55	0.56
d 39 to 52						
ADG, g	481	497	484	11.3	0.83	0.30
ADFI, g	761	788	772	18.5	0.65	0.33
G:F	0.63	0.63	0.63	0.011	0.69	0.93
d 20 to 52						
ADG, g	360	371	369	8.2	0.45	0.49
ADFI, g	530	551	541	12.4	0.52	0.30
G:F	0.68	0.67	0.68	0.011	0.96	0.58
BW, kg						
d 20[2]	5.92	6.07	6.06	0.132	0.31	0.50
d 25	7.24	7.28	7.23	0.165	0.92	0.68
d 39	11.53	11.55	11.7	0.270	0.57	0.80
d 52	17.80	18.02	18.03	0.354	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(OH)D_3, and bone mineralization of pigs pre- and postweaning.

[2] Mean differences in d 20 BW is a result of differences in the statistical model used for preweaning and nursery data analyses.

Table 1-4. Effects of oral vitamin D_3 dose on serum 25(OH)D_3, Ca, P, 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	1.15	0.99	0.99
d 10	14.7	57.3	68.5	1.19	0.01	0.01
d 20	8.0	28.1	35.8	1.19	0.01	0.01
d 30	10.4	17.8	22.5	1.21	0.01	0.36
d 52	13.9	15.0	15.4	1.21	0.36	0.82
Serum Ca, mg/dL						
Initial	11.9	12.0	12.1	0.15	0.40	0.69
d 10	11.0	11.0	11.3	0.16	0.19	0.63
d 20	10.4	10.5	10.8	0.16	0.05	0.72
d 30	10.2	10.1	10.2	0.16	0.93	0.56
d 52	10.4	10.1	10.7	0.16	0.11	0.02
Serum P, mg/dL						
Initial	13.5	13.2	14.0	0.29	0.27	0.14
d 10	10.8	10.9	10.9	0.30	0.70	0.91
d 20	9.5	9.8	9.8	0.30	0.48	0.66
d 30	8.0	8.1	8.1	0.31	0.77	0.83
d 52	9.3	9.5	9.5	0.31	0.59	0.71
Bone ash, %[2]						
d 19						
Femur	42.0	42.7	40.5	1.64	0.54	0.46
Rib	35.5	32.6	30.8	1.84	0.09	0.82
d 35						
Femur	39.0		39.7	0.64	0.47[3]	
Rib	31.5		33.0	1.71	0.55[3]	

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

[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.

Table 1-5. Effects of supplemental vitamin D_3 by an oral dose or in early nursery diets on preweaning and nursery growth performance, serum 25(OH)D_3, and PCV antibody titers Exp. 2[1]

						Probability, P <		
Oral dosage[2]:	None		40,000 IU D_3			Dose × diet		
Dietary D_3, IU/kg[3]:	1,375	13,750	1,375	13,750	SEM	interaction	Dosage	Diet
Preweaning[4]								
Weight gain, kg	3.21		3.30		0.066		0.17	
Weaning BW, kg	5.18		5.26		0.066		0.17	
Nursery[5]								
d 21 to 31								
ADG, g	158	163	166	151	9.7	0.15	0.80	0.46
ADFI, g	156	153	161	156	12.6	0.84	0.51	0.56
G:F	1.02	1.06	1.03	0.98	0.036	0.09	0.23	0.86
d 31 to 45								
ADG, g	421	407	405	420	10.3	0.17	0.85	0.95
ADFI, g	554	538	538	554	10.7	0.14	0.97	0.99
G:F	0.76	0.76	0.75	0.76	0.013	0.66	0.83	0.98
d 21 to 45								
ADG, g	311	306	305	308	7.6	0.59	0.83	0.92
ADFI, g	386	378	380	388	9.3	0.28	0.83	0.99
G:F	0.8	0.81	0.8	0.79	0.013	0.65	0.57	0.84
25(OH)D_3, ng/mL[6,7]								
d 21	7.8	7.9	26.8	21.6	2.59	0.30	0.01	0.32
d 31	21.3	33.5	28.6	35.6	2.59	0.33	0.08	0.01
d 45	10.1	14.3	15.6	13.7	2.59	0.25	0.35	0.66
PCV2 antibody titer, log [8,9]								
d 21 (weaning)	6.6	7.6	6.6	6.6	0.41	0.16	0.14	0.21
d 64 (5 wk post vaccination)	8.4	9.4	7.5	8.2	1.02	0.84	0.23	0.35
Change	1.8	1.8	0.9	1.6	1.13	0.74	0.59	0.70

[1] A total of 400 barrows from 80 litters (PIC 1050; intially 7 d of age) were used in a 45 d study to determine the effects of supplementing vitamin D3 in a single oral dose prior to weaning, or in early nursery diets on preweaning and nursery growth and 25(OH)D_3.

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

[3] Dietary vitamin D_3 levels were fed in phase 1 diets (d 21 to 31), then pigs were fed common diets containing 625 IU/lb 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.

[5] At weaning (d 21), a sub sample of 300 barrows were used in the 24-d nursery portion of the exp.

[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] Serum collected on d 21 (weaning) and 5 wk post-vaccination was sent to the K-State Veterinary Diagnostic Laboratory for indirect fluorescent antibody (IFA) assay.

[9] Endpoint antibody titers determined by indirect fluorescent antibody (IFA) assay were log 2 transformed.

Table 1-6. Effects of water supplemented vitamin D_3 on nursery growth performance and 25(OH)D_3, Exp. 3[1,2]

	Water supplemented D_3, IU/liter			
	None	1,056,700[3]	SEM	*Probability, P<*
d 0 to 10[4]				
ADG, kg	255	259	6.5	0.63
ADFI, kg	257	254	5.6	0.75
G:F	0.99	1.02	0.012	0.05
d 10 to 30				
ADG, kg	578	562	5.1	0.03
ADFI, kg	752	741	7.7	0.30
G:F	0.77	0.76	0.004	0.05
d 0 to 30				
ADG, kg	470	460	4.6	0.15
ADFI, kg	586	577	6.5	0.31
G:F	0.80	0.80	0.003	0.28
Serum 25(OH)D_3, ng/mL5,6				
d 0	11.6	16.0	2.79	0.27
d 10	27.4	90.2	2.79	0.01
d 20	17.8	47.7	2.79	0.01
d 30	21.0	32.6	2.79	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.

[2] Common diets formulated to contain 2,200 IU/lb 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 treatment level.

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

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

Table 1-7. Evaluation of nursery pig feed preference for diets formulated to varying levels of vitamin D_3, Exp. 4[1]

Feed comparison[2]:	1				2			
Dietary vitamin D_3, IU/kg:	1,378	13,780	SEM	*Probability, P<*	1,378	44,100	SEM	*Probability, P<*
Feed intake, %								
d 0 to 7	54.5	45.5	4.2	0.14	77.7	22.3	4.20	0.01
d 7 to 14	46.4	53.6	6.7	0.46	61.4	38.6	6.74	0.03
d 0 to 14	49.3	50.7	5.2	0.85	66.9	33.1	5.20	0.01

[1] A total of 72 pigs (PIC 327 × 1050; initially 28 d of age) were used in a 14-d feed comparison to evaluate nursery pig preference to diets containing varying levels of vitamin D_3.
[2] There were 6 pigs/pen and 6 pens/feed comparison.

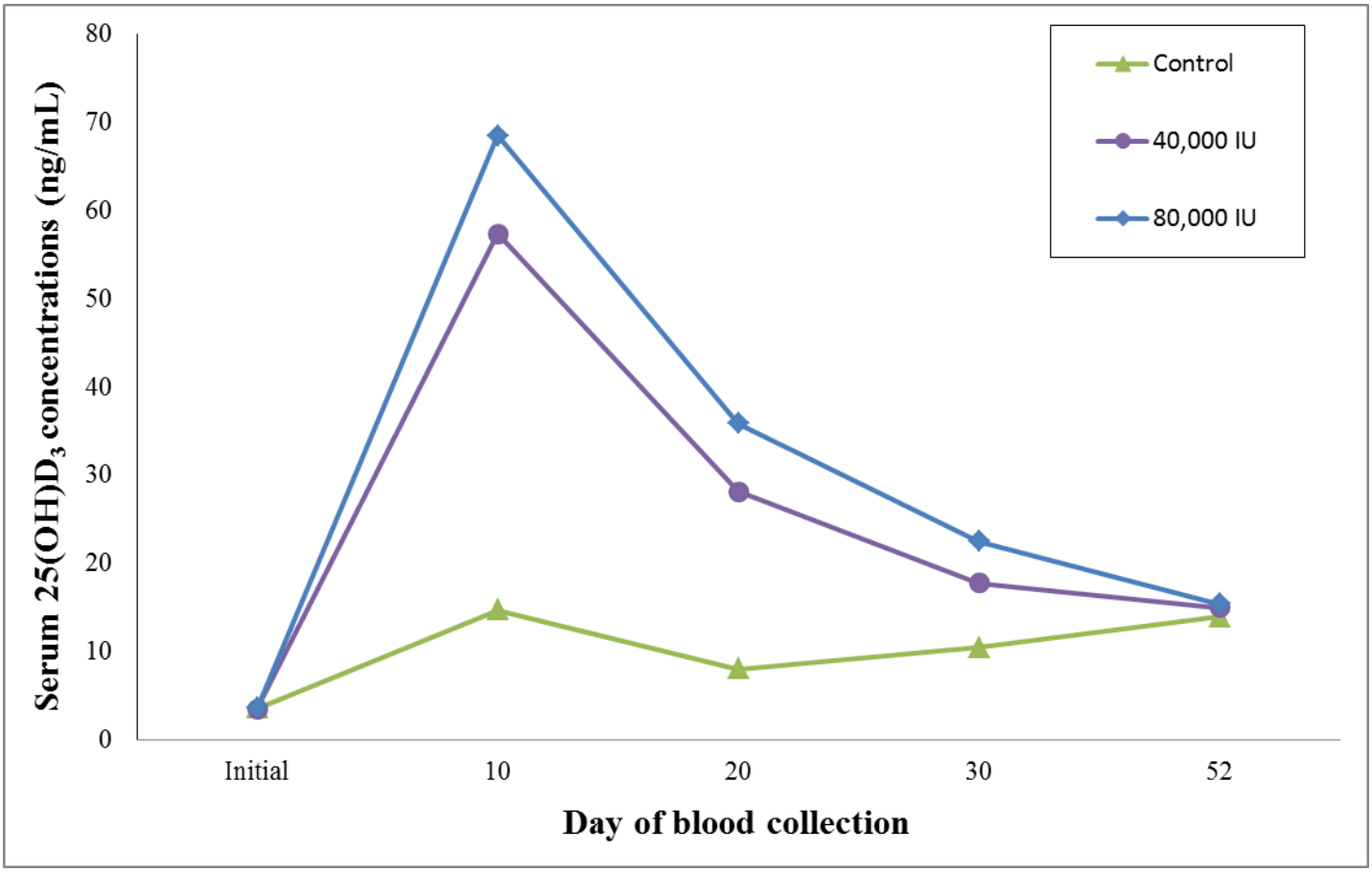

Figure 1-1. Effects of oral vitamin D_3 on serum 25(OH)D_3 concentration Exp. 1

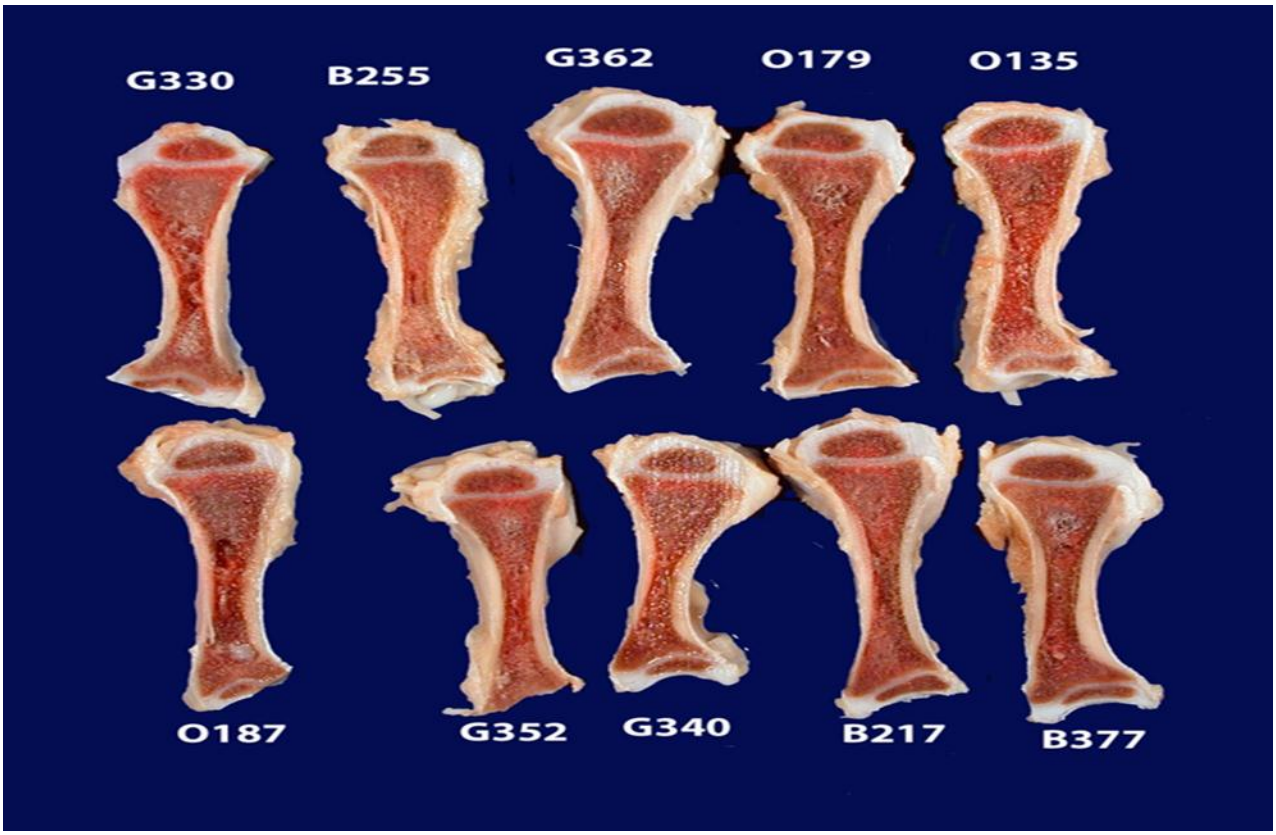

Figure 1-2. Cross section of 10 sampled tibias collected in Exp. 1, all tibias had normal bone mineralization.

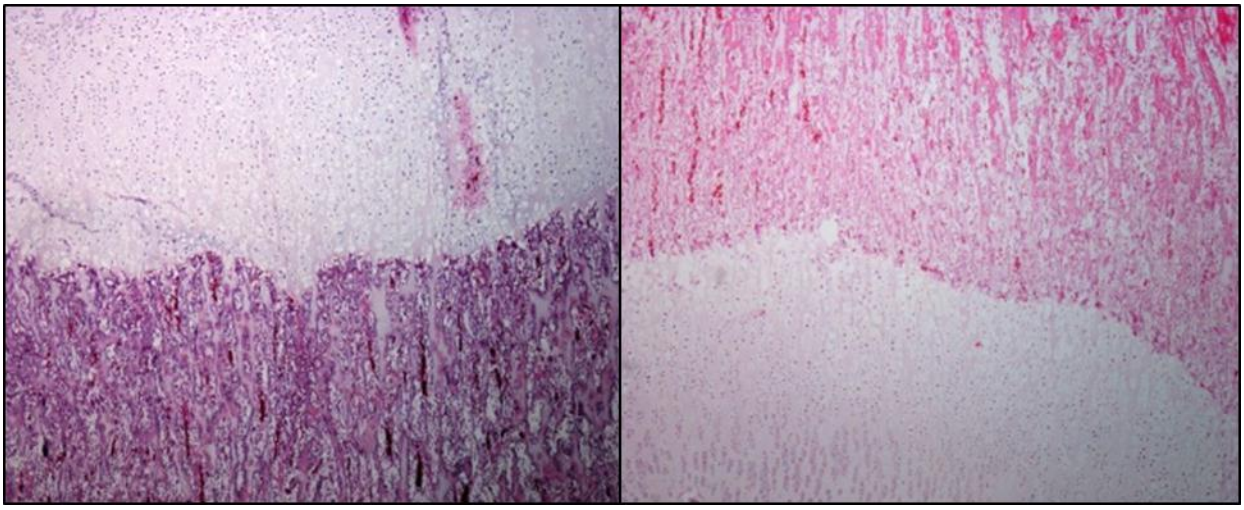

Figure 1-3. Histological evaluation of ribs from Exp. 1, all ribs were categorized as normal in cartilage and bone development

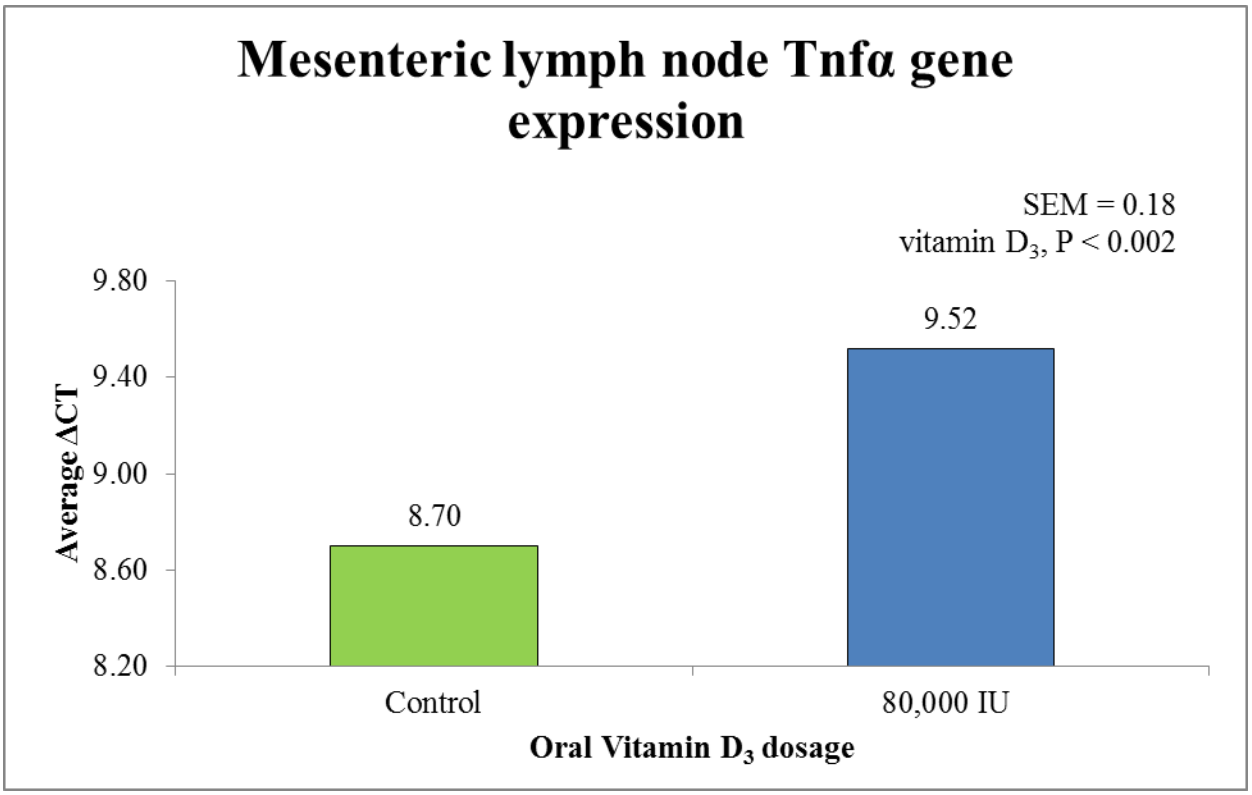

Figure 1-4. Effects of oral vitamin D_3 on relative abundance of TNFα in lymphatic tissue

Chapter 2 - An evaluation of the effects of added vitamin D_3 in maternal diets on sow and pig performance

Abstract

A total of 84 sows (PIC 1050) and their litters were used to determine the effects of supplementing high levels of dietary maternal vitamin D_3 on sow and pig performance, and serum 25(OH)D_3, milk vitamin D_3, neonatal bone mineralization and neonatal tissue vitamin D_3 concentrations. After breeding, sows were allotted to 1 of 3 dietary vitamin D_3 treatments (1,500, 3,000, or 6,000 IU/kg of complete diets) in a RCBD. Sows were bled on d 0 and 100 of gestation, and at farrowing and weaning (d 21). Pig BW was recorded at birth and weaning, and serum was collected from 2 pigs/litter at birth, on d 10, and at weaning. A total of 54 pigs (18/treatment) were euthanized at birth and necropsied to sample bones and tissues. Sow and suckling pig performance, neonatal bone ash, and bone density were not different ($P > 0.10$) among maternal vitamin D_3 treatments. However sow 25(OH)D_3 and milk vitamin D was increased (linear, $P < 0.01$) with increasing maternal vitamin D_3 supplementation. Piglet serum 25(OH)D_3 was increased (quadratic, $P < 0.03$) throughout the lactation period with increased maternal vitamin D_3. Neonatal kidney vitamin D_3 concentrations tended ($P = 0.08$) to decrease with increasing maternal dietary vitamin D_3, but liver vitamin D_3 concentrations tended (linear, $P = 0.09$) to increase with increasing maternal dietary vitamin D_3. At weaning, a subsample of 180 pigs (PIC 327 × 1050) were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 and 2 levels of dietary vitamin D_3 (1,800 or 18,000 IU/kg) from d 0 to 10 post-weaning on nursery growth and 25(OH)D_3. Overall (d 0 to 35), nursery ADG and G:F was not influenced by dietary vitamin D_3, but a tendency (quadratic, $P < 0.06$) for decreased ADFI with increased maternal vitamin D_3 was observed because pigs from sows fed 3,000 IU had lower ADFI compared to pigs from sows fed 1,500 or 6,000 IU/kg. Nursery pig serum 25(OH)D_3 also increased on d 10 and 21 with increasing maternal D_3; however, the magnitude of increase was greater when pigs were fed high dietary vitamin D_3 (maternal × nursery diet interaction; $P < 0.01$). 25(OH)D_3 was increased on d 0 (linear, $P < 0.01$) with increased maternal vitamin D_3. Maternal × diet interactions ($P < 0.01$) were observed on d 10, and 21 because 25(OH)D_3

increased with increasing maternal vitamin D_3, but increases were to a greater extent when pigs were fed high dietary vitamin D_3. In conclusion, sow and pig serum 25(OH)D_3, milk vitamin D_3, and neonatal tissue vitamin D_3 can be increased by increasing maternal vitamin D_3, and nursery pig 25(OH)D_3 can be increased by increasing dietary vitamin D_3: however, sow and pig performance, and neonatal bone mineralization was not influenced by increasing vitamin D_3 dietary levels.

Keywords: sow, vitamin D, vitamin D_3, 25(OH)D_3

Introduction

Recently, a tremendous amount of speculation has surfaced about serum 25(OH)D_3 concentrations of nursery pigs reared in modern swine production facilities. This is mainly due to documented cases where vitamin D has been absent from premixes fed to pigs (Feedstuffs, 2010; Salas, 2011). In these cases, large percentages of pigs developed metabolic bone disease, which is categorized as disturbances related to bone formation and remodeling, and can lead to bone breakages and clinical symptoms of rickets (Madsen, 2011).

Understanding and quantifying the relationship of pig serum 25(OH)D_3 as an indicator of vitamin D status to normal bone mineralization and optimal growth performance is complex due to the tightly regulated metabolic pathways associate with bone growth. However, because most pigs are housed and raised in confinement facilities, pigs no longer have access to direct sunlight which is needed for the endogenous production of vitamin D_3. Therefore, for the suckling pig, vitamin D is supplied from maternal sources until after weaning. Goff et al. (1984) reported increased piglet serum 25(OH)D_3 concentrations in newborn pigs from sows dosed with vitamin D_3 intramuscularly 20 d prior to farrowing, suggesting vitamin D and its metabolites are transferred transplacentally. Lauridsen et al. (2009) tested the effects of supplementing vitamin D_3 in maternal diets at 4 levels between 200 and 2,000 IU/kg and observed decreases in stillborns with increased supplementation levels. However, there is no published research looking at supplementing vitamin D_3 at high levels (6 to 30 times requirement, NRC, 1998) in maternal diets.

Therefore, the objectives of this experiment were to evaluate the effects of supplementing high levels of vitamin D_3 to sows on maternal performance, milk vitamin D concentrations, sow

and piglet serum 25(OH)D_3 concentrations, subsequent pig performance, neonatal pig liver and kidney vitamin D concentrations, and neonatal bone mineralization.

Materials and Methods

Experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. This experiment was conducted at the Kansas State University Swine Teaching and Research Facility in Manhattan, KS and was conducted from the months of January to August of 2012. Feed manufacturing of gestation and lactation sow diets and Phase 2 and Phase 3 nursery diets were performed at the Kansas State University Animal Science Feed Mill in Manhattan, KS. Phase 1 nursery diets were manufactured at the Kansas State University Grain Science Feed Mill. All diets were formulated to meet or exceed nutrient requirement estimates (NRC, 1998).

A total of 84 sows (PIC 1050) and their litters were used to determine the effects of supplementing high levels of dietary vitamin D_3 on maternal performance, subsequent pig performance, sow and piglet serum 25(OH)D_3, Ca and P, milk vitamin D, neonatal bone mineralization, and piglet tissue vitamin D_3 concentrations. Following breeding, sows were assigned to 1 of 3 dietary vitamin D_3 treatments (1,500, 3,000 or 6,000 IU/kg vitamin D_3) throughout 3 farrowing groups in a RCBD with parity and BW at breeding as blocking criteria. There were 27 sows per treatment and 7 to 11 replications per farrowing group. During d 0 to 110 of gestation, sows were housed in gestation stalls (1.70 × 0.61 m) and were fed 2.0 kg/d of the gestation diets. On d 110, sows were transported to the farrowing house and were housed in farrowing crates. Both the gestation and farrowing barns were totally enclosed, environmentally controlled, and mechanically-ventilated buildings. The farrowing barn contained 29 farrowing crates (2.13 × 0.46 m for the sow and 2.13 × 0.48 m for the pigs) that were each equipped with a single feeder and nipple waterer. After farrowing, sows were switched to lactation diets. Gestation and lactation diets were formulated to contain 0.56% and 0.94% standardized ileal digestible lysine, respectively (Table 2-1). Gestation and lactation diets contained 40% and 20% dried distillers grains with solubles, respectively. For the first 3 d after farrowing, sows were gradually provided increased feed according to appetite. After d 3, all sows were allowed ad

libitum access to the lactation diet. Temperature in the farrowing house was maintained at a minimum of 20°C, and supplemental heat was provided to piglets with heat lamps.

Lactation feed intake was determine by measuring feed disappearance on d 0, 7, 14, and 21 (weaning). Sow BW was measured at breeding, d 110 of gestation, within 24 h after farrowing, and at weaning to determine gestation weight gain and lactation weight loss. Sows were bled on d 0 and 100 of gestation, within 12 h after farrowing, and on d 10 and 21 (weaning) in lactation to determine serum 25(OH)D_3, Ca, and P concentrations. Milk samples were collected within 12 h after farrowing, and on d 10 and d 21 (weaning) to determine milk vitamin D_3 concentrations. Milk samples were obtained by an intravenous injection of oxytocin (1 mL, Agrilabs, St. Joseph, MO) and milk was collected from all functional glands. At birth, all piglets were weighed individually and ear tagged for identification. The 2nd and 5th pigs born within each litter were bled prior to suckling on d 0, on d 10, and at weaning to determine piglet serum 25(OH)D_3, Ca, and P. The 7th pig born from 54 litters (18 pigs per treatment, 6 replications per farrowing group) was euthanized prior to suckling and necropsied for bone and tissue sample analysis to determine neonatal pig bone ash content, bone density, and tissue vitamin D_3 concentrations. Mummified and stillborn pigs were recorded to calculate total born and live born piglets. Although minimal, cross-fostering was conducted within 24 h post farrowing to help standardize litter size within vitamin D_3 dietary treatments. Pigs were weighed after fostering to measure fostered litter weight. At weaning, piglet weights and piglet counts were recorded to determine individual and litter weight gains, along with survivability.

At weaning, a subsample of 180 multi-sex pigs (PIC 327 × 1050) from the first sow group were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D_3 concentration and 2 levels of dietary vitamin D_3 (1,800 or 18,000 IU/kg; from d 0 to 10 postweaning) on growth performance and serum 25(OH)D_3, Ca, and P. At weaning, pigs were allotted to pens based on their previously administered maternal vitamin D_3 treatments in order to maintain the integrity of weaning weights consistent with maternal vitamin D_3 effects. Pens were then randomly assigned to dietary vitamin D_3 treatments. There were 6 pigs per pen and 5 pens per treatment. Dietary vitamin D_3 treatments were provided from d 0 to 10 in the nursery and were fed in a pellet form (Table 2-2). Common Phase 2 and 3 diets were provided to pigs from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1,800 IU/kg vitamin D_3. All pens (1.2 × 1.5 m) had woven wire flooring, one 3-hole, dry self-feeder,

and a nipple waterer to allow for ad libitum access to feed and water. All pigs and feeders were weighed on d 0, 5, 10, 17, 21, 28, and 35 after weaning to determine ADG, ADFI, and G:F. Blood samples were collected from 10 pigs/treatment on d 0, 10, 24, and 35 to determine serum 25(OH)D_3, Ca, and P.

Feed and Premix vitamin D_3 analysis

Vitamin D_3 supplement (Rovimix D_3, 500,000 IU/g, DSM Nutritional Products Inc., Parsippany, NJ) was mixed with rice hulls to achieve a premix formulated to contain 2,204,620 IU/kg of vitamin D_3. Premix was then added to the control diet (1,378 IU/kg vitamin D_3) by replacing corn to achieve desired dietary treatments. Vitamin premixes and complete diet samples were collected during feed manufacturing. These samples were pooled by specific diet type or premix and were subsampled. Subsamples were sent in duplicate to DSM Nutritional Products Inc. (Parsippany, NJ) where they were analyzed for vitamin D_3 by using a combination of HPLC and mass spectrometry (Schadt et al., 2012).

Serum 25(OH)D_3, milk and tissue vitamin D, and serum Ca and P analysis

All blood, milk, and tissue sample analyses was conducted by Heartland Assays (Ames, IA). Blood samples were collected via jugular venipuncture using 25-mm (neonatal and nursery pigs) and 38-mm (sows) × 20-gauge needles and 10-mL blood collection tubes containing a gel separator for use in determining circulating serum 25(OH)D_3, Ca, and P concentrations. Six h after collection, blood was centrifuged (1,600 × g, 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 using a previously described RIA (Hollis et al., 1993), serum Ca concentrations were determined by spectrophotometry with a commercial kit (Pointe Scientific Inc., Canton, MI) using a method described by Pointe Scientific (2009a). Serum P was determined by spectrophotometry with a commercial kit (Pointe Scientific Inc., Canton, MI) using a method described by Point Scientific (2009b). Milk and whole tissue samples were frozen at -20°C until analysis. Analysis was conducted using a combination of HPLC and mass spectrometry previously described by (Schadt et al., 2012).

Necropsies and bone and tissue sampling

Necropsies were performed on site and in compliance with the college's standard operating procedures. Pigs were euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus). Right femurs and 2nd ribs were collected to determine bone ash content and left 2nd ribs were used to determine bone density. Whole liver and kidney tissues were collected and frozen immediately at -20°C until samples were prepared for specific analysis.

Bone density analysis

Bone densities were determined at the Iowa State University College of Veterinary Medicine (Ames, IA). All left 2nd ribs were stripped to the periosteum, submerged in water for 4 h under 625 mmHg vacuum and blotted dry prior to recording bone weight. Bone volume was determined using weight in air minus weight under water according to Archimedes principle (Keenan et al., 1997). Bone density values were then expressed as g of bone/mL volume.

Bone ash determination

Bone ash analysis, which was performed on the right femurs and right 2nd rib, was conducted at the Kansas State Swine Nutrition Laboratory in Manhattan, KS. Bones were cleaned to the periosteum and were split perpendicular to the long axis of the diaphysis. Fat extraction was conducted by placing bones in cellulose thimbles, and inserting thimbles in the main chambers of soxhlet extractors. The extraction solvent was petroleum ether. Fat extraction was conducted for 7 d. At the completion of the extraction period, bone samples were dried in a forced air oven at 100°C until a consistent dry weight was achieved. Then bones were ashed at 600°C for 24 h. Ash weights were recorded and expressed as a percentage of dry fat-free bone.

Statistical Analysis

Data were analyzed using the MIXED procedure in SAS (SAS Institute, Inc., Cary, NC). Treatment means were analyzed using the LSMEANS statement and pre-planned contrasts were used to determine the linear and quadratic effects of increasing vitamin D_3. Unequally spaced linear and quadratic contrasts were derived using the IML procedure in SAS. Maternal performance data were analyzed as a RCBD with sow as the experimental unit and farrowing group as a random effect. Nursery performance was analyzed as a 3 × 2 split plot design and pen was used as the experimental unit. Additional pre-planned contrasts were used to determine the

effects of early nursery vitamin D_3 treatments and the interaction of maternal vitamin D_3 and early nursery dietary vitamin D_3 treatments. Serum 25(OH)D_3, Ca, and P, and milk vitamin D data were analyzed using the REPEATED function of SAS to determine the effects of treatment variables over time, and individual pig was the experimental unit. Bone ash, bone density, and tissue vitamin D concentrations were analyzed using individual pig as the experimental unit. Differences among treatments were considered significant with $P \leq 0.05$ and trends if $P > 0.05$ and ≤ 0.10.

Results

Analysis of vitamin D_3 concentrations in the diets verified that they were within acceptable analytical error of formulated dietary values. Experimental gestation diets had analyzed vitamin D_3 concentrations of 1,505, 3,370, 8,025 IU/kg for diets formulated to contain added vitamin D_3 at 1,500, 3,000 and 6,000 IU/kg of complete diets, respectively. Lactation diets had analyzed vitamin D_3 concentrations of 1,475, 3,390, and 6,210 IU/kg for diets formulated to contain added vitamin D_3 at 1,500, 3,000 and 6,000 IU/kg of complete diets, respectively. Nursery phase 1 diets had analyzed vitamin D_3 concentrations of 1,870 and 19,300 for diets formulated to contain added vitamin D_3 at 1,800 and 18,000 IU/kg of complete diets, respectively.

Maternal Performance

Supplementation of vitamin D_3 at levels used in this study did not influence sow lactation ADFI ($P > 0.10$; Table 2-3) or sow BW throughout gestation and lactation. Additionally, high levels of maternal vitamin D_3 did not affect litter size criteria ($P > 0.10$) or suckling pig performance.

Nursery Performance

During the nursery portion of the study, no interactions of maternal vitamin D_3 and dietary vitamin D_3 were observed ($P > 0.10$; Table 1-3) with regards to nursery performance. During Phase 1 (d 0 to10), increasing maternal vitamin D_3 (quadratic, $P < 0.04$) decreased ADG and ADFI, with pigs from sows fed 3,000 IU vitamin D_3 having lower ADG, and ADFI compared to pigs from sows fed either 1,500, or 6,000 IU vitamin D_3. Additionally, G:F was reduced ($P = 0.02$) with increasing dietary vitamin D_3 in phase 1 diets. During Phase 2 (d 10 to

21), no differences were observed due to maternal or early nursery vitamin D_3 treatment, but there was a tendency ($P = 0.10$) for G:F to increase with increased supplementation of vitamin D_3 in phase 1 diets. Maternal or early nursery dietary supplemented vitamin D_3 did not influence ($P > 0.10$) ADG, ADFI, or G:F during phase 3 (d 21 to 35). Overall (d 0 to 35), there was a tendency (quadratic, $P = 0.06$) for ADFI to decrease with increasing vitamin D_3, with pigs from sows fed 3,000 IU having lower ADFI compared to pigs from sows fed either 1,500 or 6,000 IU.

Sow Serum 25(OH)D_3, Ca, P and milk vitamin D_3

A maternal × day interaction ($P < 0.01$; Table 2-5) was observed for sow serum 25(OH)D_3 because on d 0 of gestation serum 25(OH)D_3 was not different among sows; however, increasing maternal vitamin D_3 increased (linear, $P < 0.01$) serum 25(OH)D_3 on d 100 of gestation, at farrowing, and at weaning. A day effect ($P < 0.01$) was observed for Serum Ca. Serum Ca tended (linear, $P = 0.07$) to be higher on d 0 of gestation for sows assigned to the 6,000 IU vitamin D_3 treatment compared to sows assigned to lower maternal vitamin D_3 treatments which would reflect potential differences prior to initiation of maternal vitamin D_3 treatments. On d 100 of gestation, increasing maternal dietary vitamin D_3 tended to increase ($P = 0.09$) serum Ca. Serum P concentrations were not influenced ($P > 0.10$) by maternal vitamin D_3 treatments or by sampling day ($P = 0.18$). Milk vitamin D_3 concentrations were not influenced by sampling day ($P = 0.56$); however, milk vitamin D_3 increased (linear, $P < 0.01$) with increasing maternal dietary vitamin D_3 at farrowing, on d 10 in lactation, and at weaning.

Piglet serum 25(OH)D_3, Ca, and P, neonatal bone ash and bone density, and neonatal tissue vitamin D

A day effect was observed ($P < 0.01$; Table 2-5) for piglet serum 25(OH)D_3 as serum concentrations increased over time from birth to weaning, and serum 25(OH)D_3 increased (quadratic, $P < 0.03$) with increasing maternal vitamin D_3 at birth, on d 10 in lactation, and at weaning. A day effect was observed ($P < 0.01$) for serum Ca concentrations. Additionally serum Ca tended to decrease ($P = 0.08$) with increasing maternal vitamin D_3 on d 10 of lactation. Serum P concentrations were not influenced ($P > 0.10$) by maternal vitamin D_3 treatments, but they tended to be different based on day of sampling ($P = 0.08$). No differences in bone ash values ($P > 0.10$) were observed for femurs or 2nd ribs. Rib bone density was not influenced ($P > 0.56$) by

maternal vitamin D_3 concentrations. Kidney vitamin D concentrations (quadratic, $P = 0.09$) tended to decrease with increasing maternal vitamin D_3 with pigs from sows fed 3,000 IU vitamin D_3 having much lower tissue vitamin D_3 concentrations compared to pigs from sows fed 1,500 or 6,000 IU. Liver tissue vitamin D_3 concentrations tended to increase (linear, $P = 0.08$) with increased maternal dietary vitamin D_3.

Nursery pig serum 25(OH)D_3, Ca, and P

A day effect ($P < 0.01$; Table 2-6) was observed from nursery pig serum 25(OH)D_3. At weaning (d 0), pig serum 25(OH)D_3 was increased (linear, $P < 0.01$) with increasing maternal vitamin D_3. Serum 25(OH)D_3 also increased on d 10 and 21 with increasing maternal dietary vitamin D_3; however, the magnitude of the increase was greater when nursery pigs were fed high dietary vitamin D_3 (maternal × nursery diet interactions; $P < 0.01$). On d 35, serum 25(OH)D_3 concentrations were not different among maternal or nursery dietary treatments. Serum Ca and P concentrations were not influenced by maternal or nursery vitamin D_3 concentrations, except for a tendency (quadratic, $P = 0.08$) for P concentrations to increase within increasing maternal vitamin D_3. Additionally, a day effect for serum P was observed ($P < 0.01$).

Discussion

The concept of supplementing high levels of vitamin D is often discussed in combination with hypervitaminosis or toxicity. This is because many previous studies have observed soft tissue mineralization, and even death as a result of toxicity (Chineme et al., 1976; Kamio et al., 1977; Long et al., 1984). However, in these observed incidences of toxicity, supplemented levels were more than 1,000 time the animal's requirement (NRC, 2012). The NRC (1987) presumed the upper safe levels in swine to be 2,200 IU and 33,000 IU per kg of the diet when exposure time is greater than 60 or less than 60 d, respectively. These values are 10 and 150 times the animal's established requirement (NRC, 2012). Despite requirements and recommendations, most diets in the swine industry are formulated at levels of 6 to 9 times the animal's requirement (Reese and Hill, 2010). This common practice initiated the development of the experimental design for the current study, specifically to determine if potential benefits exist from the supplementation of vitamin D_3 through maternal diets at higher than typically formulated levels.

No differences were observed in the current study with regards to sow BW change or lactation ADFI from supplementing vitamin D_3 at the levels used within this study. Lauridsen et al. (2009), observed no impact on gilt BW change during the first 28 d of gestation when gilts were fed 4 levels (200, 800, 1,400, or 2,000 IU/kg) of vitamin D from 2 sources (vitamin D_3, or 25(OH)D_3). The authors also reported no influence of dose or form of vitamin D supplementation on BW changes of multiparous sows between 2 and 5 parities throughout gestation and lactation when the same dietary treatments were administered. Interestingly, the investigators did report an interaction between parity, form of vitamin D, and dose of vitamin D on total feed intake of lactating sows. This interaction was due to decreased total feed intake with increasing doses of vitamin D_3, which was mainly observed in parity 4 and 5 sows, but for sows fed 25(OH)D_3 the largest decrease in feed intake was observed with increased vitamin D supplementation from 200 to 800 IU. However, the authors speculated on the limitations of the results due to the complexity of the interaction. Levels of vitamin D_3 fed in the current experiment were 1 to 3 times the levels utilized in the fore-mentioned trial.

Viganó et al. (2003) described the potential role of vitamin D in implantation. This is due to vitamin D's ability to increase expression of calbindin, an intracellular protein involved in calcium metabolism, and HoxA genes which are shown to impact the viability of preimplantation embryos. Lauridsen et al. (2009) reported no effect of dietary form or dose of vitamin D with regard to early reproduction in terms of the number of implanted fetuses in gilts or litter size of sows; however, the authors reported reductions in the number of stillborns with increased vitamin D doses of 1,400 and 2,000 IU compared to 200 and 800 IU. Coffey et al., (2012) reported an increased number of developed fetuses from reproductive tracts harvested from first service gilts when supplemented with 25(OH)D_3 compared to vitamin D_3 at the same supplementation level. The authors speculated that this may be due to the increased efficiency of absorption in the upper portion of the intestine, which has been observed in poultry (Bar et al., 1980). However commercial use of 25(OH)D_3 has not been approved for use in swine in the U.S. and research determining the efficiency of absorption specifically in swine has not been conducted. The current study did not observe any difference in the number of stillborns or live born pigs based on vitamin D_3 treatment level. Ultimately, to evaluate the economic incentive of increasing supplemental vitamin D_3 on the basis of sow productivity, large-scale commercial

studies with increased sample sizes will be needed to increase sensitivity and reduce the experimental error that is associated with sow reproduction measurements.

In comparison to presumed upper safe guidelines established by NRC (1987), sows in the current experiment were supplemented vitamin D_3 at rates 2 to 3 times the recommended level for exposure times greater than 60 d (2,200 IU) with no adverse effects on feed intake, sow BW, or sow productivity. This may suggest that supplementation rates up to 6,000 IU/kg of complete feeds are safe to use for sows, however, sows in this study were not followed through subsequent parities to determine potential long term effects. Additionally, due to the absence of improvement in maternal performance within this study, it appears there is no benefit to increase vitamin D_3 supplementation above 1,500 IU/kg of complete diet.

Similar to sow and neonatal pig performance, overall nursery pig performance within the current experiment was not adversely influenced by vitamin D_3 supplementation. Interestingly, from d 0 to 10 G:F was worse for pigs fed 18,000 IU/kg vitamin D_3 in phase 1 diets. However, the opposite was true during the second phase, where pigs fed 18,000 IU/kg in phase 1 diets tended to have increased G:F compared to pigs fed 1,800 IU/kg in phase 1 diets. Perhaps this insinuates that supplementation of 18,000 IU/kg of vitamin D_3 to weaned pigs is above the animals metabolic tolerance; however, a previous study conducted by Wren et al. (1980) suggested reductions in ADG and ADFI, but no impacts on G:F have been described in these instances. Flohr et al. (2012) observed decreased intake of diets supplemented with 44,100 IU/kg vitamin D_3 but G:F was not determined due to the experimental design. Also, 25(OH)D_3 concentrations in pigs supplemented 18,000 IU of vitamin D_3 ranged from approximately 50 to 60 ng/mL on d 10 which is below previously described concentrations experienced in periods of vitamin D intoxication (Littledike and Horst, 1982). The minimal impact of vitamin D_3 supplementation on nursery pig performance within this study is similar to previous studies conducted by Wahlstrom and Stolte (1958) and Combs et al. (1966). Previous research concluding improvements in growth performance as a result of vitamin D_3 supplementation have consistently been reported in experiments where pigs were fed marginal Ca and P and vitamin D deficiency has been established (Johnson and Palmer, 1939; Bethke, 1946; Rorvedt and Crenshaw, 2012).

The most widely used marker of vitamin D status in humans is serum 25(OH)D (IOM, 1997). This is because serum 25(OH)D has a circulating half-life of 10 d to 3 wk. This

circulating half-life is much longer than that of vitamin D itself, or of the active metabolite $1,25(OH)_2D$, which suggests it is a better indicator of long term status. Similar to results obtained in studies conducted by Goff et al. (1984), Lauridsen et al. (2009), Witschi et al. (2011) and Coffey et al. (2012), increasing supplementation of vitamin D, either through maternal or nursery diets, resulted in increased serum $25(OH)D_3$ in sows, neonatal pigs, and nursery pigs in the current study. Increased sow and pig $25(OH)D_3$ at parturition observed with increased vitamin D_3 supplementation supports previous conclusions reported by Goff et al. (1984), who described a strong correlation of sow $25(OH)D_3$ and piglet $25(OH)D_3$ at parturition. This correlation is related to the ability of vitamin D and its metabolites, specifically $25(OH)D_3$, to be transferred transplacentally from sow to fetus. Additionally, the increases in maternal vitamin D_3 supplementation resulted in increases in milk vitamin D_3 concentrations which agrees with previous research performed in dairy cows (Hollis et al., 1983). Additionally, this increase in milk vitamin D_3 concentration contributed to increased piglet serum $25(OH)D_3$ from birth (prior to suckling) to weaning due to milk intake. Nursery pig serum $25(OH)D_3$ increased as a result of an interaction between maternal and phase 1 dietary vitamin D_3 supplementation. However by d 35, nursery pigs were similar in serum $25(OH)D_3$ concentrations affirming the half-life of $25(OH)D_3$ is between 10 d and 3 wk.

Research in rats has shown that vitamin D is accumulated in fetal tissues (Clements and Fraser, 1988) specifically, in fetal muscle as $25(OH)D_3$. The researchers reported enhancement of the placental transport of vitamin D during the third trimester of gestation in the rat, and they speculated that the relationship of neonatal rickets and maternal vitamin D deficiency in humans may be similar to this mechanism. Research by Schröder et al. (1993) concluded that newborn piglets do not rely on vitamin D-dependent Ca transport until the 4^{th} wk postpartum. In the current studies, liver vitamin D_3 concentrations tended to increase with increased maternal vitamin D_3 and kidney vitamin D_3 concentrations decreased with increasing maternal vitamin D_3. But in general, tissue concentrations were lower than expected. This may suggest that vitamin D_3 is not transferred across the placenta as effectively as $25(OH)D_3$, or that it is not stored in hydroxylating tissues at birth. More work determining concentrations of $25(OH)D_3$ in these specific tissues of new born pigs along with hydroxylating enzyme levels is needed to better understand the ability of the neonate to synthesize vitamin D metabolites, and whether it is useful for pre-weaning Ca and P absorption.

To our knowledge, previous research quantifying bone mineralization in newborn pigs has not been conducted. Vitamin D supplementation has been shown to influence fetal bone development in the human fetus when mothers are vitamin D deficient (Morley et al., 2006). In the current experiment, bone ash content of ribs and femurs of pigs euthanized at birth was not influenced by maternal treatment. Percentage bone ash of ribs and femurs were 15% lower than previously referenced bone ash values of nursery pigs 2 months of age (Crenshaw et al., 1981). This is probably a function of age which has previously been described as a predominate factor in bone mineral content (Crenshaw et al., 1981). Additionally differences in ash content of bones depending on skeletal function have been discussed by Reinhard et al. (1976), and the current study would agree with these results, because rib bone ash percentage was 1% lower than femur bone ash percentages. Bone densities were not different among sampled ribs, this agrees with research conducted by Witschi et al. (2011), who reported similar bone mineral content and bone mineral density for pigs (35 d postpartum) from sows fed 200 IU/kg vitamin D or 2,000 IU/kg vitamin D from supplemented 25(OH)D_3. Rortvedt and Crenshaw (2012) reported decreased mineral content and density of femurs at 9 wk of age from pigs that were fed marginal Ca and P levels. The decreases in mineral content and density were exacerbated to a greater degree when pigs were from sows fed vitamin D_3 deplete diets. This suggests that dietary Ca and P plays a greater role in bone mineralization of nursery pigs compared to maternal vitamin D supplementation, but vitamin D supplementation is still a factor involved in bone mineralization.

Serum Ca and P were not adversely influenced by supplemental vitamin D_3 in maternal diets or in phase 1 nursery diets. All reported results are within normally described physiological ranges (Ullrey et al., 1967; Friendship et al., 1984). In the current study, Ca and P were supplied in excess of the animal's requirements (NRC, 1998), which suggests that vitamin D_3 supplementation above 1,500 IU in maternal diets or 1,800 IU in the nursery did not influence circulating Ca and P. This conclusion agrees with results of Witschi et al. (2011). However, decreases in serum Ca and P have been associated with deficient supplementation rates of vitamin D_3 in growing pigs by Hagemoser et al. (2000).

Although not evaluated in the current experiment, the interest in vitamin D's role in immune function has increased. Human health research has observed impacts of vitamin D on both innate and acquired immunity (Bikle, 2009). Typically, vitamin D has been viewed as an immunosuppressant based on its inhibition of T cell proliferation (Lemire et al., 1995); however,

the production of cathelicidin, a potent antimicrobial, in macrophages or keratinocytes has been previously determined to be dependent on serum 25(OH)D_3 concentrations (Lui et al., 2006). Lauridsen et al. (2009) reported no differences in haptoglobin for pigs from sows supplemented varying levels of vitamin D_3 or 25(OH)D_3. Research measuring potential impacts of vitamin D on immune function of pigs reared in commercial conditions needs to be conducted in order to evaluate if supplementation of vitamin D_3 at high levels can impact swine health.

Overall, the results of this study indicate that supplementing high concentrations of vitamin D_3 to sows can increase sow and piglet serum 25(OH)D_3, milk vitamin D concentrations, and neonatal tissue vitamin D concentrations. Additionally, maternal vitamin D_3 and dietary vitamin D_3 supplementation can increase nursery pig serum 25(OH)D_3. However, supplementation of high levels of maternal vitamin D_3 or dietary vitamin D_3 did not impact sow or subsequent pig performance, neonatal bone mineralization, or serum Ca and P. This suggests that there is no benefit to supplementing vitamin D_3 levels above 1,500 IU for sows and 1,800 IU to nursery pigs.

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Tables and Figures

Table 2-1. Composition of sow diets (as-fed basis)[1]

	Gestation			Lactation		
	1,500	3,000	6,000	1,500	3,000	6,000
Ingredient, %						
Corn	52.95	52.88	52.73	52.19	52.12	51.97
Soybean meal (46.5% CP)	2.99	3.00	3.01	23.88	23.89	23.90
DDGS[2]	40.00	40.00	40.00	20.00	20.00	20.00
Monocalcium P (21% P)	0.65	0.65	0.65	0.90	0.90	0.90
Limestone	1.90	1.90	1.90	1.60	1.60	1.60
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15
L-lys HCl	0.23	0.23	0.23	0.15	0.15	0.15
Phytase[5]	0.13	0.13	0.13	0.13	0.13	0.13
Vitamin D_3 premix[6]	0.01	0.07	0.21	0.01	0.07	0.21
TOTAL	100	100	100	100	100	100
Calculated analysis						
ME, kcal/kg	3,289	3,287	3,283	3,281	3,280	3,276
CP, %	17.0	17.0	17.0	21.1	21.1	21.1
Total Lys, %	0.72	0.72	0.72	1.13	1.13	1.13
Standarized ileal digestible amino acids, %						
Lys	0.56	0.56	0.56	0.97	0.97	0.97
Thr	0.47	0.47	0.47	0.66	0.66	0.66
Met	0.28	0.28	0.28	0.32	0.32	0.32
Trp	0.10	0.10	0.10	0.20	0.20	0.20
Ile	0.49	0.49	0.49	0.74	0.74	0.74
Leu	1.64	1.64	1.64	1.78	1.78	1.78
Ca, %	0.88	0.88	0.88	0.88	0.88	0.88
P, %	0.59	0.59	0.59	0.64	0.64	0.64
Available P[7], %	0.50	0.50	0.50	0.48	0.48	0.48
Analyzed vitamin D_3, IU/kg[8]	1,505	3,370	8,025	1,475	3,390	6,210

[1] A total of 81 sows and litters were used over 3 farrowing groups to determine the effects of supplemental vitamin D_3 on maternal performance, subsequent pig performance, sow and piglet serum 25(OH)D_3, Ca and P, milk vitamin D, neonatal bone mineralization, and piglet tissue vitamin D concentrations.

[2] DDGS: dried distillers grains with solubles.

[3] Vitamin premix provided 11,023 IU vitamin A, 1,378 IU vitamin D_3, 44 IU vitamin E, 4.41mg menadione, 8.27 mg riboflavin, 27.56 mg pantothetic acid, 49.60 mg niacin, 38.5 μg vitamin B_{12}, 551 mg choline, 0.22 mg biotin, 1.65 mg folic acid, and 4.96 mg pyridoxine per kg of the complete diet.

[4] 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 per kg of the complete diet.

[5] Natuphos 600, BASF, Florham Park, NJ. Provided 752 FTU/kg of diet.

[6] Vitamin D_3 premixes were mixed to contain 2,024,620 IU/kg of premix by blending vitamin D_3 with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.

[7] Phytase provided 0.12% available P to gestation and lactation diets.

[8] Vitamin D_3 analyses were performed by DSM Nutritional Products (Parsippany, NJ), and values represent the average of 2 pooled sampled/diet.

Table 2-2. Composition of nursery diets (as-fed basis)[1]

	Phase 1[2]		Phase 2[3]	Phase 3[4]
Ingredient, % vitamin D_3 IU/kg:	1,800	18,000	1,800	1,800
Corn	39.58	38.78	44.73	65.78
Soybean meal (46.5% CP)	17.33	17.39	23.41	30.67
DDGS[5]	5.00	5.00	15.00	---
Spray-dried porcine plasma	5.00	5.00	---	---
Spray-dried blood cells	1.25	1.25	---	---
Spray dried whey	25.00	25.00	10.00	---
Select menhaden fish meal	---	---	4.50	---
Soybean oil	3.00	3.00	---	---
Monocalcium P (21% P)	0.85	0.85	0.15	1.03
Limestone	1.00	1.00	0.70	0.98
Salt	0.30	0.30	0.30	0.35
Zinc oxide	0.39	0.39	0.25	---
Trace mineral premix[6]	0.15	0.15	0.15	0.15
Vitamin Premix[7]	0.25	0.25	0.25	0.25
L-Lys HCl	0.20	0.20	0.28	0.36
DL-Met	0.13	0.13	0.05	0.13
L-Thr	0.05	0.05	0.05	0.13
L-Ile	0.10	0.10	---	---
Phytase[8]	0.13	0.13	0.17	0.17
Acidifier[9]	0.20	0.20	---	---
Vitamin E, 20,000 IU	0.05	0.05	---	---
Choline Chloride 60%	0.04	0.04	---	---
Vitamin D Premix[10]	0.02	0.76	0.02	0.02
TOTAL	100	100	100	100
Calculated analysis				
ME, kcal/kg	3,415	3,391	3,320	3,314
CP, %	21.2	21.2	23.1	20.4
Total Lys, %	1.50	1.50	1.46	1.38
Standardized ileal digestible amino acids, %				
Lys	1.35	1.35	1.30	1.25
Thr	0.86	0.86	0.81	0.78
Met	0.39	0.39	0.42	0.42
Trp	0.24	0.24	0.22	0.21
Ile	0.82	0.82	0.83	0.75
Leu	1.78	1.78	1.85	1.60
Ca, %	0.80	0.80	0.70	0.68
P, %	0.71	0.71	0.63	0.61
Available P, %[11]	0.63	0.63	0.50	0.42
Analyzed vitamin D_3, IU/kg[12]	1,870	19,300	1,855	1,911

[1] A total of 180 pigs (PIC 327 × 1050; initially 21 d of age) were used in a 3 × 2 split plot design for 35 d to determine the effects of maternal vitamin D3 and early nursery dietary vitamin D_3 on nursery growth performance and serum 25(OH)D_3 concentrations.

[2] Phase 1 diets were fed from d 0 to 10.
[3] Phase 2 diets were fed from d 10 to 24.
[4] Phase 3 diets were fed from d 24 to 35.
[5] DDGS: dried distillers grains with solubles.
[6] 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 per kg of the complete diet.
[7] Vitamin premix provided 11,023 IU vitamin A, 1,378 IU vitamin D_3, 44 IU vitamin E, 4.41mg menadione, 8.27 mg riboflavin, 27.56 mg pantothetic acid, 49.60 mg niacin, and 38.5 μg of vitamin B_{12} per kg of the complete diet.
[8] 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.
[9] KemGest, Kemin Industries Inc., Des Moines, IA.
[10] Vitamin D_3 premixes were mixed to contain 2,204,620 IU/kg of premix by blending vitamin D_3 with rice hulls. Premix replaced a percentage of corn to achieve the desired treatment vitamin D_3 concentrations.
[11] Phytase provided 0.12, 0.13, and 0.12% available P for Phase 1, 2, and 3 diets respectively.
[12] Vitamin D_3 analyses were performed by DSM Nutritional Products Inc. (Parsippany, NJ).

Table 2-3. The effects of high maternal vitamin D_3 on sow and litter performance[1,2]

	Vitamin D_3, IU/kg				*Probability, P <*	
Item	1,500	3,000	6,000	SEM	Linear	Quadratic
Sows, n	28	26	26			
ADFI, kg						
d 0 to 7	4.78	4.99	5.11	0.257	0.31	0.72
d 7 to 14	5.62	5.87	5.95	0.296	0.31	0.60
d 14 to wean	6.33	6.47	6.68	0.385	0.29	0.93
d 0 to wean	5.65	5.88	5.98	0.339	0.27	0.63
Sow BW, kg						
Gestation						
d 0	193.1	194.1	192.1	8.75	0.91	0.89
d 110	231.4	235.2	237.1	5.99	0.52	0.80
Change	+38.3	+41.1	+45.0	5.44	0.24	0.92
Lactation						
d 0	221.9	227.6	224.1	5.96	0.89	0.50
d 21 (weaning)	212.3	220.3	217.4	7.52	0.67	0.42
Change	-9.6	-7.3	-6.7	2.78	0.24	0.43
Piglets						
Litter size, n						
Mummies	0.3	0.2	0.3	0.12	0.88	0.86
Stillborn	0.6	0.4	0.8	0.34	0.60	0.37
Total born alive	13.0	12.5	13.2	0.88	0.74	0.57
Fostered	12.3	12.1	13.0	0.70	0.50	0.48
Weaned	11.2	10.8	11.5	0.652	0.48	0.32
Survivability, %	91.2	89.2	88.5	2.02	0.88	0.58
Piglet BW, kg						
birth	1.31	1.36	1.34	0.041	0.63	0.47
weaning	5.31	5.55	5.52	0.165	0.43	0.42

[1] A total of 84 sows (PIC 1050) and their litters were used. There were 2 sows removed from the 3,000 IU/kg vitamin D_3 treatment because of lameness and illness. There were 2 sows removed from the 6,000 IU/kg vitamin D_3 treatment because of late-term abortion and farrowing complications.

[2] Sow group was used as a random effect in the statistical model.

[3] Survivability was calculated by dividing the weaned litter size by the fostered litter size.

Table 2-4. The effects of maternal and early nursery vitamin D_3 supplementation on nursery pig growth performance[1]

	Maternal vitamin D_3, IU/kg							*Probability, P <*			
	1,500		3,000		6,000			Maternal × Diet	Maternal		
Early nursery vitamin D_3[2]:	1,800	18,000	1,800	18,000	1,800	18,000	SEM	Interaction	Linear	Quadratic	Diet
d 0 to 10[3]											
ADG, g	270	270	239	242	292	266	14.6	0.56	0.26	0.02	0.52
ADFI, g	285	293	250	270	298	291	16.4	0.70	0.45	0.04	0.60
G:F	0.95	0.92	0.96	0.90	0.98	0.92	0.024	0.70	0.48	0.68	0.02
d 10 to 21											
ADG, g	312	309	282	286	284	291	17.3	0.96	0.27	0.23	0.84
ADFI, g	423	414	406	372	416	408	21.5	0.78	0.98	0.16	0.34
G:F	0.73	0.75	0.69	0.78	0.68	0.71	0.032	0.51	0.12	0.71	0.10
d 21 to 35											
ADG, g	609	611	593	606	614	576	19.0	0.39	0.46	0.75	0.63
ADFI, g	964	1,010	958	939	976	917	27.1	0.17	0.20	0.30	0.64
G:F	0.63	0.61	0.62	0.65	0.63	0.63	0.014	0.20	0.56	0.36	0.95
d 0 to 35											
ADG, g	417	419	391	399	416	396	13.3	0.56	0.56	0.12	0.75
ADFI, g	597	618	577	565	602	576	17.3	0.39	0.47	0.06	0.69
G:F	0.70	0.68	0.68	0.71	0.69	0.69	0.012	0.17	0.96	0.71	0.90

[1]A total of 180 mixed-sex pigs (PIC 327 × 1050; initially 21 d of age) were weaned from the 1st sow group and used in a 3 × 2 split plot design for 35 d to determine the effects of maternal and early nursery dietary vitamin D_3 on growth performance.

[2] Dietary vitamin D_3 treatments were fed in Phase 1 diets from d 0 to 10. Common Phase 2 and 3 diets were fed from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1, 800 IU/kg vitamin D_3. Treatments are expressed as IU/kg of the complete diet.

Table 2-5. Effects of high maternal vitamin D_3 on serum 25(OH)D_3, Ca, P, milk vitamin D, neonatal bone ash content and bone density[1,2]

	Maternal vitamin D_3, IU/kg				Probability, *P* <	
	1,500	3,000	6,000	SEM	Linear	Quadratic
Sow						
25(OH)D_3, ng/mL						
d 0	30.1	26.2	32.0	4.65	0.54	0.27
d 100	33.2	36.5	57.9	4.65	0.01	0.23
Farrowing	30.1	35.4	56.9	4.65	0.01	0.38
Weaning	39.3	52.5	66.3	4.65	0.01	0.31
Ca, mg/dL						
d 0	9.1	9.1	9.5	0.20	0.07	0.50
d 100	9.2	8.7	9.1	0.20	0.95	0.09
Farrowing	8.9	9.3	9.3	0.20	0.30	0.37
Weaning	9.3	9.5	9.4	0.20	0.94	0.41
P, mg/dL						
d 0	7.3	7.1	7.1	0.34	0.67	0.74
d 100	6.4	6.3	6.6	0.34	0.59	0.50
Farrowing	7.4	7.4	6.8	0.34	0.14	0.71
Weaning	6.5	6.1	6.6	0.34	0.64	0.24
Milk vitamin D_3, ng/g						
Farrowing	1.02	2.33	3.97	0.314	0.01	0.37
d 10	0.78	2.33	3.73	0.314	0.01	0.13
Weaning	1.02	1.98	3.53	0.314	0.01	0.73
Piglet						
25(OH)D_3, ng/mL						
birth	4.5	5.9	9.4	0.75	0.01	0.03
d 10	4.4	6.2	10.6	0.75	0.01	0.01
weaning	5.6	8.0	14.0	0.81	0.01	0.01
Ca, mg/dL						
birth	10.3	10.7	10.3	0.25	0.93	0.27
d 10	10.5	10.7	9.9	0.25	0.14	0.08
weaning	10.1	10.0	9.8	0.27	0.48	0.84
P, mg/dL						
birth	6.5	6.3	6.0	0.62	0.46	0.78
d 10	12.5	12.8	13.1	0.62	0.43	0.88
weaning	10.1	10.7	10.8	0.66	0.79	0.97
Bone ash content, %						
2^{nd} rib	43.6	43.6	43.5	0.80	0.95	0.96
Femur	44.9	44.5	44.8	0.55	0.76	0.66
Bone density, g/mL						
2^{nd} rib	1.30	1.30	1.31	0.017	0.64	0.56
Tissue vitamin D_3, ng/g						
Kidney	1.68	0.10	1.37	0.842	0.99	0.09
Liver	0.04	0.04	0.19	0.050	0.08	0.16

[1] A total of 84 sows (PIC 1050) and their litters were used to determine the effects of high maternal vitamin D_3 on sow and pig performance, serum 25(OH)D_3, Ca, P, milk vitamin D, neonatal bone mineralization, and tissue vitamin D.

[2] Day effects were $P < 0.01$, $P < 0.01$, $P = 0.18$, $P = 0.56$, $P < 0.01$, $P < 0.01$, and $P = 0.08$, for sow 25(OH)D_3, Ca, P, milk vitamin D, and piglet 25(OH)D_3, Ca, and P, respectively. Maternal × day interactions were $P < 0.01$, $P = 0.68$, $P = 0.33$, $P = 0.87$, $P = 0.13$, $P = 0.86$, and $P = 0.67$ for sow 25(OH)D_3, Ca, P, milk vitamin D, and piglet 25(OH)D_3, Ca, and P, respectively.

Table 2-6. The effects of maternal and early nursery dietary vitamin D_3 on nursery pig serum 25(OH)D_3, Ca, and P[1,2]

	Maternal vitamin D_3, IU/kg							*Probability, P <*			
	1,500		3,000		6,000			Maternal × Diet	Maternal		
Nursery vitamin D_3, IU/kg[3]	1,800	18,000	1,800	18,000	1,800	18,000	SEM	Interaction	Linear	Quadratic	Diet
25(OH)D_3, ng/mL											
d 0	6.3		10.5		17.6		3.09		0.01	0.91	
d 10	20.0	53.5	21.9	49.6	24.0	60.9	2.16	0.01	0.01	0.04	0.01
d 21	13.2	26.7	13.6	23.9	14.4	31.6	2.16	0.01	0.16	0.15	0.01
d 35	16.7	18.0	14.5	19.3	14.9	19.5	2.16	0.42	0.94	0.83	0.04
Ca, mg/dL											
d 0	10.7		10.7		11.1		0.27		0.32	0.65	
d 10	9.8	10.0	9.9	10.3	10.6	10.3	0.40	0.34	0.14	0.98	0.77
d 21	11.3	10.9	10.7	10.6	10.9	10.5	0.40	0.50	0.45	0.36	0.36
d 35	10.8	10.7	11.6	11.0	10.9	10.6	0.40	0.29	0.72	0.13	0.31
P, mg/dL											
d 0	12.0		12.2		11.6		0.31		0.36	0.30	
d 10	10.9	10.8	12.0	11.4	11.2	11.6	0.44	0.14	0.36	0.08	0.78
d 21	11.8	12.1	11.6	11.1	11.5	11.7	0.44	0.44	0.56	0.25	0.96
d 35	11.2	11.9	11.6	11.1	11.7	11.8	0.44	0.66	0.53	0.50	0.81

[1]A total of 180 mixed-sex pigs (PIC 327 × 1050; initially 21 d of age) were weaned from the 1st sow group and used in a 3 × 2 split plot design for 35 d to determine the effects of maternal and early nursery dietary vitamin D_3 on growth performance. Ten pigs/treatment were bled to determine serum 25(OH)D_3, Ca, and P.

[2] Day effects were $P < 0.01$, $P = 0.12$, $P < 0.01$ for serum 25(OH)D_3, Ca and P, respectively. Maternal × diet × day interactions were $P = 0.32$, $P = 0.96$, $P = 0.92$ for 25(OH)D_3, Ca, and P, respectively.

[3] Nursery vitamin D_3 were fed in Phase 1 diets from d 0 to 10. Common Phase 2 and 3 diets were fed from d 10 to 21 and d 21 to 35, respectively. Common diets were formulated to contain 1,800 IU/kg vitamin D_3.

Chapter 3 - The effects of high sulfate water on nursery pigs; and the efficacy of non-nutritive feed additives to influence those effects

Abstract

Two experiments were conducted to investigate the effects of high sulfate water from sodium sulfate and the efficacy of non-nutritive feed additives in nursery pig diets. In Exp. 1, 320 barrows (5.4 ± 0.1 kg BW and 21 d of age) were allotted to 1 of 8 treatments for 24 d in a 2 × 4 factorial with 2 levels of sulfate water (control or 3,000 mg/L added sodium sulfate), and 4 dietary zeolite (clinoptilolite) levels (0, 0.25, 0.50, or 1%). Fecal samples were collected on d 5, 9, 16, 23, visually scored for consistency (1= firm, 5= watery), and analyzed for DM. No interactions of sulfate × zeolite were observed for any response criteria. Overall (d 0 to 24), pigs drinking high sulfate water had decreased ($P < 0.01$) ADG, ADFI, and G:F compared to pigs drinking control water. Pigs drinking high sulfate water also had increased ($P < 0.01$) fecal scores and lower ($P < 0.04$) fecal DM on d 5, 9, and 16, compared to pigs drinking control water. Increasing dietary zeolite increased (linear, $P < 0.05$) ADG and ADFI, but had no effect on G:F. In Exp. 2, 350 barrows (5.7 ± 0.1 kg BW and 21 d of age) were allotted to 1 of 10 treatments in a 2 × 5 factorial for 21 d. There were 2 levels of sulfate water (control or 2,000 mg/L added sodium sulfate) and 5 dietary treatments (control, 1 or 2% zeolite, 1% humic acid substance, and 1% humic and fulvic acid substance). Fecal samples were collected on d 5, 8, 15, 21, visually scored for consistency (1= firm, 5= watery), and analyzed for DM. Overall (d 0 to 21), a water source × diet interaction was observed for ADG and G:F because pigs fed the 1% humic acid substance had decreased ($P < 0.01$) ADG and G:F when drinking high sulfate compared to other treatments, but increased ADG and G:F when drinking control water. Pigs drinking high sulfate water had decreased ($P < 0.01$) ADG and G:F, and tended ($P < 0.08$) to have decreased ADFI compared to pigs drinking control water. Pigs drinking high sulfate water had increased ($P < 0.01$) fecal scores and decreased ($P < 0.01$) fecal DM on d 5 and 8. In conclusion, water high in sulfate concentrations decreased growth performance and increased fecal moisture in newly weaned pigs. The non-nutritive feed additives used in both experiments were unsuccessful in ameliorating the increased osmotic diarrhea observed from high sulfate water, and although

zeolite improved growth performance in the 1st experiment, it did not influence growth in the second study.

Keywords: humic substances, nursery pigs, sulfate water, zeolite

Introduction

Water quality can be compromised with increased dissolved salt concentrations. The most common dissolved salts contaminating well water throughout North America are sulfates. A survey conducted by McLeese et al. (1991) indicated that over 25% of wells in Saskatchewan used for swine production have concentrations greater than 1,000 mg/L. Another survey in Ohio (Veenhuizen, 1993) concluded that wells ranged in concentrations of 6 to 1,629 mg/L sulfate, and that sulfate levels were correlated with geographic location, well depth, and total dissolved solids. The most common form of sulfate salts are magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4), with both acting similarly when at concentrations of 1,800 mg/L in the water supply of growing pigs (Veehuizen et al., 1992). At high concentrations (> 7,000 mg/L) the incidence of non-pathogenic diarrhea is increased and reduced performance is observed in young pigs (Anderson et al., 1994). Meanwhile, at lower concentrations (< 2,650 mg/L) researchers saw no reduction in growth performance (Patience et al., 2004) but diarrhea was still prevalent.

Nutritional therapies may be a potential way to reduce osmotic diarrhea from high sulfate water. Natural zeolite (clinoptilolite) is an alumino-silicate 3-dimensional structure known for its high absorption, cation exchange capability, and its ability to bind with water (Mumpton and Fishman, 1977). Humic substances are another natural feed additive that has been used in nursery diets to decrease the incidence and severity of diarrhea (Trckova et al., 2005). Humic substances are largely made up of humic acid, fulvic acid, and humin with several other minerals.

Therefore, the objectives of these experiments were to develop a high sulfate water induced osmotic diarrhea model, and to evaluate the efficacy of non-nutritive additives in reducing negative effects associated with the high sulfate water.

Materials and Methods

Experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. Both experiments were conducted at the Kansas State

University Segregated Early Weaning Research Facility in Manhattan, KS. Each pen (1.22 × 1.22 m) contained a 4-hole dry self-feeder and 1 cup waterer to provide ad libitum access to feed and water.

All diets in Exp. 1 and 2 were fed in 2 phases; with the same feed additive inclusion rates in both phases. The first phase diets were manufactured at Kansas State University Grain Science Feed Mill and were presented in a pelleted form. The second phase diets were manufactured at the Kansas State University Animal Science Feed Mill and were fed in a meal form. All diets were formulated to meet or exceed nutrient requirement estimates (NRC, 1998; Table 3-1). Samples of the control diets were collected at the beginning and end of each feeding phase and were sent with samples of feed additives to a commercial laboratory (Ward Laboratories, Inc., Kearney, NE) for proximate analysis of moisture (AOAC, 1990; method 935.29), crude protein (AOAC, 990.03), crude fat (ANKOM, 2004), and ash (AOAC, 942.05). Samples were also analyzed for Ca, P, K, Mg, Zn, Fe, Mn, Cu, S, Na, NaCl, and Cl using methods described by Johnson and Ulrich (1959; Table 3-2).

Experimental water treatments were achieved by mixing a stock solution of sodium sulfate (Na_2SO_4) water into the water supply (Municipal Water, Manhattan, KS) by medicator (Dosatron International Inc., Clearwater, FL) at a rate of 1:10. Samples collected from experimental water treatments were taken at the end of each feeding phase. These samples were refrigerated, and sent to a commercial laboratory (Servi-Tech Laboratories Inc., Dodge City, KS) for analysis of mineral content, pH, and electrical conductivity using methods described by Martin et al. (1994; method 200.7), Pfaff (1993; method 300.0), and the American Public Health Association (1999; SM 2510b, 4500 H^+). Calculations using electrical conductivity and sulfate sulfur values were used to determine total dissolved solids and sulfate concentrations (Table 3-3).

Fecal collections were conducted in both experiments to evaluate fecal moisture and consistency by visual score and to determine DM of fecal samples. Samples were collected by rectal massage from either 2 or 3 pigs per pen. Then 5 trained individuals, blinded to treatments, scored samples based on a visual moisture content using a numeric scale discussed by Smiricky et al. (2002) in which, 1 = hard, dry pellet; 2 = firm, formed stool; 3 = soft, moist stool that retains shape of container, 5 = watery liquid that can be poured. Afterwards, scores were

averaged to determine an average score for each pen. Fecal samples were then frozen at -20°C, until they were thawed and both partial and laboratory DM techniques (Undersander et al, 1993) were conducted. Partial dry matter was achieved by drying whole fecal samples at 50°C in a forced air drying oven for 24 h. Afterwards samples were cooled and weighed, then samples were ground and stored at -20°C until laboratory DM was achieved by weighing a 1 g subsample from each fecal sample and drying at 100°C in a forced air drying oven for 12 h.

Experiment 1

A total of 320 barrows (1050; PIC, Hendersonville, TN: initially 5.4 ± 0.1 kg BW and 21 d of age) were used in a 24-d growth experiment to evaluate the potential negative effects associated with the high sulfate water, and the ability of natural zeolite (Clinoptilolite), at different levels, to lessen those effects. Upon arrival to the facility (d 0), pigs were allotted to pens by BW, and pens were assigned to 1 of 8 treatments in a CRD. The 8 experimental treatments were arranged as a 2 × 4 factorial with 2 water treatments (none or water with 3,000 mg/L sodium sulfate; $NaSO_4$), and 4 dietary zeolite levels (0, 0.25, 0.5, and 1.0%). Water treatments remained the same from d 0 to 24. First phase diets were fed from d 0 to 10, and second phase diets were fed from d 10 to 24. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 5, 10, 17, and 24 of the trial. Fecal collections were performed on d 5, 9, 16, and 23.

Experiment 2

A total of 350 barrows (1050; PIC, Hendersonville, TN; initially 5.7 ± 0.1 kg BW and 21 d of age) were used in a 21 d study to evaluate the efficacy of natural zeolite and humic substances at alleviating the negative effects associated with high sulfate water. Upon arrival to the facility (d 0), pigs were allotted to pens by BW, and pens were assigned to 1 of 10 experimental treatments in a CRD. The 10 experimental treatments were arranged as a 2 × 5 factorial with 2 water treatments (none or water with 2,000 mg/L sodium sulfate), and 5 dietary regimens (control, 1 or 2% zeolite, 1% humic acid substance [HA], 1% humic and fulvic acid substance [HFA]). Water treatments remained the same from d 0 to 21. First phase diets were fed from d 0 to 8, and second phase diets were fed from d 8 to 21. Average daily gain, ADFI, G:F were determined.

Statistical Analysis

For both experiments, data were analyzed as a CRD using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Treatment means were analyzed using the LSMEANS statement and pre-planned CONTRAST statements in SAS, with barn location as a random effect. Fecal scores and fecal DM data were analyzed using the REPEATED function in SAS to determine the effects of treatment variables over time. In Exp. 1, pre-planned contrasts included control water vs. high sulfate water, linear and quadratic effects of increasing levels of dietary zeolite, and the interactions of water treatment and dietary zeolite treatment. The coefficients for the unequally spaced linear and quadratic contrasts were derived using the PROC IML procedure in SAS. For Exp. 2, pre-planned contrasts included control vs. high sulfate water, linear and quadratic effects of increasing dietary zeolite, control diet vs. 1% humic acid substance, control diet vs. 1% humic and fulvic acid substance, 1% zeolite vs. 1% humic acid substance, 1% zeolite vs. 1% humic and fulvic acid substance, 1% humic acid substance vs. 1% humic and fulvic acid substance, and the interactions of water treatments within each dietary treatment. Differences among treatments were considered significant with $P \leq 0.05$ and trends if $P > 0.05$ and ≤ 0.10.

Results

Experiment 1

During phase 1 (d 0 to 10), a water treatment × dietary zeolite interaction (linear, $P < 0.04$) was observed for ADFI (Table 3-4), which occurred because ADFI increased as dietary zeolite increased for pigs drinking high sulfate water, but decreased with increasing dietary zeolite for pigs drinking control water. No other interactions were observed for any response criteria. Sodium sulfate addition to the water and dietary zeolite did not influence ADG, ADFI, or G:F from d 0 to 10.

During phase 2 (d 10 to 24), increasing zeolite improved (linear, $P < 0.01$) ADG and ADFI with no effect on G:F. Also, ADG, ADFI, and G:F were worsened ($P < 0.02$) for pigs drinking high sulfate water compared to pigs drinking control water.

Overall (d 0 to 24), increasing zeolite increased (linear, $P < 0.05$) ADG and ADFI, but G:F was not affected. Pigs drinking high sulfate water had decreased ($P < 0.01$) ADG, ADFI, and G:F compared with pigs drinking control water.

For fecal moisture scores, a water × day interaction ($P < 0.01$) was observed because pigs drinking high sulfate water had decreasing fecal scores over time and fecal matter became firmer whereas, pigs drinking control water had consistent fecal scores throughout the length of the study. Pigs drinking high sulfate water had ($P < 0.01$) higher fecal moisture scores on d 5, 9, 16, 23, and for overall mean fecal moisture scores compared to pigs drinking control water (Table 3-5). Dietary zeolite did not influence fecal moisture score.

A water × day interaction ($P < 0.01$) was observed for fecal DM because DM increased over time for pigs drinking high sulfate water, and pigs drinking control water had consistent fecal DM throughout the length of the study. Pigs drinking high sulfate water had decreased fecal DM ($P < 0.04$) on d 5, 9, 16, and for overall mean fecal DM compared to pigs drinking control water (Table 3-6). Dietary zeolite did not affect fecal DM score.

Experiment 2

From d 0 to 8 (Phase 1), there was a tendency for a water × dietary treatment interaction for ADG ($P < 0.06$) because pigs fed the 1% HA diet had poorer ($P < 0.01$) ADG than other treatments when drinking high sulfate water, but improved ADG when drinking control water (Table 3-7). Also a water × dietary treatment interaction ($P < 0.01$) was observed for G:F because pigs fed the 1% HA diet had decreased ($P < 0.01$) G:F when drinking high sulfate water compared to other treatments, but improved G:F when drinking control water.

During the second phase (d 8 to 21), no water × dietary treatment interactions were observed, but pigs fed the 1% HA diet had decreased ($P < 0.01$) ADG and ADFI, and tended to have decreased ($P < 0.06$) G:F when drinking high sulfate water, compared to control water. Additionally, pigs consuming diets with 1% zeolite tended ($P < 0.09$) to have lower G:F when drinking high sulfate water compared to control water. Regardless of interactions, pigs drinking high sulfate water had decreased ($P < 0.05$) ADG, ADFI, and G:F compared to pigs drinking control water. No dietary treatment main effects were observed for growth performance from d 8 to 24, but there was a tendency ($P < 0.08$) for increasing zeolite to decrease ADFI.

For overall growth performance (d 0 to 21), water × dietary treatment interactions ($P < 0.03$) were observed for ADG and G:F because pigs fed the 1% HA diet had decreased ($P < 0.01$) ADG and G:F when drinking high sulfate compared to other treatments, but improved ADG and G:F when drinking control water. Pigs consuming the 1% HA diet had decreased ($P < 0.03$) ADFI when drinking high sulfate water compared to pigs drinking control water. For main effects, pigs drinking high sulfate water had poorer ($P < 0.01$) ADG and G:F, and a tendency ($P < 0.08$) for lower ADFI compared to pigs drinking control water. Dietary treatment did not affect overall growth performance.

A water × day interaction was observed ($P < 0.01$; Table 3-8) for fecal moisture scores because fecal scores decreased over time for pigs drinking high sulfate water, but were consistent for pigs drinking control water throughout the length of the study. On d 5, there was a tendency for a water × dietary treatment interaction ($P < 0.10$) for fecal moisture scores because pigs eating the 1% HA diet had greater ($P < 0.01$) differences between control water and high sulfate water compared to other dietary treatments and pigs eating 1% zeolite tended ($P < 0.06$) to have greater differences in scores between control water and high sulfate water compared to other dietary treatments. On d 8, a water × dietary treatment interaction ($P < 0.01$) was observed because pigs eating diets containing 1 or 2 % zeolite or 1% HFB had ($P < 0.03$) greater differences in fecal moisture scores between control water and high sulfate water compared to other dietary treatments. Mean fecal scores were lower ($P < 0.03$) for pigs fed diets containing 1 or 2% zeolite, 1% HA or 1% HFB when drinking control water compared to drinking high sulfate water. Pigs drinking control water had ($P < 0.01$) lower fecal moisture scores compared to pigs drinking high sulfate water on d 5 and 8, and for mean fecal scores. No main effects of dietary treatment were observed for fecal moisture scores, except for a tendency (linear, $P < 0.09$) on d 8 for increasing zeolite to decrease fecal moisture score. These differences were most evident for pigs drinking control water (3.3, 2.8, and 2.7 for control, 1 and 2% zeolite treatments respectively), however pigs drinking high sulfate water were more variable in their respective fecal scores (3.3, 3.7, and 3.4 for control, 1 and 2% zeolite treatments respectively).

A water × day interaction was observed ($P < 0.01$) for fecal DM because pigs drinking high sulfate water had increasing fecal DM over time; whereas, pigs drinking control water had consistent fecal DM throughout the length of the study. Within d 5 fecal samples, pigs eating the diet with 1% HA had lower ($P < 0.03$) fecal DM when drinking high sulfate compared to

drinking control water (Table 3-9). On d 8, a water × dietary treatment interaction was observed ($P < 0.01$) because pigs consuming diets with 1 or 2% zeolite or 1% HFB had decreased ($P < 0.04$) fecal DM when drinking high sulfate water compared to other treatments, but had higher fecal DM when drinking control water. For mean fecal DM, pigs eating diets containing 2% zeolite or 1% HA diets had decreased ($P < 0.03$) fecal DM when drinking high sulfate water compared to control water, and pigs consuming 1% zeolite tended ($P < 0.08$) to have lower fecal DM when drinking high sulfate compared to control water. Nevertheless, pigs drinking high sulfate water had decreased ($P < 0.01$) fecal DM on d 5, 8, and for mean fecal DM compared to pigs drinking control water. Within d 8, increasing zeolite tended (linear, $P < 0.08$) to increase fecal DM, this is mainly due to the magnitude of difference observed for pigs drinking control water (23.1, 26.7, and 28.7% DM for control 1 and 2% zeolite respectively), however for pigs drinking high sulfate water (22.3, 18.8, 22.1% DM for control, 1 and 2% zeolite respectively) treatment differences were not as evident. For mean fecal scores, a diet effect ($P < 0.02$) was observed because increasing zeolite increased (linear, $P < 0.01$) fecal DM, again these differences were most evident for pigs drinking control water (23.1, 24.3, 26.4% DM for control, 1 and 2% zeolite respectively) compared to those drinking high sulfate water (22.7, 22.5, 23.7% DM for control, 1 and 2% zeolite). Additionally, pigs fed the 1% HFB diet had higher ($P < 0.01$) and tended to have higher ($P < 0.06$) fecal DM than pigs fed control and 1% zeolite diets respectively.

Fecal scoring techniques used in these experiments were performed as a quick tool to determine visual fecal moisture. We were interested in whether they were as effective at predicting differences as typical DM techniques. Based on correlations, scoring was an effective predictor of fecal moisture content (as measured by fecal DM) during collections conducted in the first feeding phase (d 5 and 9 in exp. 1, and d 5 and 8 in exp. 2), but were not accurate predictors in the second phase (d 16 and 23 in Exp. 1; d 15 and 21 in Exp. 2; Figure 3-1 and Figure 3-2).

Discussion

Maximum water sulfate recommended levels by the NRC (1998) for livestock are 1,000 mg/L. Water analysis conducted for the current studies showed sulfate levels of 2,000 (Exp. 1) and 1,700 mg/L (Exp. 2) for experimental treatments when sodium sulfate was added to the

water supply at rates of 3,000 and 2,000 mg/L respectively, compared to control water concentrations of approximately 80 mg/L sulfate in both trials. Total dissolved solids in the trials were 2,800 and 1,770 for Exp. 1 and 2, respectively, which are under the recommended maximum level of 3,000 mg/L. Work by Stothers and Palmer (1961) concluded that water with the same TDS but containing chloride rather than sulfate did not reduce performance or caused excessive diarrhea, therefore sulfates may be a better estimating compound compared to TDS alone.

The weaning process triggers distinct changes in the digestive tract of young pigs (Boudry et al. 2004). Postweaning diarrhea may be the result of these gastrointestinal alterations, but it can be exacerbated by other stressors (Pluske et al, 1997). Sulfates have been found to be poorly digested in the large intestine and can cause disruption in water and electrolyte absorption. Additionally, Roth and Crittendon (1934) proposed that sulfates are cathartic agents and help speed up the passage rate through the large intestine. In the current studies, sulfates exacerbated diarrhea up to 16 d after weaning as measured by fecal DM in Exp. 1, but visual scoring suggested higher moisture content on all fecal collection days (5, 9, 15, and 23). In Exp. 2, decreased fecal DM and increased fecal moistures scores were observed up to 8 d postweaning. Similar results have been found in previous studies (Anderson and Stothers, 1978; Paterson et al., 1979; McLeese et al., 1992), which show that initially weaned pigs have increased diarrhea, but over time negative effects of high water sulfates are reduced. This may be the result of the young pig's gastrointestinal maturity and ability to adapt to higher sulfate levels. Paterson et al. (1979) and Anderson et al. (1993) have shown that sows and finishing pigs are able to tolerate higher levels of sulfates compared to weaned pigs with no influence on performance or diarrhea. Based on the fecal moisture scores from the current studies it could be concluded that pigs adapted faster to sulfate levels supplied in Exp. 2 than in Exp. 1.

Overall growth performance was negatively influenced with increased sulfate concentrations in both experiments. Average daily gain was decreased by 11% and 8%, ADFI decreased 6% and 4%, and G:F was 4% lower in both Exp. 1 and 2, respectively. A decrease in performance was found with sulfate concentrations of 4,880 mg/L by Stothers and Palmer (1961). Additionally, Stothers (1970) and Anderson and Stothers (1978) observed trends for decreases in ADG, ADFI and G:F when pigs received higher saline water in the form of sulfates,

but these differences were not significant, perhaps due to small sample sizes. McLeese et al. (1992) observed decreases in ADG and G:F in weaned pigs drinking 2,650 mg/L sulfate water, but when medications were introduced into the diet, growth performance was not affected. A potential explanation of this response to antibiotics may be due to a reduction in mucosal lining damage and immune activation that has been found with increased concentrations of sulfate in the lower bowel (Argenzio and Whipp, 1980), or a decrease in pathogenic bacteria proliferation. Interestingly, diarrhea was still observed even with medication in the diet which shows that antibiotics did not compensate for osmotic imbalances resulting in decreased electrolyte and water absorption. Patience et al., (2004) found no effect of poor quality water with high sulfate concentrations on growth performance of weaned pigs raised in commercial settings; however, diarrhea occurrences were not measured, and complete diet compositions were not provided.

Variations in results have been found in swine growth studies when zeolite is added to the diet of swine (Shurson et al., 1984). Mumpton and Fishman, (1977) described a relationship of zeolite's growth promoting level to be based on its properties, source, and the amount supplemented in the diet. Zeolite, like many other clay based feed additives has been shown to adsorb aflatoxins and mitigate effects found in contaminated feeds (Ramos et al., 1996). In the current studies, we utilized a single source of natural zeolite at different levels in the diets. For Exp. 1, an observed linear increase in ADG and ADFI were found when levels up to 1% zeolite were fed. As a follow up, levels of zeolite to be tested in the second experiment were set at 1 and 2% of the diet, which showed no differences in growth performance criteria. In both studies, zeolites proved to be ineffective in improving fecal consistency scores, but in Exp. 2 fecal DM was increased with increasing zeolite inclusion, however based on magnitude of differences, greater improvements were observed in pigs drinking control water compared to those consuming high sulfate water (3.4% vs. 1.0% in control water and high sulfate water respectively for mean fecal DM scores). The variation in growth responses and the inability of natural zeolite to improve fecal consistency suggest that when weaned pigs are under normal conditions and provided poor quality water, zeolites are ineffective as additives.

Two forms of humic substances (peat) were utilized in Exp. 2. The first substance was high in humic acid, and the second was a blended product with both humic and fulvic acid. Different sources of humic substances can result in a variety of compositions, which are typically

a result of their humic:fulvic acid ratios, humin content, and mineral content. For classification humic acids within these substances are defined as aromatic polyfunctional compounds with medium to high molecular weight. Fulvic acids are of similar composition as humic acids but have lower molecular weights (Janos, 2003). Because of the hydrophilic nature of peat it was believed to help reduce litter build up when included in turkey feeds (Enueme et al., 1987). Interestingly, ADG was reduced (12% compared to control diet) in Exp. 2 for pigs consuming 1% humic acid substance diets and drinking 2,000 mg/L sodium sulfate, but improved (11% compared to control diet) when pigs were drinking control water and fed the same diet. Perhaps there is an interaction of sulfates and humic acid in the intestine that negatively influence normal digestive function, and ultimately growth performance, but no work has previously been done with the two substances in swine. Inclusion of 1% of the humic and fulvic acid blended product did not affect growth performance. In contrast, Ji et al (2005) observed improvements in ADG and G:F with 2 humic substances that were similar in composition to the humic and fulvic acid blended product used in this study, but these advantages were observed with inclusion rates of 0.5% in diets and when pigs were in finishing phases of production. Fecal consistency scores were not improved with the inclusion of either humic substance compared to the control diets, but again interactions of sulfate and humic substances were observed for mean fecal scores and in some fecal collection days. Fecal DM was inconsistently impacted by the inclusion of humic substances based on interactions associated with collection day, or with water treatments. The inability of the humic and fulvic acid blended product to increase growth performance and inability to consistently improve fecal scores or DM of weaned pigs suggest it is not an effective additive at a 1% inclusion rate. The same can be said for the humic acid substance that was tested and its negative interactions that were observed with the sulfate water treatment. Because little published work looking at the effects of humic substances as additives in swine diets has been conducted, it may be an area in need of further research, not only evaluating ideal inclusion rates but also to determine production periods where its inclusion is beneficial.

In conclusion, water high in sulfates caused decreased performance and increased diarrhea compared to control water when supplied to weaned pigs. The use of non-nutritive adsorbent ingredients (natural zeolite, and humic substances), for pigs receiving high sulfate water, was ineffective in mitigating the negative responses observed from high sulfate water. However; more work testing nutritional therapies in a sulfate challenge model used in the present

experiments may help to identify beneficial ingredients that can improve osmotic diarrhea and growth performance in the weaned pig.

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Tables and Figures

Table 3-1. Composition of diets, Exp. 1 and 2 (as-fed basis)

Item		
Ingredient, %	Phase 1[1]	Phase 2[2]
Corn	38.16	57.06
Soybean meal (46.5% CP)	16.99	25.90
Dried distillers grains with solubles	5.00	---
Spray-dried animal plasma	4.00	---
Select menhaden fish meal	---	4.50
Spray-dried blood cells	1.25	---
Spray dried whey	25.00	10.00
Dried porcine solubles[3]	3.00	---
Monocalcium P (21% P)	0.85	0.38
Limestone	0.85	0.58
Salt	0.30	0.30
Zinc oxide	0.39	0.25
Trace mineral premix	0.15	0.15
Vitamin premix	0.25	0.25
L-Lys HCl	0.20	0.25
DL-Met	0.13	0.13
L-Thr	0.08	0.11
Phytase[4]	0.13	0.17
Acidifier[5]	0.20	---
Vitamin E, 20,000 IU	0.05	---
Choline chloride 60%	0.04	---
Zeolite (clinoptilolite)[6]	---	---
HA[7]	---	---
HFB[8]	---	---
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible amino acids, %		
Lys	1.35	1.30
Ile:Lys	54	61
Leu:Lys	132	127
Met:Lys	30	35
Met & Cys:Lys	57	59
Thr:Lys	65	63

Trp:Lys	18	17
Val:Lys	72	68
Total Lys, %	1.51	1.43
CP, %	21.6	21.3
ME, kcal/kg	3,414	3,311
Ca, %	0.75	0.70
P, %	0.73	0.63
Available P, %	0.65	0.47
Na, %	0.75	0.25
K, %	1.07	0.97
Added trace minerals, ppm		
Zn	2,973	1,965
Fe	165	165
Mn	40	40
Cu	17	17
I	0.3	0.3
Se	0.3	0.3

[1] Phase 1 diets were fed from d 0 to 10 in Exp. 1, and d 0 to 8 in Exp. 2.

[2] Phase 2 diets were fed from d 10 to 24 in Exp. 1, and d 8 to 21 in Exp. 2.

[3] DPS-50, Nutra-Flo Company, Sioux City, IA.

[4] Natuphos 600, BASF, Florham Park, NJ. provided 354 and 446 FTU/lb of diet, respectively.

[5] Kem-gest, Kemin Industries Inc., Des Moines, IA

[6] Used in Exp. 1 and 2, St. Cloud Mining Company, Truth or Consequences, NM, replaced corn to provide 0, 0.25, 0.50 and 1% zeolite.

[7] Exp. 2, 1% humic acid substance (DPX 5800, Humatech Inc., Houston, TX) was added to the control diet.

[8] Exp. 2, 1% humic and fulvic acid substance (DPX 7702, Humatech Inc., Houston, TX) was added to the control diet.

Table 3-2. Proximate and mineral analysis of control diets and feed additive ingredients[1]

	Exp. 1[2]		Exp. 2[3]		Ingredient		
	Phase 1	Phase 2	Phase 1	Phase 2	Zeolite[4]	HA[5]	HFA[6]
Item, %							
Moisture	8.2	9.1	9.2	8.9	4.1	12	8.7
CP	20.9	22.5	21.3	21.4	0.1	4.7	2.8
Ash	---	---	---	---	91.2	24.8	55.26
Fat (oil)	4.8	2.5	4.8	2.6	---	---	---
Ca	0.93	0.75	0.81	1.00	1.79	0.47	0.56
P	0.80	0.66	0.70	0.69	0.05	0.05	0.05
K	1.18	1.09	1.21	1.07	0.86	0.07	0.09
Mg	0.17	0.17	0.15	0.17	0.44	0.06	0.13
S	0.44	0.28	0.45	0.29	0.05	0.32	0.29
Na	0.64	0.20	0.60	0.28	0.17	0.08	0.4
NaCl	1.12	0.66	1.07	0.93	0.04	0.03	0.03
Cl	0.68	0.40	0.65	0.56	0.02	0.02	0.02
Item, ppm							
Zn	2,966	1,297	2,909	2,243	45	40	79
Cu	32	15	20	27	7	14	14
Fe	593	249	414	414	6,078	5,767	9,265
Mn	117	54	80	87	255	121	148

[1] All samples were sent to Ward laboratories Inc., Kearney, NB. Values are means of 2 samples collected at the beginning or end of each feeding phase, or 2 subsamples from each additive ingredient.

[2] Phase 1 diets were fed from d 0 to 10 in a pelleted form, and phase 2 diets were fed from d 10 to 24 in a meal form.

[3] Phase 1 diets were fed from d 0 to 8 in a pelleted form, and phase 2 diets were fed from d 8 to 21 in a meal form.

[4] One source of zeolite was used for both experiments, St Cloud Mining Inc., Truth or Consequences, NM.

[5] Humic acid substance, DPX 5800, Humatech Inc., Houston, TX.

[6] Humic and fulvic acid blended substance, DPX 7702, Humatech Inc., Houston TX.

Table 3-3. Analyzed composition of water[1]

	Exp. 1[2]		Exp. 2[3]	
Item, g/L	Control water	3,000 g/L sodium sulfate	Control water	2,000 g/L sodium sulfate
TDS	321	2,773	233	1,770
SO_4	84	2,002	77	1,700
SO_4 -S	28	660	26	565
Cl	65	49	51	39
Na	38	750	34	565
Ca	25	26	13	14
Mg	12	12	10	10
K	6	7	6	6
Fe	0.06	0.1	0.1	0.1
Mn	0.01	0.01	0.01	0.01
pH, units	9.1	9.0	8.8	8.7
Electrical conductivity, µmho/cm	502	4,320	363	2,760

[1] Samples were analyzed by Servi-tech Laboratories, Dodge City, KS.

[2] Two samples were collected on d 10 and 24, and values are the mean of the sample analysis.

[3] Two samplers were collected on d 8 and 21, and values are the mean of the sample analysis.

Table 3-4. Effects of sulfate water and dietary zeolite on early nursery pig growth performance[1]

Water	Dietary	d 0 to 10			d 10 to 24			d 0 to 24		
sulfate, g/L	zeolite, %	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F	ADG, g	ADFI, g	G:F
0	0	167	166	1.01	354	497	0.71	276	359	0.77
	0.25	163	162	1.01	370	524	0.71	284	373	0.76
	0.50	151	150	0.99	388	530	0.73	283	364	0.78
	1.00	143	150	0.94	409	543	0.75	291	370	0.79
2,000	0	127	138	0.87	309	442	0.70	229	311	0.73
	0.25	168	162	1.03	324	465	0.69	259	339	0.76
	0.50	151	153	1.00	352	508	0.69	268	360	0.74
	1.00	147	163	0.90	349	508	0.69	265	364	0.73
SEM		12.9	8.8	0.053	19.9	22.2	0.018	12.5	14.7	0.015
		Probability, P <								
Interactions										
Sulfate × zeolite linear		0.17	0.04	0.24	0.68	0.49	0.14	0.43	0.08	0.18
Sulfate × zeolite quadratic		0.21	0.34	0.18	0.65	0.66	0.94	0.25	0.37	0.30
Main effects										
Sulfate		0.40	0.62	0.36	0.01	0.01	0.02	0.01	0.01	0.01
Zeolite linear		0.51	0.90	0.97	0.01	0.01	0.32	0.05	0.02	0.85
Zeolite quadratic		0.31	0.90	0.12	0.39	0.21	0.86	0.20	0.23	0.43

[1] A total of 320 weanling pigs (PIC 1050 barrows, initial BW of 11.9 lb and 21 d of age) were used with 5 pigs per pen and 8 pens per treatment.

Table 3-5. Effects of sulfate water and dietary zeolite on fecal consistency scores[1,2,3,4,5]

Water sulfate, g/L	Dietary zeolite, %	Day of collection				Mean
		5	9	16	23	
0	0	3.4	3.4	3.3	3.3	3.4
	0.25	3.3	3.3	3	3.2	3.2
	0.50	3	3.4	3.3	3.5	3.3
	1.00	3.2	3.3	3.2	3.2	3.2
2,000	0	4.1	4	3.6	3.7	3.9
	0.25	4.1	4	3.9	3.6	3.9
	0.50	4.1	4.4	3.5	3.6	3.9
	1.00	4.1	4	3.5	3.4	3.8
SEM		0.13	0.13	0.13	0.13	0.07
		Probability, P <				
Interactions						
Sulfate × zeolite linear		0.58	0.68	0.44	0.5	0.23
Sulfate × zeolite quadratic		0.26	0.12	0.72	0.53	0.8
Main effects						
Sulfate		0.01	0.01	0.01	0.01	0.01
Zeolite linear		0.55	0.74	0.37	0.25	0.14
Zeolite quadratic		0.38	0.18	0.79	0.64	0.75

[1] A total of 792 fecal samples were collected (192 per collection day, fecal samples were collected on d 5, 9, 16, and 23). 3 samples were taken per pen and were scored by 5 trained individuals, those 15 scores were then averaged and reported as pen means for each collection day.

[2] Samples were collected from 3 random pigs per pen, and samples were scored on a numerical scale from 1 to 5 and were scored by 5 trained individuals.

[3] Scoring scale guidelines: 1 = dry firm pellet, 2 = firm formed stool, 3 = soft stool that retains shape, 4 = soft unformed stool that takes shape of container, 5 = watery liquid that can be poured.

[4] Water × diet × day interaction ($P = 0.18$).

[5] Day effect ($P < 0.01$).

Table 3-6. Effects of sulfate water and dietary zeolite on fecal DM[1,2,3,4]

Water sulfate, g/L	Dietary zeolite, %	Day of collection				Mean
		5	9	16	23	
0	0	21.4	23.9	25.6	24.6	23.9
	0.25	21.0	25.0	26.4	25.8	24.6
	0.50	23.5	25.2	24.6	21.9	23.8
	1.00	23.1	26.2	26.0	25.7	25.3
2,000	0	13.5	19.0	25.6	21.9	20.0
	0.25	12.7	18.0	20.9	23.9	18.9
	0.50	14.0	17.0	24.4	24.3	19.9
	1.00	13.2	19.8	23.7	24.6	20.4
SEM		0.01	0.01	0.01	0.01	0.01
		Probability, P <				
Interactions						
Sulfate × zeolite linear		0.41	0.64	0.85	0.43	0.73
Sulfate × zeolite quadratic		0.87	0.24	0.61	0.14	0.86
Main effects						
Sulfate		0.01	0.01	0.04	0.39	0.01
Zeolite linear		0.39	0.22	0.88	0.27	0.13
Zeolite quadratic		0.71	0.39	0.29	0.72	0.34

[1] A total of 792 fecal samples were collected (192 per collection day, fecal samples were collected on d 5, 9, 16, and 23).

[2] Samples were collected from 3 random pigs per pen.

[3] Water x diet x day Interaction ($P = 0.41$).

[4] Day main effect ($P < 0.01$).

Table 3-7. Influence of dietary natural zeolite or humic acid substances (HA and HFB) and high-sulfate water on nursery pig performance[1]

Water sodium	Dietary	d 0 to 8			d 8 to 21			d 0 to 21		
sulfate, ppm	regimen	ADG, lb	ADFI, lb	G:F	ADG, lb	ADFI, lb	G:F	ADG, lb	ADFI, lb	G:F
0	Control	128	136	0.92	360	529	0.68	268	374	0.72
	1% zeolite	140	140	1.00	356	514	0.69	274	372	0.74
	2% zeolite	121	122	0.97	328	488	0.67	248	347	0.71
	1% HA	157	128	1.29	389	545	0.71	300	386	0.78
	1% HFB	142	147	0.96	357	521	0.69	274	377	0.73
2,000	Control	150	142	1.06	338	514	0.65	264	369	0.71
	1% zeolite	142	135	1.04	317	494	0.64	249	353	0.70
	2% zeolite	134	131	0.99	340	491	0.70	262	354	0.74
	1% HA	102	130	0.80	307	473	0.65	229	342	0.67
	1% HFB	119	142	0.84	344	507	0.68	255	363	0.70
SEM		15.1	12.1	0.095	18.6	19.4	0.023	13.4	14.0	0.021
Interactions		*Probability, P <*								
Sulfate × diet		0.06	0.90	0.01	0.11	0.33	0.25	0.02	0.41	0.02
Sulfate within control		0.26	0.66	0.27	0.37	0.56	0.33	0.80	0.76	0.91
Sulfate within 1% zeolite		0.95	0.67	0.72	0.12	0.44	0.09	0.16	0.31	0.22
Sulfate within 2% zeolite		0.52	0.49	0.86	0.60	0.91	0.43	0.43	0.70	0.33
Sulfate within 1% HA		0.01	0.85	0.01	0.01	0.01	0.04	0.01	0.03	0.01
Sulfate within 1% HFB		0.24	0.68	0.31	0.59	0.61	0.75	0.28	0.45	0.32
Main effects										
Sulfate		0.35	0.83	0.15	0.01	0.05	0.05	0.01	0.08	0.02
Diet		0.81	0.34	0.57	0.82	0.48	0.86	0.91	0.54	0.95
Dietary comparisons										
Zeolite linear		0.40	0.21	0.97	0.40	0.08	0.40	0.37	0.12	0.50
Zeolite quadratic		0.52	0.58	0.67	0.75	0.92	0.63	0.94	0.90	0.95
Control vs. 1% HA		0.49	0.31	0.54	0.97	0.49	0.51	0.92	0.59	0.70
Control vs. 1% HFB		0.53	0.58	0.33	0.93	0.68	0.50	0.88	0.90	0.98
1% zeolite vs. 1% HA		0.41	0.39	0.79	0.52	0.80	0.51	0.78	0.90	0.91
1% zeolite vs. 1% HFB		0.44	0.48	0.19	0.43	0.58	0.50	0.81	0.58	0.76
1% HA vs. 1% HFB		0.94	0.12	0.12	0.90	0.77	1.00	0.96	0.67	0.69

[1] A total of 350 weanling pigs (PIC 1050 barrows, initially 12.5 lb and 21 d of age) were used with 5 pigs per pen and 7 pens per treatment.

Table 3-8. Influence of dietary natural zeolite or humic acid substances (HA and HFB) and high-sulfate water on nursery pig fecal consistency[1,2,3]

Water sodium sulfate, ppm	Dietary regimen	Day of collection				
		5	8	15	21	Mean
0	Control	3.4	3.3	3.4	3.4	3.4
	1% zeolite	3.4	2.8	3.3	3.4	3.2
	2% zeolite	3.5	2.7	3.1	3.4	3.2
	1% HA	3.3	3.1	3.3	3.4	3.3
	1% HFB	3.4	3.1	3.2	3.4	3.3
2,000	Control	3.7	3.3	3.3	3.4	3.4
	1% zeolite	3.8	3.7	3.4	3.4	3.6
	2% zeolite	3.7	3.4	3.4	3.3	3.4
	1% HA	3.8	3.3	3.3	3.5	3.5
	1% HFB	3.6	3.5	3.4	3.6	3.5
SEM		0.15	0.15	0.15	0.15	0.08
		Probability, P <				
Interactions						
Sulfate × diet		0.10	0.01	0.83	0.97	0.23
Sulfate within control		0.13	0.83	0.42	0.69	0.78
Sulfate within 1% zeolite		0.06	0.01	0.65	0.96	0.01
Sulfate within 2% zeolite		0.28	0.01	0.23	0.71	0.01
Sulfate within 1% HA		0.01	0.21	0.93	0.74	0.03
Sulfate within 1% HFB		0.30	0.03	0.16	0.28	0.01
Main effects						
Sulfate		0.01	0.01	0.30	0.79	0.01
Diet		0.99	0.40	0.95	0.88	0.58
Diet comparisons						
Zeolite linear		0.85	0.09	0.48	0.73	0.20
Zeolite quadratic		0.82	0.43	0.65	0.63	0.33
Control vs. 1% HA		0.98	0.55	0.76	0.64	0.81
Control vs. 1% HFB		0.88	0.94	0.76	0.52	0.96
1% zeolite vs. 1% HA		0.76	0.66	0.73	0.82	0.67
1% zeolite vs. 1% HFB		0.66	0.92	0.73	0.69	0.89
1% HA vs. 1% HFB		0.90	0.59	0.99	0.87	0.77

[1] A total of 560 fecal samples were collected (140 per collection day; fecal samples were collected on d 5, 8, 15, and 21). Two samples were taken per pen and scored by 5 trained individuals. The 10 scores were then averaged and reported as pen means for each collection day.

[2] Scoring scale guidelines: 1 = dry, firm pellet; 2 = firmly formed stool; 3 = soft stool that retains shape; 4 = soft, unformed stool that takes shape of container; 5 = watery liquid that can be poured.

[3] Water × diet × day interaction ($P = 0.45$), water × day interaction ($P < 0.01$), diet × day ($P = 0.99$), day effect ($P < 0.01$).

Table 3-9. Influence of dietary natural zeolite or humic acid substances (HA and HFB) and high-sulfate water on nursery pig fecal DM[1,2,3]

Water sodium sulfate, ppm	Dietary regimen	Day of collection				
		5	8	15	21	Mean
0	Control	20.5	23.1	22.7	26.0	23.1
	1% zeolite	21.6	26.7	23.8	25.2	24.3
	2% zeolite	23.1	28.7	26.7	27.1	26.4
	1% HA	23.2	25.6	24.6	27.5	25.2
	1% HFB	22.7	26.5	26.9	26.8	25.7
2,000	Control	18.3	22.3	23.8	26.5	22.7
	1% zeolite	19.4	18.8	24.6	27.0	22.5
	2% zeolite	20.5	22.1	24.8	27.4	23.7
	1% HA	18.3	22.7	25.1	25.3	22.8
	1% HFB	20.7	22.0	24.9	28.3	24.0
SEM		1.70	1.70	1.70	1.70	0.92
		Probability, P <				
Interactions						
Sulfate × diet		0.19	0.01	0.73	0.93	0.60
Sulfate within control		0.32	0.70	0.63	0.82	0.74
Sulfate within 1% zeolite		0.30	0.01	0.69	0.42	0.08
Sulfate within 2% zeolite		0.24	0.01	0.38	0.88	0.01
Sulfate within 1% HA		0.03	0.19	0.83	0.32	0.03
Sulfate within 1% HFB		0.35	0.04	0.36	0.48	0.11
Main effects						
Sulfate		0.01	0.01	0.76	0.70	0.01
Diet		0.50	0.35	0.40	0.84	0.02
Diet comparisons						
Zeolite linear		0.12	0.08	0.11	0.52	0.01
Zeolite quadratic		0.94	0.34	0.83	0.61	0.38
Control vs. 1% HA		0.38	0.36	0.31	0.93	0.15
Control vs. 1% HFB		0.13	0.31	0.09	0.40	0.01
1% zeolite vs. 1% HA		0.86	0.39	0.68	0.84	0.41
1% zeolite vs. 1% HFB		0.42	0.34	0.28	0.34	0.06
1% HA vs. 1% HFB		0.54	0.95	0.51	0.46	0.30

[1] A total of 560 fecal samples were collected (140 per collection day; fecal samples were collected on d 5, 8, 15, and 21). Two samples were taken per pen and were scored by 5 trained individuals. The 10 scores were then averaged and reported as pen means for each collection day.

[2] Scoring scale guidelines: 1 = dry, firm pellet; 2 = firmly formed stool; 3 = soft stool that retains shape; 4 = soft, unformed stool that takes shape of container; 5 = watery liquid that can be poured.

[3] Water × diet × day interaction ($P = 0.69$), water × day interaction ($P < 0.01$), diet × day ($P = 0.99$), day effect ($P < 0.01$).

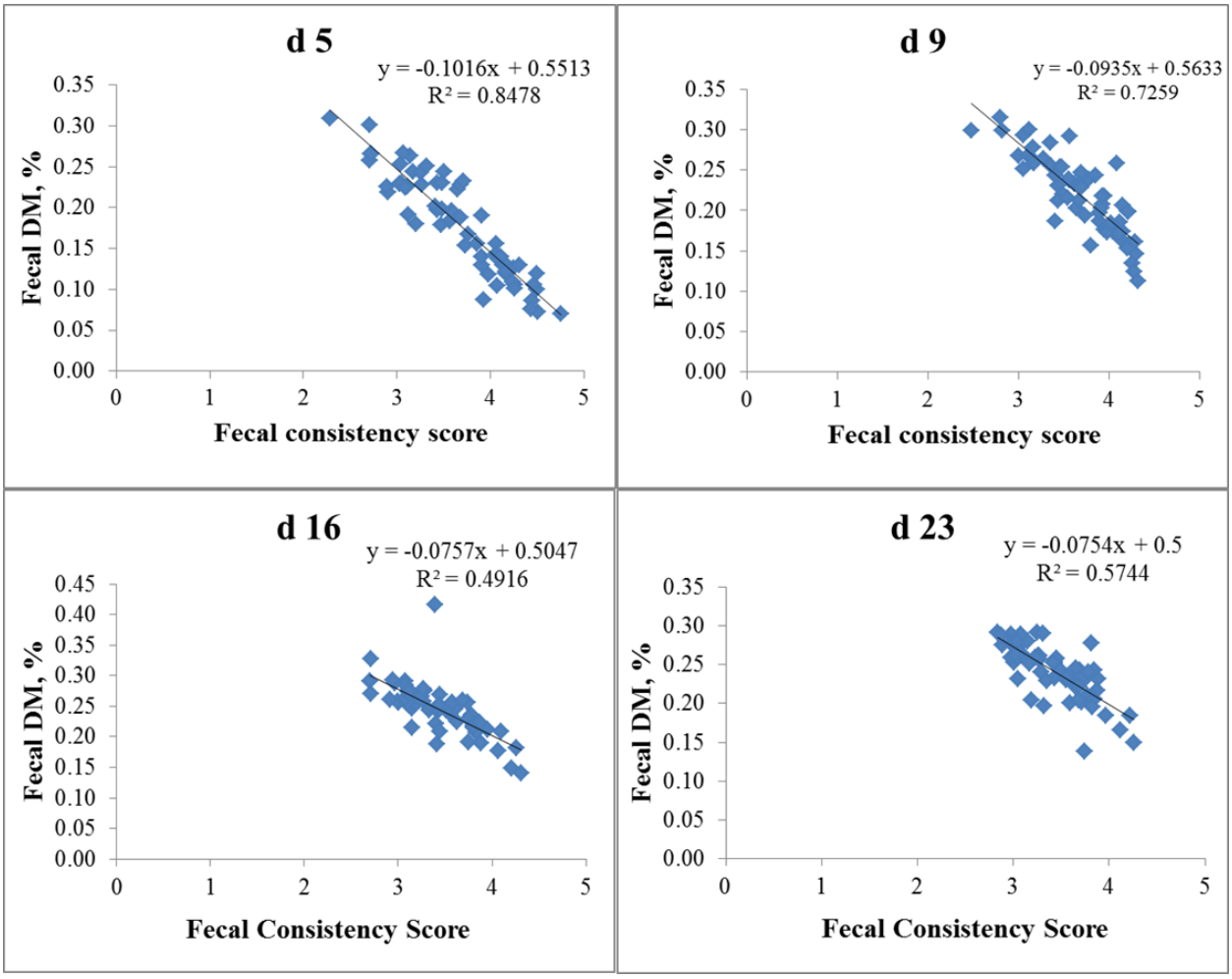

Figure 3-1. Correlation of fecal score to fecal DM, Exp. 1

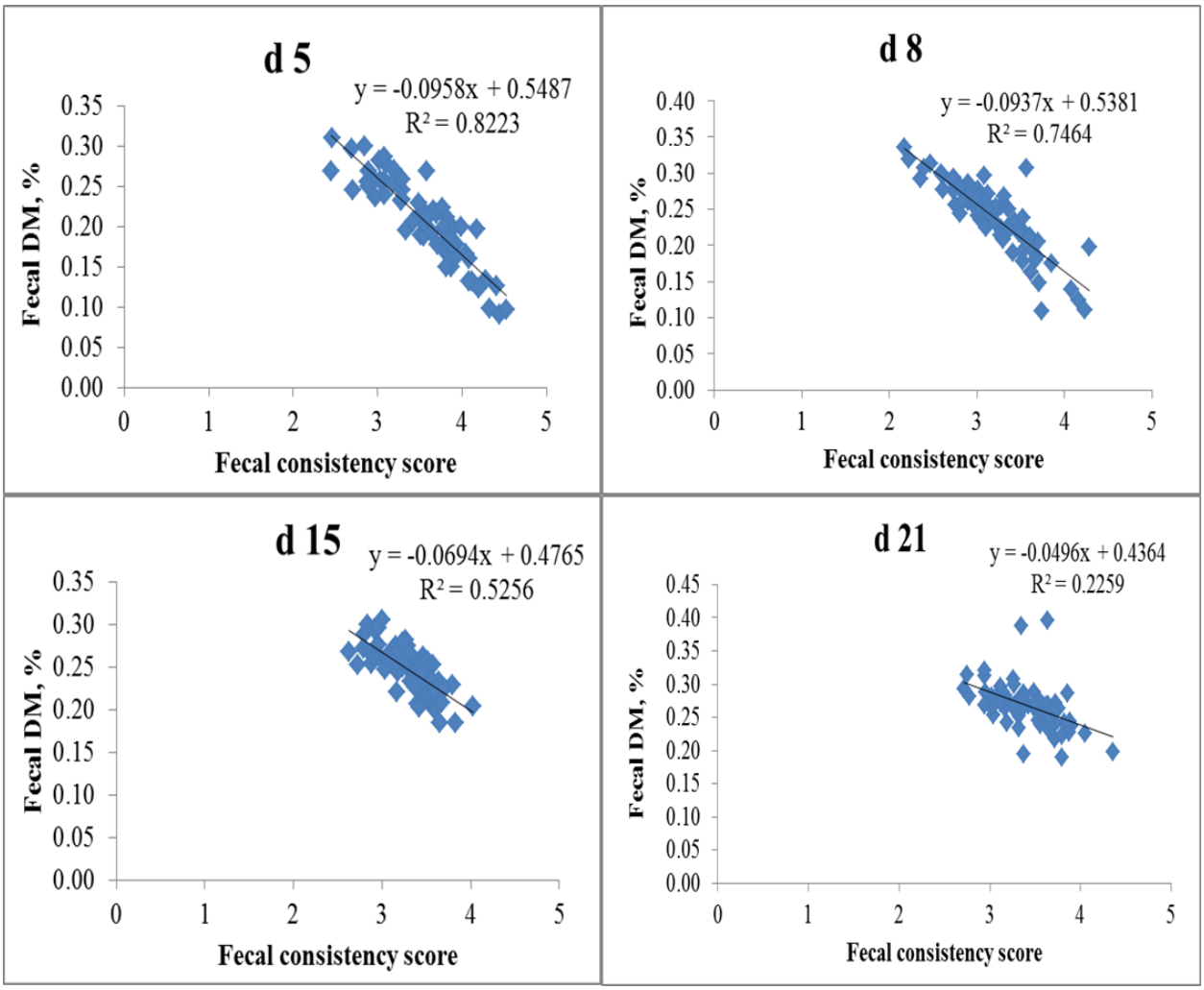

Figure 3-2. Correlation of fecal score to fecal DM, Exp. 2

Chapter 4 - Feed Efficiency of Swine – A Survey of Current Knowledge

Abstract

Pork producers and advisers to the swine industry were surveyed about their knowledge of feed efficiency. The questionnaire was designed to accomplish three objectives: (a) determine the level of knowledge related to feed efficiency topics, (b) identify production practices being used that influence feed efficiency, and (c) identify information gaps or areas requiring additional knowledge to further improve feed efficiency. Results suggest that many practices that improve feed efficiency are utilized in production, but gaps in information and knowledge exist across demographics of the industry. Extension education should be expanded to provide more information in an easy-to-access format for the swine industry.

Keywords: feed efficiency, survey, swine

Introduction

Feed represents the largest input expense for U.S. pork producers, usually totaling more than 60% of the total cost of production (Reese et al., 2010). Increased non-feed use for the U.S. corn crop (Westcott, 2012) has led to distinct rises in prices and crop supply fluctuations add to the variability in ingredient costs. Nationwide, whole-herd feed conversion (lb feed/lb pork) is approximately 3 to 1. Improving feed efficiency by one unit change (e.g., 3.00 to 2.99) represents approximately 140,000 tons of feed annually, or feed cost savings of about $28 million dollars. Efforts to fully adopt existing knowledge to optimize feed efficiency by the U.S. pork industry will improve the long-term competitiveness of the U.S. pork industry and the sustainability of food supplies.

This survey was developed to identify the current state of knowledge and the production practices used in the swine industry. The questionnaire was designed to accomplish three objectives: (1) determine the industry level of knowledge related to feed efficiency topics, (2) identify production practices being used that influence feed efficiency, and (3) identify information gaps or areas requiring additional knowledge to further improve feed efficiency.

Conclusions drawn from this study will be used to assemble extension education factsheets to rapidly disseminate information to producers and industry workers on current and innovative information that may improve feed efficiency and to aid in future research initiatives.

Procedures

This project was supported by National Research Initiative Competitive Grant no. 2011-68004-30336 from the USDA National Institute of Food and Agriculture. The procedures for this survey were approved by the Kansas State University Committee for Research Involving Human Subjects. The survey was web-based and created using the Axio Survey Creation Tool (**https://online.ksu.edu/Survey/**).

The subjects of this survey were individuals with their primary occupation in the swine industry. Most participants were from the United States, but international responses were received. The survey was made available via the internet from November 1, 2011, until March 1, 2012. Brashear et al. (2000) found that Illinois pork producers rely more on popular press material for information. Because of this, subjects targeted for the questionnaire were asked to participate through press releases advertised in popular press magazines including National Hog Farmer (**www.nationalhogfarmer.com**), Pork Magazine (**www.porknetwork.com**), and Feedstuffs Weekly Newspaper for Agribusiness (**www.Feedstuffs.com**). Emails with the press release were distributed to digital subscribers of those magazines; producer and allied industry email address lists used by K-State Swine Research and Extension, and individuals who registered for the International Conference on Feed Efficiency in Swine that was held November, 2011, in Omaha, NE. Also, a link to the survey website was available on K-State's Swine Research and Extension website (www.KSUswine.org).

Individuals who participated in the survey were not required to answer all questions; therefore, results were summarized based on responses to individual questions. Total responses for individual questions ranged from 123 to 205.

Two demographic questions were asked to identify the population of respondents and to summarize the answers received for questions within the survey. The first was designed to allow respondents to categorize themselves by the segment of the swine industry that they represented as a primary occupation (pork producer, consultant to the swine industry, education, or other; Table 4-1). Out of 205 individuals who responded to the first question, the largest percentage,

33%, identified themselves as consultants to the swine industry. An additional 28% identified themselves as producers, and 23% categorized themselves as "Other." Respondents who identified themselves as other were asked to describe their role in the swine industry. A majority of those individuals said they were graduate students, media reporters/editors, feed manufacturers, meat packers, technical support representatives for production systems, and pharmaceutical/vaccine sales representatives. The second question was designed to categorize participants by their number of years of experience working in the swine industry (0 to 5 years, 5 to 10 years, 10 to 20 years, 20+ years; Table 4-2). The greatest majority (53%) of individuals responded that they have more than 20 years of swine industry experience, and 21% had 10 to 20 years of experience. After establishing demographics of the sampled population, a series of knowledge-based, production practice, and discovery questions were asked to help achieve the objectives of the survey. Knowledge and production practice questions were delivered in a multiple-choice format, and possible answers included "Not sure" and "Other" options. Several production practice questions also branched into sub-questions depending on how respondents answered the main question. Branching sub-questions allowed for further data collection to better understand reasoning behind production practices utilized in the field, which will help extension educators identify critical control points within production systems as they pertain to feed efficiency. The discovery questions were designed so respondents could rank a predetermined topic area priority list from 1 to 10. To summarize the discovery questions, the average rank of each topic area was used to determine an overall ranking from the highest to lowest priority for future research and emphasis.

Results

Dietary energy

Participants were asked how much of an improvement in feed efficiency can be expected by increasing dietary fat by 1% (Table 4-3). In total, 138 respondents answered, with 41% answering correctly (2%), 32% answered incorrectly, and 27% responding not sure. Sixty-nine percent of producers and 83% of respondents in the "Other" category for profession answered incorrectly or not sure. When responses are sorted by years of experience, 58% of respondents with less than 5 years and 47% of individuals with 5 to 10 years of experience answered not sure.

Grinding/Particle size

A total of 164 respondents answered the question asking what cereal grain particle size is used or recommended for swine diets (Table 4-4). Most respondents (73%) indicated below 700 µm, but only 4% of respondents grind or recommend grinding grain below 400 µm, and 19% were not sure. A total of 45% of individuals who categorized their profession as "Other" (33) and 53% of individuals with 0 to 5 years of experience (17) responded "Not sure." If respondents answered with a particle size greater than 400 µm, they were asked a branched question to determine why they do not grind to a finer particle size. The most common reason (35% of responses) was that flowability or handling characteristics cause problems in the feeding system. Participants were also asked how much of an improvement in feed efficiency can result from decreasing the particle size of grain by 100 µm (Table 4-5). In total, 160 individuals answered with 36% answering correctly (1.1 to 1.4%), 31% answered "Not sure," and 33% answered incorrectly.

Pelleting

Participants were asked if they feed pelleted or recommend pelleting finishing diets. A total of 151 individuals answered with 59% replying no, and 41% replying yes (Table 4-6). Interestingly, 70% of individuals categorized as "Other" answered yes, whereas most producers, consultants, and academic participants answered no. Individuals who answered no were then asked why they do not pellet or recommend pelleting finishing diets, and respondents could check all answers that applied. A total of 148 responses were returned; 29% indicated pelleting was too expensive or that it was not available at their local feed mill. These were clearly the most common reasons why individuals do not feed pelleted finishing diets. When asked how much of an improvement can be expected from feeding high-quality pellets (Table 4-7), 70% of respondents answered correctly (2 to 6%). This result represented correct responses from more than 55% of respondents within each demographic category indicating a high knowledge level across the industry about pelleting diets for swine.

Feed Additives

Participants were asked several questions to better identify the use of feed additives and their effects on feed efficiency. The first question asked individuals if they use or recommend using copper sulfate in the nursery; 69% of 134 respondents answered yes and 31% said no (Table

4-8). More than 54% of individuals in each segment category, and 56% of participants in each age category answered yes. A branched question asked those who answered yes what percentage benefit in feed efficiency they expected from copper; those who answered no were asked why they did not recommend or use copper sulfate. Of the individuals who answered yes, 30% believed there was a 2% improvement in feed efficiency, but 20% were not sure. For those who answered no, 48% were not sure, and 29% said they did not recommend or use growth-promoting levels of copper sulfate because of environmental reasons. Richert et al. (1995) suggested that more young producers were worried about swine waste management compared to older producers, but of the individuals who said they did not feed copper sulfate because of environmental reasons, 75% (9 out of 12) had 20 or more years of experience.

Individuals were also asked if they feed or recommend feeding growth-promoting levels of antibiotics in nursery diets. A total of 134 individuals answered, with 73% saying yes and 23% saying no (Table 4-9). Demographics showed that 65% or more individuals in each industry segment, and at least 50% of each age category replied yes. Respondents were again asked branched questions depending on their answers. If they answered yes, they were asked what percentage improvement in feed efficiency they expected from its use. A total of 96 responses were received; 21% of those responded that they expected a 3% improvement, 20% responded "Not sure," 16% answered 4%, and 15% answered 5%. If survey takers answered no, they were asked why they don't use or recommend using growth-promoting levels of antibiotics in nursery diets. Forty-two responses were returned, with 33% saying it was because the potential of development of antibiotic resistance and 26% answering "Other." The most common responses for individuals who answered "Other" were that they used antibiotics only to treat unhealthy pigs and did not feed growth-promotion levels of antibiotics.

Finally, individuals were asked if they use or recommend using ractopamine (Paylean, Elanco Animal Health, Greenfield, IN), which is a β-Adrenergic-Agonist known for its ability to increase lean muscle growth in late finishing pigs. A total of 132 answered, with 70% saying yes and 30% saying no (Table 4-10). Besides individuals in the academia category (42%), more than 54% of producers, consultants, and respondents categorized as "Other" answered yes. Over 50% of each age category also answered yes. If respondents answered yes, they were asked what initial dosage they utilized; 66% of the 92 respondents answered 4.5 g/ton, and 26% answered

6.75 g/ton. They were also asked whether they utilize a step-up program or a constant level; 67% said they feed a constant level, and 33% said they use a step-up program. The step-up program was defined as feeding a lower dosage for a period of time followed by a higher dosage until pigs were marketed. If respondents said that they did not use or recommend using ractopamine, they were asked why they did not. Forty total responses were received, with 40% answering "Other," and 28% answering "Not sure". The most common reasons for individuals who replied with "Other" were that they had a niche market or special incentive not to utilize ractopamine. A knowledge-based question was also asked (Table 4-11) about the expected improvement in feed efficiency associated with the use of ractopamine. A total of 132 participants answered the question, with 49% answering correctly (5 to 15%), 24% answering incorrectly, and 22% responding "Not sure".

Sow feed efficiency

Respondents were asked approximately how much sow feed should be needed per pig weaned (Table 4-12). A total of 128 individuals answered, with 51% answering correctly (70 to 100 pounds), 26% answering "Not sure," and 22% answering incorrectly. Although more than half of the total responses were correct, only 21% of individuals in academia (24) and 41% categorized as "Other" (22) answered correctly. Based on years of experience in the swine industry, only 27% with less than 5 years (11) and 43% with 5 to 10 years (14) had correct answers.

Thermal temperature

Individuals were also asked what feed efficiency would be for finishing pigs who initially have feed conversion rates of 2.80 if the temperature is dropped 4ºF below their respective thermo-neutral zone (Table 4-13). A total of 139 individuals responded; 22% answered correctly (2.88), 4% answered incorrectly, and 30% responded "Not sure". Only 8% of individuals categorized as "Other" (24), 24% of consultants (51), 25% in academia (24), and 25% of producers (40) answered correctly. Based on years of experience, only 33% with less than 5 years, 12% with 5 to 10 years, 9% with 10 to 20 years, and 27% with 20 or more years answered the question correctly.

Discovery questions

When asked which topic areas would provide the largest opportunity to improve feed efficiency in the U.S. swine industry, total responses indicate the top three areas were health, genetics, and feed processing (Table 4-14). Additionally, dietary energy was ranked highly for several demographic segments including individuals in academia and those with 10 to 20 years of experience. Individuals were then asked to rank topic areas according to future research needs. Total responses suggest the most important areas are health, genetics, and dietary energy (Table 4-15). Also, more interest in digestive tract microbiology/health was expressed by most demographic segments. Finally, survey respondents were asked to rank topics based on their own knowledge of the topic. Overall, individuals believed they were most knowledgeable on feed processing (particle size), amino acids, and antibiotics (Table 4-16). The three topic areas that individuals were the least knowledgeable in were feed processing (extruding/expanding), digestive tract microbiology, and feed additives (other than antibiotics). However, there was a lot of variation in response depending on industry segment and years of experience. For example, producers rank health as their most knowledgeable topic area, but consultants and individuals in academia ranked health as an area that they need more knowledge in. Additionally, amino acids were listed as a topic area that consultants, academia, and individuals categorized as "Other" ranked as very knowledgeable in (2^{nd}), however producers ranked them much lower (8^{th}) in terms of knowledge.

Conclusion

Results from this survey suggest gaps in information and knowledge of feed efficiency exist across demographic segments of the industry.

Producer responses imply that they are unfamiliar with information behind the effects of fat inclusion, particle size reduction, feed additives, and thermal environment on feed efficiency. Consultants and individuals in academia had the highest percentage of correct answers for the knowledge questions, but less than half identified the correct response when asked how reducing particle size affects feed efficiency, and very few correctly answered the question about thermal environment effects associated with feed efficiency. Respondents who classified themselves as "Other" frequently replied not sure to many of the knowledge-based questions and to several production practice questions. This result may be due to the great diversity in occupation within

the group. When responses were sorted by years of experience, a majority of individuals with less experience, specifically those with 0 to 5 years, had higher percentages of not sure responses, which may be related to their unfamiliarity to specific industry practices and the knowledge behind those practices.

Regardless of demographics, most individuals were familiar with the advantages in feed efficiency associated with pelleting swine diets, and a large percentage of the industry utilizes or recommends using feed additives. Although knowledge of the benefits from pelleting is high, more access to affordable pellets is required to increase adoption of pelleting within the industry. Additionally, responses suggest that grinding cereal grains to finer particle sizes is limited mainly because of more difficult handling in feeding systems. A majority of respondents believe that topics for future research and the biggest areas of opportunity to improve feed efficiency include genetics, health, feed processing, and dietary energy. Additionally, the topic areas where most of the participants were the least knowledgeable were expanding/extruding technologies, digestive tract microbiology, and feed additives (other than antibiotics), however this question proved that there was a large amount of variation in knowledge of topic areas based on the segment of the industry and years of experience.

Extension education on current knowledge and production practices that are already proven should be expanded to provide this information in an easy-to-access format for the swine industry. Ultimately, successful dissemination will help producers and swine operations lower input costs by improving the efficiency of feed utilization.

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Tables and Figures

Table 4-1. Demographics of the survey responders[1]

	Responses	% of total
Pork producer	57	28%
Consultant to the swine industry	67	33%
Academia	33	16%
Other	48	23%
Total	205	100%

[1] The question was, "What segment of the swine industry do you represent as a primary occupation?"

Table 4-2. Demographics based on years of experience in the swine industry[1]

	Responses	% of total
0 to 5 years	23	12%
5 to 10 years	28	15%
10 to 20 years	40	21%
20+ years	101	53%
Total	192	100%

[1] The question was, "How many years of experience do you have working in the swine industry?"

Table 4-3. Effect of dietary fat inclusion on feed efficiency[1]

Industry Segment	Producers (39)	Consultants (51)	Academia (24)	Other (24)
Correct	31%	63%	33%	17%
Incorrect	25%	27%	42%	45%
Not sure	44%	10%	25%	38%
Years of Experience	0 to 5 (12)	5 to 10 (17)	10 to 20 (32)	20 or more (77)
Correct	33%	29%	31%	48%
Incorrect	8%	24%	47%	33%
Not sure	58%	47%	22%	19%
Total	(138)			
Correct	41%			
Incorrect	32%			
Not sure	27%			

[1] The question asked was, "By adding 1% fat to the diet feed efficiency is improved by approximately?" The correct answer was 2%.

Table 4-4. Particle sizes utilized by the swine industry[1]

	Responses	% of total
Greater than 800 μm[2]	1	1%
700-800 μm[2]	13	8%
600-700 μm[2]	49	30%
500-600 μm[2]	39	24%
400-500 μm[2]	24	15%
Less than 400 μm	7	4%
Not sure[3]	31	19%
Total	164	100%

[1] The question asked was, "What is the current particle size that you grind or recommend grinding cereal grains to for swine diets?"

[2] Individuals who answered with micron sizes larger than 400 μm were asked a branched question, "Why do you not grind to a finer particle size?" 35% of responses were that flowability or handling characteristics cause problems in feeding system, 18% were that ulcer rates are too high, 15% were that current mill cannot grind to a smaller particle size, and 14% were that production rate in feed mill is slowed too much.

[3] Forty-five percent of individuals categorized as "Other", and 53% of participants with 0 to 5 years of experience answered not sure.

Table 4-5. Effect of reducing grain particle size by 100 µm on feed efficiency[1]

Industry Segment	Producers (44)	Consultants (57)	Academia (28)	Other (31)
Correct	27%	46%	36%	32%
Incorrect	37%	42%	18%	23%
Not sure	36%	12%	46%	45%
Years of Experience	0 to 5 (16)	5 to 10 (21)	10 to 20 (36)	20 or more (87)
Correct	25%	48%	39%	34%
Incorrect	31%	19%	28%	38%
Not sure	44%	33%	33%	28%
Total	(160)			
Correct	36%			
Incorrect	33%			
Not sure	31%			

[1] The question asked was, "By reducing the grain particle size of a ration by 100 µm, feed efficiency improves by approximately how much?" The correct answer was 1.1 to 1.4%.

Table 4-6. Industry use of pellets for finishing diets[1]

	Responses	% of total[2]
Yes	62	41%
No[3]	89	59%
Total	151	100%

[1] The question asked was, "Do you currently pellet, or recommend pelleting finishing diets?"

[2] In total, 77% of producers (43), 55% of consultants (53), and 72% of academia answered no; 70% of individuals identified in the "Other" segment answered yes. Based on years of experience, 50% or more of each category answered no.

[3] If respondents answered no, they were asked a branched question, "Why do you not pellet finishing diets?" 29% of responses were either that it was too expensive or that pelleting capabilities were not available at their local mill. These were clearly the most common reasons why individuals do not pellet finishing diets.

Table 4-7. Effect of pelleting diet on feed efficiency[1]

Industry Segment	Producers (44)	Consultants (56)	Academia (26)	Other (31)
Correct	70%	80%	61%	52%
Incorrect	12%	13%	4%	29%
Not sure	18%	7%	35%	19%
Years of Experience	0 to 5 (16)	5 to 10 (20)	10 to 20 (36)	20 or more (85)
Correct	56%	60%	61%	76%
Incorrect	31%	30%	14%	11%
Not sure	13%	10%	25%	13%
Total	(157)			
Correct	69%			
Incorrect	14%			
Not sure	17%			

[1] The question asked was, "Although variable, feeding high quality pellets should affect feed efficiency by approximately how much? The correct answer was 2 and 6%.

Table 4-8. Use of growth promoting levels of copper sulfate in nursery diets[1]

	Responses	% of total[2]
Yes[3]	93	69%
No[4]	41	31%
Total	134	100%

[1] The question asked was, "Do you feed or recommend feeding growth promoter levels of copper sulfate in the nursery?"

[2] By industry segment; more than 54% of individuals in each category answered yes. Based on years of experience, more than 56% within each category answered yes.

[3] Individuals who answered yes were asked a branch question: What benefit in feed efficiency do you expect from its inclusion in nursery diets? 30% of responses were "2%," and 20% of responses were "Not sure."

[4] Individuals who answered no were asked a branch question, "Why do you not use growth promoting level of copper sulfate in the nursery?" 48% of responses were "Not sure," and 29% were because of environmental reasons.

Table 4-9. Use of growth-promoting levels of antibiotics in the nursery[1]

	Responses	% of total
Yes[2]	98	73%
No[3]	36	27%
Total	134	100%

[1] The question was, "Currently, do you feed or recommend feeding growth promoting levels of antibiotics in the nursery?"

[2] More than 65% of individuals in each industry segment category, and more than 50% of individuals in each age category answered yes.

[2] Individuals who answered yes were asked a branch question, "What benefit in feed efficiency do you expect from its inclusion in nursery diets?" 21% responded with "3%," 20% answered "Not sure," 16% answered "4%," and 15% answered "5% or more."

[3] Individuals who answered no were asked a branch question, "Why do you not use growth promoting level of antibiotics in the nursery?" 33% of responses were to avoid development of antibiotic resistance and 26% were "Other." The most common response for individuals who answered "Other" was because they used antibiotics only to treat sick animals and not for growth promotion.

Table 4-10. Use of ractopamine[1]

	Responses	% of total
Yes[2,3]	92	70%
No[4]	40	30%
Total	132	100%

[1] The question was, "Currently, do you feed or recommend feeding ractopamine as a growth promoter in late finishing?"

[2] More than 54% of producers, consultants, and individuals classified as "Other" answered yes; only 42% of participates in academia said yes. More than 50% of individuals in each age category answered yes.

[2] Individuals who answered yes were asked a branch question, "What initial level of ractopamine do you utilize?" 66% responded "4.5 g/ton," and 26% answered "6.75g/ton."

[3] Individuals who answered "Yes" were asked a second branched question, "Do you utilize a step-up program or do you feed a constant level?" 67% answered that they feed or recommend feeding a constant level, and 33% fed or recommend feeding a step-up program.

[4] Individuals who answered no were asked a branch question, "Why do you not use ractopamine in late finishing?" 40% of responses were "Other." The most common response for individuals who answered "Other" was because they had a niche market or special incentive not to utilize ractopamine.

Table 4-11. Effect of ractopamine on Feed Efficiency[1]

Industry Segment	Producers (33)	Consultants (51)	Academia (24)	Other (24)
Correct	48%	67%	33%	42%
Incorrect	22%	23%	46%	20%
Not sure	30%	10%	21%	38%
Years of Experience	0 to 5 (16)	5 to 10 (20)	10 to 20 (36)	20 or more (85)
Correct	50%	40%	53%	49%
Incorrect	8%	33%	17%	36%
Not sure	42%	27%	30%	15%
Total	(132)			
Correct	59%			
Incorrect	19%			
Not sure	22%			

[1] The question asked was, "How much of an improvement do you expect in feed efficiency from the inclusion of ractopamine?" The correct answer was 5 to 15%.

Table 4-12. Sow feed efficiency[1]

Industry Segment	Producers (32)	Consultants (50)	Academia (24)	Other (22)
Correct	50%	70%	21%	41%
Incorrect	12%	18%	50%	23%
Not sure	38%	12%	29%	36%
Years of Experience	0 to 5 (11)	5 to 10 (14)	10 to 20 (29)	20 or more (74)
Correct	27%	43%	52%	55%
Incorrect	9%	14%	24%	23%
Not sure	64%	43%	24%	18%
Total	(128)			
Correct	51%			
Incorrect	23%			
Not sure	26%			

[1] The question asked was, "In your opinion, approximately how much sow feed should be required per pig weaned?" The correct answer was 70 to 100 pounds.

Table 4-13. Effect of thermal temperature on feed efficiency[1]

Industry Segment	Producers (40)	Consultants (51)	Academia (24)	Other (24)
Correct	25%	24%	25%	8%
Incorrect	32%	56%	50%	54%
Not sure	43%	20%	25%	38%
Years of Experience	0 to 5 (12)	5 to 10 (17)	10 to 20 (32)	20 or more (78)
Correct	33%	12%	9%	27%
Incorrect	12%	47%	44%	55%
Not sure	50%	41%	47%	18%
Total	(139)			
Correct	22%			
Incorrect	48%			
Not sure	30%			

[1] The question asked was, "If the ambient temperature of a finishing barn is at thermo-neutrality and pigs average a feed efficiency of 2.8, what is the estimated feed efficiency after the temperature drops to 4 degrees Fahrenheit below the thermo-neutral zone? The correct answer was 2.88.

Table 4-14. Which topic areas provide the largest opportunity to further improve feed efficiency? (1=Important, 10=Least important)[1]

		Industry segment				Years of experience			
Topic	Total	Producers	Consultants	Academia	Other	0 to5	5 to 10	10 to 20	20+
Health	2.2 (1)	2.3 (1)	2.2 (1)	2.1 (1)	2.2 (1)	2.8 (1)	2.6 (1)	2.7 (1)	1.9 (1)
Genetics	3.7 (2)	2.8 (2)	4.0 (2)	4.2 (2)	3.7 (2)	5.1 (4)	3.8 (2)	3.0 (2)	3.7 (2)
Feed Processing	4.3 (3)	4.0 (3)	4.1 (3)	5.2 (4)	4.5 (3)	4.0 (2)	4.4 (4)	4.8 (4)	4.2 (3)
Dietary energy	4.6 (4)	4.3 (4)	4.4 (4)	4.9 (3)	5.4 (6)	5.1 (4)	5.3 (6)	4.3 (3)	4.6 (4)
Digestive tract microbiology/health	5.5 (5)	6.1 (6)	5.4 (5)	5.5 (7)	4.8 (4)	5.6 (6)	3.9 (3)	5.4 (5)	5.8 (6)
Environment	5.5 (5)	5.4 (5)	5.9 (6)	5.3 (5)	5.0 (5)	4.6 (3)	5.6 (7)	6.0 (7)	5.4 (5)
Amino acids	6.2 (7)	6.2 (7)	6.6 (7)	5.4 (6)	6.2 (7)	8.1 (10)	7.1 (8)	5.6 (6)	6.0 (7)
Feed additives (other than antibiotics)	6.9 (8)	7.1 (8)	6.9 (8)	7.0 (8)	6.3 (8)	6.3 (7)	5.1 (5)	7.0 (8)	7.3 (8)
Antibiotics	7.7 (9)	8.3 (9)	7.4 (9)	7.5 (9)	7.9 (9)	7.0 (9)	8.0 (9)	7.8 (10)	7.7 (9)
Alternative feed ingredients	8.1 (10)	8.1 (10)	8.0 (10)	7.6 (10)	8.7 (10)	6.4 (8)	9.2 (10)	7.6 (9)	8.2 (10)

[1] Values are average rankings and the overall rank is listed from 1-10 in parentheses.

Table 4-15. Rank the following items on the need for future research as it pertains to feed efficiency (1=Important, 10=Least important)[1]

		Industry segment				Years of experience			
Topic	Total	Producers	Consultants	Academia	Other	0 to5	5 to 10	10 to 20	20+
Health	3.2 (1)	3.0 (2)	3.5 (1)	4.1 (3)	1.8 (1)	3.4 (5)	2.5 (1)	4.0 (3)	3.0 (1)
Genetics	3.6 (2)	2.9 (1)	4.1 (4)	4.7 (8)	2.2 (2)	3.5 (6)	2.5 (1)	4.1 (4)	3.7 (2)
Dietary energy	3.7 (3)	3.7 (3)	3.8 (2)	4.1 (3)	2.8 (4)	2.9 (2)	3.2 (4)	3.8 (1)	3.8 (3)
Digestive tract microbiology/health	3.9 (4)	4.2 (4)	3.9 (3)	4.6 (6)	2.2 (2)	3.8 (7)	2.7 (3)	3.9 (2)	4.1 (4)
Alternative feed ingredients	4.1 (5)	4.3 (6)	4.4 (7)	4.0 (2)	3.2 (8)	3.9 (9)	4.1 (11)	4.3 (6)	4.1 (4)
Amino acids	4.1 (5)	4.3 (6)	4.4 (7)	3.7 (1)	3.3 (10)	3.3 (4)	3.6 (7)	4.2 (5)	4.2 (6)
Feed additives (other than antibiotics)	4.2 (7)	4.2 (4)	4.6 (9)	4.4 (5)	3.1 (6)	2.9 (2)	3.2 (4)	4.8 (7)	4.4 (8)
Feed Processing (particle size)	4.2 (7)	4.4 (8)	4.2 (5)	4.7 (8)	3.6 (11)	4.0 (10)	3.3 (6)	4.9 (9)	4.2 (6)
Feed Processing (pelleting)	4.3 (9)	5.1 (10)	4.2 (5)	4.6 (6)	3.1 (6)	2.8 (1)	3.7 (9)	4.9 (9)	4.4 (8)
Environment	4.4 (10)	4.5 (9)	4.7 (10)	5.0 (10)	3.0 (5)	3.8 (7)	4.0 (10)	4.8 (7)	4.4 (8)
Feed Processing (extruding/expanding)	4.7 (11)	5.1 (10)	5.0 (11)	5.0 (10)	3.2 (8)	4.3 (11)	3.6 (7)	5.1 (11)	4.9 (11)
Antibiotics	5.9 (12)	6.0 (12)	5.9 (12)	6.3 (12)	5.2 (12)	5.5 (12)	5.6 (12)	6.1 (12)	5.9 (12)

[1] Values are average rankings and the overall rank is listed from 1-10 in parentheses.

Table 4-16. Rank your level of knowledge on the following areas as the y pertain to feed efficiency (1=Knowledgable, 10=Need more education)[1]

		Industry segment				Years of experience			
Topic	Total	Producers	Consultants	Academia	Other	0 to5	5 to 10	10 to 20	20+
Feed Processing (particle size)	4.7 (1)	4.9 (2)	4.3 (1)	4.8 (1)	5.2 (5)	5.8 (4)	4.4 (2)	5.7 (6)	4.2 (1)
Amino acids	4.8 (2)	5.8 (8)	4.4 (2)	4.9 (2)	4.5 (2)	6.5 (7)	3.9 (1)	4.8 (1)	4.9 (5)
Antibiotics	5.0 (3)	5.6 (7)	4.7 (4)	5.3 (6)	4.3 (1)	7.4 (12)	5.4 (9)	5.2 (2)	4.6 (2)
Alternative feed ingredients	5.1 (4)	5.4 (6)	4.7 (4)	5.4 (8)	5.4 (8)	5.5 (1)	4.4 (2)	5.3 (3)	5.1 (8)
Dietary energy	5.1 (4)	5.3 (5)	5.0 (6)	5.3 (6)	4.9 (3)	6.5 (7)	4.6 (4)	5.3 (3)	5.0 (6)
Environment	5.1 (4)	5.0 (4)	5.1 (7)	5.2 (5)	5.2 (5)	6.0 (6)	5.0 (6)	5.3 (3)	5.0 (6)
Feed Processing (pelleting)	5.1 (4)	6.1 (9)	4.5 (3)	5.0 (3)	5.2 (5)	5.8 (4)	5.7 (10)	5.7 (6)	4.7 (3)
Genetics	5.2 (8)	4.9 (2)	5.3 (9)	5.0 (3)	5.8 (11)	7.1 (10)	5.3 (8)	5.9 (11)	4.8 (4)
Health	5.3 (9)	4.8 (1)	5.4 (10)	5.8 (9)	5.0 (4)	5.6 (3)	5.1 (7)	5.8 (8)	5.1 (8)
Feed additives (other than antibiotics)	5.7 (10)	6.4 (11)	5.2 (8)	6.3 (10)	5.4 (8)	5.5 (1)	4.9 (5)	5.8 (8)	5.9 (10)
Digestive tract microbiology/health	6.0 (11)	6.2 (10)	5.7 (11)	6.5 (11)	5.7 (10)	7.0 (9)	6.0 (11)	5.8 (8)	6.0 (11)
Feed Processing (extruding/expanding)	6.6 (12)	7.0 (12)	6.6 (12)	6.7 (12)	6.1 (12)	7.3 (11)	6.8 (12)	7.1 (12)	6.4 (12)

[1] Values are average rankings and the overall rank is listed from 1-10 in parentheses.