The effects of maternal dietary supplementation of cholecalciferol (vitamin D_3) in conjunction with 25(OH)D_3 on sow and pig performance

by

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Abstract

A thorough literature review on feeding vitamin D_3 and 25(OH)D_3 revealed a large amount of research conducted in swine and poultry. In general, increasing vitamin D_3 concentrations or adding 25(OH)D_3 to the maternal diet increases the vitamin D_3 status of the dam and often the progeny as well. Varying results have been reported on the practical and valuable impacts of this elevated status with some topics including improved sow performance, changes in muscle fiber morphometrics, and growth performance to market. The first experiment used a total of 69 sows and the progeny from one group of 22 sows to determine the effects of feeding a combination of vitamin D_3 and 25(OH)D_3 to the sow. Differences in sow productivity and growth performance of progeny due to dietary treatment were not observed ($P > 0.05$). When pigs were sacrificed at birth, there were no treatment effects for all fiber morphometric measures ($P > 0.170$), except primary fiber number and the ratio of secondary to primary muscle fibers ($P < 0.014$). Pigs from the CON and DL fed sows had less primary fibers than pigs from sows fed the DH treatment ($P < 0.046$), but did not differ from each other ($P = 0.732$). These results suggest progeny went through a longer prenatal period of primary myogenesis which delayed the onset of secondary myogenesis. Pigs from DL fed sows had a smaller secondary to primary muscle fiber ratio compared to pigs from sows fed the CON treatment ($P = 0.016$), with pigs from sows fed DL treatment not differing from either ($P > 0.057$). There were treatment × time interactions for all sow and pig serum metabolites ($P < 0.001$). Therefore, we chose to compare treatment means within time period. At all time periods, sow serum 25(OH)D_3 concentrations differed for all treatments with the magnitude of difference largest at weaning ($P < 0.011$).

The second and third experiment investigated the impact of adding benzoic acid and an essential oil blend to diets and creep feed. When these additives were included in growing pig diets in a 28-d trial, a main effect of time ($P < 0.001$) was detected where there was no evidence of difference during the first 3 weeks for ADG and G:F, however both responses decreased during the final week of the experiment ($P < 0.001$) and average pen BW increased ($P < 0.001$) for all time points. There was a treatment × time interaction ($P = 0.003$) for ADFI where during the first 3 weeks, there was no evidence of difference due to dietary treatment, but during the final week of the study, pigs consumed more ($P = 0.007$) of the control diet (2.38 kg/d control vs. 2.24 kg/d benzoic acid paired and essential oil blend). Fecal samples collected provided no evidence of differences ($P > 0.05$) in fecal pathogens due to dietary treatment. When these additives were included in the maternal diet and in the creep feed, they did not ($P > 0.05$) affect sow performance or preweaned piglet performance. Fecal swabbing of pigs the day before weaning showed they did not eat the creep feed and, therefore, no ($P > 0.05$) improvements in growth performance were observed in the nursery. In conclusion, adding benzoic acid and an essential oil blend to diets and creep feed did not affect growth performance and combining vitamin D_3 and 25(OH)D_3 in the maternal diet improved the vitamin D_3 status of the dam and progeny and increased primary muscle fibers at birth.

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Dedication

This thesis is dedicated in loving memory of my dad, Mike Cox.

Chapter 1 - Review of Literature

Introduction

Vitamin D_3 requirements can be met by its production in the body and/or by dietary consumption. Vitamin D_3 can be produced in the skin of the mammals when ultraviolet sunlight is present, or in the case of the production industry today with animals are reared indoors, it can be provided in the diet and absorbed in the gastrointestinal tract. The purpose of this review is to understand the roll of vitamin D_3 in swine and poultry including how it is absorbed and transported as well as its mode of action. Also, effects of supplemental vitamin D_3 or commercially available calcidiol will be evaluated across both species and phases of production.

History and Introduction to Vitamin D_3

In 1922, McCollum and his co-workers conducted experiments to cure a disease known as rickets. They noticed oxygen destroyed a previously discovered "vitamin A" activity in cod liver oil but the oil still cured rickets. McCollum et al. (1922) had discovered a separate nutrient which they called "vitamin D". A large amount of research conducted since 1922 has shined light on how the vitamin is formed and its mode of action.

Vitamin D_3, also named cholecalciferol, is a fat-soluble vitamin derived from the steroid, 7-dehydrocholesterol, found in the skin (Figure 1.1). This steroid absorbs specific wavelengths of UV light (270- to 315-nm) from sun light and is converted to previtamin D_3 (Dittmer and Thompson, 2011). This molecule thermally isomerizes to vitamin D_3 and can diffuse from the skin to the blood. Transport through the blood is aided by a vitamin D_3 binding protein, transcalciferin, which carries the bound vitamin D_3 predominately to the liver. The circulating vitamin D_3 may be picked up by other tissues such as muscle and adipose tissues (Gropper et al., 2004).

If access to sunlight is limited, dietary vitamin D_3 is required. In nursery pigs, the current dietary recommendation for vitamin D_3 is 220 IU/kg of the diet, for finishing pigs it is 150 IU/kg, and for gestating and lactating sows 800 IU/kg (NRC, 2012). In poultry, the latest published requirement by the NRC for vitamin D_3 was in 1994 and was 200 IU/kg for broilers (NRC, 1994).

Absorption and Transport

When vitamin D_3 is provided in the diet, absorption is aided by emulsification by bile salts which breaks up fat globules into smaller droplets (Tymoczko et al., 2015). These droplets incorporate into an aggregate of amphiphilic molecules known as a micelle which transports the poorly soluble vitamin D_3 in the intestinal lumen. The mixed micelle is absorbed by passive diffusion (Hollander et al., 1978) and/or active diffusion into enterocytes (Reboul, 2015). Once the vitamin has entered the enterocyte, it is packaged in chylomicrons and exocytosed into lymphatic capillaries and ultimately enters the blood which delivers vitamin D_3 is delivered to the liver (Tymoczko et al., 2015).

A cytochrome P450 enzyme encoded by the CYP2R1 gene catalyzes 25-hydroxylation of vitamin D_3 to form calcidiol in the liver (DeLuca, 2014). This enzyme hydroxylates faster when concentrations of vitamin D_3 are low. Calcidiol is released into blood where it represents the main circulating form of vitamin D_3 and reflects the vitamin D_3 status of the animal. Measurement of calcidiol is useful for determining adequacy, deficiency, or toxicity of vitamin D_3. Blood is the largest pool of calcidiol where it is transported by a binding protein mainly to the kidney (Gropper et al., 2004).

Calcidiol is hydroxylated a second time in the proximal convoluted tubule cells of the kidney at the first carbon. This is accomplished by a mitochondrial CYP27B1-hydroxylase

enzyme and forms calcitriol which is the active hormone form of the vitamin (Adams and Hewison, 2010). The activity of this hydroxylase is stimulated by parathyroid hormone and low concentrations of calcium and phosphate in the plasma. High concentrations of calcitriol inhibit the activity of the CYP27B1-hydroxylase enzyme (Dittmer and Thompson, 2011). When the activity of CYP27B1-hydroxylase decreases, another enzyme, CYP24A1- hydroxylase, increases its activity to hydroxylate carbon 24 of calcidiol and calcitriol to form 24,25-dihydroxycholecalciferol and 1,24,25-trihydroxycholecalciferol, respectively. The metabolite, 24,25-dihydroxycholecalciferol, is also a good indicator of vitamin D status of the animal (Gropper et al., 2004).

Calcitriol is the active form of the vitamin that is released from the kidney and transported in the blood by binding proteins. The proteins release calcitriol once it reaches its target tissue allowing calcitriol to bind to a vitamin D receptor (**VDR**). The metabolite, 24,25-dihydroxycholecalciferol, is also transported to tissues by binding proteins. Vitamin D_3 is stored in adipose tissue and calcidiol is stored in blood and muscle (Gropper et al., 2004).

Modes of Action

Calcitriol, the active hormone metabolite of vitamin D_3, targets the intestine, kidney, bone, heart, muscle, pancreas, brain, and also the immune system. The two main mechanisms recognized are genomic and nongenomic (Gropper et al., 2004). In the genomic mechanism, a ligand dependent nuclear VDR binds calcitriol, the ligand, to influence gene transcription and can regulate the expression of more than 900 genes (Kongsbak et al., 2013). The calcitriol-VDR interaction then dimerizes with the retinoid X receptor (**RXR**) and translocates to the nucleus where it binds to specific vitamin D response elements (**VDRE**) found in promoter regions of specific target genes (Pike et al., 2012). Co-modulatory proteins further interact with this

complex bound to the VDRE to influence transcription of genes coding for specific proteins. For the nongenomic mechanism, calcitriol uses signal transduction pathways linked to a cell membrane VDR to initiate actions including increased calcium absorption in the intestine, opening of gated calcium channels, and increased calcium uptake by bone forming cells called osteoblasts and skeletal muscle cells. (Gropper et al., 2004).

Calcitriol plays an important role, along with parathyroid hormone, in calcium homeostasis in the blood, absorption of Ca and P in the intestine, reabsorption of Ca and P in the distal renal tubule of the kidney, and mobilization or mineralization of Ca and P in bones. Also, calcitriol affects cell differentiation, proliferation, and growth in many tissues (Gropper et al., 2004). According to Tymoczko et al. (2015), some recent research also indicates vitamin D may target muscle as well to enhance muscle growth.

Vitamin D_3 in Swine

Gilts and Sows

Vitamin D_3 status of animals is typically assessed by measuring calcidiol in circulating serum or plasma. In research comparing vitamin D_3 to commercially available calcidiol in the diet, sow blood levels of calcidiol increase during gestation and at farrowing and weaning when sows are fed calcidiol (Lauridsen et al., 2010; Coffey et al., 2012; Weber et al., 2014; Zhou et al., 2016) compared to only vitamin D_3 in the diet. Flohr et al. (2016a) fed increasing levels of vitamin D_3 (800, 2,000, or 9,600 IU per kg of the diet) as well as one dietary treatment of 50 μg of calcidiol per kg of the diet and observed a linear increase in serum calcidiol with increasing vitamin D_3 on d 100 of gestation, at farrowing, and at weaning. Sows fed 50 μg of calcidiol had intermediate vitamin D_3 status, as indicated by the calcidiol concentration in serum or plasma,

between 2,000 and 9,600 IU per kg treatments. Flohr et al. (2016a) indicated the 50 μg calcidiol treatment was equivalent to 2,000 IU of vitamin D_3 in the diets and, therefore, calcidiol in the diet provides a greater serum calcidiol response than vitamin D_3 in sows. Additionally, when calcitriol, the active hormone form of the vitamin, was measured in plasma, Weber et al. (2014) found greater concentrations at d 5 of lactation in sows fed calcidiol compared to sows fed vitamin D_3. Weber et al. (2014) proposed that sows fed vitamin D_3 did not have enough calcidiol in circulation to sustain the elevated production of calcitriol.

Coffey et al. (2012) euthanized gilts on d 90 of gestation and observed increased fetal plasma calcidiol when dams were fed calcidiol compared to vitamin D_3 due to proposed improvement in maternal vitamin D_3 status transferred to the fetuses. Zhou et al. (2016; 2017) measured the vitamin D_3 status of piglets at birth and at weaning. They observed greater serum calcidiol in piglets at birth when the maternal diet contained calcidiol due to greater concentration of calcidiol in umbilical cord blood transferred to neonatal piglets. In both experiments, there was no difference in serum concentrations in piglets at weaning, which Zhou et al. (2017) suggested was due to the 21-d half-life of serum calcidiol. Similarly, Flohr et al. (2016a) observed greater serum calcidiol at birth in pigs from sows fed 50 μg of calcidiol compared to pigs from sows fed 2,000 IU vitamin D_3 per kg of feed. There were no differences in piglet vitamin D_3 status at weaning. Pigs from sows fed the greatest vitamin D_3 level, 9,600 IU, had the greatest calcidiol in serum at birth and weaning even above the calcidiol treatment, which Flohr et al. (2016a) noted to be the first to report a vitamin D_3 level high enough to increase calcidiol in the blood above a calcidiol diet.

Witschi et al. (2011) offered creep feed with vitamin D_3 supplementation from week 3 until 5 weeks postweaning. From mating, sows were fed either 5 or 50 μg of vitamin D_3 or 50 μg

of calcidiol in gestation and lactation diets and weaned on d 35 of lactation. The calcidiol treatment, fed to the sow and the piglets through creep feed, caused an increase in circulating serum calcidiol in the piglets on d 21, 33, and 77 postpartum compared to both vitamin D_3 diets. The increase in circulating calcidiol was attributed to the increase in fetal body stores.

Vitamin D_3 is thought to be transferred from the sow to the piglets via the placenta and/or milk. Milk concentrations of calcidiol increased when sows were fed calcidiol compared to vitamin D_3 (Weber et al., 2014; Zhou et al., 2017). Flohr et al. (2014a) observed milk vitamin D_3 increased linearly with increasing concentrations of vitamin D_3 supplementation to the sow and concluded milk intake contributed to increased piglet serum calcidiol from birth to weaning.

Varying results have been detected in studies investigating sow reproductive performance and piglet growth performance. Calcidiol fed to sows increased the number of piglets born alive by 1 piglet which Zhou et al. (2016) suggest is due to vitamin D_3 effecting maternal-conceptus interaction improving implantation. No differences in pig performance were observed. Zhou et al. (2017) observed an increase in piglet growth performance the first two weeks of lactation due to maternal intake of calcidiol improving fat and protein contents in milk; however, no differences in piglet growth performance was detected at weaning on d 28 of lactation. Witschi et al. (2011) fed creep feed starting at 3-wk of age until 10-wk of age and observed that after weaning, pigs had greater ADFI and tended to have increased ADG. There were no overall differences in feed efficiency when fed 50 μg regardless of vitamin D_3 source compared to dams fed 5 μg of vitamin D_3 per kg of the diet. Weber et al. (2014) reported total litter birth weight and birth weight per piglet increased when dams were fed calcidiol due to maternal calcidiol. Lauridsen et al. (2010) reported fewer stillborn piglets when sow diets contained 1,400 IU or 2,000 IU of vitamin D_3/kg of the diet compared to 200 IU and 800 IU. Although Lauridsen et al.

(2010) did not discuss why there were fewer stillborn piglets in their study, they suggested that dietary vitamin D_3 concentration of about 1,400 IU should be recommended for reproducing swine.

In 2012, Coffey et al. observed gilts euthanized at d 90 of gestation had 2.5 more fetuses per litter when fed calcidiol compared to control gilts fed only vitamin D_3. Interestingly, even though the size of the litters were larger, the mean fetal weight was similar between treatments suggesting the number of low-birth-weight pigs did not increase with the increased litter size. In contrast, Flohr et al. (2014a; 2016a) found no effect of dietary vitamin D_3 treatments on maternal or litter performance.

Vitamin D_3 is known to have metabolic impacts on Ca and P homeostasis which contributes to bone mineralization and strength. Zhou et al. (2017) found an increase in bone strength, density, and ash content of newborn piglets, but not in weaned piglets when dams were fed 50 μg calcidiol. Witschi et al. (2011) studied sows fed 5 μg of D_3, 50 μg of D_3, or 50 μg of calcidiol per kg of the diet and observed the 2 higher treatments had increased bone breaking strength, cortical bone mineral content, and density of the tibial midshaft of piglets at weaning. Controversially, in a study analyzing the dam's bones, bone ash and ultimate strength of bones of gilts was greater when fed vitamin D_3 compared to calcidiol at equivalent levels at and above 800 IU per kg of the diet (Lauridsen et al., 2010). In studies performed by Flohr et al. (2014a, 2014b, 2016a), no differences in neonatal bone ash content or bone density were observed.

Research in humans and other animal species suggests a role of vitamin D_3 in the formation of skeletal muscle. In swine, investigators have observed an increase in muscle fiber number of the LM of d 90 fetuses (Hines et al., 2013) and pigs at birth and weaning (Zhou et al., 2016) when the maternal diet contained calcidiol. The cross-sectional area of muscle fibers in the

psoas major and longissimus dorsi also increased at weaning in the study conducted by Zhou et al. (2016) and they did not discuss the reasoning behind this novel finding. In contrast, Hines et al. (2013) found a tendency for the cross-sectional area of fibers in the longissimus muscle on d 90 of gestation to be smaller than the control sow diet containing equivalent amounts of vitamin D_3. Hines et al. (2013) also cultured myoblasts from the semitendinosus muscle of d 90 fetuses and observed greater proliferation and differentiation capacity when the cells came from fetuses of gilts fed calcidiol. Flohr et al. (2016a) observed no effect of maternal dietary vitamin D_3 on neonatal muscle fiber numbers however, average number of secondary muscle fibers per primary muscle fiber decreased when dams were fed calcidiol compared to 9,600 IU vitamin D_3. Also, pigs from sows fed calcidiol had a tendency for increased hypertrophic growth of secondary muscle fibers of the LM and primary muscle fibers of the semitendinosus compared to pigs from sows fed 9,600 IU vitamin D_3.

In summary, vitamin D status is typically determined by measuring the concentration of calcidiol in the blood of the animal. When sows and gilts are fed increased levels of vitamin D_3, their serum concentration of calcidiol increases linearly. Some authors observed that feeding a commercially available calcidiol product itself to the sows compared to the same level, 2000 IU per kg, of vitamin D_3 also increased serum concentrations of calcidiol in the sow as well as their piglets at birth. No differences in calcidiol concentration were observed at weaning unless creep feed was supplied to the piglets. Increasing levels of vitamin D_3 in the diet or the substitution of calcidiol for vitamin D_3 has also increased calcidiol concentrations in the sow's milk.

Furthermore, research on sow reproductive performance and pre-weaned piglet growth performance has provided varying results. For gestating and lactating sows, the current nutrient requirement for vitamin D_3 is 800 IU/kg of the diet (NRC, 2012). Some research results include

fewer stillborn piglets and increased piglets born alive when dams were fed levels of 1,400 and 2,000 IU of vitamin D_3 or commercially available calcidiol per kg of the diet compared to 200 and 800 IU. Dams fed 2,000 IU of vitamin D_3 in some studies have improved bone strength and mineralization. Therefore, although most studies found no difference in pre-weaned piglet performance, many of the other parameters measured suggests for gilts and sows, the nutrient requirement of vitamin D_3 needs to be increased to a level above the current requirement of 800 IU/kg of the diet with most sow research testing 2000 IU/kg.

Nursery Pigs

In the nursery phase of swine production, Konowalchuk et al. (2013) observed an increase in serum calcidiol at 2 weeks of supplementation when diets are supplemented with 50 μg calcidiol compared to the equivalent of vitamin D_3. Flohr et al. (2016b) observed pig serum calcidiol increased at weaning as maternal vitamin D_3 supplementation increased. Also, serum calcidiol increased in the nursery pig due to maternal dietary supplementation of vitamin D_3 on d 17 and 35 postweaning although Flohr et al. (2016b) noted these differences might be due to the increase in ADFI and total vitamin D_3 intake of the nursery pigs.

In an earlier study, Flohr et al. (2014b) administered an ethanol solubilized oral vitamin D_3 mixed with peanut oil at a 1 mL dose to pigs at 1 or 2 days of age. On d 10, 20, and 30 of age, Flohr et al. (2014b) observed an increase in serum calcidiol as vitamin D_3 dose concentration increased from none to 80,000 IU of added vitamin D_3. Flohr et al. (2014b) also provided vitamin D_3 to 21-d old pigs in the drinking water at a concentration of 16,516 IU vitamin D_3/L from d 0 to 10 in a 30-d nursery study and found increased serum calcidiol on d 10, 20, and 30 compared to pig's drinking water with no added vitamin D_3.

Both research publications by Flohr et al. (2014b) and Konowalchuk et al. (2013) reported no improvement of growth performance in the nursery due to vitamin D_3 or calcidiol supplementation to the pigs. Flohr et al. (2016b) observed pigs from sows fed 2,000 IU of vitamin D_3 had increased ADG and ADFI in the nursery compared to pigs from sows fed 800 or 9,600 IU of vitamin D_3 per kg of the diet. Pigs from sows fed 50 μg calcidiol, which Flohr et al. (2016b) proposed to be equivalent to 2,000 IU of vitamin D_3, had improved ADG compared to pigs from sows fed 800 IU vitamin D_3 per kg of feed.

Konowalchuk et al. (2013) investigated the immunomodulatory effects of vitamin D_3 on 18-d old weaned pigs fed 50 μg calcidiol compared to 1,500 IU vitamin D_3 per kg. The pigs fed 50 μg calcidiol produced higher leukocyte numbers with an upregulated phagocytic capacity and survivability in systemic blood and peripheral bronchoalveolar mucosal compartments. Konowalchuk et al. (2013) discussed the increased leukocytes possibly being able to migrate to sites of pathogenic activity and could potentially increase the positive effect of leukocyte-driven antimicrobial mechanisms.

In swine, the current nutrient requirement for vitamin D is 220 IU/kg of the diet for nursery pigs (NRC, 2012). Increasing vitamin D_3 concentrations in an oral dose, drinking water, or dry feed, increased serum calcidiol in nursery pigs. Also, feeding weaned pigs 50 μg calcidiol, far above the NRC requirement, could potentially allow for improved antimicrobial mechanisms. Overall, although evidence did not strongly support improved growth performance in the nursery, the increased vitamin D_3 status of the animals and improved immunomodulatory effects suggest the current requirement of 220 IU/kg of the diet may not be sufficient.

Finishing Pigs

Although maternal diets and nursery diets contained supplementation of vitamin D_3 or calcidiol, Flohr et al. (2016b) placed finishing pigs on a common diet until market to evaluate subsequent growth performance. On d 70 after weaning, there was no difference in serum calcidiol due to maternal or nursery vitamin D_3 treatments. However, Flohr et al. (2016b) did observe an improved ADG and ADFI for pigs from sows fed 2,000 IU vitamin D_3 per kg of feed compared to pigs from sows fed 800 or 9,600 IU vitamin D_3. Also, pigs from sows fed 50 μg calcidiol per kg of feed achieved higher ADG than those pigs from sows fed 800 IU vitamin D_3 per kg of feed.

Regassa et al. (2015) fed finishing pigs 150 IU/kg of vitamin D_3 alone and supplemented 50 or 100 μg/kg calcidiol to the vitamin D_3. The added calcidiol did not improve growth performance or bone mineralization, however the supplemented calcidiol did improve the mRNA expression of SLC34A1, an intestinal transporter known as sodium-dependent phosphate transporter 1. Fecal Ca and P content was reduced in pigs fed added calcidiol at either level compared to vitamin D_3 (Regassa et al., 2015). Regassa et al. (2015) suggested the potential of the added calcidiol to reduce environmental pollution via increased retention of Ca and P paired with reduced excretion of these minerals.

Zhao et al. (2014) investigated the effects of increased dietary supplementation of vitamin D_3 when pigs were challenged with procine rotavirus (PRV) and observed 5,000 IU vitamin D_3 activated the retinoic acid-inducible gene I (RIG-1) signaling pathway in pigs and therefore alleviated some negative effects caused by the disease challenge compared to pigs fed the control diet with 200 IU vitamin D_3.

The current nutrient requirement for vitamin D_3 is 150 IU/kg for finishing pigs (NRC, 2012). Based on the literature reviewed, increasing the vitamin D_3 concentration in the diet of finishing pigs above the NRC requirement did not improve growth performance or bone mineralization but did alleviate some negative effects of a disease challenge. If finishing pigs are not disease challenged, the current NRC requirement is sufficient.

Swine Carcass Effects of Vitamin D_3

Flohr et al. (2016b) harvested a subsample of pigs from their study that looked at maternal and nursery vitamin D_3 supplementation with a common diet in the finishing. At marketing, pigs from sows fed 50 μg calcidiol had a heavier final live BW and HCW compared to pigs from sows fed 9,600 IU vitamin D_3. Also, as maternal vitamin D_3 increased, marketed pigs from those sows had increased carcass yield percentage and decreased loin depth and back fat thickness. Flohr et al. (2016b) discussed these responses may truly be due to maternal treatments or possibly due to numeric differences in weaning weight of pigs whose dams were fed 2,000 IU vitamin D_3.

Vitamin D_3 in Poultry

Introduction

In poultry, the latest published requirement by the National Research Council for vitamin D was in 1994 and had a value of 200 IU/kg (NRC, 1994). Broilers as well as laying and breeder hens are commercially reared indoors and therefore require vitamin D_3 in the diet.

Laying and Breeder Hens

Increasing vitamin D_3 in laying hen diets to 5,000 IU/kg of feed increased egg weight predominately due to increased albumen content (Browning and Cowieson, 2015). In contrast, Salvador et al. (2009) replaced 2,756 IU per kg of vitamin D_3 with 5.51g of calcitriol per ton of

the diet and reported no change in egg weight. Salvador et al. (2009) also observed an improved feed efficiency of laying hens when vitamin D_3 was replaced and an improvement in shell percent and thickness when calcidiol was combined in the diet with 200 and 100 ppm vitamin C respectively. Koreleski and Świątkiewicz (2005) substituted 25% of 1,500 IU vitamin D_3 per kg of the diet with calcidiol and observed eggs produced with thicker and denser shells. Koreleski and Świątkiewicz (2005) also reported increased egg shell breaking strength with partial or complete replacement of vitamin D_3 with calcidiol. Rivera et al. (2014) substituted up to 50% of 1,600 IU vitamin D_3 per kg of the diet with calcidiol and found an increase in the percentage of egg shell in the eggs compared to no substitution. Rivera et al. (2014) also reported an improved feed:egg ratio when layers were fed diets substituted with up to 75% of 1,600 IU vitamin D_3 per kg of the diet with calcidiol.

Käppeli et al. (2011) replaced half of 3,000 IU vitamin D_3 with calcidiol and reported an increase in serum calcidiol at 11, 18, and 34 weeks and a decreased phosphate in the blood at 11 and 34 weeks. Käppeli et al. (2011) observed no effect on serum calcium, performance, or shell quality.

It a study by Morris et al. (2015) where layers were challenged with coccidia oocysts, a supplemented 100 μg calcidiol per kg of feed allowed for only a 4% reduction in BW of the layer compared to a 15% reduction when 6.25, 25, or 50 μg per kg of calcidiol was fed. Also, 6 d post challenge, layers fed 100 μg calcidiol per kg of feed maintained a positive influence on their immune response with a 3.5-fold increase in IL-10 mRNA amounts in the cecal tonsils compared to birds fed 6.25 μg calcidiol per kg of feed. Morris et al. (2015) concluded that feeding birds 100 μg calcidiol per kg of feed could reduce production losses post-coccidia challenge.

Broiler breeder hens are not only evaluated on producing a functioning egg but also on the growth performance of the hatched progeny. Atencio et al. (2005) increased vitamin D_3 concentration in the maternal diets from 0 to 4,000 IU per kg of the diet. Atencio et al. observed as the maternal dietary composition increased, the progeny BW gain increased as well as the decrease of incidences of Ca rickets and tibial dyschondroplasia. When the chicks were supplemented with 0 to 40 μg per kg of calcidiol, their BW gain, tibia ash, and plasma Ca concentration increased with increasing concentrations of calcidiol. Atencio et al. noted there was no effect of dietary source of vitamin D_3 on progeny performance, only an effect due to level.

Other research on broiler breeder hens supplemented with calcidiol in the diets reported increased hatchability and immunity toward *E. coli* (Saunders-Blades and Korver, 2015), increased egg quality (Torres et al., 2009), and increased mineralization of bones in the progeny (Peng et al., 2013).

Broilers

Many studies have been done in broiler chickens looking at the impact of increasing levels of dietary vitamin D_3 as well as the potential for calcidiol to partially or completely replace vitamin D_3 in the diet. Khan et al. (2010) fed broilers 200, 1,500, 2,500, or 3,500 IU vitamin D_3 per kg of feed in an experiment designed to evaluate the NRC (1994) recommendation of 200 IU per kg compared to increasing levels of vitamin D_3. Khan et al. reported at d 21 and 42, the three highest levels of vitamin D_3 increased BW and feed conversion ratio above the NRC (1994) recommended diet with no differences in mortality. In a later study, Gomez-Verduzco et al. (2013) observed an improved BW gain and feed conversion on d 21 for broilers fed 2,000 IU of vitamin D_3 per kg of feed compared to the control 200 IU per kg.

While a large number of research experiments are evaluating the potential for calcidiol to partially or completely replace vitamin D_3 in the diet, Han et al. (2016) investigated these two commercially available vitamin D_3 sources. In 1 to 21-d old chickens, Han et al. (2016) used a slope ratio method to evaluate the relative bioavailability value (RBV) of calcidiol compared to vitamin D_3. Using BW gain as the sole contributor, they reported a RBV of 1.85. When combining the growth data with bone mineralization data, the overall RBV was about 2.03. Han et al. (2016) concluded calcidiol is 2.03 times as active as vitamin D_3 in promoting growth and mineralization of bones.

Yarger et al. (1995) replaced vitamin D_3 with calcidiol in the feed and observed improved BW and feed efficiency with no difference in mortality. In an experiment where 50 mg/L of calcidiol was administered through the water, Santiago et al. (2016) reported an increase in weight gain and lower mortality compared to broilers with no added supplement. However, they did not observe any differences in feed conversion ratio or feed intake (Santiago et al., 2016). Hutton et al. (2014) also reported no difference in feed efficiency or growth when substituting half of 5,000 IU vitamin D_3 with calcidiol.

The vitamin D_3 status of an animal is measured by the amount of calcidiol in blood. When broilers were fed calcidiol, their vitamin D_3 status improved over birds fed vitamin D_3 (Yarger et al., 1995; Hutton et al., 2014; Vignale et al., 2015). Yarger et al. (1995) reported a positive correlation between serum calcidiol and growth performance but no such correlation with serum calcitriol.

As the vitamin D_3 in the feed increased to 3,500 IU vitamin D_3 per kg, Khan et al. (2010) reported an increase in bone mineralization as seen by an increase in tibia and toe ash of the broiler's right leg. A diet containing 2,000 IU of D_3 and 69 μg of calcidiol per kg of feed

increased bone calcification of tibias in 21-d old broilers when compared to 200 IU of D_3 or 69 μg of calcidiol per kg of feed alone (Gomez-Verduzco et al., 2013). Santiago et al. (2016) also reported increased calcium in the tibias when calcidiol was provided through the water.

J. D. Starkey (2014) discussed the topic of vitamin D and its roll in skeletal muscle and growth and commented about the need to enhance vitamin D status beyond preventing deficiency and allowing for genetic potential to be reached for skeletal muscle development and hypertrophy as well as growth and reproductive performance. Hutton et al. (2014) reported an increase in breast meat yield when boiler diets partially replaced 5,000 IU vitamin D_3 with calcidiol. Hutton et al. (2014) contributed the increase in breast meat to the satellite cell-mediated skeletal muscle hypertrophy response in the pectoralis major muscle. Khan et al. (2010) also reported increased dressing percentage and breast meat yield at the two highest levels of dietary vitamin D_3, 2,500 IU and 3,500 IU vitamin D_3 per kg of feed.

When Vignale et al. (2015) fed 5,520 IU of calcidiol per kg of the diet for 42 days, they observed an increase in breast meat yield as well as an increase in the rate of protein synthesis when compared to 2,760 IU of vitamin D_3 per kg of feed. Vignale et al. (2015)stated this high dose of calcidiol increased the expression of the vitamin D receptor compared to the other diets. They conclude that the effect of calcidiol on male broiler breasts likely is mediated through the mTOR-S6K pathway. After completing their own literature review, Świątkiewicz et al. (2017) recommended about 3,000 IU of vitamin D per kg of feed be in poultry diets.

Summary of Vitamin D_3 in Swine and Poultry

A large amount of research has been conducted since the discovery of vitamin D in 1922, which has shined light on the vitamin's requirements and how it is formed. Vitamin D_3 requirements can be achieved by its production in the body and/or by being consumed in the diet.

It can be produced in the skin of the mammal when ultraviolet sunlight is present, or it can be provided in the diet and absorbed in the gastrointestinal tract of the animal.

Based on research reviewed for gilts and sows, the current NRC (2012) nutrient requirement of vitamin D_3 needs to be increased to a level above 800 IU/kg of the diet. Most of the sow research tested 2000 IU/kg vitamin D_3 or substituted a commercially available calcidiol, 25(OH)D_3. Relevant research often suggested the calcidiol product being more effective in raising vitamin D_3 status of the animal and potentially increasing total litter birth weight when compared to vitamin D_3 at the same concentration. Similarly, in nursery pigs, although evidence did not strongly support improved growth performance, the increased vitamin D_3 status of the animals and improved immunomodulatory effects suggest the current NRC (2012) nutrient requirement of 220 IU/kg of the diet in the nursery phase is not sufficient.

The current nutrient requirement for vitamin D_3 is 150 IU/kg for finishing pigs (NRC, 2012). Based on the literature reviewed, increasing the vitamin D concentration in the diet of finishing pigs above the NRC requirement did not improve growth performance or bone mineralization but it did alleviate some negative effects of a disease challenge. If the finishing pigs were not disease challenged, the current NRC requirement is sufficient.

In poultry, the latest published requirement by the NRC for vitamin D_3 was in 1994 and had a value of 200 IU/kg (NRC, 1994). Laying and breeder hen research suggests improvements in egg weight when layers were fed 5,000 IU/kg, thicker and denser shells with increased eggshell breaking strength when 25% of 1,500 IU /kg was substituted with calcidiol, and increased progeny body weight gain as maternal diets increased up to 4,000 IU of vitamin D_3 per kg of the diet. Morris et al. (2015) concluded that feeding birds 100 μg calcidiol per kg of feed

could reduce production losses post-coccidia challenge. All of these researched levels of vitamin D_3 are much higher than the current NRC recommendation.

Research completed with broilers also supports the need for elevated requirements of vitamin D_3 in poultry above 200 IU/kg. Increased BW and improved feed conversion ratio has been observed in broilers fed concentrations of vitamin D_3 above 200 IU/kg including 1,500, 2,000, 2,500, and 3,500 IU vitamin D_3 per kg of feed. Researchers have also observed an increase in bone mineralization and an increase in breast meat yield when elevated levels of vitamin D_3 was fed.

In conclusion, the current NRC requirements concerning vitamin D in sows, nursery pigs, hens, and broilers need to be re-evaluated with finishing pig requirements possibly kept the same. In swine and poultry, the commercially available calcidiol product to replace the vitamin D_3 in the diet seems to show improved vitamin D_3 status of the animals as well as possible improvements in production efficiency.

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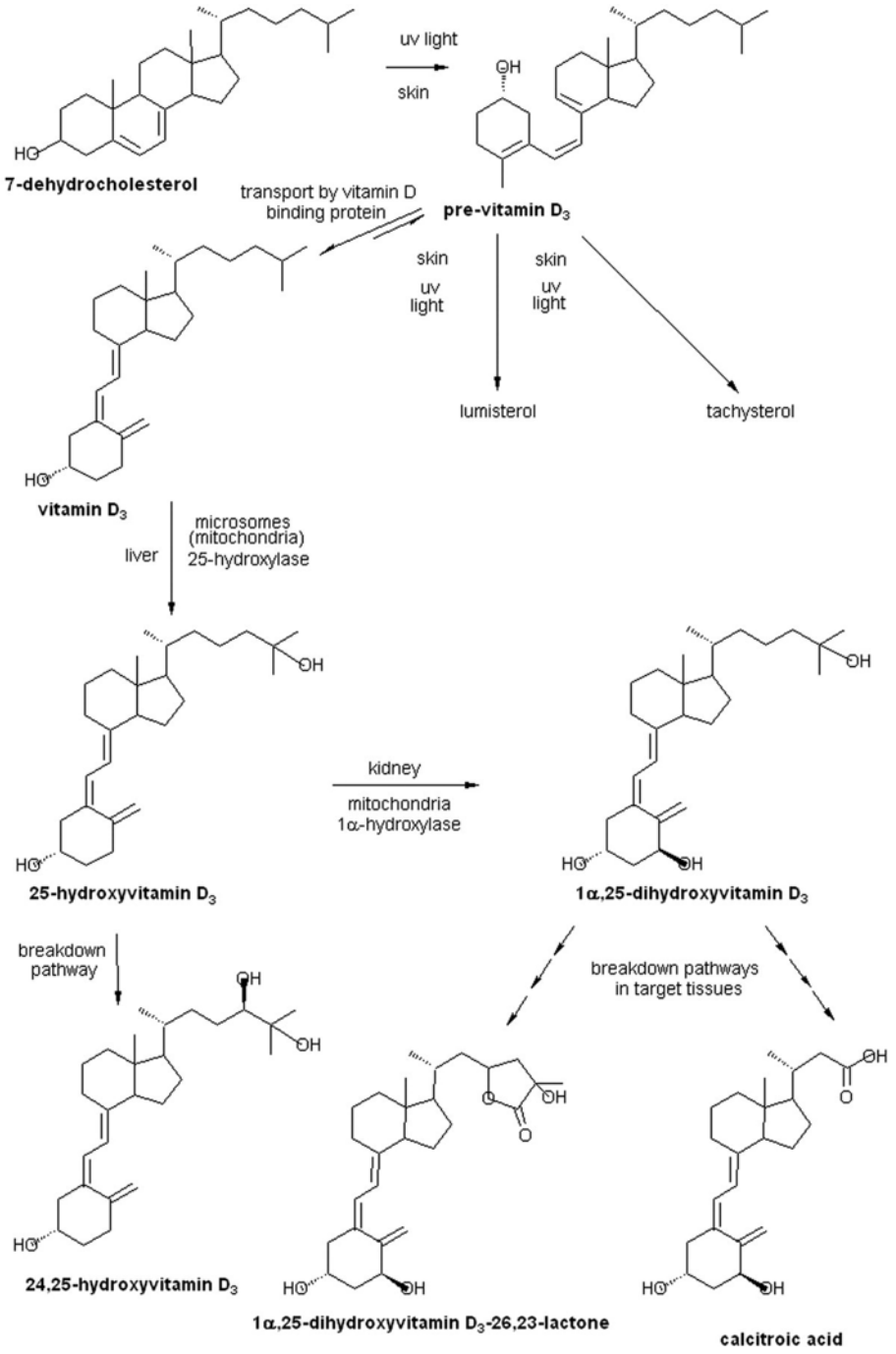
Figure 1.1 Vitamin D synthesis, activation, and breakdown (Dittmer and Thompson, 2011).



Chapter 2 - The effects of maternal dietary supplementation of cholecalciferol (vitamin D_3) in conjunction with 25(OH)D_3 on sow and pig performance

ABSTRACT: A total of 69 sows (DNA Line 200 × 400) and their progeny were used to determine if feeding a combination of vitamin D_3 (Rovimix D_3, 500,000 IU/g; DSM Nutritional Products, Parsippany, NJ) and 25(OH)D_3 (Hy-D, DSM Nutritional Products, Parsippany, NJ) influences neonatal and sow vitamin D_3 status, muscle fiber morphometrics and development of the piglets, and subsequent growth performance to market. Within 3 days of breeding, sows were allotted to 1 of 3 dietary treatments fortified with 1,500 IU/kg vitamin D_3 (CON), 500 IU/kg vitamin D_3 + 25 μg/kg 25(OH)D_3 (DL), or 1,500 IU/kg vitamin D_3 + 50 μg/kg 25(OH)D_3 (DH). Differences in sow productivity and growth performance of progeny due to dietary treatment were not observed ($P > 0.05$). When pigs were sacrificed at birth, there were no treatment effects for all fiber morphometric measures ($P > 0.170$), except primary fiber number and the ratio of secondary to primary muscle fibers ($P < 0.014$). Pigs from the CON and DL fed sows had less primary fibers than pigs from sows fed the DH treatment ($P < 0.046$), but did not differ from each other ($P = 0.732$). These results suggest progeny went through a longer prenatal period of primary myogenesis which delayed the onset of secondary myogenesis. Pigs from DL fed sows had a smaller secondary to primary muscle fiber ratio compared to pigs from sows fed the CON treatment ($P = 0.016$), with pigs from sows fed DL treatment not differing from either ($P > 0.057$). There were treatment × time interactions for all sow and pig serum metabolites ($P < 0.001$). Therefore, we chose to compare treatment means within time period. At all time periods, sow serum 25(OH)D_3 concentrations differed for all treatments with the magnitude of difference

largest at weaning ($P < 0.011$). On all three collection days, DH fed sows contained the greatest serum levels of 25(OH)D_3. For pig vitamin D_3 status, the interaction was due to serum concentrations of 25(OH)D_3 in pigs at birth from CON and DL fed sows not being different from each other, where at weaning, pigs from all sow treatments differed. At both sampling days, progeny from DH fed sows contained the greatest serum levels of 25(OH)D_3. There was no interaction for 25(OH)D_3 concentration in colostrum and milk, however a main effect of dietary treatment ($P < 0.001$) and of time ($P = 0.001$) existed. Within maternal dietary treatment, colostrum contained less ($P = 0.001$) 25(OH)D_3 compared to milk collected within 12 h and on d 21, respectively. Within sampling time, 12 h for colostrum and 21 d for milk, sows from all treatments were different ($P < 0.030$), with the largest 25(OH)D_3 concentration from DH fed sows, followed by DL, and followed by CON. In conclusion, combining vitamin D_3 and 25(OH)D_3 in the maternal diet improves the vitamin D_3 status of the dam and progeny and it increases primary muscle fibers at birth.

Introduction

Vitamin D_3 plays a major role in Ca and P absorption as well as bone calcification. This is important for mammals to have a strong skeleton to increase longevity and improve animal welfare. Transitioning livestock to living in a controlled environment indoors eliminates their access to direct sunlight, which limits endogenous production of vitamin D_3 in the animal's skin. The most common form of vitamin D fed in swine diets today is vitamin D_3.

The 25th carbon of vitamin D_3 is hydroxylated in the liver to form calcidiol (25(OH)D_3). This metabolite is then transported through the blood to the kidney where more carbon atoms are hydroxylated to form different metabolites of vitamin D_3, each with its own function in the body. Calcidiol found in blood serum is considered a good indicator of adequacy, deficiency, or

toxicity of vitamin D_3, which is communicated as the vitamin D_3 status of the animal (Soares et al., 1995).

Zhou et al. (2016) investigated improving maternal vitamin D_3 status and the impact on the sow's offspring. They evaluated muscle fiber characteristics of newborn and weaning piglets whose mothers were fed 25(OH)D_3 in combination with vitamin D_3 and observed an increase in total muscle fiber numbers of newborn and weaning piglets as well as an increase in muscle fiber cross-sectional area of weaning piglets. This promotion of skeletal muscle development before and after the piglets were born suggests the potential for better growth performance and enhanced carcass leanness and muscling when dams are fed 25(OH)D_3 in combination with vitamin D_3.

The objective of this study was to determine if feeding a combination of vitamin D_3 (Rovimix D3, 500,000 IU/g; DSM Nutritional Products, Parsippany, NJ) and its more available metabolite, 25(OH)D_3 (Hy-D, DSM), influences sow and pig performance. Performance parameters included sow performance, sow and piglet vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets.

Materials and Methods

General

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

Animals and Diets

A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used in this study. Within 3 days of breeding, each sow was assigned to 1 of 3 dietary treatments equalized for parity and BW. Gestation and lactation diets were fortified with 1,500 IU/kg vitamin D_3 (treatment **CON**), 500 IU/kg vitamin D_3 + 25 μg/kg 25(OH)D_3 (treatment **DL**), 1,500 IU/kg vitamin D_3 + 50 μg/kg 25(OH)D_3 (treatment **DH**). The total vitamin D_3 activity of the CON, DL, and DH diets were 1,500 IU, 1,500 IU, and 3,500 IU/kg of the diet, respectively. According to a survey of current vitamin and trace minerals fed in the US swine industry, Flohr et al. (2016c) combined information from 18 producer nutritionists accounting for about 40% of the US sow herd and found the median concentration of vitamin D_3 fed to gestating and lactating sows was 1,762 IU/kg. The maximum vitamin D_3 allowed in Canada for sows is 1,500 IU/kg. Therefore, our CON diet contained 1,500 IU/kg vitamin D_3. All diets were formulated to meet or exceed the Swine NRC (2012) dietary requirements for all other nutrients (Table 2.1).

During gestation, sows were housed in individual gestation stalls equipped with individual water nipples and a feed trough. During d 0 to 74 of gestation, sows were fed 2.0 kg of feed once per day at 0800 h. Feed allowance increased to 2.5 kg/d from d 75 to 110. Sows were moved into the farrowing house after consuming gestation feed on d 110. All sows were weighed within 3 d of breeding, within 24 h of farrowing, and at weaning.

In the farrowing house, sows were housed in individual farrowing crates and fed 4 times throughout the day using an electronic feeding system (Gestal Solo; JYGA Technologies, Quebec, Canada). Gestation diets were fed in the farrowing house until the sow farrowed. Once the sows gave birth, they were transitioned to lactation diets and placed on an individual feeding

curve determined by parity. Farrowing crates were also equipped with individual water nipples, snout coolers, and water misters. Lactation feed intake was recorded by feed disappearance on d 7, 14, and 21. Individual piglet weights were recorded within 24 h of birth and at weaning on d 21. The progeny from one farrowing group was double ear-tagged and followed through the nursery and finisher to market.

In the nursery, a total of 216 pigs were randomly placed in 36 pens, 6 pigs per pen, based on maternal dietary treatment. Nursery pens allowed 0.304 m^2 of floor space per pig and were equipped with a 4-hole feeder and a nipple waterer. Nursery diets were fed in 2 phases and fortified with 1,500 IU/kg vitamin D_3 (treatment CON), 500 IU/kg vitamin D_3 + 25 μg/kg 25(OH)D_3 (treatment DL), 1,500 IU/kg vitamin D_3 + 50 μg/kg 25(OH)D_3 (treatment DH). The total vitamin D_3 activity of the CON, DL, and DH diets were 1,500 IU, 1,500 IU, and 3,500 IU/kg of the diet, respectively. All diets were formulated to meet or exceed the Swine NRC (2012) dietary requirements (Table 2.2). Phase 1 was fed in meal form from d 0 to 14 postweaning. Phase 2 was fed also in meal form from d 14 to 59 postweaning. Individual pen weights and feeder weights were measured on d 7, 14, 21, 28, 35, 42, 49, and 59 to determine ADG, ADFI, and G:F.

Pigs were transferred from the nursery to the finisher on d 59 postweaning. They were moved to preserve pen integrity. Finisher pens allowed for 0.836 m^2 of slatted floor space per pig and were equipped with a dry self-feeder with 2 eating spaces and a 1-cup waterer for ad libitum access. The finisher dietary treatments were fed in 3 phases. Phase 1 diet was fed until about 61 kg BW then switched to phase 2 until about 100 kg BW. The third and final diet phase was fed from 100 kg of body weight until market. Finisher phase 1 and 2 diets, containing the same added levels of vitamin D_3, were fortified with 1,000 IU/kg vitamin D_3 (treatment CON), 25

μg/kg 25(OH)D_3 (treatment DL), 50 μg/kg 25(OH)D_3 (treatment DH). The total vitamin D_3 activity of the CON, DL, and DH diets were 1,000 IU, 1,000 IU, and 2,000 IU/kg of the diet, respectively. Finisher phase 3 diet was fortified with 800 IU/kg vitamin D_3 (treatment CON), 20 μg/kg 25(OH)D_3 (treatment DL), 40 μg/kg 25(OH)D_3 (treatment DH). The total vitamin D_3 activity of the CON, DL, and DH diets were 800 IU, 800 IU, and 1,600 IU/kg of the diet, respectively. All diets were formulated to meet or exceed the Swine NRC (2012) dietary requirements (Table 2.3). Feed was distributed and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) 4 times a day. Individual pen weights and feeder weights were measured approximately every 16 d to determine ADG, ADFI, and G:F ratio. Final live weight was collected on individual pigs one day before marketing. HCW was collected on individual pigs at Triumph Foods (St. Joseph, MO).

Chemical Analyses

All diets were prepared at the K-State O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Gestation, lactation, and nursery diets were bagged and sampled at the feed mill. Finisher diets were delivered in bulk and sampled from 80% of the feeders in the finisher facility occurring once per feed delivery. Samples were pooled, subsampled, and stored at -20°C. Feed samples were analyzed for vitamin D_3 as well as Hy-D by DSM Nutritional Products (Parsippany, NJ) using a combination of HPLC and mass spectrometry (Schadt et al., 2012). Feed samples were also analyzed for Ca (Campbell and Plank, 1991; Kovar, 2003), P (Campbell and Plank, 1991; Kovar, 2003; Wolf et al., 2003), and CP (AOAC 990.03, 2006) by Ward Laboratories (Kearney, NE). Tables 2.1 to 2.6 contain diet compositions and analyses.

Serum Collection and Analyses

Blood samples were collected via jugular venipuncture from sows within 3 d of breeding, excluding the first group of sows pending IACUC approval. All sows were bled on d 100 of gestation as well as within 24 h of farrowing and at weaning (lactation d 21) to be analyzed for serum $25(OH)D_3$. Within 24 h of birth and at weaning, blood samples were collected via mammary vein from one average BW male and female piglet per litter to be analyzed for serum vitamin D_3, $25(OH)D_3$, and $24,25(OH)_2D_3$. On d 59 of the nursery phase, about 80 d old pigs, a blood sample was collected via jugular venipuncture from one average weight gilt per pen to be analyzed for serum $25(OH)D_3$. One day before market, a blood sample was collected via jugular venipuncture from one average weight gilt per pen, preferably the same gilt bled in the nursery, to be analyzed for serum $25(OH)D_3$. Whole blood samples were stored at 4° C for 24 h after collection. They were then centrifuged (1,800 × g for 30 min at 4° C) and serum was collected for analysis. Serum samples were stored at -80°C in polypropylene tubes before being sent to Heartland Assays LLC (Ames, IA) for analysis using the following method.

Serum/plasma samples along with standard curve and controls were protein precipitated with 0.2M zinc sulfate solution (Polson et al., 2003), vortexed, followed by methanol addition and vortexed. Then d3-vitamin D_3/d3-$25(OH)D_2$/d3-$25(OH)D_3$/d6-$24,25(OH)_2D_3$ internal standards were added to appropriate samples and controls followed by vortexing. Hexane was added to all samples and controls then tubes were capped and vortexed, followed by centrifugation. The organic layer was then transferred followed by drying. All standards, controls, and samples were then reconstituted with **LC/MS** (liquid chromatography/mass spectrometry) grade methanol and water with both containing 0.1% formic acid, then loaded onto the auto-sampler for analysis. The LC/MS/MS system used was an Agilent 1290 infinity

HPLC coupled to an Agilent 6460 MS/MS with ESI source. Assay accuracy was determined to be > 95% based on NIST certified standard assessment (Makowski et al., 2017; Weidner et al., 2017; Verone-Boyle et al., 2016) for 25(OH)D and 24,25$(OH)_2$D. Controls for vitamin D in serum were also found to be > 90% accurate. Reagents, solvents, and supplies were purchased through Sigma-Aldrich/Cerilliant, Fischer Scientific, Isosciences, Agilent Technologies and Medical Isotopes.

Colostrum and Milk Collection and Analyses

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

Milk samples were weighed out along with assay controls containing 25$(OH)D_2/D_3$ and 24,25$(OH)_2D_2/D_3$ samples and controls were then spiked with d3-vitamin D_3/d3-25$(OH)D_2$/d3-25$(OH)D_3$/d6-24,25$(OH)_2D_3$ internal standards. Methanolic potassium hydroxide was then added to all samples and controls and saphonified (Larson-Meyer et al., 2017; Roseland et al., 2016) in a water bath at 60°C. After 2.0 h, samples and controls were vortexed and then liquid-liquid extracted with hexanes: methylene chloride (80:20) solution. The organic layer was dried and then reconstituted with hexanes and methylene chloride (90:10) and then applied to 1.0 g silica **SPE** (solid phase extraction) columns for further purification and isolation. Elution was then dried and derivatized with 0.75 mg/ml **PTAD** (4-Phenyl-1,2,4-triazole-3,5-dione) (Aronov et al., 2008) in acetonitrile for 2.0 h at room temperature. Samples and controls were then dried and reconstituted with LC/MS/MS mobile phase containing acetonitrile, methanol, water and 0.1% formic acid and then loaded onto the auto-sampler for analysis. The LC/MS/MS system used was

an Agilent 1290 infinity HPLC coupled to an Agilent 6460 MS/MS with ESI source. All controls were found to be > 94% accurate with %CV for inter-assay < 10.0% and intra-assay of < 5.0%. All analytes had R^2 values of > 0.99 with assay range from 0.062 to 8.000 ng/g. Reagents, solvents and supplies were purchased through Sigma-Aldrich/Cerilliant, Fischer Scientific, Isosciences, Agilent Technologies and Medical Isotopes.

Harvest and Muscle Sample Collection

One average BW male within 24 h of birth and at weaning were selected to be euthanized from 36 litters farrowed in this study. Selected pigs were euthanized by exposure to CO_2 gas for 10 min administered via a Euthanex® AgProTM system (Nutriquest, Mason City, IA). Prior to dissection, whole body measurements were collected including: crown to rump length, head width, head length, head circumference, and heart girth. During dissection, weights of the heart, lungs, liver, kidneys, and brain were collected. Carcasses were split, the left side was further fabricated to primal cuts, and weights of the boston butt, picnic shoulder, loin, ham, and belly were recorded. The right LM was removed between the fourth rib and last lumbar vertebrae, weighed, and used for immunohistochemistry procedures.

Immunohistochemistry

A 2.54-cm section of the LM was removed between the last rib and second or fourth vertebrae, depending on the age of the pig. Whole muscle cross-sectional area **(CSA)** was collected by gently placing the LM muscle on blotting paper and tracing the outline of the blot. Blots, including a reference scale, were imaged using a scanner (Hewlett-Packard, Palo Alto, CA). Using NIS Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc., Melville, NY), images were calibrated, and the area within the outline was measured.

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

Cryosections analyzed for pigs harvested at birth were incubated in blocking solution, which contained 5% horse serum and 0.2% TritonX-100 (Fisher Scientific) in phosphate-buffered saline (PBS) for 30 min to inhibit nonspecific antigen-binding sites. Cryosections were incubated in the following primary antibodies: undiluted supernatant α-Pax7 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), 1:500 α-dystrophin (Thermo Scientific, Waltham, MA), and 1:10 supernatant myosin heavy chain, slow, IgG2b (BA-D5; Developmental Studies Hybridoma Bank) for 18 h at 4°C in a humidified environment. Following incubation, cryosections were rinsed with PBS 3 times for 5 min each and incubated with the following secondary antibodies in blocking solution for 30 min protected from light and in a humidified environment: 1:1,000 Alexa-Flour 488 goat-anti-mouse IgG1 heavy and light chains (Life Technologies) for Pax7, 1:1,000 Alexa-Flour 594 goat-anti-rabbit heavy and light chains (Life Technologies) for α-dystrophin, 1:1,000 Alexa-Flour 633 goat anti-mouse IgG2b (Life Technologies) for BA-D5, and 1:1,000 Hoechst 33342 dye (Thermo Scientific) for nuclei.

The first slide for pigs harvested at weaning was used for muscle fiber type and CSA analyses, and the second slide was used for satellite cell analysis. All slides were incubated in blocking solution as described above. Muscle fiber type slides were incubated in PBS with the

following primary antibodies: 1:500 α-dystrophin (Thermo Scientific), 1:10 supernatant myosin heavy chain, slow, type I, IgG2b (BA-D5; Developmental Studies Hybridoma Bank), 1:10 supernatant myosin heavy chain, type IIA, IgGl (SC-71; Developmental Studies Hybridoma Bank), and 1:10 supernatant myosin heavy chain, type IIB, IgM (BF-F3; Developmental Studies Hybridoma Bank). Cryosections were rinsed and the following secondary antibodies and dilutions used were 1:1,000 Alexa-Flour 594 goat-anti-rabbit heavy and light chains (Life Technologies) for α-dystrophin, 1:1,000 Alexa-Flour 633 goat anti-mouse IgG2b (Life Technologies) for BA-D5, 1:1,000 Alexa-Flour 594 goat anti-mouse IgG1 (Life Technologies) for SC-71, 1:1,000 Alexa-Flour 488 Goat anti-mouse IgM (Life Technologies, Carlsbad, CA) for BF-F3, and 1:1,000 Hoechst 33342 dye (Thermo Scientific) for fiber-associated nuclei.

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

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

For muscle fiber morphometric data collection, a minimum of 1,000 fibers per animal (minimum of 2 photomicrographs per section) were analyzed with NIS-Elements Imaging

Software (Nikon Instruments Inc.). When analyzing muscle fibers of pigs harvested at birth, primary muscle fibers stained positively for BA-D5 and secondary muscle fibers stained negative for BA-D5. When analyzing muscle fibers of pigs harvested at weaning, fibers that stained exclusively positive for BA-D5, SC-71, and BF-F3 were labeled type I, type IIA, and IIB, respectively. Fibers that stained positive for both SC-71 and BF-F3 were labeled as type IIX fibers (Noel et al., 2016). The periphery of all muscle fibers was identified with α-dystrophin, Hoechst 33342 dye identified all nuclei, and Pax7 identified satellite cells for all cryosections. The total number of muscle fibers within the LM was calculated as the whole muscle CSA divided by the overall average muscle fiber CSA. The total number of a specific fiber isoform was calculated by multiplying the total number of muscle fibers by the percentage of fibers pertaining to that specific type.

Statistical Analyses

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

Results

There were no treatment effects of combining 25(OH)D_3 with vitamin D_3 in the maternal diet on sow and preweaned pig performance ($P > 0.283$; Table 2.7). When pigs were sacrificed at birth, there were no treatment effects for all fiber morphometric measures ($P > 0.170$; Table 2.8), except primary fiber number and the ratio of secondary to primary muscle fibers ($P < 0.014$). Pigs from the CON and DL fed sows had less primary fibers than pigs from sows fed the DH treatment ($P < 0.046$), but did not differ from each other ($P = 0.732$). Pigs from DL fed sows had a smaller secondary to primary muscle fiber ratio compared to pigs from sows fed the CON treatment ($P = 0.016$), with pigs from sows fed DL treatment not differing from either ($P > 0.057$). When pigs were sacrificed at weaning, there were no treatment effects for all fiber morphometric measures ($P > 0.129$; Table 2.9).

There were no treatment effects on whole body measurements of pigs sacrificed at birth or weaning ($P > 0.100$; Table 2.10), except head width at birth ($P = 0.038$). Pigs from DL fed sows had a larger head width at birth compared to pigs from sows fed the DH treatment ($P = 0.043$), with pigs from sows fed CON treatment not differing from either ($P > 0.113$).

All pig serum samples analyzed for vitamin D_3 had concentrations that were not above the detectable limit of 1.5 ng/mL. There were treatment × time interactions for all other sow and pig serum metabolites ($P < 0.001$; Table 2.11). Therefore, we chose to compare treatment means within time period. At all time periods, sow serum 25(OH)D_3 concentrations differed for all treatments with the magnitude of difference largest at weaning ($P < 0.011$), where serum 25(OH)D_3 concentration was always the greatest when sows were fed the DH diet. The interaction was due to serum concentrations of 25(OH)D_3 in pigs at birth from CON and DL fed sows not being different from each other, where at weaning, pigs from all sow treatments

differed. At birth, pigs from DH fed sows had greater serum $25(OH)D_3$ concentrations than pigs from sows fed the DL treatment ($P = 0.003$), with pigs from sows fed CON treatment not differing from either ($P > 0.061$). At weaning, serum concentrations of $25(OH)D_3$ in pigs from all sow treatments were different ($P < 0.001$), with the greatest concentration in pigs from DH sows, followed by CON, and followed by DL. There was a treatment effect on percent of detectable samples analyzed for serum $25(OH)D_3$ at birth ($P < 0.001$). The percent of samples from pigs born to all sow treatments were different ($P < 0.016$), with the largest percentage in DH progeny samples, followed by DL, and followed by CON. All samples analyzed for serum $25(OH)D_3$ at weaning were above the detectable limit of 1.5 ng/mL.

The interaction was due to serum concentrations of $24,25(OH)_2D_3$ in pigs at birth from all sow treatments were different, where pigs at weaning from CON and DL fed sows were not different from each other. At birth, serum concentrations of $24,25(OH)_2D_3$ in pigs from all sow treatments were different ($P < 0.001$), with the greatest concentration in pigs from DH sows, followed by DL, and followed by CON. At weaning, pigs from the CON and DL fed sows had serum $24,25(OH)_2D_3$ concentrations less than pigs from sows fed the DH treatment ($P < 0.001$), but did not differ from each other ($P = 0.944$). All samples analyzed for serum $24,25(OH)_2D_3$ at birth were above the detectable limit of 0.3 ng/mL. There was no treatment effect on percent of detectable samples analyzed for serum $24,25(OH)_2D_3$ at weaning ($P = 0.783$). At both time periods, grower and finisher, measuring pig serum $25(OH)D_3$ concentrations, all treatments differed from each other with the magnitude of difference larger in grower age pigs ($P < 0.001$).

There were no treatment × time interactions for all milk metabolites including the percent of detectable samples ($P > 0.068$; Table 2.12). There was a treatment effect ($P < 0.001$) and time effect ($P = 0.001$) for $25(OH)D_3$ concentrations in milk. Within maternal dietary treatment,

colostrum contained less ($P = 0.001$) $25(OH)D_3$ compared to milk collected within 12 h and on d 21, respectively. Within sampling time, 12 h for colostrum and 21 d for milk, sows from all treatments were different ($P < 0.030$), with the largest $25(OH)D_3$ concentration from DH fed sows, followed by DL, and followed by CON. There were no treatment or time main effects for $24,25(OH)_2D_3$ concentrations in milk ($P > 0.166$). There was no treatment effect on percent of detectable samples analyzed for serum $24,25(OH)_2D_3$ ($P = 0.783$), but there was a main effect of time ($P = 0.011$). Within maternal dietary treatment, the percent of samples above the detectable limit was greater ($P = 0.011$) in colostrum than milk collected within 12 h and on d 21, respectively.

When pigs were in the nursery, there were no treatment effects for all growth performance measures ($P > 0.132$; Table 2.13), except feed efficiency from d 28 to 59 and d 0 to 59 ($P < 0.015$). When pigs were in the finisher, there were no treatment effects for all growth performance measures ($P > 0.171$; Table 2.14). Also, there was no treatment effect for live weight, HCW, or dressing percentage in pigs taken to market ($P > 0.826$; Table 2.15).

Discussion

The current experiment investigated the impact of adding vitamin D_3 in combination with $25(OH)D_3$ to the maternal diet on sow performance, sow and piglet vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. Sows were fed 1 of 3 diets fortified with 1,500 IU/kg vitamin D_3 (treatment CON), 500 IU/kg vitamin D_3 + 25 μg/kg $25(OH)D_3$ (treatment DL), 1,500 IU/kg vitamin D_3 + 50 μg/kg $25(OH)D_3$ (treatment DH). The total vitamin D_3 activity of the CON, DL, and DH diets were 1,500 IU, 1,500 IU, and 3,500 IU/kg of the diet, respectively. Interestingly, in humans, orally consuming 1 μg of $25(OH)D_3$ was about 5 times more effective in raising serum $25(OH)D_3$ than the same amount of

vitamin D_3 (Cashman et al., 2012). Therefore, although the units of total vitamin D_3 activity are equivalent in CON and DL diets, 25(OH)D_3 in combination with vitamin D_3 in the DL diet could prove to be more available to the animal.

Varying results have been detected in studies investigating sow reproductive performance and preweaned piglet growth performance in response to added dietary vitamin D_3. Based on results from this experiment, there was no evidence for improved sow performance for all measurements or preweaned piglet performance which is consistent with Flohr et al. (2014a; 2016a). Peng et al. (2013) also observed no effect of added 25(OH)D_3 to the diet on reproductive performance of breeder hens. In contrast, Weber et al. (2014) detected total litter birth weight and birth weight per piglet increased when dams were fed diets replacing vitamin D_3 with 25(OH)D_3 at the same level due to maternal 25(OH)D_3 supporting intrauterine embryo development. Like the current study, combining vitamin D_3 sources, 50 μg/kg 25(OH)D_3 added to a diet already containing 50 μg/kg vitamin D_3 fed to sows (4,000 vs. 2,000 IU total vitamin D_3 activity) increased the number of piglets born alive by 1 piglet. Zhou et al. (2016) suggest the born alive increase is due to vitamin D_3 of any form effecting maternal-conceptus interaction to improve implantation, but no difference in pig performance was observed (Zhou et al., 2016). Zhou et al. (2017) combined vitamin D_3 sources and observed an increase in piglet growth performance the first 2 weeks of lactation when the combination was fed. Zhou et al. (2017) suspect the improved performance was due to maternal intake of 25(OH)D_3 improving fat and protein contents in milk. No differences in piglet growth performance was detected at weaning on d 28 of lactation (Zhou et al., 2017).

Research in humans and other animal species suggests there is a role of vitamin D_3 in the formation of skeletal muscle. In swine, investigators have observed an increase in total muscle

fiber number of the LM of d 90 fetuses (Hines et al., 2013) and pigs at birth and weaning (Zhou et al., 2016) when the maternal diet contained a combination of vitamin D_3 and 25(OH)D_3. Wigmore and Stickland (1983) determined secondary muscle fiber myogenesis begins to occur around d 50 lasting until d 90 of gestation and is why Hines et al. (2013) chose to study d 90 fetuses. In the current study, although there was no difference in total muscle fiber number at birth or weaning, which is in agreement with Flohr et al. (2016a), we did observe an increase in primary muscle fiber numbers of piglets at birth born to dams fed the DH diet compared to the pigs at birth born to DL and CON fed dams. There was also no difference in the individual fiber type numbers at weaning. For the DH treatment fed to the dams since mating, results suggest their progeny went through a longer prenatal period of primary myogenesis which delayed the onset of secondary myogenesis. Interestingly, Wigmore and Stickland (1983) also concluded that secondary fibers are more susceptible to external influences, i.e. nutritional environment of the embryo, than primary fibers. In the present experiment, it appears that we influenced primary myogenesis with maternal nutrition and not secondary.

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

The current study provided evidence for a smaller average number of secondary muscle fibers per primary muscle fiber ratio when dams were fed the DH diet (3,500 IU total vitamin D_3 activity) compared to CON (1,500 IU D_3), with a mean ratio of 11.8 and 20.4, respectively. From the experiment completed by Flohr et al. (2016a), it appears that increasing the total vitamin D_3

activity in the diet fed to sows increases the mean ratio of secondary fibers per primary, however in the current study, the ratio appears to decrease due to the increase in total vitamin D_3 activity fed to the dam, which could be partially explained by the increase in primary muscle fibers observed. Although we cannot explain why results from the two experiments were conflicting, we can highlight the differences in the amount of total vitamin D_3 activity tested in each experiment, a single or combined source of vitamin D_3 fed, the location of fiber testing, and different genetics of the pigs. Flohr et al. (2016a) fed a single source of vitamin D_3 and in a large quantity, where in this experiment, a combination of sources were used at 64% less total vitamin D_3 activity. Also, Flohr et al. (2016a) analyzed muscle fibers from the longissimus thoracis muscle of PIC pigs, however the current study analyzed muscle fibers from a different location of the same muscle, the longissimus lumborum, and of DNA genetics.

Vitamin D_3 status of the animal is typically assessed by measuring 25(OH)D_3 concentration in circulating serum or plasma. In research, evaluating the replacement of vitamin D_3 with 25(OH)D_3 or combining them in the diet, sow blood levels of 25(OH)D_3 were greater during gestation, at farrowing, and at weaning when the sow's diet included 25(OH)D_3 (Lauridsen et al., 2010; Coffey et al., 2012; Weber et al., 2014; Zhou et al., 2016), which is in agreement with the current study. At all days of collection, serum 25(OH)D_3 concentration was the greatest when sows were fed the DH diet, with the DL diet being intermediate, and sows fed the CON diet having the least amount of 25(OH)D_3 in the serum. The interaction appeared to be due to means separating wider at weaning compared to gestation d 100 and farrowing. Lauridsen et al. (2010) and Weber et al. (2014) also observed a general decrease in serum levels around farrowing and an increase as lactation days accumulate. This trend over time could simply be a reflection of the sow's feed intake pattern. Generally, the gilt or sow will limit their own feed

intake around the time of parturition and increase feed consumption as the demand for milk supply increases.

Coffey et al. (2012) euthanized gilts on d 90 of gestation and observed increased fetal plasma calcidiol when dams were fed a combination of vitamin D_3 and 25(OH)D_3 compared to vitamin D_3 alone due to proposed improvement in maternal vitamin D_3 status carried on to the fetuses. In the present experiment, the DH diet fed to dams caused a 153% increase in progeny 25(OH)D_3 from birth to weaning, where only an 80% and 124% increase was observed in DL and CON progeny, respectively. At both sampling days, the greatest concentration of 25(OH)D_3 was observed in DH progeny. In agreement, Zhou et al. (2016; 2017) measured the vitamin D_3 status of newborn piglets and those piglets at weaning and observed greater serum 25(OH)D_3 in piglets at birth when the maternal diet contained a combination of vitamin D_3 and 25(OH)D_3 due to greater concentration of 25(OH)D_3 in umbilical cord blood transferred to neonatal piglets. In contrast, Zhou et al. (2016; 2017), observed no difference in serum levels of piglets at weaning which Zhou et al. (2017) suggested was due to the short 21-d half-life of serum calcidiol. Flohr et al. (2016a) also observed no differences in piglet vitamin D_3 status at weaning.

As expected in this experiment, all of the piglet serum samples at birth did not contain an amount of vitamin D_3 above the detectable limit of 1.5 ng/mL. In agreement with these findings, Flohr et al. (2016a) fed increasing levels of vitamin D_3 (800, 2,000, or 9,600 IU per kg of the diet) as well as one dietary treatment of 50 μg 25(OH)D_3 per kg of the diet and found only the greatest supplemented dietary treatment, 9,600 IU, contained 54.2% detectable samples. The greatest supplemented dietary treatment in the present study, DH, contains the equivalency of only 3,500 IU/kg. Additionally, in the current study, 31.3, 60.9, and 97.7% of analyzed 25(OH)D_3 serum samples from piglets collected at birth were above the detectable limit of 1.5

ng/mL. The 25(OH)D_3 means presented were calculated from only those samples above the detectable limit.

Seo et al. (1997) conducted research feeding 24,25(OH)$_2D_3$ to chickens and concluded that when this metabolite is present at physiological levels, it is essential for normal bone integrity and healing of bone fractures in chicks. Zhou et al. (2017) found an increase in bone strength, density, and ash content of newborn piglets when dams were fed 50 μg 25(OH)D_3 in combination with 50 μg vitamin D_3. These improvements were not found in weaned piglets and serum 24,25(OH)$_2D_3$ was not evaluated in the study by Zhou et al. (2017). From this research, the current study analyzed piglet serum samples for 24,25(OH)$_2D_3$ concentration at birth and at weaning in which nearly all samples had concentrations above the detectable limit, 0.3 ng/mL, for this metabolite. Pigs born to dams fed the CON diet had concentrations of 24,25(OH)$_2D_3$ decrease by 47% from birth to weaning; however, pigs from sows fed the DL and DH diets had larger decrease at 61% and 50%, respectively. At both sampling days, the greatest concentration of 24,25(OH)$_2D_3$ was observed in DH progeny. To our knowledge, this is the first study investigating the effect of improving maternal vitamin D_3 status on serum 24,25(OH)$_2D_3$ of the piglets.

Progeny from 1 farrowing group, 22 sows, was followed through the nursery and finisher to market. At the conclusion of the nursery period, d 59 postweaning, and the day before marketing, d 156 postweaning, blood was collected and analyzed for serum 25(OH)D_3 concentration. No evidence of difference was observed in pigs fed the CON diets, however the DL and DH fed pigs produced a 18% and 12% decrease in serum 25(OH)D_3 concentration over time, respectively. A decrease in serum 25(OH)D_3 concentration in the finisher may be due to the decreased amount of 25(OH)D_3 added to the finisher phase diets. Although, this might not be

the case when considering how the increase in ADFI of pigs at these two ages would increase the daily consumption of total vitamin D_3 activity. Flohr et al. (2016b) observed a quadratic effect of serum 25(OH)D_3 of nursery pigs on d 35 and a tendency on d 17 postweaning due to increasing maternal dietary supplementation of vitamin D_3, but noted these differences might be due to the increase in ADFI and total vitamin D_3 intake of the nursery pigs. Flohr et al. (2016b) placed pigs on a common diet on d 35 postweaning and on d 70 after weaning, there was no difference in serum 25(OH)D_3 due to maternal or nursery vitamin D_3 treatments.

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

Analyses of 1,25(OH)2D_3 in colostrum and milk was of interest to us in this study because it is known to be the active form of vitamin D_3. However, Hollis et al. (1983) investigated bovine milk vitamin D_3 metabolites and reported relative concentrations of each based on the percentage of serum levels in the cow. Their conclusions suggest that 1,25(OH)2D levels would be very low and have little significance to the piglet at such low levels. We found

the matrix noise and low endogenous levels at or around limit of detection proved to be difficult to overcome for quantitation and was not reported. Although novel, the application for $1,25(OH)_2D_3$ levels at such low concentrations in both milk and colostrum are questionable and therefore is not justified.

Progeny feed efficiency in the nursery from d 0 to 59 was improved when dams and nursery pigs were fed the DH diet compared to pigs from dams fed DL diet with the control diet not different than the previously two mentioned. In contrast, Flohr et al. (2014b) and Konowalchuk et al. (2013) reported no significant improvement of growth performance in the nursery due to vitamin D_3 or $25(OH)D_3$ supplementation. Also, Flohr et al. (2016b) observed pigs from sows fed 2,000 IU of vitamin D_3 had increased ADG and ADFI in the nursery, but not feed efficiency, compared to pigs from sows fed 800 or 9,600 IU of vitamin D_3 per kg of the diet.

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

There was no difference in HCW due to combining vitamin D_3 with $25(OH)D_3$ in the feed of the pigs or maternal diet. In contrast, Flohr et al. (2016b) observed pigs from sows fed 50 μg $25(OH)D_3$ had a heavier final live BW and HCW compared to pigs from sows fed 9,600 IU vitamin D_3. Also, as maternal vitamin D_3 increased, marketed pigs from those sows had increased dressing percentage and decreased loin depth and back fat thickness. Flohr et al.

(2016b) discussed these responses may truly be due to maternal treatments or possibly due to numeric differences in weaning weight of pigs whose dams were fed 2,000 IU vitamin D_3.

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

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Table 2.1 Sow diet composition (as-fed basis)

Ingredient, %	Gestation[1]	Lactation[2]
Corn	80.33	63.04
Soybean meal	15.60	30.20
Monocalcium P (21% P)	1.48	1.48
Limestone	1.15	1.05
Salt	0.50	0.50
L-Lys-HCL	------	0.20
DL-Met	------	0.05
L-Thr	0.03	0.075
Choice white grease	------	2.50
Trace mineral premix[3]	0.15	0.15
Vitamin premix without vitamin D[4]	0.25	0.25
Sow add pack[5]	0.25	0.25
Phytase[6]	0.015	0.015
Vitamin D premix[7]	0.25	0.25
Total	100.00	100.00
Calculated analysis[8]		
SID lysine, %	0.56	1.07
NE NRC, kcal/kg	2475	2506
CP, %	14.10	19.90
Ca, %	0.76	0.77
Avail. P, %	0.46	0.48
Standardized digestible P, %	0.48	0.52

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

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

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

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

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

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

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

[8] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

Table 2.2 Analyzed sow diet composition (as-fed basis)[1]

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

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

Table 2.3 Nursery diet composition (as-fed basis)[1]

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

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

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

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

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

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

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

[7] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

Table 2.4 Analyzed nursery diet composition (as-fed basis)[1]

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

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

Table 2.5 Finisher diet composition (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	74.40	83.42	83.42
Soybean meal	22.85	14.30	14.30
Monocalcium P (21% P)	0.55	0.33	0.33
Limestone	0.95	0.85	0.85
Salt	0.35	0.35	0.35
L-Lys-HCL	0.31	0.25	0.25
DL-Met	0.06	0.02	0.02
L-Thr	0.09	0.05	0.05
Trace mineral premix[2]	0.15	0.13	0.13
Vitamin premix without Vitamin D[3]	0.15	0.15	0.15
Phytase[4]	0.015	0.015	0.015
Vitamin D premix[5]	0.15	0.15	0.15
Total	100.00	100.00	100.00
Calculated analysis[6]			
SID lysine, %	0.98	0.73	0.73
NE NRC, kcal/kg	2,479	2,537	2,537
CP, %	17.40	14.0	14.0
Calcium, %	0.55	0.45	0.45
Avail. P, %	0.27	0.21	0.21
Standardized digestible P, %	0.33	0.26	0.26

[1] Phase 1 diets were fed from d 0 to 35, phase 2 diets were fed from d 35 to 67, and phase 3 diets were fed from d 67 to 97 in the finisher.

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

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

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

[5] Vitamin D premixes contain either vitamin D_3 (Rovimix D3-500, DSM Nutrition Products) mixed with corn or 25(OH)D_3 (Hy-D Premix 137.5 mg/kg, DSM Nutritional Products) mixed with corn to achieve desired vitamin D concentrations for each treatment.

[6] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

Table 2.6 Analyzed finisher diet composition (as-fed basis)

Item	Phase 1			Phase 2			Phase 3		
	1,000 IU D_3	25 µg 25(OH)D_3	50 µg 25(OH)D_3	1,000 IU D_3	25 µg 25(OH)D_3	50 µg 25(OH)D_3	800 IU D_3	20 µg 25(OH)D_3	40 µg 25(OH)D_3
Formulated									
Vitamin D_3, IU/kg	1,000	---	---	1,000	---	---	800	---	---
25(OH)D_3, µg/kg	---	25	50	---	25	50	---	20	40
CP, %	17.4	17.4	17.4	14.0	14.0	14.0	14.0	14.0	14.0
Ca, %	0.55	0.55	0.55	0.45	0.45	0.45	0.45	0.45	0.45
P, %	0.47	0.47	0.47	0.39	0.39	0.39	0.39	0.39	0.39
Analyzed									
Vitamin D_3, IU/kg	1,490	---	---	970	181	---	840	---	170
25(OH)D_3, µg/kg	---	33	57	---	34	56	---	28	53
CP, %	17.4	17.4	19.2	13.5	12.7	13.9	14.0	13.9	14.3
Ca, %	0.82	0.67	0.73	0.51	0.56	0.54	0.58	0.61	0.56
P, %	0.49	0.47	0.48	0.37	0.38	0.38	0.38	0.43	0.38

[1] Samples were collected from 80% of the feeders in the finishing facility occurring once per feed delivery, pooled by diet, subsampled, and stored at -20°C. Samples were shipped to DSM Nutritional Products (Parsippany, NJ) for vitamin D_3 and 25(OH)D_3 analysis and to Ward Laboratories (Kearney, NE) for proximate analysis.

Table 2.7 Effects of feeding vitamin D_3 alone or in combination with 25(OH)D_3 on sow and preweaned piglet performance[1]

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

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

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

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

Table 2.8 Whole Longissimus lumborum and muscle fiber characteristics of pigs at birth from sows fed vitamin D_3 alone or in combination with 25(OH)D_3[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 & 25 μg 25(OH)D_3	1,500 IU D_3 & 50 μg 25(OH)D_3	SEM	Probability, $P <$
Pigs, *n*	12	12	12	---	---
Live birth weight, kg	1.43	1.44	1.35	0.061	0.517
Whole muscle CSA[3], mm^2	192	195	186	11.7	0.838
All fiber characteristics[4]					
Number[5]	1,846,503	1,994,031	1,869,073	176,468	0.805
CSA, μm^2	108	106	103	6.7	0.875
Myonuclei	598	675	632	30.0	0.170
Satellite cells	28.1	32.7	34.8	3.84	0.390
Fiber type characteristics[6]					
Primary					
Number[7]	96,957^b	111,124^b	174,054^a	22,206	0.007
CSA, μm^2	222	240	215	20.3	0.661
Secondary					
Number[7]	1,749,488	1,882,926	1,694,981	169,975	0.702
CSA, μm^2	101	99	93	6.4	0.614
Secondary fibers per primary fiber[8]	20.4^a	18.8^{ab}	11.8^b	2.06	0.014

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

[1] A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. Thirty-six piglets were sacrificed within 24 h of birth.

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

[3] CSA = cross-sectional area.

[4] Overall fiber characteristics independent of fiber type.

[5] Total number of muscle fibers was calculated as the whole muscle CSA divided by the overall average muscle fiber CSA.

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

[7] Total number of a specific fiber isoform was calculated by multiplying the total number of muscle fibers by the percentage of fibers pertaining to that specific type.

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

Table 2.9 Whole Longissimus lumborum and muscle fiber characteristics of pigs at weaning from sows fed vitamin D_3 alone or in combination with 25(OH)D_3[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 & 25 μg 25(OH)D_3	1,500 IU D_3 & 50 μg 25(OH)D_3	SEM	Probability, $P <$
Pigs, *n*	11	12	12	---	---
Live birth weight, kg	1.46	1.43	1.28	0.091	0.323
Live weaning weight, kg	5.82	5.64	5.24	0.276	0.334
Whole muscle CSA[3], mm^2	656	604	541	57.3	0.360
All fiber characteristics[4]					
Number[5]	1,159,100	1,158,383	1,077,460	103,812	0.791
CSA, μm^2	563	565	505	45.6	0.553
Myonuclei	262	285	270	12.1	0.371
Satellite cells	16.2	15.8	17.3	1.21	0.676
Fiber type characteristics[6]					
Type I					
Number[7]	140,371	149,587	124,585	16,341	0.494
Distribution[8], %	12.2	12.7	11.6	0.83	0.646
CSA, μm^2	434	393	416	21.5	0.384
Type IIA					
Number[7]	180,675	204,471	193,385	19,725	0.678
Distribution[8], %	15.7	17.7	17.9	0.81	0.129
CSA, μm^2	401	360	345	26.1	0.291
Type IIX					
Number[7]	311,590	314,033	269,263	36,853	0.567
Distribution[8], %	26.8	26.3	24.6	1.42	0.498
CSA, μm^2	539	532	469	37.2	0.329
Type IIB					

Number[7]	526,465	490,294	490,224	44,418	0.785
Distribution[8], %	45.7	42.9	45.8	1.44	0.256
CSA, μm^2	671	727	613	74.4	0.537

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

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

[3] CSA = cross-sectional area.

[4] Overall fiber characteristics independent of fiber type.

[5] Total number of muscle fibers was calculated as the whole muscle CSA divided by the overall average muscle fiber CSA.

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

[7] Total number of a specific fiber isoform was calculated by multiplying the total number of muscle fibers by the percentage of fibers pertaining to that specific type.

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

Table 2.10 Whole body measurements of pigs at birth and weaning from sows fed vitamin D_3 alone or in combination with $25(OH)D_3$[1]

	Diet[2]				
	1,500 IU D_3	500 IU D_3 & 25 μg $25(OH)D_3$	1,500 IU D_3 & 50 μg $25(OH)D_3$	SEM	Probability, $P <$
Birth measurements					
Crown to rump, cm	29.7	29.3	29.4	0.54	0.866
Head width, mm	48.3[ab]	51.3[a]	47.7[b]	1.02	0.038
Head length, mm	88.8	90.0	85.6	1.63	0.157
Head circumference, cm	21.0	20.9	20.3	0.27	0.202
Heart girth, cm	23.8	23.5	21.4	0.97	0.176
Boston butt, g	47.8	48.1	47.0	3.57	0.975
Loin, g	63.2	65.9	62.7	4.11	0.842
Ham, g	111.7	111.2	102.2	6.37	0.501
Belly, g	62.4	67.1	61.1	3.83	0.513
Picnic shoulder, g	86.6	87.0	81.0	4.31	0.550
Right longissimus lumborum, g	11.6	11.5	10.5	0.863	0.622
Heart, g	12.1	12.4	11.1	0.60	0.257
Lungs, g	24.0	23.8	22.3	1.26	0.596
Liver, g	43.9	47.2	46.4	2.68	0.676
Kidneys, g	12.9	12.0	12.4	0.91	0.763
Brain, g	32.4	31.4	33.0	0.92	0.471
Weaning measurements					
Crown to rump, cm	45.0	42.1	43.1	1.26	0.279
Head width, mm	67.5	67.7	65.9	1.97	0.777
Head length, mm	122.8	124.3	117.3	3.43	0.296
Head circumference, cm	30.1	31.6	29.2	0.98	0.212
Heart girth, cm	38.9	38.3	37.3	0.81	0.351
Boston butt, g	193.2	193.7	189.1	17.59	0.978

Loin, g	320.3	295.4	270.8	23.74	0.333
Ham, g	544.0	544.5	486.6	36.01	0.409
Belly, g	363.8	325.5	309.4	23.77	0.254
Picnic shoulder, g	337.7	333.9	310.1	21.59	0.603
Right longissimus lumborum, g	76.0	70.9	65.0	6.79	0.511
Heart, g	43.8	43.4	40.8	2.47	0.634
Lungs, g	110.0	105.9	102.5	7.35	0.764
Liver, g	219.4	233.2	195.6	12.63	0.100
Kidneys, g	44.0	44.3	37.5	2.93	0.169
Brain, g	46.5	45.8	44.6	1.16	0.476

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

[1] A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. Thirty-six piglets were sacrificed at birth and an additional 35 pigs at weaning.

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

Table 2.11 Effects of feeding 25(OH)D_3 on serum concentrations of vitamin D_3 metabolites[1]

	Maternal diet[2]				Probability, *P* <		
	1,500 IU D_3	500 IU D_3 & 25 μg 25(OH)D_3	1,500 IU D_3 & 50 μg 25(OH)D_3	SEM	Trt	Time	Trt × Time
Sow serum[3]							
25(OH)D_3, ng/mL					<0.001	<0.001	<0.001
Gestation, d 100	21.2^{c}	31.4^{b}	52.1^{a}	1.90			
Farrowing	17.8^{c}	25.3^{b}	43.3^{a}	1.46			
Weaning	27.6^{c}	48.8^{b}	82.3^{a}	2.82			
Piglet serum[4]							
25(OH)D_3, ng/mL					<0.001	<0.001	<0.001
Birth	2.1^{ab}	2.0^{b}	3.0^{a}	0.27			
Weaning	4.7^{b}	3.6^{c}	7.6^{a}	0.19			
24,25$(OH)_2D_3$, ng/mL					<0.001	<0.001	<0.001
Birth	1.9^{c}	2.8^{b}	4.8^{a}	0.15			
Weaning	0.9^{b}	1.1^{b}	2.4^{a}	0.09			
Detectable samples[5], %							
25(OH)D_3							
Birth	31.3^{c}	60.9^{b}	97.9^{a}	7.27	<0.001	---	---
Weaning	100.0	100.0	100.0	---	---	---	---
24,25$(OH)_2D_3$							
Birth	100.0	100.0	100.0	---	---	---	---
Weaning	95.7	97.8	100.0	2.47	0.783	---	---
Pig serum							
25(OH)D_3, ng/mL					<0.001	<0.001	<0.001
Grower[6]	16.6^{c}	36.4^{b}	61.3^{a}	1.63			
Finisher[7]	17.8^{c}	30.0^{b}	53.4^{a}	1.76			

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

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

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

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

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

[5] Detectable sample represents the percentage of samples above the detectable limit for each piglet serum metabolite (1.5 ng/mL for 25(OH)D_3 and 0.3 ng/mL for 24,25$(OH)_2D_3$). The means were calculated using only samples above the detectable limit.

[6] Grower serum was collected immediately after being transferred to the finisher, 59 d postweaning.

[7] Finisher serum was collected the day before marketing, 156 d postweaning.

Table 2.12 Effects of feeding 25(OH)D_3 on colostrum and milk concentrations of vitamin D_3 metabolites[1,2]

	Maternal diet[3]				Probability, $P <$		
	1,500 IU D_3	500 IU D_3 & 25 μg 25(OH)D_3	1,500 IU D_3 & 50 μg 25(OH)D_3	SEM	Trt	Time	Trt × Time
25(OH)D_3, ng/g					<0.001	0.001	0.518
Colostrum, d 0	0.333[b]	0.537[ab]	0.852[a]	0.091			
Milk, d 21	0.487[c]	0.728[b]	1.180[a]	0.070			
24,25(OH)$_2D_3$, ng/g					0.619	0.166	0.068
Colostrum, d 0	0.118	0.262	0.382	0.081			
Milk, d 21	0.242	0.211	0.114	0.048			
Detectable samples[4], %							
24,25(OH)$_2D_3$					0.357	0.011	0.344
Colostrum, d 0	25.0	58.3	75.0	14.9			
Milk, d 21	18.2	16.7	18.2	12.2			

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

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

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

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

[4] Detectable sample represents the percentage of samples above the detectable limit for 24,25(OH)$_2D_3$ (0.062 ng/g). All colostrum and milk samples had values for 25(OH)D_3 above the detectable limit. The means were calculated using only samples above the detectable limit.

Table 2.13 Effects of feeding 25(OH)D_3 on nursery pig growth performance[1,2]

	Maternal and Nursery Diet[3]				
	1,500 IU D_3	500 IU D_3 & 25 μg 25(OH)D_3	1,500 IU D_3 & 50 μg 25(OH)D_3	SEM	Probability, $P <$
d 0 to 7					
ADG, kg	0.025	0.047	0.044	0.010	0.225
ADFI, kg	0.104	0.110	0.113	0.009	0.761
G:F	0.181	0.398	0.337	0.091	0.233
d 7 to 14					
ADG, kg	0.262	0.281	0.269	0.012	0.512
ADFI, kg	0.326	0.337	0.316	0.013	0.529
G:F	0.803	0.836	0.857	0.027	0.389
d 0 to 14					
ADG, kg	0.143	0.161	0.156	0.009	0.385
ADFI, kg	0.215	0.223	0.215	0.009	0.762
G:F	0.665	0.712	0.729	0.022	0.132
d 0 to 21					
ADG, kg	0.230	0.251	0.233	0.009	0.242
ADFI, kg	0.383	0.397	0.377	0.009	0.287
G:F	0.601	0.630	0.619	0.017	0.496
d 21 to 28					
ADG, kg	0.502	0.521	0.523	0.014	0.513
ADFI, kg	0.723	0.750	0.725	0.016	0.421
G:F	0.694	0.694	0.721	0.013	0.277
d 28 to 59					
ADG, kg	0.702	0.675	0.713	0.014	0.149
ADFI, kg	1.217	1.211	1.196	0.021	0.773
G:F	0.577[ab]	0.557[b]	0.596[a]	0.007	0.002
d 0 to 59					
ADG, kg	0.508	0.504	0.517	0.010	0.643
ADFI, kg	0.858	0.863	0.845	0.014	0.649
G:F	0.593[ab]	0.583[b]	0.613[a]	0.007	0.015
BW, kg					

d 0	5.71	5.67	5.71	0.058	0.827
d 7	5.88	6.00	6.02	0.088	0.522
d 14	7.72	7.98	7.90	0.156	0.472
d 21	10.70	11.03	10.61	0.225	0.398
d 28	14.22	14.75	14.35	0.262	0.341
d 59	35.98	35.68	36.59	0.575	0.529

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

[1] A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. A total of 216 weaned pigs were used in a 59-d nursery growth trial with 6 pigs per pen and 12 pens per treatment.

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

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

Table 2.14 Effects of feeding 25(OH)D_3 on finishing pig growth performance[1,2]

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

[1] A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. A total of 216 weaned pigs were used to continue the nursery growth trial into the finisher with consistent pen integrity of 6 pigs per pen and 12 pens per treatment.

[2] Experimental diets were fed from finisher d 0 to 97 in 3 phases.

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

divided into maternal dietary treatments from which the progeny inherited their treatment. Finishing pigs were fed 3 phases of diets with different concentrations of vitamin D_3 and 25(OH)D_3 than the dams.

Table 2.15 Effects of feeding 25(OH)D_3 on HCW[1]

	Maternal Diet[2]				
	1,500 IU D_3	500 IU D_3 & 25 µg 25(OH)D_3	1,500 IU D_3 & 50 µg 25(OH)D_3	SEM	Probability, $P <$
Live weight, kg	134.0	134.0	134.4	1.39	0.967
HCW[3], kg	101.3	101.1	101.4	1.25	0.987
Dressing[4], %	75.6	75.4	75.4	0.29	0.826

[1] A total of 69 sows (DNA Line 200 × 400) and their progeny over 3 consecutive farrowing groups were used to determine if feeding a combination of vitamin D_3 and 25(OH)D_3 influences neonatal and sow performance and vitamin D_3 status, muscle fiber morphometrics, and subsequent growth performance of the piglets to market. A total of 168 market pigs were used for these calculations out of the 202 pigs that made it to the plant. The remaining pigs either could not be identified or were skinned, causing incorrect recording of HCW.

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

[3] HCW= Hot carcass weight.

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

Chapter 3 - The effects of feeding benzoic acid and essential oils on growth performance, mortality, and selected fecal pathogens of grower pigs

ABSTRACT: A total of 200 pigs (DNA Line 200 × 400) with an initial BW of 36.7 kg were used to determine the effects of feeding benzoic acid and an essential oil blend on growth performance and selected fecal pathogens of growing pigs. Pigs were allotted by BW to 20 pens (n = 10 pigs/pen) and randomly assigned to 1 of 2 dietary treatments that were fed for 28 days. Dietary treatments included a control diet or the control diet with added benzoic acid paired with an essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.3% and 0.01% inclusion, respectively. Fecal samples were collected on d 1, 14, and 28. Although there was no treatment × time interaction for ADG, G:F, or BW, there was a time effect (P < 0.001) for all. There was no evidence of difference during the first 3 weeks for ADG and G:F, however both responses decreased during the final week of the experiment (P < 0.001). Average pen BW increased (P < 0.001) for all time points. There was a treatment × time interaction (P = 0.003) for ADFI where during the first 3 weeks, there was no evidence of difference due to dietary treatment, but during the final week of the study, pigs consumed more (P = 0.007) of the control diet (2.38 kg/d control vs. 2.24 kg/d benzoic acid paired and essential oil blend). Throughout the duration of this study, a trend was found for an increased (P = 0.065) loss from death and removals in pigs fed the control diet. *Lawsonia intracellularis* was detected in all d-28 fecal samples collected, except for one, but the mean Ct values were not different (P > 0.05) due to dietary treatments. *Salmonella* was not detected in any of the fecal samples. Although there was no hemolytic *Escherichia coli* detected, there were other coliforms that were

present sporadically. There was no treatment × time interaction or main effects ($P > 0.05$) when analyzing fecal samples for *E. coli*, but when comparing fecal samples collected on d 14, pigs fed the control diet had a numerically larger ($P > 0.05$) proportion of fecal samples containing *E. coli*.

Introduction

Alternative feed ingredients with growth-promoting properties are becoming more important than ever in the swine industry. One such alternative being studied is organic acid for acidification. Knarreborg et al. (2002) observed, *in vitro*, benzoic acid was superior to 5 other organic acids in exhibiting bactericidal effects on coliform and lactic acid bacteria in the stomach as well as the small intestine of piglets. Benzoic acid is considered to be a growth-promoting feed additive because Guggenbuhl et al. (2007) reported improved nutrient digestibility and Kluge et al. (2006) found it to have strong antimicrobial effects in the piglet gastrointestinal tract.

Another alternative being studied is a class of phytogenic feed additives known as essential oils. Essential oils are volatile lipophilic compounds extracted from plants by steam or alcohol distillation, or cold expression (Windisch et al., 2008). This class of alternatives are known to sometimes have beneficial properties including flavoring, stimulation of enzyme secretion, antioxidant properties, and/or microbiome-stabilizing effect. Additionally, some are synthetically made to be "naturally identical" (Weber et al., 2012). Li et al. (2012) demonstrated that encapsulated essential oils can improve gut microflora, immune function, and performance of newly weaned pigs. Also, in grower pigs, Yan et al. (2010) observed increased daily gain and feed efficiency as well as increased digestibility of nitrogen and energy when pigs were fed

essential oils. Therefore, benefits of essential oils have the potential to improve digestive efficiency and performance in swine.

The objective of this study was to determine if feeding benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) and an essential oil blend (CRINA Piglets AF, DSM Nutritional Products, Parsippany, NJ) can affect growth rate and improve survival rate for growing pigs.

Materials and Methods

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State Swine Teaching and Research Center, Manhattan, KS.

A total of 200 pigs (DNA Line 200 × 400) with an initial BW of 36.7 kg were used in a 28-d grower trial. Pigs were housed 10 pigs per pen and 10 replicates per treatment with pen as the experimental unit. The pigs were allotted by BW to pen and pens were randomly assigned 1 of 2 dietary treatments in a completely randomized design. The two dietary treatments included a control grower diet without any growth-promoting feed additives, and the control diet with added benzoic acid and essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.3% and 0.01% inclusion, respectively. The essential oil blend product in this study, CRINA Piglets AF, contained thymol, 2-methoxyphenol, eugenol, piperine, and curcumin. All diets were formulated to meet or exceed the Swine NRC (2012) dietary requirements. Pigs were provided *ad libitum* access to water and feed in meal form.

Pigs and feeders were weighed on a weekly basis including d 0, 7, 14, 21, and 28 of the trial to determine ADG, ADFI, and G:F. The average total for pen live gain, pen feed disappearance, and pen live G:F were determined for d 0 to 28. Pen weights were collected after

the initial allotment weigh period on d 0. Feed was distributed and recorded by a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) 4 times a day. Diet samples were taken from all 20 feeders, pooled, subsampled, and stored at -20°C. Table 3.1 contains both diet formulations and the calculated analysis. All diets were prepared at the Kansas State O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). Fecal samples were collected from 3 barrows per pen on d 1, 14, and 28. Samples were sent to the Iowa State Veterinary Diagnostic Lab in Ames, IA, where they were pooled before being analyzed. Day 1, 14, and 28 samples were analyzed for *Salmonella* and *Escherichia coli*. Day 28 samples were also analyzed for *Lawsonia intracellularis*.

Growth performance data were analyzed using the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Inc., Cary, NC). The experiment was a completely randomized design with repeated measures. The pen was the experimental unit and dietary treatment was considered as the fixed effect. Time points were the repeated measure factor at which the pens were measured repeatedly over time. The covariance structure for repeated measure factor was chosen to be autoregressive model of order 1 [AR(1)] as the best fit based on Bayesian Information Criterion. Individual time periods d 0 to 14, d 0 to 21, and d 0 to 28 were analyzed separately from the repeated measures analysis by normal distribution using with identity link function. Total pen live gain and total pen feed disappearance were analyzed by Gamma distribution with log link function. ADG, ADFI, G:F, and BW were analyzed under normal assumption. The *E. coli* data were analyzed consistent with the previously mentioned repeated measures description using binary data measuring the probability of presence with the default logit link function. *Lawsonia intracellularis* data was analyzed under normal assumption and without repeated measures due to only 1 time point. The death/removal rate data were analyzed using the Fisher's Exact Test to

test the relationship between deaths/removals and feed categorical variables. Differences were considered significant at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$.

Results

Although there was no treatment × time interaction for ADG, G:F, or BW, there was a time effect for all ($P < 0.001$; Table 3.2). There was no evidence of difference during the first 3 weeks for ADG and G:F, however both responses decreased during the final week of the experiment ($P < 0.001$). Average pen BW increased ($P < 0.001$) for all time points. There was a treatment × time interaction ($P = 0.003$) for ADFI where during the first 3 weeks, there was no evidence of difference due to dietary treatment, but during the final week of the study, pigs consumed more ($P = 0.007$) of the control diet (2.38 kg/d control vs. 2.24 kg/d benzoic acid paired and essential oil blend). Benzoic acid and essential oil supplemented diet did not effect ($P > 0.05$) total pen (d 0 to 28) live gain, pen feed disappearance, or live G:F compared to pigs fed the control diet (Table 3.3).

Throughout the duration of this study, a trend was found for an increased ($P = 0.065$) loss from death and removals in pigs fed the control diet (Table 3.2). One pig fed the control diet died or was removed on d 12, 14, 16, 19, and 28, and two pigs died on d 20 of the trial. One pig fed the benzoic acid and an essential oil blend diet died on d 18.

Salmonella was not detected in any of the samples on d 1, 14, or 28. Although there was no hemolytic *E. coli* detected on d 1, 14, or 28, there were other coliforms of *E. coli* that were present in varying concentrations (Table 3.4). Although there was no treatment × time interaction or main effects ($P > 0.05$) when analyzing fecal samples for *E. coli*, when comparing fecal samples collected on d 14, pigs fed the control diet had a numerically larger ($P > 0.05$) proportion of fecal samples containing *E. coli*. There were also no differences in fecal *E. coli*

presence on d 1 or 28 of the experiment ($P > 0.05$). All 20 fecal samples collected on d 28, except one, tested PCR positive for *Lawsonia intracellularis*. There was no evidence of difference on d 28 average *Lawsonia intracellularis* cycle-threshold (**Ct**) values ($P > 0.05$) due to dietary treatment.

Discussion

The essential oil blend product in this study contained thymol, 2-methoxyphenol, eugenol, piperine, and curcumin. Based on results from this experiment, there was no evidence due to dietary treatment for improved growth performance, feed efficiency, or BW of pigs throughout the duration of the study. In agreement with these findings, Yan and Kim (2012) fed eugenol and cinnamaldehyde to growing pigs and observed no effect on any growth performance parameters. Ilsley et al. (2005) fed curcumin to weaned pigs and observed no influence on pig performance or immune status. In contrast, Chen et al. (2016) observed improved final BW and improved ADG and G:F, from days 15 to 42 postweaning and the entire 42-d trial period when pigs were fed benzoic acid. Chen et al. (2016) mentioned the improved growth performance in their study may be partially explained by the improved intestinal development and intestinal barrier function of the weaned pigs fed benzoic acid.

Although there were no dietary treatment main effects or treatment × time interactions in this study for ADG, G:F, and BW, there was a main effect of time where as time passed, BW increased and ADG and G:F decreased. As the animal's BW increases over time, it needs to consume more energy and nutrients in the diet to perform maintenance needs and deposit protein and fat. Smith et al. (1999) developed weight and composition curves as pigs aged which depicted as BW increases with age, protein accretion quadratically increases then decreases and lipid accretion linearly increases. At the same time protein accretion is starting to decrease, ADG

is still increasing but by smaller amounts. In this study, the observed decrease in ADG during the last week was not a typical growth response and we speculate the pigs were under disease challenge as also seen by the decreased feed efficiency and watery bacteria.

In the present study, there was a treatment × time interaction for ADFI. During the first 3 weeks, there was no evidence for difference due to dietary treatment, but during the final week of the trial, pigs fed the control diet consumed more feed per day (2.38 kg/d control vs. 2.24 kg/d benzoic acid paired and essential oil blend). This shift in feed intake during the last week of the trial may be due to increased death loss/removals. Five pigs fed the control diet died or were removed, compared to 1 pig fed the benzoic acid and an essential oil blend diet, during the d 14 to 21 period. The assumption is that pigs in the same pen eat about the same amount of feed per day, but this could very well become an incorrect assumption when pigs become ill. During the last week of the trial, remaining pigs with a better health status were more likely to consume their preferred amount of feed per day thus increasing the pen's ADFI. Feed intake for the pigs fed benzoic acid paired with the essential oil blend did increase from the third to fourth week but not as much as the control fed pigs.

Some literature suggests a blend of essential oils in combination with benzoic acid will have an added or synergistic effect. In poultry, Weber et al. (2012) fed a diet with a blend of essential oils (0.006% combination of thymol, eugenol, and piperine) with 80% benzoic acid at 300 mg/kg of the combination product compared to a negative control diet and found an improvement in BW on d 21 and d 42 for those broilers fed essential oils paired with benzoic acid. They also reported an increase in ADG for the entire poultry starter and grower trial period with no differences in mortality compared to the control. Also, Li et al. (2012) attributed an improvement in feed intake of nursery pigs mainly to the pleasant odor and flavor of essential

oils in the diet when fed at 150 g/1000 kg, which is equal to 0.015% of the diet. Pigs are known to have an extensive olfactory apparatus as seen by food seeking and detection of sex steroids at the time of mating. Also, pigs have taste buds throughout the oral cavity with the highest concentration on the tongue and they are known to have taste preferences when using water or dry feed to convey flavor (Moran, 1982). Therefore, we speculate it is possible the concentration of the essential oils could have been too low in the present experiment, 0.01%, fed to swine and thus no pleasant odor or flavor of the essential oils fed was detected.

An unexpected finding in this experiment was a tendency for increased death/removal losses in the control fed pigs compared to the benzoic acid and essential oil fed pigs. Cao et al. (2010) fed thymol and cinnamaldehyde essential oils to broilers and observed a decreased mortality in the first 3 weeks of a 6-wk study. In contrast, Weber et al. (2012) fed a blend of essential oils to broiler chicks and found mortality to be considered normal and not affected by dietary inclusion or absence of essential oils. Guggenbuhl et al. (2007) fed weaned pigs 0.5% benzoic acid and observed no differences in mortality compared to the negative control fed pigs. The increased death/removal rate in this study lead us to test the fecal samples collected throughout the trial period.

The question of presence versus the impact of a pathogen in an animal is very important for disease diagnosis. An animal may have the pathogen present in the body, but the presence only becomes relevant when disease is associated with the pathogen and consequential effects become obvious. Burrough et al. (2015) found a strong negative correlation between the Ct value of a positive fecal PCR sample and the quantity of *Lawsonia intracellularis* antigen in proliferative intestinal lesions detected by immunohistochemistry (**IHC**). In other words, smaller PCR Ct values correlate with more abundant bacteria within the intestine. In the current study,

although all samples excluding one on d 28 tested PCR positive for *Lawsonia intracellularis*, 5 of the total 20 pooled pen samples contained Ct values less than 20. This low Ct value corresponds to a high likelihood of this pathogen causing clinical significance (Burrough et al., 2015). There were no differences in Ct value of *Lawsonia intracellularis* on d 28 due to dietary treatment.

When comparing fecal samples from collection on d 14 of the current study, pigs fed VevoVitall and CRINA had a numerically smaller proportion of samples containg *E. coli* detected. Guggenbuhl et al. (2007) fed weaned pigs 0.5% benzoic acid and observed a reduction in almost all bacterial populations in the gastrointestinal tract of the piglet, specifically for gastric lactic acid bacteria and caecal *E. coli*. Kluge et al. (2006) observed benzoic acid improved feed conversion, body weight gain, and nitrogen balance in pigs and suggested this effect might be associated with the decrease in the number of bacteria found in the gastrointestinal tract of the pigs. Li et al. (2012) demonstrated encapsulated essential oils reduced the occurrence of diarrhea and decreased *E.coli* counts in feces of newly weaned pigs.

We hypothesize the numerical decrease in *E. coli* bacteria in the current experiment might have occurred due to the decrease in pH of the digesta on d 14 although it was not directly measured in this experiment. Diao et al. (2015) fed pigs 2,000 mg/kg benzoic acid+100 mg/kg thymol and observed a tendency for decreased colon digesta pH values while also observing lower diarrhea score and improved feed to gain ratio compared to the control unsupplemented diet. Chen et al. (2016) found a lower jejunal pH value when nursery pigs were supplemented with dietary benzoic acid while also observing a suppressed intestinal *E. coli* growth on d 14.

In conclusion, adding benzoic acid and an essential oil blend to the grower diet did not affect overall growth performance in this study. However, the increased losses from deaths and

removals in the control pigs throughout the study warranted further testing of the fecal samples collected. The presence of *Lawsonia intracellularis* was only determined for d 28 samples and was found to be present in every pen but one. More research is needed to confirm and understand the reduced death loss found for pigs fed benzoic acid and an essential oil blend.

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Table 3.1 Diet composition (as-fed basis)[1]

Ingredient	%
Corn	71.51
Soybean meal	25.70
Monocalcium P (21% P)	0.55
Limestone	1.125
Salt	0.35
L-Lys-HCL	0.305
DL-Met	0.06
L-Thr	0.085
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.15
Phytase[4]	0.015
Benzoic acid[5]	---
Essential oil blend[6]	---
Total	100.00
Calculated analysis	
SID lysine, %	1.05
NE, kcal/kg	2,457
CP, %	18.50
Ca, %	0.60
Available P, %	0.28
Standardized digestible P, %	0.34

[1] Diets were fed from d 0 to 28.

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

[3] Provided per kg of premix: 4,409,171 IU vitamin A, 551,146 IU vitamin D3, 17,637 IU vitamin E, 15 mg vitamin B12, 1,764 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

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

[5] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.3% of the diet.

[6] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

Table 3.2 Effects of feeding benzoic acid and an essential oil blend on pig growth performance and mortality[1]

	Diet[2]			Probability, *P* <		
	Control	Benzoic acid[3] and essential oil blend[4]	SEM	Trt	Time	Trt , Time
Item[5]						
ADG				0.452	<0.001	0.767
ADFI				0.932	<0.001	0.003
G:F				0.430	<0.001	0.993
d 0 to 7						
ADG, kg	0.96	0.96	0.045			
ADFI, kg	1.81	1.83	0.035			
G:F	0.53	0.53	0.023			
d 7 to 14						
ADG, kg	0.90	0.89	0.045			
ADFI, kg	1.90	1.95	0.035			
G:F	0.47	0.46	0.023			
d 14 to 21						
ADG, kg	0.98	0.99	0.045			
ADFI, kg	2.06	2.12	0.035			
G:F	0.48	0.47	0.023			
d 21 to 28						
ADG, kg	0.73	0.65	0.045			
ADFI, kg	2.38[a]	2.24[b]	0.035			
G:F	0.31	0.29	0.023			
d 0 to 14						
ADG, kg	0.93	0.93	0.033	0.941	---	---
ADFI, kg	1.85	1.89	0.034	0.510	---	---
G:F	0.50	0.49	0.020	0.705	---	---
d 0 to 21						
ADG, kg	0.95	0.95	0.021	0.976	---	---
ADFI, kg	1.92	1.96	0.030	0.301	---	---

G:F	0.50	0.48	0.012	0.506	---	---
d 0 to 28						
ADG, kg	0.89	0.87	0.014	0.348	---	---
ADFI, kg	2.03	2.03	0.026	0.942	---	---
G:F	0.44	0.43	0.009	0.399	---	---
Mortality and removals[6], %	7.00	1.00	---	0.065	---	---
BW, kg				0.920	<0.001	0.835
d 0	36.7	36.8	0.579			
d 7	43.4	43.6	0.579			
d 14	49.7	49.8	0.579			
d 21	56.5	56.7	0.579			
d 28	61.6	61.3	0.579			

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

[1] A total of 200 grower pigs (DNA Line 200 × 400, initially 36.7 kg BW) were used in a 28-d growth trial with 10 pigs per pen and 10 pens per treatment.

[2] Diets were fed from d 0 to 28.

[3] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.3% of the diet.

[4] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

[5] Weekly intervals measured consecutively were analyzed as repeated measures and time intervals with more than one week combined were analyzed individually. Weekly average pen BW was also analyzed as repeated measures.

[6] The mortality and removal data were analyzed using the Fisher's Exact Test to test the relationship between deaths/removals and feed categorical variables for the entire trial period d 0 to 28.

Table 3.3 Effects of feeding benzoic acid and an essential oil blend on total pen live performance[1]

	Diet[2]			
	Control	Benzoic acid[3] and essential oil blend[4]	SEM	Probability, $P <$
Average pigs per pen				
d 0	10	10	---	---
d 28	9.3	9.9	---	---
d 0 to 28				
Average total pen live gain, kg	213.2	238.3	11.81	0.150
Average total pen feed disappearance, kg	554.8	566.8	9.06	0.366
Average total pen live G:F	0.38	0.42	0.018	0.151

[1] A total of 200 grower pigs (DNA Line 200 × 400, initially 36.7 kg BW) were used in a 28-d growth trial with 10 pigs per pen and 10 pens per treatment.

[2] Diets were fed from d 0 to 28.

[3] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.3% of the diet.

[4] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

Table 3.4 Effects of feeding benzoic acid and an essential oil blend on selected fecal pathogens in grower pigs[1,2]

	Diet[3]			Probability, $P <$		
Bacteria[6]	Control	Benzoic acid[4] and essential oil blend[5]	SEM	Trt	Time	Trt × Time
E. coli[7]				0.3848	0.4640	0.2842
d 1	0.50	0.50	0.167			
d 14	0.60	0.20	0.163			
d 28	0.30	0.30	0.153			
Lawsonia intracellularis[8], Ct						
d 28	22.58	24.35	1.478	0.4082	---	---

[1] A total of 200 grower pigs (DNA Line 200 × 400, initially 36.7 kg BW) were used in a 28-d growth trial with 10 pigs per pen and 10 pens per treatment.

[2] Fecal samples were collected from 3 random barrows per pen on d 1, 14, and 28 of the study and pooled before analysis.

[3] Diets were fed from d 0 to 28.

[4] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.3% of the diet.

[5] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

[6] *Salmonella* was not detected in any of the fecal samples on d 1, 14, or 28.

[7] *E. coli* was analyzed by repeated measures using binary distribution modeling the probability of presence.

[8] PCR to detect *Lawsonia intracellularis* was only conducted on d 28 samples and measured in cycle-threshold (Ct) value. Only 1 of 20 samples, which was fed the benzoic acid and essential oil blend diet, tested negative for *Lawsonia intracellularis*.

Chapter 4 - The effects of feeding benzoic acid and essential oils on sows and litter performance

ABSTRACT: A total of 48 sows (DNA Line 200 × 400) and their progeny was used to determine if feeding sows and/or piglets benzoic acid paired with an essential oil blend enhances sow and pig performance during lactation, piglet weight gain in the nursery, and survivability to market. Sows and their litters were randomly allotted to 1 of 2 lactation dietary treatments based on parity and BW 5 to 7 d before farrowing. Dietary treatments included a control diet or the control diet with added benzoic acid and essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.5% and 0.01% inclusion, respectively. Whole litters of piglets were assigned to a creep treatment or no creep was offered 7 d before scheduled weaning (d 21). The creep treatments included a control creep diet or a diet with benzoic acid and essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.225% and 0.01% inclusion, respectively. Both creep diets contained 1% chromic oxide as an indigestible marker. Adding benzoic acid and essential oil blend to the maternal diet did not affect ($P > 0.05$) sow performance or preweaned piglet performance in the farrowing house. Fecal swabbing of pigs the day before weaning showed they did not eat the creep feed. Nursery pigs weighed on d 12 or d 45 postweaning were not heavier ($P > 0.05$) due to the maternal diet or the presence/absence of creep feed in the farrowing crate. Also, survivability of pigs from weaning to market was not affected ($P > 0.05$) by maternal diet. From this study, it appears that benzoic acid paired with an essential oil blend does not affect sow and pig performance or survivability to market.

Introduction

Many nursery pigs are challenged at weaning with considerable enteric diseases. This common challenge leads to decreased piglet growth rate caused by various pathogenic bacteria in the environment. Unfortunately, the use of feed ingredients with growth promoting properties are limited for use in the nursery phase of swine production. One such alternative being studied is a class of phytogenic feed additives known as essential oils. Essential oils are lipophilic compounds extracted from plants by steam or alcohol distillation, or cold expression (Windisch et al., 2008). This class of alternatives are known to sometimes have beneficial properties including flavoring, stimulation of enzyme secretion, antioxidant properties, and/or microbiome-stabilizing effects. Additionally, some are synthetically made to be "naturally identical" (Weber et al., 2012).

Another alternative being studied is benzoic acid for acidification of the intestines to lessen the impact of pathogenic disease harming the pig. Benzoic acid is considered to be a growth-promoting feed additive because Guggenbuhl et al. (2007) reported improved nutrient digestibility and Roth and Kirchgessner (1998) and Kluge et al. (2006) found it to have strong antimicrobial effects in the gastrointestinal tract leading to improved performance. Chen et al. (2016) investigated the effects of benzoic acid on growth performance and intestinal development of 90 weaned pigs and observed increases in BW, daily growth, and feed efficiency for the overall trial period (d 1 to 42). Giannenas et al. (2014) investigated benzoic acid and essential oil compounds separately or in combination fed to turkey poults and suggested that it is the combination of both that exert a positive effect on performance of turkey poults and improved intestinal microbiota.

Sulabo et al. (2010a) researched the effects of lactation feed intake and creep feeding on sow and piglet performance. They suggested the lack of postweaning performance differences between litters offered creep-feed or not offered creep-feed may be due to the assumption that every piglet consumed the creep-feed offered. To further investigate this suggestion, Sulabo et al. (2010a) categorized individual piglets as eaters, non-eaters, and non-creep-fed pigs. The "eaters" had greater overall postweaning ADG and total body weight gain from d 0 to 28 compared to non-eaters or non-creep-fed pigs.

The objective of this study was to determine if feeding sows and/or piglets benzoic acid (VevoVitall, DSM Nutritional Products, Parsippany, NJ) paired with an essential oil blend (CRINA Piglets AF, DSM Nutritional Products, Parsippany, NJ) can affect sow and pig performance during lactation, piglet weight gain in the nursery, and survivability to market.

Materials and Methods

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the K-State Swine Teaching and Research Center, Manhattan, KS.

A total of 48 sows (DNA Line 200 × 400) and their progeny over 2 consecutive farrowing groups were used in this study. Sows were fed diets 5 to 7 d before farrowing and throughout lactation with weaning on d 21. Sows and their litters were randomly allotted to lactation treatments based on sow parity and BW. The lactation dietary treatments included a control lactation diet without any feed additives, and the control diet with added benzoic acid and an essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.5% and 0.01% inclusion, respectively. At 7 d before weaning, whole litters of piglets were assigned to 1 of 2 creep treatments or no creep was offered. The creep treatments

included a control creep diet without any feed additives, and the control diet with added benzoic acid and an essential oil blend (VevoVitall and CRINA Piglets AF; DSM Nutritional Products, Parsippany, NJ) at 0.225% and 0.01% inclusion, respectively. Both creep diets contained 1% chromic oxide as an indigestible marker. The essential oil blend product used in this study, CRINA Piglets AF, contained thymol, 2-methoxyphenol, eugenol, piperine, and curcumin. All diets were formulated to meet or exceed the Swine NRC (2012) dietary requirements. All diets were prepared and sampled at the Kansas State O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). Samples were pooled, subsampled, and stored at -20°C. Diet compositions and calculated analyses can be found in Tables 4.1 and 4.2.

In the farrowing house, sows were housed in individual farrowing crates and fed 4 times per d using an electronic feeding system (Gestal Solo; JYGA Technologies, St-Lambert-de-Lauzon, Quebec, Canada). Sows were fed dietary treatments at 2.75 kg/d as soon as they entered the farrowing house. Once the sows gave birth, they were placed on a feeding curve determined by their parity. Feed consumption in lactation was recorded by feed disappearance. Sow weights were collected on d 107 of lactation, within 24 h of birth, and at weaning. Individual piglet weights were recorded within 24 h of birth and at weaning. Creep feeders were installed on d 14 of lactation only to farrowing crates assigned one of the two creep treatments. Piglets were fecal swabbed the day before weaning (d 20) to visually detect the chromic oxide present or not present in the feces in order to determine if piglets consumed the creep feed. A subset of 540 pigs from the 48 sows were weighed on d 12 and 45 postweaning in the nursery. All pigs were monitored for survivability from weaning to market.

The experimental design for sow performance was a completely randomized design, as sows were balanced for parity and d 107 BW then randomly allotted to dietary treatments. Thus,

data were analyzed as 1-way ANOVA using the GLIMMIX procedure in Statistical Analysis Software (SAS) version 9.4 (SAS Institute, Inc., Cary, NC). The treatment factor was the sow diet with 2 levels. Response variables measured from sows that are counts of piglets were analyzed in negative binomial distribution with log link function, likewise, measurements which are percentages were analyzed in gamma distribution with log link function. The rest were analyzed in normal distribution with identity link function. Subsequently, preweaned and nursery pig performance data were analyzed as a split-plot design with the 2-level sow dietary treatment as the whole plot treatment factor and 2-level, presence or absence, piglet creep feed as the subplot treatment factor. Sows were recognized as the whole plot experimental units and individual piglets were experimental units for the subplot treatment. The survivability data from weaning to market were analyzed using the Chi-square test for contingency table to assess the association between survivability and maternal diet categorical variables. Differences were considered significant at $P \leq 0.05$ and trends at $0.05 > P \leq 0.10$.

Results

Adding benzoic acid and an essential oil blend to the maternal diet had no effect ($P > 0.05$) on sow performance, piglet preweaning survivability, or piglet birth weight (Table 4.3). Fecal swabbing of litters the day before weaning to detect chromic oxide in the feces revealed only 7 pigs total out of 433 creep fed pigs weaned had ingested creep feed. Therefore, overall pigs did not eat the creep feed or not enough time had passed since ingesting the feed for it to be shown in the feces.

There were no differences ($P > 0.05$) in preweaned piglet performance due to maternal diet or the presence/absence of creep feed in the farrowing crate (Table 4.4). Additionally, there were no differences ($P > 0.05$) in nursery pig weights on d 12 or 45 due to the maternal diet or

the presence/absence of creep feed (Table 4.5). Survivability of pigs from weaning to market was not affected ($P > 0.05$) by maternal diet (Table 4.6).

Discussion

The essential oil blend product in this study contained thymol, 2-methoxyphenol, eugenol, piperine, and curcumin. Results from this experiment indicate there was no evidence for improved sow performance during lactation as seen by similar feed intake and body weight loss among treatments. In agreement with these findings, Kluge et al. (2010) observed no negative effects on feed intake when sow diets were supplemented with 0.5% benzoic acid. Additionally, Devi et al. (2016) fed a blend of protected organic acids to the sow at 0.1% or 0.2% of the diet did not observe an influence on sow BW loss during lactation. In contrast, when Balasubramanian et al. (2016) fed microencapsulated organic acids and essentials oils (**MOE**) to sows during lactation, there was a tendency for reduced BW loss for sows fed increasing levels of MOE from 0.0 to 0.1%. Balasubramanian et al. (2016) suggested the tendency for reduced BW loss could be due to improved intake and increased utilization of supplements because the microencapsulation protects the organic acids and essential oils to be delivered to the most beneficial sites of the gastrointestinal tract.

A review completed by Roth and Kirchgessner (1998) attributed the primary mode of action behind feeding organic acids to be their effect on the ecosystem of microbiota in the intestinal tract and therefore controlling potential pathogens from proliferating. With a more stabilized intestinal health, pigs are less exposed and harmed by bacterial toxins and metabolites. Devi et al. (2016) observed at farrowing, sows fed protected organic acids since d 95 of gestation had decreased fecal *E. coli* contents compared to sows with no supplementation and a trend existed for the same observation at weaning. Kluge et al. (2010) observed a decrease in sow

urinary pH when the diet was supplemented with 0.5% benzoic acid. Reduced bacterial shedding and more acidic urine from the sow in the farrowing crate provided a cleaner environment for the piglets in hopes there would be better growth potential achieved pre and postweaning due to fewer negative impacts from environmental stressors.

In the current study, adding benzoic acid and an essential oil blend to the maternal diet had no effect on litter performance or piglet weaning weight. Likewise, piglet preweaning survival, birth weight, and ADG were not affected by addition of protected organic acids in a study by Devi et al. (2016). In contrast, Balasubramanian et al. (2016) detected pigs from sows fed microencapsulated organic acids and essentials oils had higher ADG during the suckling period than pigs from negative control fed sows. Also, a decrease in the number of diarrheal piglets existed for sows fed 1000 mg/kg compared to negative control fed sows for about a week in the middle of the 25-d lactation period. Authors suggest the enhanced growth performance of MOE sow litters may be due to organic acid and essential oil benefits being transferred to the piglets through the milk, which can also reduce the presence of diarrhea (Balasubramanian et al., 2016).

Pigs in this study were provided creep feed 7 d before weaning. Sulabo et al. (2010a) allowed creep feeding for 18 d, which had no effect on preweaning growth performance. In a later study by Sulabo et al. (2010b) investigating the effects of creep feed duration, there was no differences in weaning weights, total BW gain, or daily BW gain for pigs fed creep feed for 13, 6, or 2 d. It appears for the current study, providing pigs with creep feed for a longer duration than 7 d may not have improved preweaning growth performance compared to non-creep-fed pigs.

Sulabo et al. (2010a) observed no differences in postweaning performance between creep-fed and non-creep-fed pigs. They suggested the lack of growth performance post weaning was because pigs were penned in the nursery depending on if the litter was supplied creep feed or not, when they should have been penned by individual creep feed consumption. Sulabo et al. (2010a) realized their incorrect assumption that every piglet consumed the creep-feed offered and therefore designated pigs as eaters of the creep feed if green chromic oxide was visible in the feces. For piglets fed creep feed, 59% were categorized as eaters (n = 254) and 41% were categorized as non-eaters (n = 173). The pigs designated as eaters in lactation had greater overall postweaning ADG and total body weight gain, contributed to increased feed intake postweaning, compared to non-eaters or non-creep-fed pigs. Similarly, Kuller et al. (2007) labeled pigs as eaters in lactation, as seen by chromic oxide in feces, and those pigs possessed heavier BW and ADG than the non-eaters for 4 wk postweaning. In the current study, piglets were rectally swabbed to determine if green chromic oxide was visible in the feces consistent with Kuller et al. (2007) and Sulabo et al. (2010a). Out of the total 433 creep fed pigs weaned in this experiment, only 7 pigs were designated as eaters of the creep feed regardless of the creep treatment. Therefore, pigs did not eat the creep feed or not enough time had passed since ingesting the feed for it to be shown in the feces. Individual creep feed consumption categories could not be used as nursery pen allotment criteria in this experiment. The presence/absence of creep feed during lactation did not affect pig BW measured on d 12 and 45 postweaning which is in agreement with Sulabo et al. (2010a).

Although nursery pigs were not fed diets containing organic acids or essential oils, BW was collected on d 12 and 45 postweaning and no difference was observed in pig BW at either time point due to maternal diet or the presence/absence of creep feed. Blavi et al. (2016) added

feed flavoring, which included > 10% eugenol, to maternal diets in late gestation through lactation and observed weaned piglets had a preference for the same flavored feed which enhanced voluntary feed intake after weaning. Had we fed the same essential oils and organic acid combination to the nursery pigs, we may have observed an increase in feed intake due to flavor preference. Survivability of piglets from weaning to market was also monitored and we observed no differences due to maternal diet.

In conclusion, adding benzoic acid and an essential oil blend to diets fed to the sows and/or piglets did not enhance sow or pig performance during lactation. It also did not affect nursery pig performance or survivability to market. Although some research suggests the lack of benefit from a longer duration of creep feeding, maybe adding essential oils and benzoic acid to the nursery diets would have improved performance postweaning.

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Table 4.1 Sow lactation diet composition (as-fed basis)[1]

Ingredient	%
Corn	63.285
Soybean meal	30.20
Monocalcium P (21% P)	1.475
Limestone	1.05
Salt	0.50
L-Lys-HCL	0.20
DL-Met	0.05
L-Thr	0.075
Choice white grease	2.50
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.25
Sow add pack[4]	0.25
Phytase[5]	0.015
Benzoic acid[6]	------
Essential oil blend[7]	------
Total	100.00
Calculated analysis	
SID lysine, %	1.07
NE NRC, kcal/kg	2,506
CP, %	19.90
Ca, %	0.77
Available P, %	0.48
Standardized digestible P, %	0.52

[1] Diets were fed 5 to 7 days before parturition and throughout lactation.

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

[3] Provided per kg of premix: 4,409,171 IU vitamin A, 551,146 IU vitamin D3, 17,637 IU vitamin E, 15 mg vitamin B12, 1,764 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

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

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

[6] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.5% of the diet.

[7] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

Table 4.2 Piglet creep diet composition (as-fed basis)[1]	
Ingredient	%
Corn	8.84
Soybean meal	2.32
Spray-dried whey	25.00
Fine ground oat groats (steamed)	30.00
HP 300	10.00
Spray-dried animal plasma	6.00
Select menhaden fish meal	6.00
Lactose	5.00
Choice white grease	4.00
Monocalcium P, 21% P	0.35
Limestone	0.40
Salt	0.30
L-Lysine HCl	0.15
DL-methionine	0.15
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.25
Phytase[4]	0.04
Vitamin E, 20,000 IU	0.05
Chromic oxide	1.00
Benzoic acid[5]	-----
Essential oil blend[6]	-----
Total	100.00
Calculated analysis	
SID lysine, %	1.40
NE NRC, kcal/kg	2,743
CP, %	23.20
Ca, %	0.70
Available P, %	0.66
Standardized digestible P, %	0.66

[1] Diets were fed 7 days before weaning.

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

[3] Provided per kg of premix: 4,409,171 IU vitamin A, 551,146 IU vitamin D3, 17,637 IU vitamin E, 15 mg vitamin B12, 1,764 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

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

[5] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.225% of the diet.

[6] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

Table 4.3 Effects of feeding benzoic acid and an essential oil blend on sow performance[1]

	Maternal diet[2]			
	Control	Benzoic acid[3] and essential oil blend[4]	SEM	Probability, $P <$
Sow, *n*	24	24	---	---
Parity	2.42	2.29	---	---
Lactation ADFI, kg	5.8	5.7	0.13	0.689
Sow BW, kg				
Gestation				
d 107	243.3	241.5	5.71	0.821
Lactation				
d 0	229.1	226.3	5.70	0.730
d 21	219.6	216.9	5.34	0.724
BW loss, kg	-9.5	-9.4	1.69	0.962
Litter characteristics				
Total born, *n*	17.1	16.3	0.99	0.551
Born alive, %	91.2	90.7	1.76	0.835
Stillborn, %	7.3	7.9	3.84	0.909
Mummies, %	1.4	1.3	1.05	0.954
Standardized liter size[5], *n*	15.1	14.5	0.80	0.554
Weaning liter size, *n*	13.8	13.2	0.76	0.585
Survivability, %	92.1	92.1	1.67	0.990
Piglet BW, kg				
Birth	1.4	1.5	0.04	0.287

[1] A total of 48 sows (DNA Line 200 × 400) and their progeny over 2 consecutive farrowing groups were used in this study.

[2] Diets were fed to the sows 5 to 7 days before parturition and throughout lactation.

[3] VevoVitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.5% of the diet fed to the sow.

[4] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the sow diet.

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

Table 4.4 Effects of feeding benzoic acid and an essential oil blend on preweaned piglet performance[1,2]

Maternal diet:	Control		Benzoic acid[3] and essential oil blend[4]			Probability, $P <$	
Creep feed[5]:	No	Yes	No	Yes	SEM[6]	Maternal diet	Creep feed
Piglet, *n*	110	219	102	214	---	---	---
Piglet BW, kg							
d 0	1.48	1.41	1.52	1.47	0.047	0.255	0.119
weaning	5.84	5.64	5.84	5.70	0.190	0.860	0.262

[1] A total of 645 piglets (DNA Line 200 × 400) from 48 sows were used to determine preweaning performance. Piglets were weighed within 24 h of birth and again at weaning on d 21.

[2] There was no maternal diet × creep feed interaction ($P < 0.654$). Only main effect p-values are presented.

[3] Vevovitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.5% of the maternal diet.

[4] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the maternal diet.

[5] Whole litters of piglets were assigned to 1 of 2 creep treatments or no creep was offered 7 d before weaning. The 2 creep treatments were combined in this table in the "yes" category.

[6] Presented value is the largest interaction standard error of the mean.

Table 4.5 Effects of feeding benzoic acid and an essential oil blend on nursery pig performance[1,2]

Maternal diet:	Control		Benzoic acid[3] and essential oil blend[4]			Probability, $P <$	
Creep feed[5]:	No	Yes	No	Yes	SEM[6]	Maternal diet	Creep feed
Pig, *n*	93	178	86	183	---	---	---
Pig BW, kg							
weaning	6.01	5.95	5.94	5.86	0.163	0.569	0.576
d 12	6.97	6.88	6.84	6.78	0.189	0.495	0.627
d 45	25.91	25.77	25.27	26.00	0.587	0.682	0.546

[1] A total of 540 nursery pigs (DNA Line 200 × 400) from 48 sows were used to determine postweaning performance. Pigs were weaned at 21-d of age, fed a common diet, and weighed on d 12 and 45 in the nursery.

[2] There was no maternal diet × creep feed interaction ($P < 0.367$). Only main effect p-values are presented.

[3] Vevovitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.5% of the maternal diet.

[4] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the maternal diet.

[5] Whole litters of piglets were assigned to 1 of 2 creep treatments or no creep was offered 7 d before weaning. The 2 creep treatments were combined in this table in the "yes" category.

[6] Presented value is the largest interaction standard error of the mean.

Table 4.6 Effects of feeding benzoic acid and essential oil blend on survivability to market[1]

	Maternal diet		SEM	Probability, $P <$
	Control	Benzoic acid[2] and essential oil blend[3]		
Pig, *n*	331	314	---	---
Survivability[4], %	96.98	97.13	---	0.907

[1] A total of 645 pigs (DNA Line 200 × 400) from 48 sows were used to determine survivability. Pigs were weaned at 21-d of age, fed a common diet in the nursery, and monitored for mortality from weaning to market.

[2] Vevovitall (DSM Nutritional Products, Parsippany, NJ) is an ultra-pure source of benzoic acid added at 0.5% of the diet.

[3] CRINA Piglets AF (DSM Nutritional Products, Parsippany, NJ) is a blend of essential oils added at 0.01% of the diet.

[4] Survivability data from weaning to market were analyzed using the Chi-square test to assess the association between survivability and maternal diet categorical variables.