ABSTRACT

BECERRA ROEL. Improving Sow Productivity and Nursery Pig Performance by Injecting Sows with d- α -Tocopherol, Retinyl palmitate, and Cholecalciferol (Under the direction of Dr. Eric van Heugten).

Vitamins A, E, and D play important roles in preventing cell damage, enhancing immune function, and improving fertility. We hypothesized that highly productive sows require supplemental fat-soluble vitamins to maximize reproductive performance and the growth and health of their offspring.

In experiment 1, fifty-four sows were allotted by parity to 3 treatments: 1) control (C; 5 mL i.m. saline injection on d 107 of gestation; 2) 2 injections (V2) of 5 mL i.m. of a multi-vitamin product containing 200,000, 300, and 100,000 I.U./mL of vitamin A, E, and D, respectively on d 107 of gestation and d 4 of lactation; and 3) same as V2 plus a third 5 mL i.m. injection of vitamins on d 14 of lactation (V3). The doses of vitamins used and the injection schedule were based on previous research. Blood samples from sows were collected on d -7, 1, 7, and 17 relative to farrowing and from piglets on d 0 (before suckling), 3, 7, 17, and 25 (4 d post-weaning). Results showed that serum retinol, retinyl palmitate and α -tocopherol concentrations in piglets before suckling were not detectable (<0.2 ppm). Serum retinol concentrations in sows and piglets increased over time ($P<0.001$) but were not impacted by injection. Serum α -tocopherol in sows increased as lactation progressed ($P<0.001$); V2 increased ($P<0.05$) serum α -tocopherol on d 7 compared to control and V3 tended ($P<0.10$) to increase serum α -tocopherol on d 17 compared to control and V2. Piglet serum α -tocopherol decreased ($P<0.001$) from d 3 to 25 (7.67 to 2.19 $\mu\text{g/mL}$) but was not affected by treatment. Vitamin injection increased ($P<0.05$) serum 25(OH)D₃ in sows on d 1, 7, and 17 and V3 increased ($P<0.05$) serum 25(OH)D₃ compared to V2 on d 17. In piglets,

serum 25(OH)D₃ increased ($P<0.05$) on d 7 and 17 with V2 compared to control. Pigs from V3 sows had greater ($P<0.05$) serum 25(OH)D₃ on d 7, 17, and 25 than control (12.6, 26.7, 19.8, and 15.4 vs. 6.4, 7.2, 9.0, and 13.4 ng/ml for d 3, 7, 17, and 25, respectively) and had greater ($P<0.05$) serum 25(OH)D₃ on d 17 (32.2 vs. 19.8 ng/ml) and 25 (24.6 vs. 15.4 ng/ml) compared to V2. Piglet BW at birth and weaning was greater ($P<0.05$) with V3 compared to V2, whereas BW after 14 d ($P=0.06$; 8.76, 8.83, and 9.33 kg for control, V2, and V3, respectively) and 35 d in the nursery was improved with V3 compared to V2 and control ($P=0.005$; 18.03, 18.31, and 19.73 kg for control, V2, and V3, respectively).

In experiment 2, a large field study was conducted to determine the effectiveness of injecting sows with vitamin A, E, and D before farrowing and during lactation to increase litter size and improve pig performance during lactation and nursery. Sows ($n=337$) were allotted by parity to 3 treatments; the protocol was the same as experiment 1, except sows in the control treatment did not receive a saline injection at any point. Total pigs born alive, stillborn pigs, and mummies were not different among treatments ($P>0.52$). Litter weight at d 3 and individual BW of pigs were not different among treatments ($P>0.61$). Total number of pigs at d 3 and at weaning were not significantly differently among treatments ($P>0.15$). However, the number of sows that were assisted during farrowing was greater ($P < 0.05$) for control sows than sows that were injected. Litter weaning weight was greater in V2 compared to V3, but not different from C ($P=0.05$; 68.45, 64.13, and 67.19 kg, respectively). ADG from d 3 to weaning was greater in V2 and C compared to V3 (0.25, 0.25 and 0.23 kg, respectively, $P=0.04$). Body weight at 7 d in the nursery was greater for C and V2 compared to V3 ($P=0.03$), but no differences were observed between C and V2. Nevertheless, weight per pen at d 7 was not different among treatments ($P=0.28$). At the end of the nursery (d 37),

weight per pen, average individual pig BW, ADG, and mortality were not significantly different among groups ($P>0.19$). In summary, in experiment 1, injecting fat-soluble vitamins in sows improved piglet performance through the nursery; however, this was not the case in experiment 2. The impact of injectable vitamins A, D, and E may depend on the production level and health status of the herd. In experiment 1, the total number of pigs weaned was 9.6 and mortality in the nursery was 8.5%, whereas in experiment 2, total number of pigs weaned was 11.5 and mortality in the nursery was only 0.9%. In addition, assistance with farrowing at the commercial farm in experiment 2 was high and was higher in control sows. Intensive sow management may have minimized the opportunity for a positive response of injectable vitamins in this second experiment. This suggests that more research needs to be conducted in different farms to evaluate and compare the outcomes of injecting sows with these three fat soluble vitamins under different production conditions.

Key Words: Sow, Vitamin A, E, and D, Nursery pigs

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Improving Sow Productivity and Nursery Pig Performance by Injecting Sows with d- α -
Tocopherol, Retinyl palmitate, and Cholecalciferol

by
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DEDICATION

To my mother and father who are my two heroes and my piglets who are smarter than my friends.

BIOGRAPHY

Roel Becerra was born on June 10, 1987, in Venaditos Ojocaliente Zacatecas, México. In his home country, Mexico, his family had poultry, swine and, sheep on their family farm. When the animals were ill, there was a high probability that they would not survive. This was really frustrating because he did not know how to tend to them or maintain their health. There was not one veterinarian in his hometown, which was a huge void. Although there were some veterinarians located far away, the fees for their services were often more than the value of the animals and could not be justified. Memories of these events allow him to reflect on how important it is to gain knowledge about farm animals and inspired him to study Animal Science. His personal experiences both in Mexico and the United States have shaped the person he is today and helped him establish what he wants to be in the next few years. When he came to the USA at the age of 18, he did not speak English; however, his determination to follow his dreams forced him to learn English so he could attend college. He earned his high school diploma in two years from Pontiac Central High School in Pontiac, Michigan. After receiving his high school diploma, he went to Oakland Community College to get his Associated Degree in Science. After finishing at Oakland Community College, he transferred to Michigan State University where he obtained his Bachelor's Degree in Animals Science. In fall 2015 he started his Master's program at North Carolina State University. After finishing his Master's, he is going to Purdue University to work on his DVM. What started off as a desire for him to learn English became earning an associate degree, which in turn became obtaining a BS degree then an MS degree and finally attending veterinary school in

fall 2017. His call to work in animal science and the veterinary profession has been not only his dream but has served as his inspiration.

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CHAPTER 1: Literature review

Introduction

Modern genetics, new technologies in housing and feeding, and advances in reproduction and nutrition have brought about an improvement of reproductive performance in sows. Modern genetic strains of pigs have also allowed the capacity for faster body growth at younger ages than many years ago (McDowell, 2000). This could be related to the improved nutritional status; however, enhancements of diets and technology have not reduced the number of pigs born dead (stillborn) nor reduced farrowing problems. In fact, modern sows are farrowing larger litters than in the past, and at the same time this may have contributed to a higher number of stillborn pigs, greater pre-weaning mortality, and an increased number of excessively light pigs.

Much of the research to establish vitamin requirements of lactating sows has been conducted over 30 years ago with low producing sows. We propose that vitamins A, E, and D need to be supplemented at higher levels to sows, so they can improve reproductive performance and the growth and health of their pigs in lactation and the nursery, and ultimately increase profitability. Indeed, water soluble vitamins may need to be supplemented at higher levels than what is recommended. Vitamins A, E, and D play important roles in preventing cell damage, enhancing the immune system, and improving fertility (Zile and Cullum, 1983; Babinszky et al., 1990; Coffey et al., 2012) In piglets, vitamin A, E, and D start to decline after weaning, but they can be increased by either including higher amounts of these fat-soluble vitamins in their diets or by giving higher amounts of these vitamins to sows during lactation (Malm et al., 1976; Valentine and

Tanumihardjo, 2005; Wilburn et al., 2008; Heying et al., 2013; Flohr et al., 2014b). It is suggested (McDowell, 2000) that modern sows need higher dietary concentrations of vitamin A, E, and D than what the NRC (1998) or the present NRC (2012) recommend. The NRC (2012) provides suggestions and estimations of vitamin levels to be included in sow diets, but it is based on research conducted many years ago. Nevertheless, there is not enough research to support requirement estimates for new genetic lines of sows that are highly productive. Although information is available for the suggested vitamin requirements for piglets and sows, the long-term or whole life cycle requirements of pigs need to be explored more in order to know the true fat-soluble vitamins requirements for optimal production (Cunha 1997; McDowell 2000).

Vitamin A

History. Vitamin A belongs to the group of lipid soluble vitamins and is an unsaturated compound that is related to retinol. Retinol, retinal, retinoic acid, and provitamin A carotenoids are part of the vitamin A group. 3500 years ago the Egyptians used liver to cure blindness, even when they did not know that liver was high in vitamin A and it was important for vision. It was not known that vitamin A was stored in the liver, and until 1913, vitamin A was known to form part of the fat-soluble vitamins. In 1931, Paul Karrer showed the chemical structure of vitamin A by isolating retinol from liver oil from a fish (Friedrich, 1988). In addition, Paul Karrer worked on β -carotene and demonstrated its activity and with the help of his colleague Kuhn, they isolated active carotenoids. Research conducted in the 1920s and 1930s demonstrated that animal species need vitamin A in their diets (McDowell, 2000).

Chemical Structures, Properties, and Nomenclature. Vitamin A can exist in many different isomers. Different compounds such as retinol, which is the alcohol form of vitamin A can be converted to retinal by removing the alcohol group and replacing it with an aldehyde group. By replacing it with an acid group, it yields retinoic acid (Figure 1). All of these compounds form vitamin A (McDowell, 2000). Vitamin A can be found in many sources such as meat, especially liver tissue. From plants, vitamin A can come from carotenoids that are synthesized by pigments coming from orange, yellow and green plants. The most commonly found form of vitamin A in meat is in the form of retinol ester, which can become retinol (Combs, 2012). In vegetables, the most commonly found structures of vitamin A are carotenes, which are considered provitamin A. This provitamin is converted to retinal by the enzyme β -carotene dehydrogenase and retinal is converted into retinol by the enzyme retinal reductase (Blomhoff, 1994). Meat, milk, and eggs contain the most prevalent form of vitamin A in the form of all-trans-retinyl palmitate and other fatty acid esters derived from retinol. There are some carotenoid pigments that are active as provitamin A, such as β -cryptoxanthin, zeaxanthin, and lutein, which contain hydroxyl groups on their ring structure; however, the only one that has some provitamin activity is β -cryptoxanthin. On the other hand, lutein and zeaxanthin cannot be used as provitamin A, but they are involved in oxidative stress (Combs, 2012). Lycopene is another carotenoid pigment related to vitamin A, but with no provitamin activity (McDowell, 2000; Combs, 2012). In this group (carotenoid pigments), the α and β carotene have high provitamin A activity, while lycopene works as an antioxidant and helps to protect against degenerative disease (Combs, 2012).

Provitamin A can be broken down by one of the many enzymes known that can yield retinal. This happens when β -carotene is ingested in food and travels through the small intestine for absorption. The intestinal mucosa and liver have a specific enzyme found in the cytosol called β -carotene 15, 15-dioxygenase that cleaves the β -carotene (Fidge et al., 1969). Also, the enzyme carotene dioxygenase can cleave to form other carotenoids and yield retinal (Diplock, 1985). The enzyme needs iron as a cofactor and the reaction needs oxygen to react with the two central carbons that are located in the *beta*-carotene between carbon C-15 and C15' (Fidge et al., 1969; Combs, 2012). This enzyme can be inhibited by sulfhydryl groups, which are composed of sulfur bonded to hydrogen atoms. Also, this enzyme is inhibited by chelators of ferrous iron. Vitamin A is also sensitive to oxidation caused by light, heat, metal catalysts, and oxidized oils (Olson, 1991; Glover and Glover, 2007). Nevertheless, many antioxidants, such vitamin E can protect vitamin A from oxidation. Carotenoids can be cleaved nonspecifically at different sites of hydrolysis and yield retinal, or retinoic acid. When cleavage of carotenoids does not occur at the C-15 and C15' site, it yields apo-carotenones and apo-carotenals which can be converted to retinoic acid (Combs, 2012). The cleavage of carotenoids can be done by different enzymes and lead to *cis* and *trans* retinoic acid. Nevertheless, the *trans* isomers are better utilized than the *cis* isomers (Diplock, 1985). After the cleavage of carotenoids, most of them will yield retinal and may be converted to retinoic acid and retinol. Retinal and retinol can be interconverted, while retinal can be converted to retinoic acid and used for growth, but not storage and it cannot be converted back to retinal (Combs, 2012). The bioavailability of vitamin A in food is around 45%;

however, the bioavailability of carotenoids as provitamin A can be less than that. The bioavailability of vitamin A depends on digestibility, chemical structure and how much carotenoids are present in the source of food.

Figure 1. Chemical structure of vitamin A and its derivatives. (Kono and Arai, 2015), © 2014 The Authors. Traffic published by John Wiley & Sons Ltd.

Absorption, Transport, and Tissue Delivery. Retinyl esters are the most preformed vitamin A in the diet. When retinyl ester is hydrolyzed in the small intestine, it yields retinol, which is taken up by enterocytes of the small intestine, and approximately 75% of the retinol is absorbed (Combs, 2012). Vitamin A in plant is in the form of carotenoids or provitamin A. β -carotene is a provitamin A, which can be converted to two moles of retinal. One of the isomers coming from β -carotene that has the highest biological activity is all-*trans*-retinol. In this case one microgram of all-*trans* retinol is equivalent to one microgram of retinol and 0.3 micrograms of all-*trans*-retinol is equivalent to one I.U. (Blomhoff, 1994). In the lining of the intestinal wall, most animals can convert β -carotene to vitamin A. However, cats and mink cannot convert β -carotene to vitamin A due to the lack of the intestinal enzyme that cleaves β

carotene (McDowell, 2000). In swine, there is low conversion to vitamin A when intake of β -carotene is high (Wellenreiter et al., 1969). In addition, van Vliet et al (1996) showed that rats fed with high levels of β -carotene had lower conversion to vitamin A than rats fed low levels of β -carotene. However, cattle and humans absorb high amounts of β -carotene (Parker, 1989). The acid forms can be found as all-*trans*-retinoic acid and 13-*cis* retinoic acid. The aldehyde forms can be found as all-*trans*-retinal and 11-*cis*-retinal (Blomhoff, 1994).

Vitamin A is mostly absorbed as retinyl esters, but also as carotenoids. Vitamin A is a fat-soluble vitamin, so it needs micellar solubilization, so it can travel in the small intestine. It is important to include fat in the diet so retinol can be incorporated into micelles and taken up by cells. In the brush border of the small intestine, hydrolases will hydrolyze retinyl esters into retinol (Blomhoff et al., 1991). In pigs and most mammals, vitamin A is absorbed via the lymphatic system, and because birds, rats, and reptiles, do not have a well-developed lymphatic system, the only way to absorb vitamin A is via the portal system (Combs, 2012). When retinol is derived from β -carotene, it uses specific transporters to be transported via facilitated diffusion. When retinol is inside the enterocyte, some long chain fatty acids, such as lineolate, oleate, stearate, and palmitate can re-esterify retinol into retinyl ester (Blomhoff et al., 1990; Blomhoff, 1994). Half of the total esters are composed of retinyl palmitate (Blomhoff, 1994). After conversion into retinyl esters, retinol is secreted from the mucosal cells in the small intestine into chylomicron particles (Diplock, 1985). Chylomicrons transport retinyl esters to the liver via the lymphatic circulation. Another way to transport retinol is via the serum retinol-binding protein (RBP) (Blaner et al., 1985). The role of RBP

is to transport retinol, which is released through ester hydrolysis from liver and extra hepatic tissues into the circulatory system (Blaner, 1989).

Storage Site and Excretion. The liver contains around 90% of the total body vitamin A and the rest is found in kidneys, lungs, adrenal glands, blood, and other organs and tissues (McDowell, 2000). In humans, and probably farm animals, the site of storage in the liver is primary in the stellate cells (Blomhoff et al., 1990). When vitamin A has intact carbon chains, it is excreted into the feces; however, when acidic chains are shortened, the products are excreted into the urine (Olson, 1991). During high vitamin A intake levels, the fecal excretion may be two times more as urinary excretion (Combs, 2012).

Figure 2. Major pathways for vitamin A transport and metabolism in the body. βC , β -carotene; RE , retinyl ester; ROH , retinol; RAL , retinal; RA , retinoic acid; $CRBP$, cellular retinol-binding protein; $CRABP$, cellular retinoic acid-binding protein; RBP , retinol-binding protein; TTR , transthyretin; RAR , retinoic acid receptor; RXR , retinoid X receptor; CM , chylomicron; CMR , chylomicron remnant. (Kono and Arai, 2015), © 2014 The Authors. Traffic published by John Wiley & Sons Ltd.

Deficiency Signs, Recommended Levels, and Toxicity. Vitamin A is necessary for normal growth of any species; therefore, it is important to have it in the diet. In animals, vitamin A may be required in small amounts because animals can store vitamin A during times of excess intake (Blomhoff et al., 1991). Also, when vitamin A is deficient in an animal or human being, signs of deficiencies may not be seen immediately due to reserves of vitamin A in the body (liver). However, when there is a lack of vitamin A in the body and liver, loss of appetite, loss of weight, and reduced fertility are some of the signs that may be seen (McDowell, 2000). In addition, vitamin A deficiency can result in decreased antibody production and negatively affect the immune system to fight infective agents (Davis and Sell, 1983).

The recent suggested requirements of vitamin A listed in the NRC (2012) for growing pigs weighing from 5 to 135 kg fed at libitum are 585 to 3,623 IU per day, while for gestating and lactating sows the suggested requirements are 8,398 and 11,932 IU per day, respectively (Table 1). Toxicity in animals and humans due to vitamin A is remote; nevertheless, excess of vitamin A has shown to have toxic effects in animals and humans. The safe levels of vitamin A are 4 to 10 times the nutritional requirements (McDowell, 2000).

Vitamin A in Swine. During early lactation, piglets depend on colostrum and milk to obtain vitamin A. There are no established vitamin A requirements for piglets at the age of 1 d to 21 d, and it is not really known if sows are passing enough vitamin A via colostrum and milk to meet the requirements of the nursing pig. Deficiencies in vitamin A in reproducing

sows and gilts can lead to estrus failure, reabsorption of the fetuses, or piglets born dead (Cunha, 1997). The amount of vitamin A that is passed on to the fetus during gestation is highly regulated by the mother (Bates, 1983; Marceau et al., 2007). During low and high intake of vitamin A in rats and rabbits, not much vitamin A is passed to the offspring (Moore, 1971). Therefore, it is important to supplement sows with high levels of vitamin A before farrowing so the newborn pigs can obtain the vitamin A in the colostrum. It was reported by Lindemann et al. (2008) that an intramuscular injection of vitamin A in sows at weaning and at breeding improved the subsequent number of pigs born alive and pigs weaned per litter, and decreased embryonic mortality. Valentine and Tanumihardjo (2005) suggested that one-time vitamin A supplementation of lactating sows is enough to increase liver vitamin A in their offspring. However, they suggested that more research is needed to determine the correct dose of vitamin A given to sows to improve or meet the requirements of vitamin A in the offspring. Coffey and Britt (1993) showed that injecting sows with 200 mg of β -carotene or 50,000 IU of vitamin A (palmitate) at the day of weaning, the day of mating and d 7 post-mating, increased litter size. Whaley et al. (1997) suggested that the increase in litter size may be related to an increase in embryonic survival, but not due to increasing ovulation rate. Likewise, Whaley (1995) showed that vitamin A increased embryonic survival during early gestation, which may be related to increased progesterone secretion in the peri- and post-ovulatory period of early gestation.

Vitamin E

History. In 1922, Evans and Bishop (University of California, Berkeley) discovered vitamin E as an unidentified factor found in vegetable oil that was required for reproduction in female rats (Evans and Bishop, 1922). When Evans and Bishop experimented with female rats, they observed that rats were able to maintain pregnancy at the beginning, but fetuses died and were reabsorbed if the diets were not supplemented with small amounts of wheat germ, dried alfalfa, leaves or fresh lettuce, which all contained vitamin E. In addition, male rats were also affected by vitamin E deficiency, which caused testicular degeneration, so it affected their reproductive performance. The name vitamin E was proposed by Sure in 1924 and Evans in 1924 (Evans and Burr, 1924; Sure, 1924). It was called vitamin E because it was the next serial alphabetical designation following vitamin D. The first isolated form of vitamin E was an α -tocopherol isomer (Evans et al., 1936). The word tocopherol was derived from the Greek *tokos*, which means offspring, the Greek *pherein*, which means to bring forth, and *ol* to designate an alcohol.

Chemical Structures, Properties, and Nomenclature. The activity of vitamin E can be derived from a series of compounds of plant origin that contain tocotrienols and tocopherols. A hydroquinone nucleus and an isoprenoid side chain are part of the structure of both the tocopherols and tocotrienols. There are eight forms of vitamin E that can be found in nature, and four of them are tocopherols (α , β , δ , and γ) and the other four are tocotrienols (α , β , δ , and γ) (Figure 3). Tocopherols have a saturated side chain, while the tocotrienols have an unsaturated side chain containing three double bonds (Kamal-Eldin and Appelqvist, 1996).

Differences between the α , β , δ , and γ in the tocopherols and tocotrienols are due to the presence of the methyl groups on the positions of the 5, 7 or 8 carbon of the chromanol ring (Hoffmann-La Roche Inc., 1991). When one or all of the methyl groups in the 5, 7 or 8 position of the chromanol ring is lost, it reduces the vitamin E activity of the structures (McDowell, 2000). The most biologically active form of vitamin E in feedstuffs is α -tocopherol (Traber, 2007; Combs, 2012). The natural activity of α -tocopherol and d - α -tocopherol, also known as RRR-tocopherol, is 1.49 IU/mg, the vitamin E acetate form is 1.36 IU/mg, while for synthetic-free tocopherol, dl- α -tocopheryl, also called all- rac - α -tocopheryl the activity is 1.1 IU/mg (McDowell, 2000).

Figure 3. Chemical structures of vitamin E homologs. Tocopherols and tocotrienols each have four isomers (α , β , γ and δ). (Kono and Arai, 2015), © 2014 The Authors. Traffic published by John Wiley & Sons Ltd.

Absorption, Transport, and Tissue Delivery. Vitamin E is absorbed by passive diffusion from the small intestine into the enterocyte (Combs, 2012) (Figure 4). The mechanism of absorption does not require energy, is non-saturated, and is not carrier-mediated. Because vitamin E is fat soluble, its absorption depends on fat absorption. In low

fat diets, vitamin E absorption can be compromised (Bjorneboe et al., 1990; Kayden and Traber, 1993; Moreira and Mahan, 2002). Tocopheryl palmitate, tocopheryl acetate, and tocopheryl succinate, which are tocopheryl esters, are converted to free tocopherol during absorption, probably by mucosal esterase (Mathias et al., 1981). When tocopherol is inside the enterocyte it is incorporated into chylomicrons and very low density lipoproteins (VLDL) to then be secreted into the lymphatic system and subsequently into the blood stream. In contrast to vitamin A, this transfer mechanism does not require a specific binding protein. In general, the vitamin E that is incorporated into micelles can be enhanced by the absorption process of medium-chain triglycerides, while long chain polyunsaturated fatty acids, such as linoleic acid reduce the absorption of vitamin E (Horwitt, 1962; Bjorneboe et al., 1987).

After vitamin E is delivered to the liver, it is incorporated into very low-density lipoproteins (VLDLs) and secreted into plasma for further absorption by peripheral tissues, with selectivity in favor of α -tocopherol over γ -tocopherol (Traber and Kayden, 1989).

Tocopherol is transported in all lipoproteins. For example, during the conversion of VLDL to low-density lipoproteins (LDL) in the circulation, part of vitamin E is transferred to LDL (Traber et al., 1992). Nevertheless, the more abundant lipoproteins are LDL and high-density lipoproteins (HDL) (Chow, 2001). The mechanism of how vitamin E is taken up by tissues is still unclear. In the liver, vitamin E is taken up by the parenchymal cells or stellate cells via the remnant receptor that is located on the surface of hepatocytes (Bjorneboe et al., 1990; Kayden and Traber, 1993).

Storage Site and Excretion. The α -tocopherols can be stored in most tissues, especially liver, skeletal muscle, and adipose tissue. where together they represent 92% of α -tocopherol found in these three tissues (Machlin and Gabriel, 1982; Bjorneboe et al., 1986; Traber and Kayden, 1987). Traber and Kayer (1987) showed that α -tocopherol in adipose tissue is located in bulk lipid droplets and has a low turnover. Most of the tocopherol is found in parenchymal cells in the outer membrane of the mitochondria, while in red blood cells that carry vitamin E in the blood stream, it is found in the membrane of the cell (Chow, 1975; Bjorneboe et al., 1991). The excretion of vitamin E and most of the tocopherols is via fecal elimination. When there is an excess of α -tocopherol and other tocopherols, they are excreted first into the bile and then into the feces (Chow, 2001)

Figure 4. Major pathways for vitamin E transport in the body. α , α -tocopherol; γ , γ -tocopherol; α -TTP, α -tocopherol transfer protein; CM, chylomicron; CMR, chylomicron remnant; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein. (Kono and Arai, 2015), © 2014 The Authors. Traffic published by John Wiley & Sons Ltd.

Deficiency Signs, Recommended Levels, and Toxicity. Vitamin E deficiency can result from insufficient intake of vitamin E, but can also be associated with high levels of polyunsaturated fatty acids (PUFA), and low selenium and sulfur (McDowell, 2000; Chow, 2001). PUFA do not only reduce the vitamin E levels in sows, but they can also reduce it in their offspring (Hidiroglou et al., 1993). Blaxter (1962) reported that one of the syndromes commonly associated with vitamin E deficiency across species is muscular dystrophy in the skeletal, heart and smooth muscle.

The recent suggested requirements of vitamin E listed in the NRC (2012) for growing pigs 5 to 135 kg of body weight fed at libitum (90% dry matter) are 4.3 to 30.7 IU per day, respectively, while for gestating and lactating sows it is 92.4 and 262.5 IU per day, respectively (Table 1). Vitamin E is generally nontoxic; however, vitamin E toxicity has been demonstrated in swine (NRC, 2012). Studies in rats, chicks, and humans have shown that maximum levels of vitamin E are in the range of 1,000 to 2,000 IU/kg of diet (NRC, 1987). In addition, Bonnette et al. (1990) showed in growing pigs that levels as high as 550 mg/kg of diet did not have toxic effects.

Vitamin E in Swine. One of the first experiments conducted in swine (Adamstone et al., 1944) showed that feeding gilts with a diet deficient in vitamin E reduced reproductive performance and piglets from sows fed the diet deficient in vitamin E displayed muscular incoordination and abnormalities in the liver. Research in reproductive sows has demonstrated that supplemental vitamin E helps to prevent deficiencies in young pigs, especially post-weaning, which is one of the most stressful phases of their life because they

are separated from the mother and fail to eat for the first three days in the nursery. In addition, research has demonstrated that supplemental vitamin E improved sow litter size, increased sow serum α -tocopherol concentrations, promoted their health and the health of their offspring (Mahan, 1991a; Mahan, 1994; Wuryastuti et al., 1993). Moreover, research suggest that selenium (Se) to some degree can replace vitamin E, thereby preventing tissue deterioration in swine, muscular degeneration in young ruminants and avoiding exudative diathesis in poultry (McDowell, 2000). In a similar way as vitamin A, vitamin E is passed in very low amounts via placenta to the fetus; however, research shows that it can be passed in high amounts in colostrum (Mahan, 1991a). Therefore, it is important to supplement sows with high levels of vitamin E before farrowing so the newborn pigs can obtain vitamin E in the colostrum. In newborn pigs, it is very important to increase their intake of α -tocopherol via colostrum because they are born deficient, and colostrum is the only source of vitamin E for newborn pigs (Mahan, 1991a; Lauridsen et al., 2002a). The first days for a newborn piglet are very critical. They depend on antibodies that are absorbed as immunoglobulins (IG) from colostrum. Research conducted in lactating sows showed that by increasing vitamin E in the diet, the health of piglets was improved by increasing IG (Hayek et al., 1989; Babinszky et al., 1990). Vitamin E provides protection against oxidative damage to newborn pigs (Debier, 2007). Therefore, it is important to supplement sows with a form of vitamin E that can be passed in the milk or supplement creep feed containing vitamin E to the pigs during lactation. Nevertheless, it is important to mention that serum vitamin E declines after weaning (Chung et al., 1992). Therefore, weaned pigs may be susceptible to free radical

and oxidative damage. Amazan et al. (2014) reported that sows fed natural vitamin E (*d*- α -tocopherol)) produced piglets with higher BW at weaning compared to synthetic vitamin E (*dl*- α -tocopherol), but these differences were not maintained at 42 days of age.

Vitamin D

History. Vitamin D is a fat soluble vitamin that is involved in maintaining bone and calcium and phosphorus balance in the body. In 1919, Sir Edward Mellanby showed that cod liver oil could be used as a cure for rickets when he experimented with puppies by feeding synthetic diets to over 400 dogs (Mellanby, 1921). In addition, Trousseau (1865) in his book discussed that getting enough sunlight is an important source of vitamin D and explains how rickets is one form of osteomalacia in older people; however, they did not know it was vitamin D at that time (Collins and Norman, 2001). In 1936, Adolf Windaus, using cod liver oil, was able to identify the structure of vitamin D (Windaus et al., 1936). Vitamin D can be an essential and non-essential nutrient. Vitamin D can be obtained from many dietary sources, such as fish, milk, cheese, and cereals. Vitamin D can also be synthesized by the body upon exposure to sun light from steroids (Combs, 2012). Nevertheless, people or animals that are not able to get enough sun light need a dietary source of vitamin D. People that live in the northern hemisphere and animals that are raised in closed facilities need vitamin D in their diets.

Chemical Structures, Properties, Nomenclature, and Metabolism. Vitamin D, also known as calciferol, has several forms or vitamers. There are two major forms of vitamin D, one is vitamin D₂ (ergocalciferol) and the other is vitamin D₃ (cholecalciferol) (Figure 5).

The only difference between the two forms of vitamins is that D₂ has a double bond in the noncyclic carbon chain. 7-dehydrocholesterol is a provitamin D that is formed from cholesterol and a precursor to form vitamin D₃. 7-dehydrocholesterol is synthesized in the sebaceous glands of the skin and absorbed in the multiple layers of epidermis. 7-dehydrocholesterol can be absorbed in the diet and converted to cholecalciferol in the skin. The formation of vitamin D in animals is performed by ultraviolet (UV) light (295-300) that activates 7-dehydrocholesterol in the epidermis (McDowell, 2000). The activation of 7-dehydrocholesterol to vitamin D₃ depends on the absorption of UV light that promotes the B ring on the 5,7-diene to open and isomerize, so a stable *s-trans*, *s-cis*-provitamin D₃ can be made (Combs, 2012). The efficiency to convert vitamin D₃ from sterol depends on many factors, such as skin type, species, environment, season, and latitude. Vitamin D₃ made in the skin and dietary vitamin D₃ absorbed in the intestine goes to the liver where they are converted to 25(OH)D₃, which is the most abundant form of vitamin D in the body. The enzyme vitamin D 25-hydroxylase catalyzes the conversion of vitamin D₃ into 25(OH)D₃ by hydroxylation of the side chain carbon C-25 (Saaremaa et al., 1984). After hydroxylation of 25(OH)D₃, it is further hydrolyzed into 1,25-(OH)₂-D₃ in the kidney and this is the active form of vitamin D. The enzyme 25-OH-vitamin D 1-hydroxylase catalyzes this reaction by hydroxylation at the C-1 position of the A ring (Henry and Norman, 1974). Furthermore, by hydroxylating 25(OH)D₃ at the C-24 of the side chain, it can yield 24,25(OH)₂-D₃ (Collins and Norman, 2001; Combs, 2012). Moreover, 1,25-(OH)₂-D₃ can produce 1,24,25-(OH)₃ by hydroxylation at the C-24 side chain (Combs, 2012). The 24-hydroxylase has greater affinity

for $1,25-(\text{OH})_2\text{D}_3$ than for $25(\text{OH})\text{D}_3$, but because there is more $25(\text{OH})\text{D}_3$ in the body the 24-hydroxylase has a 1000-fold greater capacity to hydrolyze $25(\text{OH})\text{D}_3$ (Haddad and Walgate, 1976; Mallon et al., 1980).

Figure 5. Structures of vitamin D₂ and D₃. Notice that the difference between D₂ and D₃ is the double bond found in the red circle. Adapted from Jatlas (2014).

Regulation of Vitamin D Metabolism. As mentioned, $25(\text{OH})\text{D}_3$ is the most abundant form in the body while $1,25-(\text{OH})_2\text{D}_3$ is the most active form. Regulation of vitamin D metabolism is affected by serum calcium levels, serum phosphorus levels and two hormones; calcitonin and parathyroid hormone (PTH), and it is also influenced by $1,25-(\text{OH})_2\text{D}_3$. The amount of $1,25-(\text{OH})_2\text{D}_3$ that is produced is dependent on dietary intake and the amount of UV light received. PTH stimulates the production of $1,25-(\text{OH})_2\text{D}_3$ in the kidney, which in turn promotes absorption of calcium in the intestine (Hibberd and Norman, 1969). $1,25-$

(OH)₂-D₃ in the intestine stimulates the absorption of calcium (Ca) and phosphorus (P) and promotes the synthesis of Ca-binding protein (calbindin), so calcium is absorbed. Increasing Ca concentrations in plasma will reduce 1,25-(OH)₂-D₃ production, which reduces Ca absorption and suppresses PTH (Combs, 2012). When calcium is low, it will stimulate PTH which will stimulate 25-OH-vitamin D 1-hydroxylase to make more 1,25-(OH)₂-D₃ so more calcium is released from bones. However when both calcium and phosphorus are low, 25-OH-vitamin D 1-hydroxylase gets super-stimulated (Combs, 2012). The mechanism by which vitamin D stimulates Ca absorption is not well known. However, Weber et al. (1971) suggested that 1,25-(OH)₂-D₃ is found in the nuclei of bone cells; therefore, 1,25-(OH)₂-D₃ is able to bring mineralization to the bone matrix. Rickets in young children and osteomalacia in older people is caused by a vitamin D deficiency; therefore, without vitamin D the organic matrix in bone is not able to be mineralized (Weber et al., 1971). Although the most active form of vitamin D is synthesized in the kidney, the kidney also converts 25(OH)D₃ to 24,25-(OH)₂-D₃, 25-26-(OH)₂-D₃ and 1,24,25-(OH)₃-D₃; nevertheless, the role of these compounds have not been fully evaluated (McDowell, 2000).

Absorption, Transport, and Tissue Delivery. The absorption of vitamin D in the small intestine depends on bile salts that help in emulsification and subsequently absorption in the small intestine by non-saturated passive diffusion (Heymann, 1938). The absorption of vitamin D depends on the fat content of the diet; when there is a low concentration of fat in the diet, it is not well absorbed. This is because fat is needed to make chylomicrons and to allow for emulsification of the fat soluble vitamins, which is the way they travel in the small

intestine to be subsequently taken up by the enterocytes in the brush border of the small intestine. After absorption by enterocytes, vitamin A, E, as well as vitamin D are transferred by chylomicrons via the lymphatic system, but only when these vitamins come from the diet. When vitamin D is made endogenously in the skin by 7-dehydrocholesterol (Holick, 1981), it is transported by a plasma protein called vitamin D binding protein (DBP), also called transcalferrin (DeLuca, 1967; Bouillon et al., 1976; Combs, 2012). In mammals, vitamin D, 25(OH)D₃, 1,25-(OH)₂-D₃ and 24,25-(OH)₂-D₃ are transported by the DBP (Silver and Fainaru, 1979; Mallon et al., 1980). There is a higher affinity for 25(OH)D₃ than for 1,25-(OH)₂-D₃, and this is the reason why in the body 25(OH)D₃ is the predominant form of vitamin D in the body (Mallon et al., 1980).

Storage Site and Excretion. Vitamin D can be stored in the liver, similar to vitamin A and E. Nevertheless, high concentrations of vitamin D can be found in the blood in contrast to other tissues in the body (Hay and Wastson, 1977). In pig tissue, it was found that 1,25-(OH)₂-D₃ concentrations in adipose tissue were seven-fold higher than plasma levels (Rungby et al., 1993). The catabolism of vitamin D is not well known; however, excretion of vitamin D and its metabolites occurs mostly in feces and very little in the urine. The catabolism of vitamin D can occur by the production of trihydroxylated products by hydroxylation of 1,25-(OH)₂-D₃, like 1,24,25-(OH)₂-D₃, 1,25,26-(OH)₂-D₃ and 1,25-(OH)₂-D₃-23,26-lactone. In addition, catabolism can occur by oxidative cleavage of the side chain (Kumar, 1986).

Figure 6. vitamin D metabolism (Deeb et al., 2007)

Deficiency Signs, Recommended Levels, and Toxicity. A reduction in serum calcium and phosphorus is one of the first symptoms of vitamin D deficiency. The results of a deficiency in vitamin D are abnormal growth rate and alteration of bones that can lead to rickets, osteoporosis and other diseases. The recent suggested requirements of vitamin D listed in the NRC (2012) for growing pigs weighing 5 to 135 kg fed at libitum (90% dry matter) are 59 to 418 IU per day. For gestating and lactating sows it is 1,680 and 4,773 IU per day, respectively (Table 1). Excess consumption of vitamin D can result in toxicity and even death (Chineme et al., 1976; Long, 1984). Nevertheless, the supplemental levels in these studies were more than 1,000 times the requirements suggested by the NRC (2012). Many of the effects caused by excessive intake of vitamin D are associated with abnormal

elevation of blood calcium (McDowell, 2000). However, natural sources of commonly used feed ingredients do not have excessive amounts of vitamin D. In case of intoxication, it can be reversible by just withdrawing vitamin D and reducing calcium levels (Collins and Norman, 2001).

Vitamin D in swine. In contrast to vitamin A and E, vitamin D can be transferred via the placenta in higher amounts compared to vitamin A and E. It was reported by Coffey et al. (2012) that feeding 25(OH)D₃ to gilts before farrowing increased fetal vitamin D, meaning that vitamin D can be passed to the fetus in higher amounts via the placenta compared to vitamin A and E. Flohr et al. (2014a) reported that when sows were fed with vitamin D₃ after breeding (1,500, 3,000, or 6,000 IU/kg of added vitamin D₃ in the complete diet) piglet serum 25(OH)D₃ concentrations were increased due to colostrum and milk intake, and also because vitamin D that was passed transplacentally. Research showed that the number of stillborn pigs were reduced when sows were fed at early gestation with vitamin D at doses of 1,400 and 2,000 IU compared to 200 and 800 IU (Lauridsen et al., 2010). Coffey et al. (2012) reported that when first-service gilts were supplemented at the same level of 2,500 IU/kg of the complete diet with 25(OH)D₃ compared to vitamin D₃, a higher number of developed fetuses were found in the reproductive tracts of gilts fed with 25(OH)D₃. Viganò et al. (2003) reported that vitamin D potentially can be involved in implantation because it is capable of increasing calbindin *HoxA* genes, which affect the viability of preimplantation embryos.

Rortvedt and Crenshaw (2012) reported that nursery pig performance was reduced when pigs were fed with low concentrations of vitamin D and marginal Ca and P

concentrations and when their dams were fed maternal diets with no vitamin D₃. Flohr et al. (2016) reported that vitamin D₃ or 25(OH)D₃ fed to sows at the same recommended level did not affect growth performance of nursery pigs. However, when feeding 2,000 IU of vitamin D₃/kg, piglets performed better compared to 800 or 9,600 IU of vitamin D₃/kg. Moreover, Flohr et al. (2014b) showed that supplementing formulated dietary concentrations of any form of vitamin D₃ did not affect growth performance in nursery pigs. Witschi et al. (2011) showed that when sows in lactation were fed with 50 µg/kg (2,000 IU) of vitamin D₃ and 25(OH)D₃ compared to 5 µg/kg (200 IU) of vitamin D₃ and 25(OH)D₃, ADFI was greater and growth performance tended to improve when sows were fed with 50 µg/kg regardless of the form of vitamin D.

Current Research Focus.

The purpose of this thesis was to determine the impact of vitamins A, D, and E injected in sows and gilts on sow productivity, livability and performance of pigs from birth throughout the nursery, and the number of full value pigs produced per sow. There are many positive benefits that these three soluble vitamins can have on the health of pigs or sows. Nevertheless, the administration of these three vitamins in combination and at higher concentrations than NRC (2012) recommendations to sows to improve the performance of sows and piglets, including the nursery phase, has not been previously evaluated. Vitamin E is important in sows and piglets because it constitutes an efficient nutrient in the body to regulate oxidative stress by capturing free radicals (Halliwell, 1994). It may help to increase the immune response in piglets and sows by protecting cells that are exposed to high levels of

free radicals (Babinszky et al., 1990). Moreover, increased oxidative and metabolic stress in the sow (e.g. due to high productivity or heat stress) results in excessive production of reactive oxygen species, which can overwhelm antioxidant defense systems resulting in oxidative damage of proteins, lipids, and DNA, with obvious negative consequences on fetal and mammary gland development and milk production (Black et al., 1993, Zhao et al., 2011). Interestingly, the antioxidant capacity in sows during late gestation and lactation is greatly reduced (Zhao, 2011). This is not surprising because: 1) gestating sow diets contain little fat and fat is required for absorption of fat soluble vitamins; 2) the tremendous production level of modern lactating sows which increases requirements of vitamins; and 3) the use of coproducts (such as DDGS) that contain high concentrations of unsaturated lipids that are potentially highly oxidized (resulting in degradation of vitamins).

Vitamins A, E, and D help to prevent damage of cells during high (oxidative) stress, provide protection to the immune system, and are involved in reproductive processes. Data show that individual fat soluble vitamins (A, D, and E) have many benefits in reproductive sows and improve the health status of their piglets; nevertheless, combined effects of these vitamins on the response of sows and piglets during lactation and the nursery have not been evaluated. The aim of the present research project was to administer vitamin A, D, and E via intermuscular injection to sows and gilts and determine the response in sow productivity and the performance of their piglets during lactation and the nursery.

Table 1. Daily vitamin requirements of gestating sows, lactating sows, and growing pigs (90 % dry matter) by the NRC (2012).

	Requirements (amount per day)	
Item	Gestation	Lactation
sows		
vitamin A (IU) ¹	8,398	11,932
vitamin E (IU) ²	92.4	262.5
vitamin D (IU) ³	1,680	4,773
Item	BW range, 5-135 kg	
Growing pigs		
vitamin A (IU) ¹	585 to 3,623	
vitamin E (IU) ²	4.3 to 30.7	
vitamin D (IU) ³	59 to 418	

¹1 IU vitamin A = 0.30 µg retinol or 0.344 µg retinyl acetate.

²1 IU vitamin E = 0.67 mg of d- α -tocopherol or 1 mg of dl- α -tocopheryl acetate.

³1 IU vitamin D₂ or D₃ = 0.025 µg vitamin D.

Adapted from NRC (2012).

Literature Cited

- Adamstone, F. B., L. Krider, and M. F. Jamest. 1944. Response of swine to vitamin E-deficient rations. *Dep. Zool. Anim. Sci.*:260–268.
- Amazan, D., G. Cordero, C. J. López-Bote, C. Lauridsen, and a I. Rey. 2014. Effects of oral micellized natural vitamin E (D- α -tocopherol) v. synthetic vitamin E (DL- α -tocopherol) in feed on α -tocopherol levels, stereoisomer distribution, oxidative stress and the immune response in piglets. *Animal* 8:410–9.
- Babinszky, L., D. J. Langhout, M. W. A. Vestegen, L. A. den Hartog, P. Joling, and M. Nieuwland. 1990. Effect of vitamin E and fat source in sows' diets on immune response of suckling and weaned piglets. *J. Anim. Sci.* 69:1833–1842.
- Bates, C. J. 1983. Vitamin A in pregnancy and lactation. *Proc. Nutr. Soc.* 42:65–79.
- Bjorneboe, A., G. E. Bjorneboe, E. Bodd, B. F. Hagen, N. Kveseth, and C. A. Drevon. 1986. Transport and distribution of alpha-tocopherol in lymph, serum and liver cells in rats. *Biochim Biophys Acta* 889:310–315.
- Bjorneboe, A., M. S. Nenseter, B. F. Hagen, G. E. Bjorneboe, K. Prydz, and C. A. Drevon. 1991. Effect of dietary deficiency and supplementation with all-rac-alpha-tocopherol on hepatic content in rats. *J. Nutr.* 121:1208–1213.
- Bjorneboe, A., E. Soyland, G. E. Bjorneboe, G. Rajka, and C. A. Drevon. 1987. Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *Br. J. Dermatol.* 117:463–469.
- Bjorneboe, a, G. E. Bjorneboe, and C. a Drevon. 1990. Absorption, transport and distribution of vitamin E. *J. Nutr.* 120:233–242.
- Blaner, W. S. 1989. Retinol-Binding Protein: The Serum Transport Protein for Vitamin A. *Endocr. Rev.* 10:308–316.
- Blaner, W. S., H. F. Hendriks, A. Brouwer, A. M. de Leeuw, D. L. Knook, and D. S. Goodman. 1985. Retinoids, retinoid-binding proteins, and retinyl palmitate hydrolase distributions in different types of rat liver cells. *J. Lipid Res.* 26:1241–51.
- Blaxter, L. 1962. Vitamin E in Health and Disease of Cattle and Sheep. *Hannah Dairy Res. Institute, Ayr, Scotl.*:633–641.
- Blomhoff, Rune, Green, H Michael, Balmer Green, Joanne, Berg, Trond, and R. N. K. 1991. *Physiological Reviews*.

Blomhoff, R. 1994. Vitamin A in the Health and Disease. Marcel Dekker, Inc., New York, USA.

Blomhoff, R., M. H. Green, T. Berg, and K. R. Norum. 1990. Transport and Storage of Vitamin A. *Sci. New Ser.* 250:399–404.

Blomhoff, R., M. H. Green, J. B. Green, T. Berg, and K. R. Norum. 1991. Vitamin A metabolism: new perspectives on absorption, transport, and storage. *Physiol Rev* 71:951–990.

Bonnette, E. D., E. T. Kornegay, M. D. Lindemann, and C. Hammerberg. 1990. Humoral and cell-mediated immune response and performance of weaned pigs fed four supplemental vitamin E levels and housed at two nursery temperatures. *J. Anim. Sci.* 68:1337–1345.

Bouillon, R., H. Van Baelen, W. Rombauts, and P. De Moor. 1976. The Purification and Characterisation of the Human-Serum Binding Protein for the 25-Hydroxycholecalciferol (Transcalciferin). *Eur. J. Biochem.* 66:285–291.

Chineme, C. N., K. L., and W. G. Pond. 1976. Bone pathology in hypervitaminosis D; an experimental study in young pigs. *Cornell Vet.* 66:387–412.

Chow, C. K. 1975. Distribution of tocopherols in human plasma and red blood cells. *Am. J. Clin. Nutr.* 28:756–760.

Chow, C. K. 2001. Handbook of vitamins. Third Edit. (R. B. Rucker, J. W. Suttie, D. B. McCormick, and L. J. Machlin, editors.). New York, NY USA.

Chung, Y. K., D. C. Mahan, and A. J. Lepine. 1992. Efficacy of Dietary D- α -Tocopherol and DL- α -Tocopheryl Acetate for Weanling Pigs. *J. Anim. Sci.* 70:2485–2492.

Coffey, J. D., E. A. Hines, J. D. Starkey, C. W. Starkey, and T. K. Chung. 2012. Feeding 25-hydroxycholecalciferol improves gilt reproductive performance and fetal vitamin D status. *J. Anim. Sci.* 90:3783–3788.

Coffey, M. T., and J. H. Britt. 1993. Enhancement of Sow Reproductive Performance by B-Carotene or Vitamin A. *J. Anim. Sci.* 71:1198–1202.

Collins, E. D., and A. W. Norman. 2001. Handbook of vitamins. Third edit. (R. B. Rucker, J. W. Suttie, D. B. McCormick, and L. J. Machlin, editors.). Marcel Dekker, Inc., New York, NY USA.

Combs, G. F. 2012. The vitamins, Fourth Edition. Fourth Ed. Elsevier/Academic Press.

- Cunha, T. J. 1997. *Swine Feeding and Nutrition*. Academic Press, Inc., New York, USA.
- Davis, Y., and J. L. Sell. 1983. Effect of all-trans Retinol and Retinoic Acid Nutriture on the Immune System of Chicks. *J. Nutr.* 113:1914–1919.
- Debier, C. 2007. Vitamin E During Pre- and Postnatal Periods. *Vitam. Horm.* 76:357–373.
- Deeb, K. K., D. L. Trump, and C. S. Johnson. 2007. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat.Rev.Cancer* 7:684–700.
- DeLuca, H. F. 1967. Action and Metabolic Fate of vitamin D. In: *Vitamins Hormones*. p. 316–367.
- Diplock, A. T. 1985. *Fat-Soluble Vitamins*. Technomic Publishing Co., Inc, Lancaster, Pennsylvania, USA.
- Evans, H. M., O. H. Emerson, and G. A. Emerson. 1936. The isolation from wheat germ oil of an alcohol, alpha tocopherol, having the properties of vitamin E. *J. Biol. Chem.* 113:319–332.
- Evans, M. H., and K. S. Bishop. 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Sci. New Ser.* 56:650–651.
- Evans, M. H., and G. B. Burr. 1924. The anti-sterility vitamin fat soluble E. *Proc. Natl. Acad. Sci. United States Am.* 209:334–341.
- Fidge, B. N. H., F. R. Smith, and D. S. Goodman. 1969. Vitamin A and Carotenoids. *J. Biol Chem* 114:689–694.
- Flohr, J. R., M. D. Tokach, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, J. L. Nelssen, and J. R. Bergstrom 2014a. An evaluation of the effects of added vitamin D₃ in maternal diets on sow and pig performance^{1,2}. *J. Anim. Sci.* 92:594–603.
- Flohr, J. R., M. D. Tokach, S. S. Dritz, J. M. Derouchey, R. D. Goodband, J. L. Nelssen, S. C. Henry, L. M. Tokach, M. L. Potter, J. P. Goff, N. J. Koszewski, R. L. Horst, E. L. Hansen, and E. D. Fruge. 2014b. Effects of supplemental vitamin D₃ on serum 25-hydroxycholecalciferol and growth of preweaning and nursery pigs. *J. Anim. Sci.* 92:152–163.
- Flohr, J. R., J. C. Woodworth, J. R. Bergstrom, M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. M. DeRouchey. 2016. Evaluating the impact of maternal vitamin D supplementation on sow performance: II. Subsequent growth performance and carcass

characteristics of growing pigs. *J. Anim. Sci.* 94:4643–4653.

Friedrich, W. 1988. *Vitamins*. Walter de Gruyter & Co., Berlin, NY.

Glover, J. 2007. Factors affecting vitamin A transport in animals and man. *Proc. Nutr. Soc.* 42:19–30.

Haddad, J.G., Walgate, J. 1976. 25-Hydroxyvitamin D Transport in Human Plasma. *J. Biol. Chem.* 251:4803–4809.

Halliwel, B. 1994. Free radicals and antioxidants: a personal view. *Nutr. Rev.* 52:253–65.

Hay, W. M. A., and G. Wastson. 1977. The binding of 25-hydroxycholecalciferol and 25-hydroxyergocalciferol to receptor proteins in a new world and an old world primate. *Int. Sociol.* 9:131–148.

Hayek, M. G., G. E. Mitchell, R. J. Harmon, T. S. Stahly, G. L. Cromwell, R. E. Tucker, and K. B. Barker. 1989. Porcine immunoglobulin transfer after prepartum treatment with selenium or vitamin E. *J. Anim. Sci.* 67:1299–1306.

Henry, H. L., and A. W. Norman. 1974. Studies on Calciferol Metabolism. IX. Renal 25-hydroxyl-vitamin D₃-1-Hydroxylase. Involvement of cytochrome P-450 and other properties. *J. Biol. Chem.* 249:7529–7535.

Heying, E. K., M. Grahn, K. V Pixley, T. Rocheford, and S. a Tanumihardjo. 2013. High-provitamin A carotenoid (Orange) maize increases hepatic vitamin A reserves of offspring in a vitamin A-depleted sow-piglet model during lactation. *J. Nutr.* 143:1141–6.

Heymann, W. 1937. Metabolism and mode of action of vitamin D. IV. Importance of bile in the absorption and excretion of vitamin D. *J. Biol Chem* 122:249–256.

Hibberd, A., and A. W. Norman. 1969. Comparative biological effects of vitamins D₂ and D₃ and dihydrotachysterol₂ and dihydrotachysterol₃ in the chick. *Biochem. Pharmacol.* 18:2347–2355.

Hidiroglou, M., E. Farnworth, and G. Butler. 1993. Vitamin E and fat supplementation of sows and the effect on tissue vitamin E concentrations in their progeny. *Reprod. Nutr. Dev.* 33:557–565.

Hoffmann-La Roche Inc. 1991. *Vitamin Nutrition for Swine*. Animal Nutrition, Hoffmann-La Roche, Nutley, NJ.

Holick, M. F. 1981. The cutaneous photosynthesis of previtamin D₃: A unique

photoendocrine system. *J. Invest. Dermatol.* 77:51–8.

Horwitt, M. K. 1962. Interrelations between Vitamin E and Polyunsaturated Fatty Acids in Adult Men. *Vitam. Horm.* 20:541–558.

Jatlas. 2014. Vitamin D biosynthesis in fungi and animals. :1.

Kamal-Eldin, A., and L. Å. Appelqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* 31:671–701.

Kayden, H. J., and M. G. Traber. 1993. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J. Lipid Res.* 34:343–358.

Kono, N., and H. Arai. 2015. Intracellular Transport of Fat-Soluble Vitamins A and E. *Traffic* 16:19–34.

Kumar, R. 1986. The metabolism and mechanism of action of 1,25-dihydroxyvitamin D3. *Kidney Int.* 30:793–803.

Lauridsen, C., H. Engel, S. K. Jensen, A. M. Craig, and M. G. Traber. 2002. Lactating Sows and Suckling Piglets Preferentially Incorporate RRR- over All-rac- α -Tocopherol into Milk, Plasma and Tissues. *J. Nutr.* 132:1258–1264.

Lauridsen, C., U. Halekoh, T. Larsen, and S. K. Jensen. 2010. Reproductive performance and bone status markers of gilts and lactating sows supplemented with two different forms of vitamin D. *J. Anim. Sci.* 88:202–213.

Lindemann, M. D., J. H. Brendemuhl, L. I. Chiba, C. S. Darroch, C. R. Dove, M. J. Estienne, and A. F. Harper. 2008. A regional evaluation of injections of high levels of vitamin a on reproductive performance of sows. *J. Anim. Sci.* 86:333–338.

Long, G. G. 1984. Acute toxicosis in swine associated with excessive dietary intake of vitamin D. *J. Am. Vet. Med. Assoc.* 184:164–170.

Machlin, L. J. 1991. Handbook of vitamins. Thrid Edit. (R. B. Rucker, J. W. Suttie, D. B. McCormick, and L. J. Machlin, editors.). New Yok, NY USA.

Machlin, L. J., and E. Gabriel. 1982. Kinetics of tissue a-tocopherol uptake and depletion following administration of high levels of vitamin E. *Ann.NY Acad.Sci.* 393:48–60.

Mahan, D. C. 1991a. Assessment of the Influence of Dietary Vitamin-e on Sows and Offspring in 3 Parties - Reproductive-Performance, Tissue Tocopherol, and Effects on Progeny. *J. Anim. Sci.* 69:2904–2917.

Mahan, D. C. 1991b. Dietary Vitamin E on Sow Reproductive Performance over a Five-Parity Period. *Anim. Sci.*:2870–2879.

Mallon, J. P., D. Matuszewski, and H. Sheppard. 1980. Binding specificity of the rat serum vitamin D transport protein. *J. Steroid Biochem.* 13:409–413.

Malm, A., W. G. Pond, E. F. Walker, M. Homan, A. Aydin, and D. Kirtland. 1976. Effect of polyunsaturated fatty acids and vitamin E level of the sow gestation diet on reproductive performance and on level of alpha tocopherol in colostrum, milk and dam and progeny blood serum. *J. Anim. Sci.* 42:393–399.

Marceau, G., D. Gallot, D. Lemery, and V. Sapin. 2007. Metabolism of Retinol During Mammalian Placental and Embryonic Development. *Vitam. Horm.* 75:97–115.

Mathias, P. M., J. T. Harries, T. J. Peters, and D. P. R. Muller. 1981. Studies on the In vivo Absorption of Micellar Solutions of Tocopherol and Tocopheryl Acetate in the Rat - Demonstration and Partial Characterization of a Mucosal Esterase Localized to the Endoplasmic-Reticulum of the Enterocyte. *J. Lipid Res.* 22:829–837.

McDowell, L. 2000. *Vitamins in Animal and Human Nutrition*. Second Edi. Iowa State University Press, Ames, IA.

Mellanby, E. 1921. *Experimental rickets*. HM Stationery Office.

Moore, T. 1971. Vitamin A transfer from mother to offspring in mice and rats. *Int. J. Vitam. Nutr. Res.* 41:301–306.

Moreira, I., and D. C. Mahan. 2002. Effect of dietary levels of vitamin E (all- rac-tocopheryl acetate) with or without added fat on weanling pig performance and tissue alpha-tocopherol concentration. *J. Anim. Sci.* 80:663–669.

NRC. 1987. *Vitamin Tolerance of Animals*. National Academies Press, Washington, DC.

NRC. 2012. *Nutrient Requirements of Swine*. 11 th ed. National Academies Press, Washington, DC.

Olson, J. A. 1991. *Handbook of Vitamins*. (L. J. Machlin, editor.). New York, NY USA.

Parker, R. S. 1989. Carotenoids in Human Blood and Tissues. *J. Nutr.* 119:101–104.

Rortvedt, L. A., and T. D. Crenshaw. 2012. Expression of kyphosis in young pigs is induced by a reduction of supplemental vitamin D in maternal diets and vitamin D, Ca, and P

concentrations in nursery diets. *J. Anim. Sci.* 90:4905–4915.

Rungby, J., L. Mortensen, K. Jakobsen, A. Brock, and L. Mosekilde. 1993. Distribution of hydroxylated vitamin D metabolites [250HD₃ and 1,25(OH)₂D₃] in domestic pigs: Evidence that 1,25(OH)₂D₃ is stored outside the blood circulation? *Comp. Biochem. Physiol. Part A Physiol.* 104:483–484.

Saarem, K., S. Bergseth, H. Oftebro, and J. I. Pedersen. 1984. Subcellular localization of vitamin D₃ 25-hydroxylase in human liver. *J. Biol. Chem.* 259:10936–10940.

Silver, J., and M. Fainaru. 1979. Transport of vitamin D sterols in human plasma: effect of excess vitamin D, 25 hydroxyvitamin D and 1,25 dihydroxyvitamin D. *Eur. J. Clin. Invest.* 9:433–438.

Sure, B. 1924. Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction.

Traber, M. G. 2007. Vitamin E Regulatory Mechanisms. *Annu. Rev. Nutr.* 27:347–362.

Traber, M. G., and H. J. Kayden. 1987. Tocopherol distribution and intracellular localization in human adipose tissue. *Am. J. Clin. Nutr.* 46:488–495.

Traber, M. G., and H. J. Kayden. 1989. Preferential incorporation of alpha-tocopherol vs gamma-tocopherol in human lipoproteins. *Am. J. Clin. Nutr.* 49:517–26.

Traber, M. G., J. C. Lane, N. R. Lagmay, and H. J. Kayden. 1992. Studies on the transfer of tocopherol between lipoproteins. *Lipids* 27:657–663.

Valentine, A. R., and S. A. Tanumihardjo. 2005. One-time vitamin A supplementation of lactating sows enhances hepatic retinol in their offspring independent of dose size. *Am. J. Clin. Nutr.* 81:427–433.

Viganò, P., S. Mangioni, F. Pompei, and I. Chiodo. 2003. Maternal-conceptus Cross Talk—A Review. *Placenta* 24:56–61.

Weber, B. J. C., V. Pons, and E. Kodicek. 1971. The Localization of 1,25-Dihydroxycholecalciferol in Bone Cell Nuclei of Rachitic Chicks. *J. Biol. Chem.* 125:147–153.

Wellenreiter, R. H., D. E. Ullrey, E. R. Miller, and W. T. Magee. 1969. Vitamin A activity of corn carotenes for swine. *J. Nutr.* 99:129–136.

Whaley, S. L. 1995. Effects of Intramuscular Injection of Vitamin A on Increased

Litter Size in Swine.

Wilburn, E. E., D. C. Mahan, D. A. Hill, T. E. Shipp, and H. Yang. 2008. An evaluation of natural (RRR- α -tocopheryl acetate) and synthetic (all-rac- α -tocopheryl acetate) vitamin e fortification in the diet or drinking water of weanling pigs. *J. Anim. Sci.* 86:584–591.

Windaus, A., F. Schenck, and V. f. Werder. 1936. “Über das antirachitisch wirksame Bestrahlungsprodukt ans 7-Dehydro-cholesterin.” *Hoppe-Seyler’s Zeitschrift für Physiol. Chemie*:101–103.

Witschi, A. K. M., A. Liesegang, S. Gebert, G. M. Weber, and C. Wenk. 2011. Effect of source and quantity of dietary vitamin D in maternal and creep diets on bone metabolism and growth in piglets. *J. Anim. Sci.* 89:1844–1852.

Wuryastuti, H. J., H. D. Stowe, R. W. Bull, and E. R. Miller. 1993. Effects of Vitamin E and Selenium on Immune Responses of Peripheral Blood , Colostrum , and Milk Leukocytes of Sows. *J. Anim. Sci.* 71:2464–2472.

Zile, M. H., and M. E. Cullum. 1983. The Function of Vitamin A: Current Concepts. *Proc. Soc. Exp. Biol. Med.* 152:139–152.

CHAPTER II:
Improving Nursery Pig Performance by Injecting Sows with d- α -Tocopherol, Retinyl palmitate, and Cholecalciferol

ABSTRACT

Vitamins A, D, and E play important roles in preventing cell damage, enhancing immune function, and improving fertility. It is hypothesized that highly productive sows require supplemental fat-soluble vitamins to maximize reproductive performance and the growth and health of their offspring. Fifty-four sows were allotted by parity to 3 treatments: 1) control (C; 5 mL i.m. saline injection on d 107 of gestation; 2) 2 injections (V2) of 5 mL i.m. of a multi-vitamin product containing 200,000, 300, and 100,000 I.U./mL of vitamin A, E, and D, respectively on d 107 of gestation and d 4 of lactation; and 3) same as V2 plus a third 5 mL i.m. injection of vitamins on d 14 of lactation (V3). The doses used and the days chosen to inject sows were based on previous research. Blood samples from sows were collected on d -7, 1, 7, and 17 relative to farrowing and from piglets on d 0 (prior to nursing), 3, 7, 17, and 25 (4 d post-weaning). Results showed that serum retinol, retinyl palmitate and α -tocopherol concentrations in piglets before suckling were not detectable (<0.2 ppm). Serum retinol concentrations in sows and piglets increased over time ($P < 0.01$) but were not impacted by injection. Serum α -tocopherol in sows increased as lactation progressed ($P < 0.01$); V2 increased ($P < 0.05$) serum α -tocopherol on d 7 compared to control and V3 tended ($P < 0.10$) to increase serum α -tocopherol on d 17 compared to control and V2. Piglet serum α -tocopherol decreased ($P < 0.001$) from d 3 to 25 (7.67 to 2.19 $\mu\text{g/mL}$) but was not affected by treatment. Vitamin injection increased ($P < 0.05$) serum 25(OH)D₃ in sows on d 1, 7, and 17 and V3 increased ($P < 0.05$) serum 25(OH)D₃ compared to V2 on d 17. In piglets, serum 25(OH)D₃ increased ($P < 0.05$) on d 7 and 17 with V2 compared to control. Pigs from V3

sows had greater ($P<0.05$) serum 25(OH)D₃ on d 7, 17, and 25 than control (12.6, 26.7, 19.8, and 15.4 vs. 6.4, 7.2, 9.0, and 13.4 ng/ml for d 3, 7, 17, and 25, respectively) and had greater ($P<0.05$) serum 25(OH)D₃ on d 17 (32.2 vs. 19.8 ng/ml) and 25 (24.6 vs. 15.4 ng/ml) compared to V2. Piglet BW at birth and weaning was greater ($P<0.05$) with V3 compared to V2, whereas BW after 14 d ($P=0.06$; 8.76, 8.83, and 9.33 kg for control, V2, and V3, respectively) and 35 d in the nursery was improved with V3 compared to V2 and control ($P=0.005$; 18.03, 18.31, and 19.73 kg for control, V2, and V3, respectively). In conclusion, injecting fat-soluble vitamins in sows improved piglet performance through the nursery, which may be related to improved vitamin status of pigs.

Introduction

Essential fat-soluble vitamins such vitamins A, E, and D, are critical to maintaining growth, reproduction, and good health in intensively housed farm animals. One of the primary roles of vitamin A is to regulate vision under low light (Dowling and Wald, 1960). Retinoids which are analogs of vitamin A or retinol are involved in the differentiation of epithelial cells in the digestive tract. Vitamin A participates in the regulation of keratin expression which controls the structure of the cells; therefore, a vitamin A deficiency can lead to squamous metaplasia (hyper-keratinization) of the columnar cells in the intestinal tract (Dowling and Wald, 1960), potentially leading to endotoxemia. Vitamin E is a free radical scavenger that protects the membrane of cells from oxidation. In addition, research has shown that increasing vitamin E in the diet improved the health of piglets by increasing colostrum and milk immunoglobulins that provide passive immunity to the highly susceptible

newborn (Hayek et al., 1989; Babinszky et al., 1990). In addition, vitamin E provides protection against oxidative damage to young pigs (Debier, 2007) and supplementation of sows with vitamin E can supply vitamin E to nursing piglets via the milk. Serum vitamin E concentrations decline after weaning (Chung et al., 1992); therefore, weaned pigs may be compromised in their ability to reduce free radicals and consequently suffer oxidative damage. Vitamin D is needed for bone growth and maintenance of normal calcium (DeLuca, 1967; DeLuca, 2008). Research showed that postnatal growth was positively related to the total number of muscle fibers at birth, which originate during fetal muscle development (Dwyer et al., 1994; Dwyer et al., 1993). Endo et al (2003) showed that knockout mice had smaller muscle fibers at 3 weeks of age when the vitamin D receptor was not present compared to wild-type. In addition, Hines et al. (2013) reported that there were alterations in fetal muscle characteristics in fetuses from bred gilts fed with 25(OH)D₃ compared to fetuses from bred gilts that were fed D₃. We hypothesize that based on the importance of these individual vitamins that supplying them in combination to sows through intramuscular injection will exert positive effects on the performance of piglets when nursing their dams and further enhance growth performance after weaning. Thus, the objective of the present study was to investigate the effects of injecting vitamins A, E, and D in sows before farrowing and during lactation on colostrum, milk, sow and pig serum concentrations of vitamins A, E, and D and performance of pigs pre- and post-weaning.

Materials and Methods

All procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). Animal use protocols were approved by North Carolina State University Institutional Animal Care and Use Committee.

Experimental Design. A total of 54 sows and gilts (average parity was 3) were assigned to 1 of 3 treatments, consisting of 1) control (intramuscular (i.m.) injection with a saline); 2) i.m. injection of vitamins on day 107 of gestation and the second injection on day 4 of lactation; and 3) i.m. injection of vitamins on day 107 of gestation, second injection on day 4 of lactation and the third injection on day 14 of lactation. Injections consisted of 5 mL of a saline for control or 5 mL of a commercially available multivitamin product containing 3 naturally derived fat-soluble vitamins (200,000 IU/mL of vitamin A (as retinyl-palmitate), 300 IU/mL of vitamin E (as d-alpha-tocopherol), and 10,000 IU/mL of vitamin D₃, in an emulsified matrix). Thus, injections provided a total of 1,000,000 IU of vitamin A, 1,500 IU of vitamin E, and 50,000 IU of vitamin D₃ on the day of administration. The timing of injections was selected specifically to provide vitamins to piglets via colostrum and milk at birth and during lactation. The post-farrowing injection prior to weaning was administered to improve vitamin status of pigs at weaning and to supply vitamin A to sows to improve fertility and subsequent litter size (Coffey and Britt, 1993).

General Procedures. Sows in gestation were fed according to their body condition score to maintain an ideal score of 3 on a 1 to 5 body condition scoring scale. Sows were fed on ad libitum basis during the lactation phase. Gestation and lactation diets (Table 3) met or

exceeded the minimum recommended nutrients suggested by the NRC (2012). One week prior to parturition, sows were moved into individual crates (1.67 m wide by 2.33 m long) where they were fed 1.8 kg of lactation feed twice per day until farrowing. Sows had ad libitum access to water via two water nipples, one on top and one on the bottom of the farrowing crate. Sows were induced 3 days before farrowing by giving an intramuscular injection of 2 cc of prostaglandin F₂ α (Lutalyse). Pigs were processed on d 1 and castrated on d 7 according to the standard farm protocol. For the newborn pigs, each crate contained one heat lamp for supplemental heat and rubber floor mats for the entire lactation period. Creep feed was not fed to piglets. After weaning, 314 total pigs were blocked by size and placed within treatment groups in pens of 8 to 10 mixed sex pigs, resulting in 19 pens for the control and the three injection treatments and 18 pens for the two injection treatment. In the nursery, two-phase diets were fed to pigs. The first diet was fed for the first 2 weeks in the nursery and the second diet for 3 wks. Both diets (Table 3) were formulated to meet the NRC (2012) nutrient requirements. Each pen contained two feeders and 2 water nipples that allowed pigs to eat and drink on an ad libitum basis. All diets were the same for all treatments.

Measurements. Litter and individual piglet BW for each litter was recorded on d 1 and at weaning (20 $\pm$ 2 d of age). Piglets were cross-fostered if needed only within treatments. Growth performance data for all weaned pigs was collected until the end of the nursery (55 $\pm$ 2 d of age), including BW, feed intake, feed efficiency, mortality, and morbidity.

Blood, colostrum, and milk samples were collected from a subset of lactating sows (parity 1 to 4; n=6 per treatment) to determine their vitamin status. Blood samples from sows

were collected 7 d before farrowing, and on d 1, 7, and 17 of lactation. Sows were snared and blood was collected via jugular venipuncture in 10 ml tubes. Blood was refrigerated at 23°C to allow it to clot and allowed to stay at room temperature for 30 minutes before centrifugation. Blood was centrifuged ($3,500 \times g$ for 20 min at 21°C) and serum was harvested into 5 ml tubes and frozen at -80°C before chemical analysis was conducted. Colostrum was collected 1 to 24 hours after parturition. Milk samples were collected on d 7 and 17 of lactation. Before colostrum and milk collection, the udder was cleaned with a paper towel. Both colostrum and milk samples were collected from any randomly available teat in the udder. Oxytocin (1 mL) was injected i.m. to stimulate milk let down; however, in the case that not enough milk could be collected, an extra 0.5 mL was used. A total of approximately 15 mL of colostrum or milk was collected in a 30 ml tube and placed on ice. Immediately after finishing collecting the milk, each sample was distributed into 3 tubes and then frozen at -80°C.

Blood samples from piglets from sows of parity 1 to 4 were collected 1 to 2 minutes after piglets were born. Pigs were not allowed to nurse the sows prior to blood collection. Blood from 2 pigs per sow was collected ($n=12$ per treatment), and after serum was harvested, samples from the 2 pigs per sow were combined and mixed. In addition, blood samples from piglets were collected (from sows of parity 1 to 4) at 3, 7, 17, and 25 days of age. The protocol for blood collection of pigs was performed in a similar way as it was in sows, except that 4 ml of blood was collected from each piglet. The schedule of injections (3 treatments), milk, colostrum, blood collections, and timeline are shown below (Table 2).

Table 2. Injections, sample collection, and timeline

Item	Pre-Farrowing 1...107...114	Days on Lactation 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21																					Nursery 1 2 3 4 5...14...35													
<u>Injections</u>																																				
Control ¹	↑																																			
2-injections ¹	↑																																			
3-injections ¹	↑																																			
Blood sows	↑																																			
Colostrum/milk																																				
Blood piglets																																				
Pig BW																																				

¹Vitamin injection: 5 ml i.m. to sows and gilts (Vital E – Repro), Stuart Products, Bedford, TX) or saline (control). The vitamin product contained 300 IU vitamin E (as d- α -tocopherol), 200,000 IU vitamin A (as retinyl-palmitate) and 100,000 IU vitamin D₃ compounded with 18% ethyl alcohol and 1% benzyl alcohol in an emulsifiable base.

Chemical Analysis. Determination of vitamin A and E concentrations in serum, colostrum and milk was performed by the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA). Serum α -tocopherol, retinol, and retinyl palmitate concentrations were determined by HPLC using a photodiode array detector. Samples were deproteinized followed by extraction with a mixture of 95% hexane and 5% chloroform. The upper layer was transferred to a 7 mL vial and concentrated to dryness using N₂. Samples were reconstituted in 100 μ L of methanol and a 20 μ L portion was injected on an HPLC with a photodiode array detector. Separation was achieved using a Perkin Elmer Pecosphere C18 (3 μ M, 4.6 x 33mm) column (PerkinElmer, Inc., Waltham, MA) and a mobile phase with 95% of a 90/10 methanol/chloroform mixture and 5% water with a flow rate of 1 mL/min.

Detection wavelengths were 292 nm for α -tocopherol and 325 for retinol and retinyl palmitate. Milk analysis for α -tocopherol, retinol, and retinyl palmitate were performed in the same manner as the serum protocol, except that a 50/50 methanol/chloroform was used as the mobile phase.

Serum concentrations of 25(OH)D₃ were analyzed quantitatively by Heartland Assays (Ames, IA) using a commercial RIA kit (68100E, DiaSorin, Inc., Stillwater, MN).

Statistical Analyses. All data were analyzed by ANOVA using Proc GLM (SAS, Cary, NC). The model for sow and growth performance data included sow group (2 groups of sows were used, 1 month apart) and treatment. Vitamin concentrations in serum and milk were measured at multiple points and were analyzed using the Mixed procedure using repeated measures analysis. For statistical differences a $P \leq 0.05$ was used to declare significance and for statistical tendencies, $0.05 < P \leq 0.10$ was used. Fisher's LSD method was used in ANOVA to compare treatment means.

Results

General Observations. After three days in the nursery, pigs started to show diarrhea. The area of the anus in male and vulva and anus on female pigs was reddish after showing symptoms of diarrhea. Some pigs became lethargic and some died after 3 or 4 days (Figure 7). Mycotoxin contamination of feed was suspected, but analyzed aflatoxin and vomitoxin (or deoxynivalenol) levels were within acceptable ranges in phase I nursery diets (0.0 ppb and 360.9 ppb, respectively) and phase II diets (0.0 ppb, 181.3 ppb respectively). Additional

mycotoxins were detected at low levels. Necropsies were performed on sick pigs and contemporary pigs, showing significant irritation of the intestine (Figure 8). During the trial, one 4-week old female pig that was showing signs of diarrhea and lethargy was sent to Rollins Animal Disease Laboratory (Raleigh, NC). The pig had a good body condition (score of 5 on a scale of 1 to 9), weighed approximately 10 kg, and was not treated with any medicine. The pig had diarrhea and was coughing. The lab reported that the pig had petechial hemorrhages which were scattered around the lungs, and a few pinpoint hemorrhages were found in the cortex of each kidney. The intestinal contents and feces had dark yellow contents. The reported cause of death was most likely a bacterial infection of the heart valve. Mycotoxin contamination was not confirmed.

Figure 7. Observations in sick pigs during the nursery. The first picture on the left shows a pig with irritated vulva and a pig with a normal vulva. The picture in the middle shows a pig with chronic diarrhea. The third picture on the right shows a lethargic pig.

Figure 8. Necropsy of a sick nursery pig and a healthy pig. The first picture shows a sick pig with significant gut irritation. The second picture shows a contemporary, healthy pig.

Litter performance. The number of pigs born alive, stillborn pigs, and total weaned pigs were similar ($P>0.60$) for all treatments (Table 4). Pigs whose dams received three injections (V3) were heavier at birth compared to pigs whose dams received two injections (V2) (1.45 vs. 1.35 kg respectively, $P=0.02$); however, these two treatments were the same at birth because sows in both treatment groups were injected with the vitamin combination on d 107 of gestation. At birth there was a significant tendency for pigs from V3 sows to be heavier than pigs from control sows (1.39 vs. 1.45 kg, $P=0.06$), but there were no differences between C and V2 sows ($P=0.22$). Pig body weight ($n=514$) at weaning tended to be different ($P=0.09$) among treatments. Weaning BW of pigs from sows in V3 was greater than that from sows in V2 (6.38 vs. 6.02 kg, $P=0.032$) but not C. Average daily gain (ADG) from birth to weaning was not different between treatments ($P=0.24$).

Nursery Pig Performance. Body weight at 14 days in the nursery showed a tendency among treatments ($P=0.06$), where BW of pigs born to V3 sows was greater ($P\leq 0.056$) compared to V2 and C (9.33, 8.83, and 8.76 kg, respectively), but V2 and C were not different ($P=0.793$). Similarly, at 5 wk in the nursery ($n=481$ pigs), V3 pigs were heavier ($P\leq 0.005$) than V2 and C (19.73, 18.31, and 18.03 kg, respectively), but there was no difference between V2 and C ($P=0.579$). From weaning to the end of the nursery, ADFI, ADG, G:F and mortality were not significantly different among treatments ($P>0.40$), (Table 4).

Sow Serum Vitamin Concentrations. Before any injections were given, serum vitamin A, E, and D concentrations in gilts and sows ($n=6$ per treatment) were not different among treatments, showing that sows started with a similar vitamin status (Table 5). Sow serum retinol concentrations were not different ($P\geq 0.089$) between treatments when measured on d -7, 1, 7, and 17 (mean concentrations of 0.251, 0.255, 0.372, and 0.366 $\mu\text{g/ml}$, respectively); however, on d 1, there was a tendency for treatment differences, showing that retinol concentrations for V3 were greater ($P=0.033$) than C (0.290 vs 0.211 $\mu\text{g/ml}$) only (Table 5). Serum concentrations of α -tocopherol in sows were not different among treatments for any collection day ($P\geq 0.143$). Concentrations of 25(OH)D₃ were not different in sow serum due to treatments ($P=0.170$) initially on d -7 d (0.046, 0.041, and 0.048 $\mu\text{g/ml}$ respectively), but it was higher on d 1, 7, and 17 in V2 and V3 sows compared to control ($P\leq 0.001$).

Pig Serum Vitamin Concentrations. Piglet serum retinol, retinyl palmitate, and α -tocopherol concentrations on d 0 (prior to nursing) were not detectable (< 0.2 ppm),

suggesting that the sows pass minimal amounts of these vitamins to the pigs via the placenta. However, pig serum 25(OH)D₃ concentrations on d 0 (prior to nursing) from sows that received two or three injections (combined treatments) had higher levels of 25(OH)D₃ compared to pigs from control sows ($P=0.008$) (Figure 19). Serum retinol concentrations in pigs at d 3, 7, 17, and 25 did not differ ($P\geq 0.191$) among treatments (Table 6). Retinol concentrations among treatments were constant throughout the lactation period with a mean average of 0.30 µg/ml. Similarly, serum concentrations of α-tocopherol in pigs were not different among treatments at any collection point ($P\geq 0.154$). In fact, serum concentrations of α-tocopherol in pigs decreased over time (Table 6 and Figure 11). Serum concentrations of 25(OH)D₃ in pigs on d 3, 7, and 17 were significantly increased for V2 and V3 compared to C ($P\leq 0.001$); however, on d 25, V3 was significantly different from V2 and C ($P\leq 0.01$), but V2 and C were not different from each other (0.015 vs 0.024 µg/ml, $P=0.528$). 25(OH)D₃ in pigs correlated with the sows serum 25(OH)D₃; as vitamin D₃ concentrations were increased in sows, it was also increased in their piglets.

Colostrum and milk constituents. Retinyl palmitate concentrations in colostrum (Table 7) were higher ($P\leq 0.05$) for V2 and V3 than C (3.15, 2.57, and 0.97 µg/ml, respectively), but were not different between V2 and V3 ($P=0.436$). For milk samples collected on d 7, retinyl palmitate concentrations were higher for V2 and V3 ($P\leq 0.001$) than C (1.50, 1.25, and 0.43 µg/ml, respectively), but not between V2 and V3 ($P=0.264$). Retinyl palmitate concentrations in milk on d 17 were higher for V3 ($P\leq 0.001$) compared to C and V2 (1.05, 0.300, and 0.367 µg/ml, respectively), but not between C and V2 ($P=0.495$) (Table 7).

There were no significant differences in retinol concentrations in milk between treatments on d 1, 7, and 17 ($P \geq 0.398$) (0.107, 0.109, and 0.116 $\mu\text{g}/\text{ml}$, respectively; Table 7). Colostrum α -tocopherol concentrations on d 1 tended to be higher ($P=0.07$) for V2 compared to V3 and C (12.433, 9.267, and 5.583 $\mu\text{g}/\text{ml}$, respectively), but was not different between V3 and C ($P=0.170$). On d 7, α -tocopherol in milk was higher only for V2 ($P=0.024$) compared to C and V3 (7.433, 5.033, and 6.66 $\mu\text{g}/\text{ml}$, respectively). On d 17, α -tocopherol concentrations in milk were higher in V3 ($P=0.018$) compared with V2, but were not different ($P=0.474$) from C (5.433, 3.617, and 4.933 $\mu\text{g}/\text{ml}$ respectively; Table 7).

Discussion

The aim of this project was to determine the impact of supplemental vitamin A, E, and D injected in sows on performance of their offspring. The timing of the combined injection of these 3 vitamins 7 d prior to farrowing was to prepare sows before they farrow so that they can pass the vitamins to the piglets via colostrum and milk. The injection on d 7 was to continue elevated concentrations of the vitamins in milk for transfer to their piglets for storage in the body. The injection on d 17 of lactation was to supply supplemental vitamins to piglets immediately before weaning. At the end of the nursery, pigs from sows that received the three vitamin injections (V3) were heavier compared to pigs from sows receiving control and two injections (V2), but it is not known if the improvement in BW was related to a particular single vitamin or to the combination of all three vitamins. Results in this experiment showed that when serum and milk samples were analyzed, the vitamin that

appeared to be impacted the most at any time point of collection was 25(OH)D₃. Circulating 25(OH)D₃ levels are directly indicative of vitamin D₃ intake (Flohr et al., 2014; Flohr et al., 2016). Also, because in piglets, 25(OH)D₃ was the vitamin that seemed to be most responsive following the injection of vitamins, it may be speculated that the growth performance observed could be mostly related to vitamin D₃ rather than the other two vitamins. It should be considered; however, that 25(OH)D₃ represents the circulating form of vitamin D₃, whereas 1,25-(OH)₂-D₃ is the active form that is directly involved in calcium regulation and bone growth. Although injecting sows 3 times with vitamins A, E, and D, increased BW at birth and at the end of the nursery compared with only 2 injections and control sows, treatment differences were not significant for ADG, ADFI, and G:F when considering pigs housed in pens from weaning until the end of the nursery.

Vitamin A. Some of the functions of vitamin A in pigs are related to growth, immune function and differentiation of the epithelial structure and bone (Olson et al., 1981; Zile and Cullum, 1983; Wolf, 1984). It was reported by Coffey and Britt (1993) that when sows were injected with 200 mg of beta-carotene and 50,000 IU of vitamin A at weaning and d 7 after mating, it increased total litter weight; however, individual pig BW was not affected. Similarly, Pusateri et al. (1998) showed in a study with 1,375 sows that injection with 106 IU of vitamin A vs control injections with oil at weaning, d 0, 2, 6, 10, 13, 19, 30, 70, and 110 after breeding that there were no significant differences due to injection with vitamin A. Brief and Chew (1985) showed an increase of litter weight at birth and weaning when gilts were injected with 12,300 IU of vitamin A and 32.6 mg beta-carotene compared

to gilts fed 0, 2,100, 12,300 IU of vitamin A and 0, 32.6, 65.2 mg of beta-carotene. In that experiment, gilts had been fed a diet free of vitamin A and β -carotene for 5 weeks before being assigned to treatments. In the present study, serum retinol concentrations in sows and piglets were not significantly different among treatments at any collection point. In contrast, Heying et al. (2013) conducted a study with sows fed either a high provitamin A orange maize diet or white maize diet supplemented with a 1.05-mmol retinyl palmitate oral dose at the beginning of gestation throughout pregnancy and lactation and reported that pigs whose dams were fed the white maize diet supplemented with an oral dose of retinyl palmitate had greater serum retinol concentration on d 10 to d 28 compared to the orange maize diet. However, in milk collected on d 0, 5, 10, 20 and 28, retinol concentration was higher in sows fed with orange maize compared to white maize. Similarity, Valentine and Tanumihardjo (2005) reported that when sows were injected with 200,000, or 400,000 IU of ?? on d 2 to d 4 after farrowing, there were no differences in pig serum retinol between maternal treatment groups at either time, which agrees with the current study. Moreover, in the present study, it was observed that on d -7 the concentrations of retinol were approximately 0.25 $\mu\text{g/ml}$ and increased to 0.37 $\mu\text{g/ml}$ on d 7 and remained constant for the rest of lactation in sows. In piglets, the levels of retinol in serum were similar to sows, and there were no significant differences among treatment groups. In milk, retinol concentrations were constant over time in all treatments. However, milk concentrations of retinyl palmitate were greater when sows were given two or three vitamin injections during lactation when measured on d 1, 7, and 17. Concentrations of milk retinyl palmitate decreased as lactation progressed. The form of

vitamin A that was injected in sows was retinyl palmitate and data indicate that retinol palmitate was directly transferred to colostrum and milk as evidenced by higher concentrations of retinol palmitate, but not retinol.

Vitamin E. Piglets during the first days in the nursery have high levels of stress due to their separation from their mother, the transition from milk to solid food, being placed in a new environment, and being combined with other pigs. Vitamin E (as well as selenium) plays an important role in protecting pigs from stress. During oxidative stress, vitamin E acts as an efficient antioxidant by reducing free radicals in the cell membrane, protecting cells from being compromised by free radicals and other reactive substances (Halliwell, 1994; Debier, 2007). In the current study, pigs whose dams received three injections of vitamins A, E, and D were heavier than pigs from control sows or sows receiving two injections. Amazan et al. (2014) reported that sows receiving a natural form of vitamin E (RRR- α -tocopherol) weaned piglets at a higher body weight than sows fed α -tocopheryl acetate, but this difference was not maintained at d 42 of age. In a second experiment, Amazan et al. (2014) they reported no significant differences among treatments in pigs at d 28 and 42 when the synthetic or natural vitamin E was fed to sows during gestation and lactation. Wilburn et al. (2008) was not able to detect significant differences in ADG or ADFI when adding natural vitamin E to the diet or drinking water of weanling pigs for a 21 d period. However, there was an improvement in G:F ratio when vitamin E was added to the diet, but not to the water. In a second study, Wilburn et al. (2008) reported no differences in ADG, ADFI, or G:F when two different forms of vitamin E (synthetic v. natural) were added to the drinking water at

various levels for a d 21 period after weaning. In the present study, serum α -tocopherol in sows was similar on d -7 (7 d prior to farrowing) and d 1 of lactation (1.517 and 1.256 $\mu\text{g/ml}$, respectively), then increased to an average mean of 2.66 $\mu\text{g/ml}$ on d 7 of lactation, and then stayed constant at d 17 of lactation. Amazan et al. (2014) showed that serum alpha-tocopherol concentrations in sows decreased when sows were fed either a synthetic or a natural form (at 1/2 and 1/3 of the concentration of the synthetic form) of vitamin E d -7 pre-partum to d 28 postpartum. For milk, α -tocopherol concentrations in the current study started high in sows in V2 and V3 compared to control (12.433, 9.267, and 5.583 $\mu\text{g/ml}$, respectively). However, over time the concentrations in V3 and V2 started to decrease, and at d 17 of lactation the V3 and V2 plateaued to a similar concentration as the control, with a mean average of 4.661 $\mu\text{g/ml}$. Amazan et al. (2014) showed that α -tocopherol decreased over time in milk. Mahan et al. (2000) and Amazan et al. (2014) reported that colostrum α -tocopherol concentrations were higher than milk, which agrees with the present study. In the present study, serum α -tocopherol in pigs started high at birth then decreased over time in all treatments and no significant differences were observed among treatments. On d 0, the average mean was at 7.669 $\mu\text{g/ml}$, and at d 25 of age (4 days after weaning) the concentration dropped to 2.794 $\mu\text{g/ml}$. Amazan et al. (2014) reported that serum α -tocopherol concentrations in pigs decreased during the course of lactation. Likewise, Lauridsen et al. (2002) indicated that α -tocopherol concentration in pigs decreased after weaning because of low concentrations of α -tocopherol being passed through the milk from the sow to the piglet. The present research suggests that sows control the amount of vitamin E that is passed to

their piglets. Because vitamin E is stored in the liver, obtaining liver tissue samples may be a better indicator of how much vitamin is passing from dams to piglets during lactation.

Vitamin D. In most cases vitamin D is not needed in the diet if there is enough exposure to sunlight. However, because most pigs are raised in closed facilities, they need to be supplemented with vitamin D in the diet. In the current study, an improvement in BW at birth and at the end of the nursery was more pronounced in pigs whose dams received the three injections. Rortvedt and Crenshaw (2012) reported that nursery pig performance was reduced when pigs were fed with low concentrations of vitamin D and marginal Ca and P concentrations when sows were fed with no vitamin D₃. Witschi et al. (2011) indicated growth performance of pigs tended to be greater when sows were fed with 50 µg/kg compared to 5 µg/kg of vitamin D, regardless of whether the source of vitamin D was vitamin D₃ or 25(OH)D₃.

Piglets as many other newborn animals are born with a low concentration of vitamin D₃, which increases the risk of being deficient in vitamin D (Horst and Littledike, 1982). In the present study, sows in the control treatment had low and constant serum concentrations of 25(OH)D₃ for all time points (0.046 µg/mL). As soon as the first injection in V2 sows was given, serum concentrations of 25(OH)D₃ increased from 0.041 µg/mL at d -7 (before farrowing) to 0.133 µg/mL at d 7 and then stayed constant until d 17. For sows in V3, 25(OH)D₃ increased after the first injection and continued to increase after the second and third injections. Clearly, injecting a combination of vitamins A, E, and D increased serum vitamin D concentrations in sows. Similarly, serum concentrations of 25(OH)D₃ increased in

piglets whose dams were in V2 and V3 treatments. In a similar study, Goff et al. (1984) reported that when sows were injected with 5 million IU of cholecalciferol d 20 pre-partum, vitamin D in piglets increased. Also, Flohr et al. (2014) reported that when sows were injected with vitamin D₃, piglet serum 25(OH)D₃ concentrations increased due to vitamin D that was passed transplacentally and increased vitamin D concentrations in colostrum and milk. In a similar study, Witschi et al. (2011) showed that vitamin D status was improved in piglets when their dams received 25(OH)D₃. Flohr et al. (2014) reported that vitamin D₃ in milk increased at farrowing, on d 10 of lactation, and at weaning when feeding sows with high levels of vitamin D₃ during gestation. Overall, feeding sows with vitamin D₃ during gestation and lactation can increase vitamin levels in newborn pigs as shown in previous and the present research.

Vitamin A, D, and E Interactions and Effects on Performance. Aburto and Britton (1998) conducted a study to determine the interactive effects of dietary levels of vitamin A, D, and E on performance in broiler chickens. They conducted four experiments. In the first experiment, chicks were fed marginal dietary vitamin D₃ (500 IU/kg) and increasing dietary levels of vitamin A (5,000, 10,000, 20,000, 40,000, 80,000, and 160,000 IU/kg) and showed that vitamin A levels above 20,000 IU/kg of diet reduced body weight. In the second experiment, two levels of vitamin A (1,500 and 15,000 IU/kg) and six levels of vitamin E (10, 500, 1,000, 2,500, 5,000, and 10,000 IU/kg) were supplemented and a significant reduction in BW was reported when vitamin E was fed at high levels and this was more severe when vitamin A was fed at lower levels. In the third experiment, combinations of two

levels of vitamin A (1,500 and 15,000 IU/kg of vitamin A) and six levels of vitamin D₃ (500, 1,000, 1,500, 2,000, 2,500, and 3,000 IU/kg) were added to a basal diet that contained 10,000 IU/kg of vitamin E. Chickens fed 10,000 IU/kg of vitamin E and high levels of vitamin A had a greater BW at all levels of vitamin D in the diet and there was no interaction between vitamin A and D for BW. In the final experiment, three levels of vitamin A (1,500, 15,000, and 45,000 IU/kg), three levels of vitamin D₃ (500, 1,500, and 2,500 IU/kg), and three levels of vitamin E (10, 5,000, and 10,000 IU/kg) were added to the basal diet in a 3 x 3 factorial. Vitamin A and D₃ improved BW, but BW was reduced by feeding the high levels of vitamin E (5,000 or 10,000 IU/kg). Abawi and Sullivan (1989) evaluated potential interactive effects of vitamin A, D₃, E, and K in the diet of broiler chickens. Three levels of each vitamin representing deficient, optimum, and excessive were included for a total of 81 dietary treatments. Vitamin D had a significant quadratic effect on body weight gain and the lowest level of vitamin A resulted in the most efficient feed utilization. Interaction for A x D showed improves weight gain when high levels of vitamin A and E were fed. Therefore, it may be suggested that increasing vitamin D to 1,000 IU/kg increased BW gain in chickens. Ching et al. (2002) evaluated the relationship of vitamin E (source and level) and vitamin A in weaning pigs. Two levels of vitamin A (2,200 or 13,200 IU/kg), vitamin E (15 or 90 IU/kg), and two vitamin E sources (D- α -tocopheryl acetate or DL- α -tocopheryl acetate) were evaluated in a 35 d study, showing no differences in pig performance due to vitamin supplementation. In the second experiment, three levels of vitamin A (2,200, 13,200, or 26,400 IU/kg) and two sources of vitamin E (D- α -tocopheryl acetate or DL- α -tocopheryl

acetate) each provided at 40 IU/kg diet were evaluated in a 35 d study and no effects on pig performance were observed due to dietary treatments. Anderson et al. (1995) reported no improvements in performance when growing-finishing pigs were supplemented in the diet with 2,000 or 20,000 IU/kg of vitamin A and 0, 15, and 150 IU/kg of vitamin E in a 2 x 3 factorial arrangement. Copeland et al. (2017) studied the effects on nursery pig performance of adding vitamin E and D in drinking water. In the study, 150 pigs were assigned to two treatments in which pigs received vitamin E and D₃ through the water system to provide 120 IU vitamin E and 7,200 IU vitamin D per 3.79 L (1 gallon) drinking water vs. control pigs. The results showed no differences in ADG (0.46 vs 0.45 kg/d; $P = 0.52$), ADFI (0.62 vs 0.62 kg/d; $P = 0.98$), or G:F (0.75 vs 0.73; $P = 0.16$) in control vs. vitamin-treated pigs, respectively. In previous work, Becerra et al. (2015) evaluated the impact of a 5 ml i.m. injection of 300 I.U. of vitamin E, 200,000 I.U. of vitamin A, and 100,000 I.U. of Vitamin D₃ compared to control 7 days before farrowing. The authors reported that there were no differences in number of total pigs born, number of live births, number of pigs weaned, mean litter weight at birth or weaning, and plasma vitamin E concentrations. However, pigs born to sows injected with vitamins had greater weights at 21 and 42 days than pigs from saline (control) injected sows.

In conclusion, injecting sows with vitamin A, D, and E pre-farrowing and during lactation improved body weight of piglets and these differences may be related to improved vitamin status of sows and piglets. Future studies should be conducted to determine the impact of each of the vitamins separately on sow and pig performance. Overall, the weight

increase of 0.57 kg at weaning and 1.7 kg at d 35 in the nursery potentially translates to a decrease of total days on feed and a significant savings for producers marketing thousands of pigs per year.

Literature cited

- Abawi, F. G., and T. W. Sullivan. 1989. Interactions of vitamins A, D₃, E, and K in the diet of broiler chicks. *Poult. Sci.* 68:1490–8.
- Aburto, A., and W. M. Britton. 1998. Effects and interactions of dietary levels of vitamins A and E and cholecalciferol in broiler chickens. *Poult. Sci.* 77:666–673.
- Amazan, D., G. Cordero, C. J. López-Bote, C. Lauridsen, and a I. Rey. 2014. Effects of oral micellized natural vitamin E (D- α -tocopherol) v. synthetic vitamin E (DL- α -tocopherol) in feed on α -tocopherol levels, stereoisomer distribution, oxidative stress and the immune response in piglets. *Animal* 8:410–419.
- Anderson, L. E., R. Myer, and J. H. Brendemuhl. 1995. fed diets varying in dietary vitamin E . The Effect of Excessive Dietary Vitamin A on Performance and Vitamin E Status in Swine Fed Diets Varying in Dietary Vitamin E. *J. Anim. Sci.* 73:1093–1098.
- Babinszky, L., D. J. Langhout, M. W. A. Vestegen, L. A. den Hartog, P. Joling, and M. Nieuwland. 1990. Effect of vitamin E and fat source in sows' diets on immune response of suckling and weaned piglets. *J. Anim. Sci.* 69:1833–1842.
- Becerra, R., J. E. Link, K. C. Turner, J. T. Gebhardt, R. L. Stuart, and G. M. Hill. 2015. Are porcine epidemic diarrhea virus (PEDv) exposed gilts and sows farrowing problems improved by vitamin E? *J. Anim. Sci.* 93.
- Brief, S., and B. P. Chew. 1985. Effects of Vitamin A and β -Carotene on Reproductive Performance in Gilts. *J. Anim. Sci.* 60:998–1004.
- Ching, S., D. C. Mahan, T. G. Wiseman, and N. D. Fastinger. 2002. Evaluating the antioxidant status of weanling pigs fed dietary vitamins A and E. *J. Anim. Sci.* 80:2396–2401.
- Chung, Y. K., D. C. Mahan, and A. J. Lepine. 1992. Efficacy of Dietary D- α -Tocopherol and DL- α -Tocopheryl Acetate for Weanling Pigs. *J. Anim. Sci.* 70:2485–2492.
- Coffey, M. T., and J. H. Britt. 1993. Enhancement of Sow Reproductive Performance by β -Carotene or Vitamin A. *J. Anim. Sci.* 71:1198–1202.
- Copeland, K. R., D. B. Scales, G. M. Hill, J. E. Link, K. C. Turner, C. J. Rozeboom, and R. L. Stuart. 2017. Effects of vitamins E and D on performance and antioxidant enzymes in nursery pigs. *J. Anim. Sci.* 95.
- Debier, C. 2007. Vitamin E During Pre- and Postnatal Periods. *Vitam. Horm.* 76:357–

373.

DeLuca, H. F. 1967. Action and Metabolic Fate of vitamin D. In: *Vitamins Hormones*. p. 316–367.

DeLuca, H. F. 2008. Evolution of our understanding of vitamin D. *Nutr. Rev.* 66:73–87.

Dowling, J. E., and G. Wald. 1960. the Biological Function of Vitamin a Acid. *Proc. Natl. Acad. Sci. U. S. A.* 46:587–608.

Flohr, J. R., M. D. Tokach, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, J. L. Nelssen, and J. R. Bergstrom. 2014a. An evaluation of the effects of added vitamin D₃ in maternal diets on sow and pig performance. *J. Anim. Sci.* 92:594–603.

Flohr, J. R., M. D. Tokach, S. S. Dritz, J. M. DeRouchey, R. D. Goodband, J. L. Nelssen, and J. R. Bergstrom. 2014b. An evaluation of the effects of added vitamin D₃ in maternal diets on sow and pig performance. *J. Anim. Sci.* 92:594–603.

Flohr, J. R., J. C. Woodworth, J. R. Bergstrom, M. D. Tokach, S. S. Dritz, R. D. Goodband, and J. M. DeRouchey. 2016. Evaluating the Impact of Maternal Vitamin D Supplementation on Sow Performance , Serum Vitamin Metabolites , Neonatal Muscle and Bone Characteristics , and Subsequent Pre-weaning Pig Performance Evaluating the Impact of Maternal Vitamin D Supplementation. *J. Anim. Sci.* 1:4629–4642.

Halliwell, B. 1994. Free radicals and antioxidants: a personal view. *Nutr. Rev.* 52:253–65.

Hayek, M. G., G. E. Mitchell, R. J. Harmon, T. S. Stahly, G. L. Cromwell, R. E. Tucker, and K. B. Barker. 1989. Porcine immunoglobulin transfer after parturition treatment with selenium or vitamin E. *J. Anim. Sci.* 67:1299–1306.

Heying, E. K., M. Grahn, K. V Pixley, T. Rocheford, and S. a Tanumihardjo. 2013. High-provitamin A carotenoid (Orange) maize increases hepatic vitamin A reserves of offspring in a vitamin A-depleted sow-piglet model during lactation. *J. Nutr.* 143:1141–6.

Horst, R. L., and E. T. Littledike. 1982. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. *Comp Biochem Physiol B* 73:485–489.

Lauridsen, C., H. Engel, S. K. Jensen, A. M. Craig, and M. G. Traber. 2002. Lactating Sows and Suckling Piglets Preferentially Incorporate RRR- over All-rac- α -Tocopherol into Milk, Plasma and Tissues. *J. Nutr.* 132:1258–1264.

Mahan, D. C., Y. Y. Kim, and R. L. Stuart. 2000. Effect of vitamin E sources (RRR- or all-rac- α -tocopheryl acetate) and levels on sow reproductive performance, serum, tissue, and milk α -tocopherol contents over a five-parity period, and the effects on the progeny. *J. Anim. Sci.* 78:110–119.

NRC. 2012. Nutrient Requirements of Swine. 11 th ed. National Academies Press, Washington, DC.

Olson, J. A., W. Rojanapo, and A. J. Lamb. 1981. The effect of vitamin A status on the differentiation and function of goblet cells in the rat intestine. *Ann. N. Y. Acad. Sci.* 359:181–191.

Pusateri, A. E., M. A. Diekman, and W. L. Singleton. 1999. Failure of Vitamin A to Increase Litter Size in Sows Receiving Injections at Various Stages of Gestation. *J. Anim. Sci.* 77:1532–1535.

Rortvedt, L. A., and T. D. Crenshaw. 2012. Expression of kyphosis in young pigs is induced by a reduction of supplemental vitamin D in maternal diets and vitamin D, Ca, and P concentrations in nursery diets. *J. Anim. Sci.* 90:4905–4915.

Valentine, A. R., and S. A. Tanumihardjo. 2005. One-time vitamin A supplementation of lactating sows enhances hepatic retinol in their offspring independent of dose size. *Am. J. Clin. Nutr.* 81:427–433.

Wilburn, E. E., D. C. Mahan, D. A. Hill, T. E. Shipp, and H. Yang. 2008. An evaluation of natural (RRR- α -tocopheryl acetate) and synthetic (all-rac- α -tocopheryl acetate) vitamin e fortification in the diet or drinking water of weanling pigs. *J. Anim. Sci.* 86:584–591.

Witschi, A. K. M., A. Liesegang, S. Gebert, G. M. Weber, and C. Wenk. 2011. Effect of source and quantity of dietary vitamin D in maternal and creep diets on bone metabolism and growth in piglets. *J. Anim. Sci.* 89:1844–1852.

Wolf, G. 1984. Multiple functions of vitamin A. *Physiol. Rev.* 64:873–937.

Zile, M. H., and M. E. Cullum. 1983. The Function of Vitamin A: Current Concepts. *Proc. Soc. Exp. Biol. Med.* 152:139–152.

Table 3. Composition of the experimental diets as feed basis¹

Sows Diets			Nursery Diets		
Item	Lactation	Gestation	Item	Phase 1	Phase 2
Ingredients %			Ingredients %		
Corn, Yellow Dent	74.16	80.8	Corn, Yellow Dent	33.75	50.25
Soybean Meal, 48%	17.52	13.85	Soybean Meal, 48%	20	24
Poultry Fat	4	1	Whey Permeate	20	10
Monocalcium P, 21 %	2.1	2.05	Poultry Meal, 60% CP	6	3
Limestone, Ground	1.08	1.1125	Cookie Meal	10	5
L-Lysine HCl	0.16	0	Blood Plasma	7	3
L-Threonine	0.04	0	Poultry Fat	0	1
Vitamin Premix ²	0.03	0.0375	Dicalcium P 18.5%	0.4	0.9
Mineral Premix ³	0.15	0.15	Limestone, Ground	0.9	0.85
Salt	0.5	0.5	L-Lysine, 78%	0.45	0.5
Grd corn HA	0.25	0.5	DL- Methionine	0.2	0.2
			L-Threonine	0.13	0.15
			L-Tryptophan		
			Vitamin Premix ²	0.03	0.03
			Mineral Premix ³	0.15	0.15
			Salt	0.22	0.22
			Grd corn HA	0.52	0.5
			Zinc oxide, 72%	0.25	0.25

¹Diets were formulated based on NRC (2012) requirements.

²Supplied per kg of complete diet: 6,171 IU of vitamin A, 879.6 IU of vitamin D₃ as D-activated animal sterol, 35.25 IU of vitamin E, 0.02 mg of vitamin B₁₂, 4.4 mg of riboflavin, 26.5 mg of niacin, 17.6 mg of d-pantothenic acid as calcium pantothenate, 2.9 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 1.3 mg of folic acid, 0.18 mg of d-biotin.

³Supplied per kilogram of complete diet: 16.5 mg Cu as CuSO₄, 0.2970 mg I as ethylenediamine dihydriodide, 165.3 mg Fe as FeSO₄, 39.6 mg Mn as MnSO₄, 0.2976 mg Se as Na₂SeO₃, and 165.3 mg Zn as ZnO.

Table 4. Effects on litter performance and nursery performance by injecting sows with vitamins A, E, and D¹

Item	Maternal Treatments			SEM	P-V
	Control ⁴	2-injections ⁴	3-injections ⁴		
Total born alive	13.2	13.05	12.06	0.96	0.65
Stillborn	1.8	1.42	1.28	0.53	0.77
Total weaned	9.46	9.81	9.56	0.28	0.67
Birth BW (kg/pig) ²	1.39 ^{ba}	1.35 ^b	1.45 ^a	0.02	0.01
Weaning BW(kg/pig), d 21 ²	6.14 ^{ba}	6.02 ^b	6.38 ^a	0.12	0.09
ADG (birth to d 21) ²	0.34	0.33	0.35	0.01	0.24
Nursery (5 wks.)					
Number of pens	19	18	19		
Nursery BW (kg/pig), d 14 ²	8.76 ^b	8.83 ^b	9.33 ^a	0.18	0.06
ADFI, d 14 ³	0.24	0.24	0.25	0.01	0.57
ADG, d 14 ³	0.2	0.21	0.21	0.01	0.76
G:F, d 14 ³	0.81	0.87	0.89	0.06	0.65
Nursery BW (kg/pig), d 35 ²	18.03 ^b	18.31 ^b	19.73 ^a	0.35	<0.05
ADFI, d 35 ³	0.77	0.78	0.81	0.03	0.61
ADG, d 35 ³	0.41	0.43	0.46	0.03	0.41
G:F, d 35 ³	0.54	0.54	0.55	0.03	0.90
Weaning to end of nursery					
Total ADFI ³	0.55	0.56	0.58	0.02	0.51
Total ADG ³	0.32	0.34	0.36	0.02	0.40
Total G:F ³	0.58	0.59	0.6	0.03	0.81
Mortality, d 14	7.36	5.04	4.94	2.84	0.79
Mortality, d 35	3.01	2.73	3.42	1.67	0.96
Total mortality	10.43	7.61	7.31	3.18	0.74

¹A total of 514 pigs from 54 litters in two farrowing groups were used from birth to d 35 in the nursery.

²For performance at birth, weaning, weaning ADG, and d 35 in the nursery, individual pig BW was the experimental unit.

³For ADFI, ADG, and G:F performance, pen was the experimental unit.

BW, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical tendency

^{a, b} Values within birth, weaning, nursery, d 14, and nursery, d 35 with different superscripts are different.

⁴Vitamin injection: 5 ml i.m. to sows and gilts (Vital E – Repro), Stuart Products, Bedford, TX) or saline (control). The Product contained 300 IU vitamin E (as d- α -tocopherol), 200,000 IU vitamin A (as retinyl-palmitate) and 100,000 IU vitamin D₃ compounded with 18% ethyl alcohol and 1% benzyl alcohol in an emulsifiable base.

Table 5. Serum vitamin concentrations in sows¹

Item	Maternal Treatments			SEM	P-value
	Control	2-injection	3-injections		
Retinol µg/ml					
d -7	0.251	0.253	0.247	0.014	0.939
d 1	0.212 ^b	0.263 ^{ba}	0.290 ^a	0.024	0.090
d 7	0.370	0.378	0.367	0.031	0.965
d 17	0.340	0.357	0.402	0.030	0.353
α-tocopherol µg/ml					
d -7	1.500	1.433	1.617	0.167	0.738
d 1	1.100	1.267	1.400	0.244	0.691
d 7	2.200	2.967	2.733	0.298	0.210
d 17	2.300	2.067	2.917	0.294	0.143
25(OH)D ₃ µg/ml					
d -7	0.046	0.041	0.048	0.002	0.170
d 1	0.043 ^b	0.086 ^a	0.094 ^a	0.008	0.0006
d 7	0.047 ^b	0.133 ^a	0.133 ^a	0.011	<0.0001
d 17	0.047 ^c	0.125 ^b	0.193 ^a	0.012	<0.0001

¹Serum vitamin concentrations in sows (n=6 per treatment) collected before vitamin injection (107 d of gestation), 1-24 hrs. after parturition, 7 d after parturition, and 17 d in lactation.

^{a, b} Values within retinol, α-tocopherol, and 25(OH)D₃ with different superscripts are different.

Serum, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical.

Table 6. Serum vitamin concentrations in pigs¹

Item	Maternal Treatments			SEM	P-value
	Control	2-injection	3-injections		
Retinol µg/ml					
d 3	0.279	0.267	0.276	0.015	0.852
d 7	0.298	0.341	0.335	0.018	0.191
d 17	0.306	0.303	0.321	0.017	0.714
d 25	0.374	0.331	0.365	0.018	0.226
α-tocopherol µg/ml					
d 3	7.600	7.758	7.650	0.622	0.983
d 7	6.575	7.800	7.500	0.562	0.289
d 17	4.542	4.992	5.325	0.597	0.652
d 25	1.600	2.825	2.158	0.436	0.154
25(OH)D ₃ µg/ml					
d 3	0.006 ^b	0.013 ^a	0.015 ^a	0.001	<0.001
d 7	0.007 ^b	0.027 ^a	0.034 ^a	0.003	<0.001
d 17	0.009 ^c	0.020 ^b	0.032 ^a	0.002	<0.001
d 25	0.013 ^b	0.015 ^b	0.025 ^a	0.002	0.003

¹Serum vitamin concentrations in pigs (n=12 per treatment) collected at 3 d of age, 7 d, 17 d of age and 25 d of age.

^{a, b} Values within retinol, α-tocopherol, and 25(OH)D₃ with different superscripts are different.

Serum, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical.

Table 7. Vitamin concentrations in milk¹

Item	Maternal Treatments			SEM	P-value
	Control	2-injection	3-injections		
Retinol µg/ml					
d 1	0.117	0.103	0.100	0.015	0.722
d 7	0.103	0.110	0.115	0.008	0.612
d 17	0.122	0.107	0.118	0.007	0.400
Retinyl palmitate µg/ml					
d 1	0.967 ^b	3.150 ^a	2.566 ^a	0.515	0.024
d 7	0.433 ^b	1.500 ^b	1.250 ^a	0.152	<0.001
d 17	0.300 ^b	0.366 ^b	1.050 ^a	0.067	<0.001
α-tocopherol µg/ml					
d 1	5.583 ^b	12.433 ^a	9.267 ^{ab}	1.805	0.053
d 7	5.033 ^b	7.433 ^a	6.667 ^{ab}	0.676	0.064
d 17	4.933 ^{ab}	3.617 ^b	5.433 ^a	0.481	0.046

¹Milk vitamin concentrations in sows (n=6 per treatment) collected before vitamin injection (107 d of gestation), 1-24 hrs. after parturition, 7 d after parturition, and 17 d in lactation.

^{a, b} Values within retinol, retinyl palmitate, and α-tocopherol with different superscripts are different.

Milk, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical.

Figure 9. Effects on pig BW from birth to 35 d of age by injecting sows with vitamin A, E and D.

^{a, b} bars within birth, d 21, d 35, and d 56 with different superscripts are different.

Figure 10. Serum α -tocopherol concentrations in sows (n=6 per treatment) collected before vitamin injection (107 d of gestation), 1-24 hrs. after parturition, 7 d after parturition, and 17 d in lactation. Symbols represent least square means $\pm$ SEM.

Figure 11. Serum α -tocopherol concentrations in pigs (n=12 per treatment) collected at 3 d, 7 d, 17 d, and 25 d of age. Symbols represent least square means $\pm$ SEM.

Figure 12. Serum retinol concentrations in sows (n=6 per treatment) collected before vitamin injection (107 d of gestation), 1-24 hrs. after parturition, 7 d after parturition, and 17 d in lactation. Symbols represent least square means $\pm$ SEM.

Figure 13. Serum retinol concentrations in pigs (n=12 per treatment) collected at 3 d, 7 d, 17 d, and 25 d of age. Symbols represent least square means $\pm$ SEM.

Figure 14. Serum 25(OH)D₃ concentrations in sows (n=6 per treatment) collected before vitamin injection (107 d of gestation), 1-24 hrs. after parturition, 7 d after parturition, and 17 d in lactation. Symbols represent least square means $\pm$ SEM.

Figure 15. Serum 25(OH)D₃ concentrations in pigs (n=12 per treatment) collected at 3 d, 7d, 17 d, and 25 d of age. Symbols represent least square means $\pm$ SEM.

Figure 16. Colostrum and milk retinol concentrations in sows (n=6 per treatment) collected at 1 d, 7 d, and 17 d in lactation. Symbols represent least square means $\pm$ SEM.

Figure 17. Colostrum and milk retinyl palmitate concentrations in sows (n=6 per treatment) collected at 1 d, 7d, and 17 d in lactation. Symbols represent least square means $\pm$ SEM.

Figure 18. Colostrum and milk α -tocopherol concentrations in sows (n=6 per treatment) collected at 1 d, 7d, and 17 d in lactation. Symbols represent least square means $\pm$ SEM

Figure 19. Serum 25(OH)D₃ concentrations in pigs (n=12 per treatment) collected on d 0 (prior to nursing).

CHAPTER III:
Improving Sow Productivity and Piglet Performance by Injecting Sows with
Retinyl palmitate, α -Tocopherol and Cholecalciferol

ABSTRACT

To determine the effectiveness of injecting sows with vitamin A, E, and D before farrowing and during lactation to increase litter size and improve pig performance during lactation and nursery, 337 sows were assigned to 3 treatments. Sows were allotted by parity to 3 treatments: 1) control (C; no injection; 2) 2 injections (V2) of 5 mL i.m. of a multi-vitamin product containing 200,000, 300, and 100,000 I.U./mL of vitamin A, E, and D, respectively on d 107 of gestation and d 4 of lactation; and 3) same as V2 plus a third 5 mL i.m. injection of vitamins on d 14 of lactation (V3). The doses used for this project and the days chosen to inject sows were based on previous research. Results showed that gestation length was longer in V3 compared to V2, but not compared to C (115.66, 115.28, and 115.46 d, respectively, $P=0.05$). Total pigs born alive, stillborn pigs, and mummies were not different among treatments ($P>0.52$). Litter weight at d 3 or BW per pig was not significantly different among treatments ($P>0.61$). Total number of pigs on d 3 and at weaning was not different among treatments ($P>0.15$). Litter weaning weight was greater in V2 compared to V3, but not different from C ($P=0.05$; 68.45, 64.13, and 67.19 kg, respectively). ADG from d 3 to weaning was greater in V2 and C compared to V3 (0.25, 0.25 and 0.23 kg, respectively, $P=0.04$). After weaning, all weaned pigs were blocked by size and placed within treatment groups in pens of 24 mixed sex pigs, resulting in 50 pens for the control treatment, 49 pens for two injections treatment and 46 pens for the three injections treatment. At 7 d in the nursery, BW per pig in the nursery was greater for C and V2 compared to V3 ($P=0.03$), but no differences were observed between C and V2. Nevertheless, weight per pen at d 7 was not

significant among treatments ($P=0.28$). At the end of the nursery (d 37), weight/pen, average BW per pig, ADG, and mortality was not significantly different among groups ($P>0.19$). In conclusion, injecting fat-soluble vitamins to sows did not improve sow productively during lactation nor piglet performance throughout the nursery. In fact, pigs whose mother had not received any injections performed better at weaning and d 7 in the nursery; however, at the end of the nursery body weight as pigs were not different.

Introduction

Vitamin supplementation is required to maintain health in animals and humans. Failure to provide adequate vitamin in animals or over-dose vitamins in animals can cause health problems, which sometimes can be irreversible. There are two classes of vitamins, water soluble and fat soluble. Water soluble vitamins are involved in many catalytic functions in the body, such as the one carbon metabolism and citric acid cycle. In contrast, fat soluble vitamins do not provide energy to the body, but they have specific functions in the development and maintenance of tissue structures.

In swine production, the number of pigs born alive per sow is very important for swine producers. New technology and advances in reproduction and nutrition have improved reproductive performance. However, enhancements of diet and technology have not reduced the number of stillborn nor reduced farrowing problems. In fact, modern sows are farrowing larger litters than in the past, and at the same time this may have contributed to a higher number of stillborn pigs, greater pre-weaning mortality, and an increased number of

excessively light pigs. Interestingly, research done in sows using fat soluble vitamin, such as vitamin A, E, and D has shown that they can improve reproductive performance, such as reducing stillborn pigs and increasing number of pigs born alive.

Cunha (1997) showed that deficiencies in vitamin A in reproducing sows and gilts can lead to estrus failure, reabsorption of the fetuses, or increased numbers of piglets born dead. Lindemann et al. (2008) reported that an intramuscular injection of vitamin A at weaning and at breeding improved the subsequent number of pigs born alive, pigs weaned per litter, and decreased embryonic mortality. Coffey and Britt (1993) showed that injecting sows with β -carotene or vitamin A (palmitate) at the day of weaning, the day of mating, and d 7 post-mating, increased litter size. Whaley et al. (1997) and Whaley (1995) showed that vitamin A increased embryonic survival during early gestation; they suggested that the increase in litter size may be related to an increase in embryonic survival, but not due to increasing ovulation rate.

Research by Mahan (1994) showed that by feeding sows with higher levels of dl- α -tocopheryl acetate (22, 44, or 66 IU/kg) rather than the NRC (1988) recommendation increased the number of pigs born alive. Mahan (1991), in a study where sows were fed with different levels of dl- α -tocopheryl acetate (0.3 ppm Se and 0, 16, 33, or 66 IU/kg), concluded that in order to improve reproductive performance in sows, they need to be fed more than 16 IU/kg. Even though vitamin E can increase the number of pigs born alive, it is not well known the mechanism underlying this observation. The function of vitamin E is to reduce free radicals that destroy cell membranes, especially of very active cells; therefore,

the mechanism of how vitamin E helps in increasing the number of pigs born alive could be related to the fact that it protects muscle cells from free radicals when they are very active during farrowing or it can protect the cell membranes of embryos during the early stage of gestation.

Lauridsen et al. (2010) showed that the numbers of stillborn pigs were reduced when sows were fed during early gestation with vitamin D at higher doses (1,400 and 2,000 IU) compared to lower doses (200 and 800 IU). Coffey et al. (2012) reported that when first-service gilts were supplemented at the same level of 2,500 IU/kg of the complete diet with 25(OH)D₃ compared to vitamin D₃, a higher number of developed fetuses were found in the reproductive tracts of gilts fed with 25(OH)D₃. Viganò et al. (2003) reported that vitamin D potentially can be involved in implantation of embryos.

Although research has shown that vitamin A, E, and D can improve reproductive performance in sows individually, however, the effects of supplementation of a combination of these three vitamins to sows at higher levels than what the NRC (2012) recommends on performance of sows and pigs housed under commercial conditions has not been evaluated. Therefore, the purpose of this study was to determine the impact of vitamins A, E, and D injected in higher doses in sows and gilts and to observe productivity, livability of pigs from birth throughout the nursery, and the number of full value pigs produced per sow.

Materials and Methods

All procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). Animal use protocols were approved by North Carolina State University Institutional Animal Care and Use Committee.

Experimental Design. A total of 337 sows and gilts (average parity was 2) were assigned to 1 of 3 treatments, consisting of 1) control (no injection); 2) i.m. injection of vitamins on day 107 of gestation and the second injection on day 4 of lactation; and 3) i.m. injection of vitamins on day 107 of gestation, second injection on day 4 of lactation and the third injection on day 14 of lactation. Injections consisted of 5 mL of a commercially available multivitamin product containing 3 naturally derived fat-soluble vitamins (200,000 IU/mL of vitamin A (as retinyl-palmitate), 300 IU/mL of vitamin E (as d-alpha-tocopherol), and 10,000 IU/mL of vitamin D₃, in an emulsified matrix). Thus, injections provided a total of 1,000,000 IU of vitamin A, 1,500 IU of vitamin E, and 50,000 IU of vitamin D₃ on the day of administration. The timing of injections was selected specifically to provide vitamins to piglets via colostrum and milk at birth and during lactation. The post-farrowing injection prior to weaning was administered to improve vitamin status of pigs at weaning and to supply vitamin A to sows to improve fertility and subsequent litter size (Coffey and Britt, 1993).

General Procedures. The field study was conducted from September, 2016 to February, 2017 in a commercial farm with 1500 sows. Sows in gestation were fed according to their body condition score to maintain an ideal score of 3 on a 1 to 5 body condition

scoring scale. Sows were fed on ad libitum basis during the lactation phase. Gestation and lactation diets met or exceeded the minimum recommended nutrients suggested by the NRC (2012). Three days prior to parturition, sows were moved into individual crates (1.67 m wide by 2.33 m long) where they were fed 1.8 kg of lactation feed twice per day until farrowing. Sows had ad libitum access to water via two water nipples, one on top and one on the bottom of the farrowing crate. Pigs were processed on d 1 and castrated on d 3 according to the standard farm protocol. For the newborn pigs, each crate contained one heat lamp for supplemental heat and two rubber floor mats for the entire lactation period. Creep feed was not fed to piglets. During farrowing, as soon as a sow started to farrow and had the first piglet, 1 ml of oxytocin injection i.m. was given sows. Sows were constantly checked to make sure they did not have any farrowing problems (assisted sows) during the day only. If sows were having problems during farrowing, such as not able to push out a piglet or piglets were coming in the wrong position, the employee in charge of the farrowing sows were there to assist the sows immediately after see the sows having difficulties to farrow. Any sow that was sleeved during farrowing was marked as an assisted sow. After weaning, all weaned pigs (n=3,480) were blocked by size and placed within treatment groups in pens of 24 mixed sex pigs, resulting in 50 pens for the control treatment, 49 the two injection treatment and 46 pens for the three injection treatment. Three groups of weaning pigs were placed in two different farms (farm 1 and farm 2) with 15 days difference between each group. The first group of pigs was placed in farm 1, resulting in 11 pens for the control treatment, 10 the two injection treatment and 9 pens for the three injection treatment. In farm 2 the next two groups were

placed. In the nursery, three-phase diets were fed to pigs. The first diet was fed for the first week, the second diet for 2 weeks in the nursery and the third diet for 3 weeks. All diets were formulated to meet the NRC (2012) nutrient requirements. Each pen contained two feeders and 2 water nipples that allowed pigs to eat and drink on an at libitum basis. All diets were the same for all treatments.

Measurements. Number of pigs born alive, mummies, stillborn pigs, and assisted sows data during farrowing was collected for all treatments. Litter weight for each litter was recorded on d 3 and at weaning (20 ± 2 d of age). Piglets were cross-fostered if needed only within treatments. Growth performance data for all weaned pigs was collected until the end of the nursery (55 ± 2 d of age), including BW, mortality, and morbidity.

Table 8. Injections, pig BW collection, and timeline-field study

Item	Pre-Farrowing 1...107...114	Days on Lactation 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21																					Nursery 1 2 3 4 5 6 7....37							
<u>Injections</u>																														
Control ¹																														
2-injections ²	↑																													
3-injections ²	↑																													
Pig BW																														

¹Control sows did not receive any injection.

²Vitamin injection: 5 ml i.m. to sows and gilts (Vital E – Repro), Stuart Products, Bedford, TX). The product contained 300 IU vitamin E (as d- α -tocopherol), 200,000 IU vitamin A (as retinyl-palmitate) and 100,000 IU vitamin D₃ compounded with 18% ethyl alcohol and 1% benzyl alcohol in an emulsifiable base.

Statistical Analyses

All data were analyzed by ANOVA using Proc GLM (SAS, Cary, NC). The model for sow and growth performance data included sow group, parity, and treatment. For statistical differences a $P \leq 0.05$ was used to declare significance and for statistical tendencies, $0.05 < P \leq 0.10$ was used. Fisher's LSD method was used in ANOVA to compare treatment means

Results

Litter performance. Results showed that gestation length was slightly longer in V3 compared to V2, but not compared to C (115.66, 115.28, and 115.46 d, respectively, $P=0.05$). Total number of pigs born alive (mean was 12.53; $P=0.89$), number of stillborn pigs (mean of 0.43), and mummies (mean of 0.46) were not different among treatments ($P>0.52$). Interestingly, sows in V3 and V2 received less help during farrowing due to farrowing problems than C ($P=0.04$). Litter weight at d 3 was not significantly different among treatments ($P>0.61$; 21.17, 21.23, and 20.79 kg for C V2 and V3 respectively). There were no significant differences in mean pig BW at d 3 among groups ($P=0.61$). Total number of pigs at d 3 and at weaning was not significantly different among treatments ($P>0.15$). However, the number of sows assisted during farrowing was lower in sows injected with vitamins compared to control sows (data not shown). Litter weaning weight was greater in V2 compared to V3, but not different from C ($P=0.05$; 68.45, 64.13, and 67.19 kg, respectively). ADG from d 3 to weaning was greater in V2 and C compared to V3 (0.25, 0.25

and 0.23 kg, respectively, $P=0.04$), but there were no differences between C and V2 (Table 9).

Nursery Pig Performance. At 7 d in the nursery, weight per pen was greater for C and V2 compared to V3 ($P=0.03$; 161.84, 151.19, and 154.66, respectively), but no differences were observed between C and V2. Nevertheless, average pig BW at d 7 was not different among treatments ($P=0.28$). In addition, at the end of the nursery (d 37), weight per pen was not different among treatments ($P=0.29$, 161.84, 151.19, 154.66 for C, V2, and V3, respectively). Also, average pig BW, ADG, and mortality were not significantly different among groups ($P>0.19$) (Table 9).

Discussion

In the present study, injecting sows before farrowing and during lactation did not improve reproductive performance in sows. By the same token vitamin injections did not improve performance in pigs, which contradict with the first experiment presented in this thesis where pigs whose mother received three injections were heavier at the end of the nursery compared to control and two injections.

Vitamin A. Vitamin A is important for swine reproduction and health protection. Failure to supplement vitamin A in swine diets can result in failure of estrous, reabsorption of fetuses and stillbirths (Brief and Chew, 1985; McDowell, 2000). In the current study, injecting sows with the three vitamins did not increase litter size. Brief and Chew (1985) examined the reproductive performance of gilts by feeding or injecting vitamin A and β -

carotene and with a diet depleted of vitamin A. For 5 weeks these authors fed gilts 12 hours apart after breeding with a deficient diet in vitamin A, and then subsequently gave weekly injections of β -carotene (32.6 mg) or 12,300 IU of vitamin A. Results showed that the injections decreased embryonic mortality, so more pigs were born alive and more pigs were weaned. Pusateri et al. (1998) showed that a single injection with 1×10^6 IU of vitamin A dissolved in corn oil at weaning or on d 0, 2, 6, 10, 13, 19, 30, 70, or 110 after breeding did not improve reproductive performance in sows. This agrees with the present study; however, in the present study the injections were done at different times compared to Pusateri et al. (1998) and sows received more than one injection.

Vitamin E. Vitamin E requirements for reproductive swine have been increased in the last 3 decades from 10 to 44 IU/kg diet (NRC, 1973, 1979, 1988, 1998);(Mahan et al., 2000). In the present study the levels of vitamin E injected in sows were above the recommendation levels by the NRC (2012). During the present study there were no differences in pigs born alive, stillborn pigs and mummies. Mahan (1994) evaluated the effects of feeding sows with vitamin E in a study with 360 farrowing sows over a five-parity period. In that study, sows were fed during gestation and lactation with three dietary levels of dl- α -tocopherol acetate (22, 44, or 66 UI/kg of diet). The results showed that litter weight and weaning weights were not significantly different from dietary vitamin E levels, which agrees with the present study. Nevertheless, there was an increase in number of pigs (total, $P < 0.01$; live, $P < 0.10$) when the dietary levels of vitamin were increased, which does not agree with the present study. However, Mahan (1994) used a different approach than the present study, because they fed

sows with high amounts of vitamin E during the entire period of lactation, while in the present study sows received only one injection before farrowing. Amazan et al. (2014) conducted two research studies in which they gave only vitamin E in the water. The first experiment consisted of three treatments, 12 sows per treatment received 74 mg/day of a synthetic form of vitamin E (α -tocopheryl acetate) on the feed during lactation and 150 mg/day during lactation (28 d). In the other two treatments, sows received a natural form of vitamin E (RRR- α -tocopherol) 1/3 or 1/2 lower levels compared to the synthetic concentrations according to the feeding period, but in the water. The results showed that piglet BW at weaning was significantly increased ($P=0.036$) when sows were fed with the vitamin E natural form. In the current study, even though pigs whose mother received three injections with a natural vitamin E form, they had lower body weight at weaning compared to control. However, in the Amazan et al. (2014) study, differences in BW at d 42 of age and ADFI were not statistically significant, which the results are similar as in the current study where at d 7 and d 37 on nursery pig BW was not significantly different among treatments. In a second experiment, Amazan et al. (2014) used the similar approach, but starting 30 d of gestation and finish at d 42 in lactation. In these studies, more vitamin E was fed because the feeding period of vitamin E was longer compared to experiment 1, but there were not significant differences among treatments in pigs at d 28 and 42 when the synthetic or natural vitamin E was fed to sows during lactation and gestation. Wilburn et al. (2008) were not able to detect significant differences in ADG or ADFI when adding natural vitamin E to the diet or drinking water of weanling pigs for a 21 d period. However, there was an improvement in

G:F ratio when vitamin E was added to the diet, but not to the water. In a second study, Wilburn et al. (2008) reported no differences in ADG, ADFI, or G:F when two different forms of vitamin E (synthetic v. natural) were added to the drinking water at various levels for a d 21 period after weaning.

Vitamin D. According to the NRC (2012), there is a need for research in vitamin D requirements of sows during gestation or lactation. Research has shown that postnatal growth was positively was related to total muscle fibers at birth (Dwyer et al., 1994; Dwyer et al., 1993). Endo et al. (2003) showed that knockout mice had smaller muscle fibers at 3 weeks of age when the vitamin D receptor was not present compared to wild-type. In addition, Hines et al. (2013) reported that there were alterations in fetal muscle characteristics in fetuses from bred gilts fed with 25(OH)D₃ compared to fetuses from bred gilts that were fed D₃. However, it is not known if alteration of fetal muscle can reduce embryo mortality. Lauridsen et al. (2010b) showed that reproductive performance was not influenced by feeding gilts 4 concentrations of 1 of 2 different vitamin D sources (200, 800, 1,400, and 2,000 IU/kg of D₃ or corresponding doses of 5, 20, 35, and 50 µg/kg of feed from 25(OH)D₃) to a total of 160 gilts from the first estrus until d 28 of gestation. At the same time in another experiment, the same 8 dietary treatments were provided to 160 multiparous sows from the first day of mating until weaning. In that experiment, the number of stillborn pigs was reduced ($P=0.03$) with the larger doses of D₃ (1,400 and 2,000 IU) compared to low doses of vitamin D₃ (200 and 800 IU).

Vitamin A, E, and D Interactions and Effects on Performance. In a previous experiment at Michigan State University, Becerra et al. (2015) showed no effects on the number of pigs born alive, stillborn pigs, and litter weight at birth when sows were injected with 5 mL of vitamin A, E, and D 7 days before farrowing compared to control sows. The vitamin levels were the same as the present study. However, in a subsample of pigs, body weight at weaning and at 42 d of age was greater in pigs from sows injected with vitamins compared to control sows. Jang et al. (2014) was unable to detect differences in BW and ADG of pigs during suckling periods between control and vitamin treatments containing 500 IU of vitamin E as d- α -tocopherol, 50,000 IU of vitamin A as retinyl palmitate, and 50,000 IU of vitamin D₃ per mL) given as an injection of 0.8 mL or the same doses given orally.

In conclusion, in the present study, injection of sows with vitamins A, E, and D did not improve sow reproductive performance or pig performance during lactation and the nursery which contradict the results from experiment I presented in Chapter 2, where the pigs whose mother received the three injections were heavier at weaning and at the end of the nursery compared to control and two injections. Also, further research is necessary to examine the performance in pigs from birth to market to determine if the differences in weights of pigs from vitamin injected sows will carry through to market weight or to determine vitamin injection may have long-term positive consequences after moving the pigs to the finishing phase. In addition, in the high level of sow productivity (compared to experiment 1) and the intensive sow management in the commercial farm may have minimized the opportunity for a positive response of injectable vitamins in the present,

commercial sow study. Therefore, further research to explore the impact of injectable vitamins in commercial farms with lower productivity (due to disease challenge or compromised production levels) may be warranted.

Literature cited

Amazan, D., G. Cordero, C. J. López-Bote, C. Lauridsen, and a I. Rey. 2014. Effects of oral micellized natural vitamin E (D- α -tocopherol) v. synthetic vitamin E (DL- α -tocopherol) in feed on α -tocopherol levels, stereoisomer distribution, oxidative stress and the immune response in piglets. *Animal* 8:410–419.

Becerra, R., J. E. Link, K. C. Turner, J. T. Gebhardt, R. L. Stuart, and G. M. Hill. 2015. Are porcine epidemic diarrhea virus (PEDv) exposed gilts and sows farrowing problems improved by vitamin E? *J. Anim. Sci.* 93.

Brief, S., and B. P. Chew. 1985. Effects of Vitamin A and β -Carotene on Reproductive Performance in Gilts. *J. Anim. Sci.* 60:998–1004.

Coffey, J. D., E. A. Hines, J. D. Starkey, C. W. Starkey, and T. K. Chung. 2012. Feeding 25-hydroxycholecalciferol improves gilt reproductive performance and fetal vitamin D status. *J. Anim. Sci.* 90:3783–3788.

Coffey, M. T., and J. H. Britt. 1993. Enhancement of Sow Reproductive Performance by β -Carotene or Vitamin A. *J. Anim. Sci.* 71:1198–1202.

Cunha, T. J. 1997. *Swine Feeding and Nutrition*. Academic Press, Inc., New York, USA.

Jang, Y. D., M. D. Lindemann, H. J. Monegue, and R. L. Stuart. 2014. The Effects of Fat-soluble Vitamin Administration on Plasma Vitamin Status of Nursing Pigs Differ When Provided by Oral Administration or Injection. *Asian-Australasian J. Anim. Sci.* 27:674–682.

Lauridsen, C., U. Halekoh, T. Larsen, and S. K. Jensen. 2010. Reproductive performance and bone status markers of gilts and lactating sows supplemented with two different forms of vitamin D. *J. Anim. Sci.* 88:202–213.

Lindemann, M. D., J. H. Brendemuhl, L. I. Chiba, C. S. Darroch, C. R. Dove, M. J. Estienne, and A. F. Harper. 2008. A regional evaluation of injections of high levels of vitamin a on reproductive performance of sows. *J. Anim. Sci.* 86:333–338.

Mahan, D. C. 1991. Assessment of the Influence of Dietary Vitamin-e on Sows and Offspring in 3 Parties - Reproductive-Performance, Tissue Tocopherol, and Effects on Progeny. *J. Anim. Sci.* 69:2904–2917.

Mahan, D. C. 1994. Effect of Dietary Vitamin E on Sow Reproductive Performance over a Five-Parity Period. *Anim. Sci.*:2870–2879.

Mahan, D. C., Y. Y. Kim, and R. L. Stuart. 2000. Effect of vitamin E sources (RRR-

or all-rac- α -tocopheryl acetate) and levels on sow reproductive performance, serum, tissue, and milk α -tocopherol contents over a five-parity period, and the effects on the progeny. *J. Anim. Sci.* 78:110–119.

McDowell, L. 2000. *Vitamins in Animal and Human Nutrition*. Second Edi. Iowa State University Press, Ames, IA.

NRC. 1988. *Nutrient Requirements of Swine*. National Academies Press, Washington, DC.

NRC. 2012. *Nutrient Requirements of Swine*. 11 th ed. National Academies Press, Washington, DC.

Pusateri, A. E., M. A. Diekman, and W. L. Singleton. 1999. Failure of Vitamin A to Increase Litter Size in Sows Receiving Injections at Various Stages of Gestation. *J. Anim. Sci.* 77:1532–1535.

Viganò, P., S. Mangioni, F. Pompei, and I. Chiodo. 2003. Maternal-conceptus Cross Talk—A Review. *Placenta* 24:56–61.

Whaley, S. L. 1995. Effects of Intramuscular Injection of Vitamin A on Increased Litter Size in Swine.

Wilburn, E. E., D. C. Mahan, D. A. Hill, T. E. Shipp, and H. Yang. 2008. An evaluation of natural (RRR- α -tocopheryl acetate) and synthetic (all-rac- α -tocopheryl acetate) vitamin e fortification in the diet or drinking water of weanling pigs. *J. Anim. Sci.* 86:584–591.

Table 9. Effects on litter performance and nursery performance by injecting sows with vitamins A, E, and D- field study¹

Item	Maternal Treatments			SEM	P-V
	Control	2-injections ²	2-injections ²		
Sows, n	115	113	109		
Average parity	2.27	2.34	2.34	0.17	0.95
Gestation length	115.46 ^{ab}	115.28 ^b	115.66 ^a	0.11	0.05
Total pigs born alive	12.58	12.43	12.56	0.25	0.89
Stillborn pigs	0.45	0.40	0.43	0.07	0.86
Mummies	0.45	0.53	0.40	0.08	0.52
Assisted Sows	0.19 ^a	0.09 ^{bc}	0.10 ^{bc}	0.31	0.04
Litter Birth W 3 d	21.17	21.23	20.79	0.38	0.68
Total pigs 3 d	12.01	12.09	12.10	0.09	0.76
Avg. individual BW, d 3	1.77	1.76	1.73	0.03	0.61
Litter Weaning Weight	67.19 ^{ab}	68.45 ^a	64.13 ^b	1.28	0.05
Total weaned	11.31	11.55	11.56	0.10	0.15
Avg. individual Weaning Weight	5.92 ^a	5.92 ^a	5.54 ^b	0.10	0.007
ADG (d 3 to d 21)	0.25 ^a	0.25 ^a	0.23 ^b	0.01	0.04
Nursery pig Performance					
Number of pens	50	49	46		
Avg. number of pigs, d 28	25	24	25	0.77	0.61
Nursery Weight/pen d 28	161.84	151.19	154.66	4.88	0.28
Avg. individual BW, d 28	6.49 ^a	6.31 ^{ab}	6.20 ^b	0.08	0.03
Nursery Weight/pen, d 58	486.55	451.38	465.17	16.05	0.29
Avg. individual BW, d 58	20.14	19.41	19.34	0.34	0.19
ADG, d 7 to d 37	0.36	0.35	0.35	0.01	0.31
Avg. Number of pigs, d 58	24	23	24	0.70	0.64
Total mortality	0.84	0.80	1.02	0.20	0.70

¹A total of 336 litters were used from birth to d 35 in the nursery.

BW, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical tendency

^{a, b} Values within birth, weaning, nursery, d 14, and nursery, d 35 with different superscripts are different.

²Vitamin injection: 5 ml i.m. to sows and gilts (Vital E – Repro), Stuart Products, Bedford, TX). The Product contained 300 IU vitamin E (as d- α -tocopherol), 200,000 IU vitamin A (as retinyl-palmitate) and 100,000 IU vitamin D₃ compounded with 18% ethyl alcohol and 1% benzyl alcohol in an emulsifiable base.

Table 10. Group by parity by treatment and group by parity effects on litter performance and nursery performance by injecting sows with vitamins A, E, and D-field study¹

Item	Maternal Diets						Grp*Pry*Trt		Grp*Pry
	Mature Sows (parity 3+)			Young Sows (parity 1 & 2)			SEM	P-V	P-V
	Control ⁴	Two injections	Three injections	Control	Two injections	Three injections			
Sows, n	45	44	42	70	69	67			
Gestation length	115.56	115.38	115.73	115.28	115.13	115.55	0.16	0.96	0.07
Total born alive	12.81	12.88	13.04	12.36	11.98	12.08	0.36	0.73	0.008
Stillborn pigs	0.44	0.55	0.48	0.47	0.25	0.38	0.10	0.26	0.13
Mummies	0.33	0.58	0.37	0.58	0.48	0.43	0.11	0.29	0.46
Birth litter weight, d 3	22.25	22.30	22.13	20.08	20.17	19.45	0.53	0.86	<0.0001
Total pigs, d 3	11.96	12.09	12.07	12.07	12.09	12.12	0.13	0.92	0.61
Avg. individual BW, d 3	1.87	1.85	1.84	1.67	1.68	1.61	0.04	0.80	<0.0001
Litter weaning weight	70.86	74.51	66.84	63.52	65.40	61.42	1.80	0.86	<0.0001
Total pigs weaned	11.48	11.60	11.50	11.13	11.52	11.62	0.15	0.27	0.42
Avg. Ind. weaning BW	6.18	6.19	5.81	5.67	5.66	5.27	.14	0.99	<0.0001
ADG (d 3 to d 21)	0.26	0.26	0.25	0.24	0.24	0.22	0.01	0.92	<0.0001

¹A total of 336 litters were used from birth to d 37 in the nursery.

BW, measured at multiple time points were compared using repeated measures analysis. $P < 0.05$ = statistical difference and $0.05 < P < 0.1$ = statistical tendency

^{a, b} Values within birth, weaning, nursery, d 7, and nursery, d 37 with different superscripts are different.

Figure 20. Effects on sow productivity by injecting sows with vitamin A, E, and D-field study.

^{a, b} bars within born alive, stillborn, and mummies with different superscripts are different.

Figure 21. Effects on pig average individual BW from d 3 to 37 d of age by injecting sows with vitamin A, E, and D-field study.

^{a, b} bars within d 3, weaning, d 28, and d 58 with different superscripts are different.