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Feeding 25-hydroxycholecalciferol improves gilt reproductive performance and fetal vitamin D status¹

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ABSTRACT: Little information is available regarding the effects of vitamin D and its metabolites on reproduction in swine. To investigate the effects of feeding the circulating metabolite of vitamin D, 25-hydroxycholecalciferol (250HD3, ROVIMIX Hy•D, DSM Nutritional Products, Basel, Switzerland) on maternal and fetal circulating 25OHD3 concentration and gilt reproductive performance, a total of 40 PIC Camborough-22 gilts (BW on d -6 = 138 kg) in 4 replicates were randomly assigned to 1 of 2 corn-soybean meal-based diets. The control diet (CTL) was formulated to contain 2,500 IU D3/kg diet, and the experimental diet (250HD3) was formulated to contain 500 IU D3/kg diet + 50 µg 25OHD3/kg diet. Gilts were fed 2.7 kg of their assigned diet once daily beginning 43 d before breeding. Gilt BW were measured on gestational d -6 and d 90. Gilts were artificially inseminated with PIC 337-G semen 12 h and 24 h after showing signs of estrus. Blood samples were collected from the jugular vein on gestational d -43, -13, 46, and 89 for analysis of circulating 25OHD3 plasma concentration and overall vitamin D status of the gilts. At gestational d 90 \pm 1, gilts were harvested and reproductive tracts were removed. Fetal weight, sex, crown-to-rump length (CRL), as well as the number of mummified fetuses were recorded. As expected, circulating plasma concentrations of 25OHD3 were not different among treatment groups at d -43 (CTL = 53.8 ng/mL, 25OHD3 = 57.4 ng/mL; P = 0.66). However, gilts fed 25OHD3 had greater (P < 0.001) circulating plasma concentrations of 25OHD3 on d -13 (89.7 vs. 56.7 ng/mL), d 46 (95.8 vs. 55.7 ng/mL), and d 89 (92.8 vs. 58.2 ng/mL) of gestation compared with CTL-fed gilts. Circulating 25OHD3 was also greater in fetuses from 25OHD3-fed gilts on d 90 (P < 0.001). A 23% increase in pregnancy rate was observed in 25OHD3-fed gilts compared with CTL (78% vs. 55%, respectively; P = 0.21). Maternal BW gain (without conceptus), number of mummified fetuses, mean fetal weight, and mean fetal CRL were similar among treatments (P > 0.05). However, litter size was larger (CTL = 10.2; 250HD3 = 12.7; P = 0.04) in 25OHD3-fed gilts compared with CTL-fed gilts. Notably, mean fetal weight was not decreased in 25OHD3-fed gilts as frequently occurs when litter size is increased. Overall, feeding 25OHD3 to first-service gilts before and during gestation improved both maternal and fetal vitamin D status and improved maternal reproductive performance.

Key words: 25-hydroxycholecalciferol, gilt, performance, reproduction, swine, vitamin D

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INTRODUCTION

Little is known regarding the requirement for vitamin D and effects of feeding different sources of vitamin D on the reproductive performance of breeding swine. Vitamin D deficiency is well known to result in rickets, retarded skeletal growth, and myopathy in growing pigs, whereas in mature swine a deficiency results in osteomalacia. Less well studied are the consequences of vitamin D deficiency on reproduction. It appears that vitamin D may have a significant role in maternal-conceptus cross talk (Vigano et al., 2003).

According to the most recent NRC (NRC, 1998), no studies have been reported regarding vitamin D requirements of gilts or sows during gestation or lactation. Supplemental vitamin D is commonly added to animal feed in the form of cholecalciferol,

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also known as vitamin D_3 (D3), which is absorbed in the intestine and transported to the liver where it is hydroxylated to 25-hydroxycholecalciferol (25OHD3).

A commercial source of 25OHD3 is available and has been approved for use in poultry (Fritts and Waldroup, 2003). Although this commercial source of 25OHD3 has not been approved for use in swine in the United States, it is currently used in the swine industries of many other countries. Data from work conducted with young broiler chickens demonstrates that 25OHD3 is more efficiently absorbed in the upper portion of the intestine (Bar et al., 1980). To our knowledge, whether this is also true for swine has yet to be determined. However, recent reports in swine showing that feeding 25OHD3 resulted in significant increases in circulating 25OHD3 concentrations suggest that this may also be the case for swine (Lauridsen et al., 2010; Witschi et al., 2011). Circulating concentration of 250HD3 is generally used as an indicator of vitamin D status (Horst and Littledike, 1982). The aim of the current study was to test the hypothesis that feeding 25OHD3 would improve both maternal and fetal vitamin D status and positively influence the reproductive performance of breeding gilts.

MATERIALS AND METHODS

All procedures relating to animal use were reviewed and approved by the Texas Tech University Institutional Animal Care and Use Committee.

Animals and Dietary Treatments

Forty PIC Camborough-22 gilts (Hendersonville, TN; BW on d -6 = 138 kg) in 4 replicates of 10 gilts each were transported from Lexington, NE, to the Texas Tech University Swine Research Center, New Deal, TX. The study was conducted from April 2009 through January 2010. Twelve hours after arrival (d-43), 5 mL of PG-600 (400 IU PMSG and 200 IU HCG, Merck Animal Health, Summit, NJ) was administered subcutaneously, and gilts were randomly assigned to 1 of 2 corn-soybean meal based diets (Table 1). The control diet (CTL) was formulated to contain 2,500 IU of D3 per kilogram of diet, and the experimental diet (250HD3) contained 500 IU D3 per kilogram of diet plus 50 µg 25OHD3 per kilogram of diet in the form of ROVIMIX Hy•D (DSM Nutritional Products Ltd, Basel, Switzerland). To ensure against any vitamin D deficiency, both diets contained a minimum of 500 IU of D3. This allowed for the direct comparison of an additional 2,000 IU of D3 and 50 µg of 25OHD3. Gilts were fed 2.7 kg of their assigned diet once daily beginning 43 d before breeding through d 90 (±1) of gestation. All gilts were housed in individual gestation crates in an environmentally controlled facility on a 12-h light-dark cycle and had ad libitum access

to water. Gilt BW were measured on gestational d -6 and d 90. On d -20, a 14-d Matrix treatment was initiated for estrus synchronization (altrenogest, 15 mg/d, Merck Animal Health, Summit, NJ). Twelve and 24 h after showing signs of estrus, gilts were artificially inseminated with semen from PIC 337-G boars. A total of 2 gilts were removed from the study. One gilt died during the isolation period and another was removed due to severe morbidity during gestation that resulted in a single piglet litter on d 90.

Analysis of Circulating 25-Hydroxycholecalciferol Concentration

On gestational d -43, -13, 46, and 89, blood samples (8 mL) were collected from the jugular vein of all gilts using 38-mm × 20-gauge needles and 10-mL blood collection tubes containing 15 mg of EDTA(K₃) for use in determining circulating 25OHD3 plasma concentration. Within 1 h of collection, blood was centrifuged (1,000 × g, 15 min at 20°C), plasma was

 Table 1. Composition of gestation diets fed to gilts,

 as-fed basis¹

	Trea	tment ²
Item	CTL	250HD3
Ingredient, %		
Corn	79.50	79.50
Soybean meal	15.85	15.85
Dicalcium phosphate	2.45	2.45
Limestone	0.65	0.65
Dyna KCl	0.60	0.60
Salt	0.50	0.50
Mineral premix ³	0.15	0.15
Vitamin premix ⁴	0.10	0.10
Choline chloride	0.10	0.10
Calculated values		
Vitamin D ₃ , IU/kg of diet	2,500	500
25OHD3, µg/kg of diet	0	50
Lysine, %	0.65	0.65
ME, Mcal/kg of diet	3.27	3.27
СР, %	13.7	13.7
Ca, %	0.85	0.85
P, %	0.75	0.75
Analyzed values		
Ca, %	0.74	0.74
P, %	0.73	0.73
Vitamin D ₃ , IU/kg of diet	2,610	761
25OHD3, µg/kg of diet	0	51

¹Each gilt was provided 2.7 kg/d of her assigned diet.

²CTL = control-fed; 25OHD3 = 25-hydroxycholecalciferol-fed

³Supplied per kilogram of diet: 12,500 IU of vitamin A, 500 IU of vitamin D₃, 70 IU of vitamin E, 1.50 mg of menadione, 1.50 mg of thiamin, 7.0 mg of riboflavin, 4.0 mg of vitamin B6, 0.03 μ g of vitamin B12, 35.0 mg of niacin, 22.0 mg of d-pantothenic acid, 4.0 mg of folic acid, 0.40 mg of biotin.

⁴Supplied per kilogram of diet: 1.71 g of calcium carbonate, 1.48 g of ferrous sulfate, 0.59 g of zinc oxide, 0.18 g of manganous oxide, 0.02 g of copper sulfate, 0.02 g of selenium, 1.05 mg of EDDI (ethylene diamine dihydriodide).

harvested, and stored at -80°C until analysis. Gilt plasma 250HD3 concentrations were determined by DSM Nutritional Products AG Analytical Research Center (Kaiseraugst, Switzerland), using a previously described isotope dilution assay (Jakobsen et al., 2007; Lauridsen et al., 2010; Witschi et al., 2011). Assays conducted by this laboratory have a lower detectable limit for 25OHD3 of 5 ng/mL. Fetal blood samples (500 μ L) were collected from the heart of individual fetuses after gilts were harvested using a 5-mL syringe fitted with a 38-mm \times 20-gauge needle. Blood from all fetuses in each litter were pooled into the same 10-mL blood collection tube containing 15 mg of $EDTA(K_3)$ before plasma collection performed exactly as described above. Fetal plasma 250HD3 concentrations were determined by Heartland Assays (Ames, IA) using a previously described RIA (Hollis et al., 1993). Assays conducted by this laboratory have a lower detectable limit for 25OHD3 of 2.5 ng/mL.

Gilt Harvest and Fetus Collection

On d 90 (\pm 1) of gestation, gilts were weighed and then transported to the Texas Tech University Gordon W. Davis Meat Laboratory, Lubbock, TX, where they were harvested in random order by electrical stunning followed immediately by exsanguination. Twenty minutes after exsanguination, reproductive tracts of all gravid gilts (CTL; n = 11 and 25OHD3; n = 14) were extracted by midventral laparotomy, weighed, and transported to a laminar flow hood for extraction of the fetuses in an aseptic environment. The sex, BW, position in the uterine horn, and crown-to-rump length (CRL) were recorded for each individual fetus (n = 292; CTL; n = 113 and 25OHD3; n = 179). Because 25OHD3 is not approved for use in swine in the United States, carcasses and offal from all gilts fed 25OHD3 were destroyed and not allowed to enter the food chain.

Statistical Analysis

Statistical analysis was performed using the GLIMMIX and MIXED procedures (SAS Inst., Inc., Cary, NC). The GLIMMIX procedure of SAS was used for analysis of pregnancy rate. Treatment was the fixed effect, and gilt replicate was included as the random effect. Maternal BW gain (without the conceptus), total litter weight, total fetuses per litter, number of female fetuses per litter, fetal BW, female fetal BW, male fetal BW, fetal CRL, female fetal CRL, and number of mummies per litter were analyzed using the MIXED procedure of SAS with treatment as the fixed effect. The random effects were replicate and gilt nested within treatment by replicate.

Gilt served as the experimental unit and the Kenward-Roger adjustment was used to correct the degrees of freedom. Fetal circulating 25OHD3 concentrations were analyzed using the MIXED procedure of SAS, with treatment as the fixed effect with the random effects exactly as described above. Fetal CRL, male fetal CRL, and female fetal CRL were analyzed using the respective fetus weight as a covariate, and treatment as a fixed effect. The effects of sex and position in the uterine horn on fetal BW and CRL also were analyzed using PROC MIXED. For each fetus, its position in the uterine horn was defined as proximal, medial, or distal. Fetuses located in Position 1 to 3 (closest to the ovary) of either uterine horn were designated *proximal*. Those in Positions 4 to 6 of either uterine horn were designated medial. Fetuses in Positions 7 to 10 (closest to cervix) of either uterine horn were designated *distal* (Perry and Rowell, 1969). Means were separated using the PDIFF option and were considered significantly different when P < 0.05. If the P-value was between 0.05 and 0.10, the difference was considered a trend.

RESULTS AND DISCUSSION

Relatively little can be found in the literature regarding the supplementation of breeding swine with 25OHD3. The dietary vitamin D₃ requirement listed in the most recent NRC for gestating and lactating sows is 200 IU per kg of feed (NRC, 1998). Yet, commercial gestation diets typically contain at least 10 times that amount (C. Sparks, Devenish Nutrition, Alden, IA, personal communication). Research conducted with rats established that dams with low vitamin D status had 75% reduced fertility, 30% smaller litters, and their offspring exhibited retarded neonatal growth (Halloran and DeLuca, 1980). In addition, the observation that vitamin D receptor-null mice exhibit uterine hypoplasia and decreased ovarian size also supports a significant role for vitamin D in reproduction (Yoshizawa et al., 1997). As a result of the lack of information regarding the effects of vitamin D and its metabolites on swine reproduction, we conducted an experiment to determine whether supplementation of gilts with 250HD3 before and during gestation could improve maternal and fetal vitamin D status and reproductive performance in comparison with a similar concentration of D3.

Gilt Circulating Plasma 25-Hydroxycholecalciferol Concentration

Circulating plasma 25OHD3 concentration was measured in plasma from gilts (n = 38) on gestational d -43, -13, 46, and 89. Concentration of 25OHD3 did not differ between treatment groups on gestational d -43

(CTL = 53.8 ng/mL, 250HD3 = 57.4 ng/mL; P = 0.66;Figure 1). However, gilts fed 25OHD3 had greater (P <0.001) concentrations of 25OHD3 in their plasma on gestational d -13 (89.7 vs. 56.7 ng/mL), d 46 (95.8 vs. 55.7 ng/mL), and d 89 (92.8 vs. 58.2 ng/mL) compared with gilts fed the CTL diet (Figure 1). These results are consistent with previous work on feeding 250HD3 to breeding swine (Lauridsen et al., 2010). The mechanism by which supplementation of swine with 25OHD3 causes such a dramatic increase in circulating concentrations of 25OHD3 is not completely clear. However, it has been demonstrated that 25OHD3 is absorbed more efficiently than D3 in the upper portion of the intestine of young broiler chickens (Bar et al., 1980). Our results, combined with the previous work of others in swine showing remarkable increases in circulating 25OHD3 concentrations when 25OHD3 is fed, suggest that this may also be true in swine (Lauridsen et al., 2010; Witschi et al., 2011). Experiments similar to those conducted in birds are necessary to determine whether 25OHD3 is more efficiently absorbed in the upper intestine of swine.

Fetal Circulating Plasma 25-Hydroxycholecalciferol Concentration

Circulating plasma concentration was significantly greater (P < 0.001) in fetuses from 25OHD3-fed gilts (8.23 ng/mL; n = 14) as compared with that of fetuses from CTL gilts (6.46 ng/mL; n = 9; Figure 2). These

in gilts [control-fed (CTL); n = 20 and 25-hydroxycholecalciferol-fed (25OHD3); n = 18] from d –43 to d 89 of gestation. Bars are least squares means ± SEM. *Treatment means within day of gestation differ (P < 0.001).

Table 2. Effect of sex on BW and crown-to-rump lengthof fetuses on d 90 of gestation

	Se	ex		
Item	Female ¹	Male ²	SEM	P-value
Fetal BW, g	605.5	657.3	22.3	< 0.01
Crown-to-rump-length, cm	25.0	25.5	0.8	0.05
¹ Female fetuses ($n = 139$)).			

²Male fetuses (n = 153).

12-

10

8

6

2

0

25-Hydroxycholecalciferol, ng/mL

data suggest that maternal nutrition and vitamin D status can indeed affect fetal vitamin D status. Feeding gilts 25OHD3 before and during gestation resulted in significantly greater circulating plasma concentrations of 25OHD3 in both dams and fetuses. These findings are consistent with work performed by others in humans, swine, cattle, sheep, rabbits, and rats (Hillman and Haddad, 1974; Goff et al., 1982; Kubota et al., 1982; Goff et al., 1984; Smith et al., 1987; Clements and Fraser, 1988). These results demonstrate that improving maternal vitamin D status by feeding 25OHD3 also results in improvement of fetal vitamin D status, which could have important implications for postnatal growth of the offspring of 25OHD3-fed gilts.

Figure 2. Circulating plasma 25-hydroxycholecalciferol concentration in fetuses from control-fed gilts (CTL; n = 9 litters) and 25-hydroxycholecalciferol-fed gilts (25OHD3; n = 14 litters) on d 90 of gestation. Blood samples were collected from the heart of individual fetuses and pooled by litter. Bars are least squares means \pm SEM. *Treatment means differ (P < 0.001).

Treatment

250HD3

CTL



Table 3. Effect of position in the uterine horn on BW andcrown-to-rump-length of fetuses on d 90 of gestation

		Position ¹			
Item	Proximal ²	Medial ³	Distal ⁴	SEM	P-value
Fetal BW, g	655.5 ^a	615.1 ^b	623.7 ^{ab}	28.2	0.01
Crown-to-rump-length, cm	25.4	25.1	25.3	0.9	0.61
1					

^{a,b}Within a row, means without a common superscript differ (P < 0.05).

 ${}^{1}\mathrm{Fetuses}$ were classified based on location in the uterine horn relative to the ovary.

 2 Proximal fetuses (n = 149) were in Position 1 to 3 (closest to the ovary) of either uterine horn.

³Medial fetuses (n = 110) were in Position 4 to 6 of either uterine horn.

 4 Distal fetuses (n = 33) were in Position 7 to 10 (closest to cervix) of either uterine horn.

Effects of Sex and Position in the Uterine Horn on Fetal BW and Crown-to-Rump Length

The effect of sex on fetal BW and CRL were determined independent of maternal dietary treatment. Male fetuses were significantly heavier than female fetuses on d 90 of gestation (P < 0.01; Table 2). In addition, male fetuses tended to have greater CRL than their female littermates on d 90 of gestation (P = 0.05; Table 2).

The consequence of fetal position in the uterine horn on fetal BW and CRL, independent of maternal dietary treatment, was also evaluated. Each fetus was categorized based on its position in the uterine horn relative to the ovary as described earlier in the Materials and Methods. Fetal position in the uterine horn did not significantly influence fetal CRL (P = 0.61; Table 3). However, fetuses in positions both proximal and distal from the ovary had the heaviest BW, whereas those in the medial portion of the uterine horn had the lightest BW on d 90 of gestation (P = 0.01; Table 3). These results are in agreement with previous work and appear to be due to variations in the vascular supply along the uterine horn (Waldorf et al., 1957; Perry and Rowell, 1969).

Gilt Reproductive Performance

Pregnancy rate was not different in 25OHD3-fed gilts compared with CTL (77.8 % vs. 55.0 %, respectively; P = 0.21; Table 4). Importantly, this study was conducted during the hottest months of the year when gilt breeding efficiencies are typically the poorest (Teague et al., 1968; Christenson, 1986). Although not statistically significant, the implications of any improvement in reproductive efficiency are important to the swine industry. Reproductive failure is one of the top reasons for culling of gilts from the herd (Stalder et al., 2004). If more firstservice gilts successfully farrow litters as a result of 25OHD3 supplementation, the overall herd culling rate can be improved at a significant economic benefit to the producer. In this study, the lack of statistical significance

Table 4. Effect of feeding 25-hydroxycholecalciferol on gilt reproductive performance

	Treatment			
Item	CTL ¹	250HD3 ²	SEM	P-value
Pregnancy rate, %	55.0	77.8	11.1	0.21
Maternal BW gain, kg ³	38.8	44.1	3.0	0.19
Fetuses per litter	10.2	12.7	1.0	0.04
Female fetuses per litter	4.6	6.3	0.6	0.05
Male fetuses per litter	5.6	6.5	0.6	0.26
Mummified fetuses per litter	0.4	0.4	0.2	0.83
Litter weight, kg	6.5	8.2	0.8	< 0.01
Fetal BW, g	626.2	636.6	31.7	0.80
Female fetal BW, g	606.1	604.9	34.1	0.98
Male fetal BW, g	646.2	668.4	34.7	0.62
Fetal crown-to-rump length, cm	25.0	25.5	0.9	0.37
Female crown-to-rump length, cm	24.7	25.3	0.9	0.28
Male crown-to-rump length, cm	25.3	25.7	0.9	0.56

¹Control-fed (CTL) gilts (n = 11).

²25-hydroxycholecalciferol-fed (25OHD3) gilts (n = 14).

 3 Maternal BW gain calculated from d 0 of gestation to d 90 of gestation with the weight of the uterus, ovaries, and conceptus removed.

can be partially explained by the small number of gilts per replicate. Much larger-scale studies with significantly greater numbers of females per dietary treatment must be conducted to determine if feeding 25OHD3 at least 30 d before breeding results in significant increases in pregnancy rates in a commercial setting.

Gilts fed 25OHD3 before breeding throughout d 90 of gestation had significantly greater litter weights (P < 0.01; Table 4) compared with CTL gilts. The increase in litter weight observed in 25OHD3-fed gilts can be explained by a significant increase in litter size of 2.5 fetuses per litter compared with unsupplemented gilts (P = 0.04; Table 4). In addition, 25OHD3 supplementation tended to increase the number of female fetuses per litter (P = 0.05; Table 4). The number of mummified fetuses observed per litter was not different among treatment groups (P = 0.83; Table 4). Importantly, fetuses from the 25OHD3-fed gilts had similar BW (P = 0.80) and CRL (P = 0.37) compared with those from CTL-fed gilts (Table 4).

The observation that 25OHD3-fed gilts had more fetuses per litter and those fetuses were numerically heavier than those from CTL-fed gilts at d 89 of gestation has significant implications for the swine industry. Typically, when litter size is increased in a herd, the volume of low-birth-weight piglets also increases and becomes a concern for producers (Waldorf et al., 1957; Beaulieu et al., 2010; Berard et al., 2010a,b). Small piglets are a challenge to the swine industry because they have fewer muscle fibers, fatten at a younger age, and ultimately have lower meat yields than their larger littermates (Bee, 2004).

Overall, the results of this study indicate that supplementing first-service gilts with 25OHD3 before and during gestation can improve both maternal and fetal vitamin D status, pregnancy rate, and litter size without sacrificing average fetal BW as compared with gilts fed a diet containing only D3. Further research using large-scale, commercial studies will ultimately determine whether there is economic incentive for 25OHD3 supplementation based on improvements in vitamin D status and reproductive performance.

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