Effect of source and quantity of dietary vitamin D in maternal and creep diets on bone metabolism and growth in piglets

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ABSTRACT: Piglets are born with reduced plasma concentrations of 25-hydroxycholecalciferol (25-OH-D₃) and are thus highly predisposed to vitamin D deficiency. Furthermore, sow milk contains little vitamin D, and the slow intestinal vitamin D absorption of sows limits the efficacy of dietary vitamin D supplementation. Hence, the neonate depends, to a large extent, on the vitamin D stores built up in fetal tissues from maternal sources. The current study was undertaken to evaluate whether the source and quantity of dietary vitamin D provided to the gestating and lactating sow, and also directly in the form of creep feed to the piglet, would influence the vitamin D status, growth performance, and skeletal development of piglets. A total of 39 primiparous and multiparous sows were randomly assigned to 1 of 3 dietary treatments (13 in each treatment), supplemented with either 5 or 50 μg of the commonly used cholecalciferol (vitamin D₃) or 50 μg of 25-OH-D₃ per kilogram of feed. By wk 3 of lactation, piglets were offered a creep diet with vitamin D supplementation according to the treatment of the dam, and they were offered the same creep diets after weaning at d 35 of age until they reached a BW of approximately 20 kg. When dietary 25-OH-D₃ was provided, circulating concentrations of 25-OH-D₃ in piglet serum increased (P < 0.05) as early as d 21 and later at d 33 and 77, indicating greater body stores in those animals. Bone-breaking strength and cortical bone mineral content and density at the tibial midshaft of piglets were reduced (P < 0.05) when vitamin D₃ was supplemented at 5 μg/kg compared with the bone traits of other groups, but no differences (P > 0.05) were observed between the 2 other groups. After weaning, ADFI was greater (P < 0.05) and growth performance tended (P = 0.08) to improve when doses of 50 μg/kg were administered, regardless of the vitamin D source. In conclusion, supplementation of the diet with 50 μg/kg of either source of vitamin D was proved to be adequate in meeting the needs of gestating sows and in permitting the accumulation of vitamin D in fetal tissues, as well as for normal skeletal mineralization and growth in the offspring. Furthermore, the markedly improved vitamin D status of piglets whose mothers received 25-OH-D₃ possibly resulted from greater tissue reserves present at birth and a greater availability of vitamin D when released from those stores.

Key words: blood, bone, growth, 25-hydroxycholecalciferol, pig, vitamin D₃

INTRODUCTION

Vitamin D deficiency is rare in pigs housed outside because sunlight stimulates sufficient endogenous production of vitamin D to prevent abnormal bone metabolism. However, total confinement of sows and their offspring during lactation is widespread and necessitates an adequate vitamin D supply via the diet (Littledike and Goff, 1987). Because blood 25-hydroxycholecalciferol (25-OH-D₃) in mammalian offspring at birth is closely related to that of the mother (Hillman and Haddad, 1974; Goff et al., 1984), neonatal rickets can occur when maternal vitamin D status is low (Watney et al., 1971). Of the common farm animals, the piglet is born with the least plasma concentration of 25-OH-D₃ and thus is highly predisposed to vitamin D deficiency (Horst and Littledike, 1982). Moreover, vitamin D deficiency causes poor mineralization of the skeleton in most mammals (Holick, 2006), and as early as in utero, long bone growth of the human fetus is clearly reduced when mothers are vitamin D deficient (Morley et al., 2006). However, similar to several other species (Hal-
loran and DeLuca, 1980; Hsu and Levine, 2004), active intestinal Ca absorption in newborn piglets does not rely on vitamin D-dependent transport until the fourth week postpartum (Lachenmaier-Currrle and Harmeyer, 1988; Schröder et al., 1993).

Furthermore, Clements and Fraser (1988) demonstrated, in experiments carried out in rats, that vitamin D is accumulated and stored in fetal tissues. Vitamin D is predominantly stored as 25-OH-D$_3$, and the greatest concentrations can be found in muscle, where vitamin D is readily available to neonatal pigs during the first weeks after birth. Because transfer of vitamin D to breast milk, and thus the vitamin D content of the milk, is very low, the neonate depends heavily on those adaptations to maintain homeostasis of Ca and P.

Supplementing the creep diet represents a potential approach to provide adequate amounts of vitamin D to suckling piglets when their regulation of Ca absorption becomes vitamin D dependent. Provision of a creep diet to suckling pigs is common, but the starting point, as well as the amount of feed ingested, is highly variable among individual animals (Bruininx et al., 2002). To optimize the vitamin D supply, using vitamin D sources of greater bioavailability and of adequate quantity might be necessary. Therefore, the aim of the present study was to conduct a comparative evaluation of the indirect (fetal tissue stores and milk of the sow) and direct (creep feed) effects of 2 amounts and sources of vitamin D (vitamin D$_3$ and 25-OH-D$_3$) on growth, blood status, and various bone traits of pigs from birth to 77 d of age.

### MATERIALS AND METHODS

All experimental procedures involving animals were approved by the official veterinary authority of the canton of Zug (Switzerland).

**Experimental Design, Animals, and Sampling Procedure**

In a previous study (A.-K. M. Witschi, unpublished data), 39 primiparous and multiparous Large White sows from the herd of the ETH research farm Chamau (Hiünenberg, Switzerland) were randomly allotted to 3 dietary treatments on the day of mating, and they were kept for 4 reproductive cycles. The gestation and lactation diets were fortified with either 5 μg (low, DL) or 50 μg (normal, DN) of vitamin D$_3$ (Rovimix D3-500, DSM Nutritional Products, Basel, Switzerland) per kilogram, representing the estimated requirement by NRC (1998) and the common practical amount in Switzerland, respectively. The third diet (HD) contained 50 μg of 25-OH-D$_3$ (Rovimix Hy-D 1.25%, DSM Nutritional Products) per kilogram.

One week before parturition, the animals were moved into individual farrowing pens with heated nests for the piglets and straw bedding according to Swiss animal welfare guidelines. From the third week of lactation, piglets were offered a creep diet in pelleted form that was supplemented with 1 of the 3 types of vitamin D supplementation, which were included in the vitamin and mineral premix according to the treatment of the dam (Table 1). At weaning (35 d) and at 70 d, individual piglet BW were obtained. Water was available ad libitum via piglet nipple drinkers. In each parity, 5 mL of serum and 5 mL of EDTA tube whole-blood samples from randomly selected piglets from each treatment were collected by jugular punctation on d 21, 33 (just before weaning), and 77 of age, respectively. Blood was centrifuged (3,500 × g for 15 min at 4°C), and serum was harvested and frozen (−80°C).

Immediately after weaning at d 35, a total of 54 randomly selected piglets from parity 1 and 2 sows (18/treatment) were slaughtered at the abattoir of the Faculty of Veterinary Medicine, Zurich, Switzerland. The tibia and the internal and external metatarsal bones of the left hind leg were excised and cleaned of extraneous tissues. Bones were stored at −20°C before analysis.

After the piglets were separated from their dams, the litters within the same treatment were mixed and moved to pens with a sun-shaded run, where the animals were fed the same creep diet as during the suckling

<table>
<thead>
<tr>
<th>Item</th>
<th>DL</th>
<th>DN</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D$_3$, μg/kg</td>
<td>5</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>25-OH-D$_3$, μg/kg</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

1DL = low vitamin D$_3$ (5 μg/kg; NRC, 1998); DN = normal vitamin D$_3$ (50 μg/kg; practical amount; Rovimix D3-500, DSM Nutritional Products, Basel, Switzerland); and HD = 25-hydroxycholecalciferol (25-OH-D$_3$) at the recommended quantity (50 μg/kg; Rovimix Hy-D 1.25%, DSM Nutritional Products).

2Supplied the following per kilogram of diet: 18,000 IU of vitamin A (acetate); 77 IU of vitamin E; 5 mg of vitamin K; 21 mg of d-pantothenic acid; 6 mg of riboflavin; 44 mg of niacin; 3.0 mg of folic acid; 0.2 mg of b-hidroxy b-citrate; 35 μg of vitamin B$_12$; 0.98 g of choline; 108 mg of Fe as ferrous sulfate; 105 mg of Zn as Zn oxide; 37 mg of Mn as Mn oxide; 16 mg of Cu as copper sulfate; 1.1 mg of I as calcium iodate; and 450 μg of Se as sodium selenite.

3Vitamin D supplement included in the vitamin and mineral premix.
period for another 5 wk. To ensure proper functioning of the feeding system used (Jetmix feeder, Zanotelli, Gipf-Oberfrick, Switzerland), the animals had to be housed in groups of a maximum of 25 animals per pen. The number of pens occupied at the same time depended solely on the number of litters weaned at the same time.

Analytical Methods

Determination of 25-OH-D₃ in serum and feed samples was performed by a specialized laboratory (DSM Nutritional Products Ltd.). The method was based on an isotope dilution assay using a reversed-phase HPLC-mass spectrometry system with a trapping column for quantification. Calcium and P in serum were determined by colorimetry with a UV-visible-recording spectrophotometer (UV-160A, Shimadzu Corporation, Kyoto, Japan), using commercial kits [Calc 20 (Axonlab AG, Baden, Switzerland) for Ca and ABX Pentra (Horiba ABX, Montpellier, France) for P]. Analyses were based on the methylthymol blue method for Ca and the phosphomolybdate method for P. Serum concentrations of osteocalcin (OC) and crosslaps (CL) were measured using commercially available ELISA test kits (Metra OC, Quidel Corporation, San Diego, CA; CrossLaps, and IDS Immunodiagnostics Systems Ltd., Boldon, UK, respectively).

Lengths of the tibial bone were determined with a digital caliper before the total cortical bone mineral density (BMD) and cortical bone mineral content (BMC) were measured in the middle of the diaphyses by using peripheral quantitative computed tomography (Stratec XTC 960A bone scanner, Stratec Medizinaltechnik GmbH, Pforzheim, Germany). The 3-point bending test at fracture was used to determine the mechanical properties of the bone according to the method of Crenshaw et al. (1981a). The force applied to the midpoint of the bone until breaking was determined with a texture analyzer (TA-HD Texture Analyzer, StableMicrosystems Ltd., Boldon, UK, respectively).

Statistical Analyses

All data were analyzed using a statistical program (version 2.8.1; R Development Core Team, 2009). Because the data were not normally distributed, the non-parametric Kruskal-Wallis test was used to examine the differences in outcomes between the 3 treatment groups. If the differences detected were significant, pairwise comparisons between groups were conducted with the Wilcoxon signed-rank test. The experimental unit was the litter for the data recorded during the suckling period and was the pen for the data obtained during the postweaning period.

RESULTS

The analyzed energy and nutrient contents of the creep diet provided in this study are presented in Table 2 and generally corresponded well to the expected values. The vitamin D contents were determined separately for each batch of feed. In the feed samples of treatment DN, the analyzed contents agreed very well with the expected values. In the feed samples of the other treatments, however, the determined vitamin D contents exceeded (treatment DL) or were below (treatment HD) the values provided by the manufacturer, probably because of the combined effect of losses during feed manufacturing and feed storage during the experimental period.

Postweaning Growth Performance

Body weights at weaning were similar for all groups, whereas the 70-d BW and total BW gain for the period between weaning and d 70 tended (P = 0.08) to be less in piglets receiving 5 μg of vitamin D₃/kg (treatment DL) than in those provided with 50 μg/kg of either vitamin D source (treatments DN and HD; Table 3). Moreover, the piglets in the DL treatment demonstrated a tendency (P = 0.08) toward decreased ADG, and ADFI was less (P < 0.05) in this group compared with piglets in treatments DN and HD. Nevertheless, the G:F was similar (P > 0.05) among the groups.

Table 2. Analyzed energy and nutrient contents of creep diets and vitamin D content of supplement (as-fed)¹

<table>
<thead>
<tr>
<th>Item</th>
<th>DL</th>
<th>DN</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creep diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, g/kg</td>
<td>895</td>
<td>895</td>
<td>895</td>
</tr>
<tr>
<td>Crude ash, g/kg</td>
<td>630</td>
<td>630</td>
<td>630</td>
</tr>
<tr>
<td>Crude fat, g/kg</td>
<td>63.2</td>
<td>63.2</td>
<td>63.2</td>
</tr>
<tr>
<td>CP (N × 6.25), g/kg</td>
<td>175</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>NDF, g/kg</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>ADF, g/kg</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Ca, g/kg</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>P, g/kg</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>DE, MJ/kg</td>
<td>14.0</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Vitamin D₃ supplement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of batches analyzed</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin D₃, μg/kg</td>
<td>14 ± 9</td>
<td>49 ± 9</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>25-OH-D₃, μg/kg</td>
<td>2Not detected.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹DL = low vitamin D₃ (5 μg/kg; NRC, 1998); DN = normal vitamin D₃ (50 μg/kg; practical amount; Rovimix D3-500, DSM Nutritional Products, Basel, Switzerland); and HD = 25-hydroxycholecalciferol (25-OH-D₃) at the recommended quantity (50 μg/kg; Rovimix Hy-D 1.25%, DSM Nutritional Products).

²Not detected.
Concentrations of 25-OH-D₃ in the offspring of sows provided with 25-OH-D₃ were greater \((P < 0.05)\) at all sampling times (Figure 1). The circulating concentrations of 25-OH-D₃ decreased numerically between d 21 and 33 (just before weaning) in treatments DL and DN, but not in treatment HD, followed by the greatest values at d 77 in all treatment groups.

Calcium concentrations were not affected \((P > 0.05)\) by treatment at d 21 or 33, but d 77 serum Ca was less \((P < 0.05)\) in piglets receiving 25-OH-D₃ (Figure 2). Similarly, in all treatment groups, Ca concentrations decreased between d 21 and before weaning, followed by an increase at d 77 in treatments DL and DN, but not in treatment HD. Serum P decreased numerically in all groups over time and was not affected \((P > 0.05)\) by treatment at any sampling point.

Serum OC, the marker of bone formation, was similar among treatments at d 21 (Figure 3). However, before weaning (d 33), concentrations for piglets in treatments DN and HD were greater \((P < 0.05)\) than those for piglets in treatment DL. However, OC concentrations at d 77 were less \((P < 0.05)\) in piglets receiving 25-OH-D₃ (treatment HD) than in those receiving vitamin D₃ (treatment DN). The serum OC of piglets in treatment DL remained similar between d 21 and 33, followed by a numerical increase at d 77, whereas concentrations in piglets in treatment HD increased from d 21 until weaning, followed by a decrease to an intermediate concentration. Only the piglets in treatment DN showed a constant numerical increase in their serum concentrations of OC from d 21 to 77. Circulating concentrations of CL, the marker of bone resorption, were similar among the groups at d 21, which was followed by an increase to greater \((P < 0.05)\) concentrations for piglets in treatments DN and HD around weaning. The CL concentrations for animals in treatment DL remained the same at weaning as on d 21 and increased between weaning and d 77, similar to those of the other groups.

Bone Measurements in Piglets

The bone quality traits analyzed in the tibial and metatarsal bones are presented in Tables 4 and 5. There was a clear treatment effect \((P < 0.05)\) on bone weights and bone-breaking strength as well as a tendency \((P = 0.07)\) toward a treatment effect on the bending moments of tibial bones; they were less in the offspring of dams provided 5 μg of vitamin D₃/kg of diet (treatment DL) than in the 2 other groups. Accordingly, the measurements of the midshaft of the tibia with peripheral quantitative computed tomography revealed less \((P < 0.05)\) total and cortical BMC and BMD for piglets in the DL group (5 μg of D₃/kg) compared with those in the DN and HD groups.
Furthermore, fresh weight, DM content, ash weight, and total amount of Ca were less ($P < 0.05$) in the metatarsal bones of piglets in treatment DL (Table 5). Percentages of metatarsal ash, P, and Ca relative to DM; total amount of P; and Ca:P were not affected by treatment.

**DISCUSSION**

As reported by Brooke et al. (1981), the postnatal BW gain of infants was clearly improved when mothers received supplemental vitamin D, reflecting the relatively prolonged action of the vitamin and the benefits provided by increased stores. Additionally, more recent findings revealed that vitamin D deficiency is a well-known accompaniment of various infectious diseases (Ustianowski et al., 2005), and susceptibility to infection was shown to be greater when vitamin D status was low (Hayes et al., 2003). To a certain extent, the results of the present study confirmed the beneficial effect of vitamin D on development in young pigs. The ADFI was greater when vitamin D was provided at 50 μg/kg (treatments DN and HD), regardless of the source used. Similarly, BW gain between weaning and d 70, BW at weaning and d 70, and ADG in the postweaning period tended to be less in piglets fed 5 μg of vitamin D$_3$ (treatment DL). As mentioned previously, vitamin D stores were found to be depleted at weaning. Therefore, the tendency toward improved postweaning growth performance (as a possible consequence of better immune function and health) in treatments DN and HD was more likely a result of differences in dietary supply than of differences in the extent of vitamin D mobilization from tissue stores. Nevertheless, environmental stressors, such as stocking density, are also known to have a marked effect on feed intake and hence growth in piglets (Hyun et al., 1998). Although the number of animals per pen was increased in treatment groups DL.
and DN in the present study, G:F was similar among the 3 treatment groups.

The piglet is born with relatively low plasma concentrations of 25-OH-D3 (Horst and Littledike, 1982). Compared with values in previous reports (Horst and Littledike, 1982; Goff et al., 1984), 25-OH-D3 concentrations of the piglets in this study were even less. Nevertheless, piglets of sows fed 25-OH-D3 had greater serum concentrations at both d 21 and 33. In a previous study (A.-K. M. Witschi, unpublished data), the milk 25-OH-D3 content of sows in treatment HD was also increased. The transfer of vitamin D and its metabolites across the placenta and into breast milk in humans is small (Hollis and Wagner, 2004). This transfer does not affect the vitamin D status of the infant unless the mother is consuming large doses of supplemental vitamin D (Specker, 1994).

In addition to the vitamin D content of milk, researchers have demonstrated that another biological strategy exists in meeting the vitamin D requirement of the neonate. In rats, vitamin D is accumulated during the last trimester of gestation particularly in fetal muscle, where the vitamin is available for the neonate in the first weeks after birth (Clements and Fraser, 1988). Like rat pups, piglets have little subcutaneous fat, and muscle tissue thus forms the greatest proportion of the total fetal mass. Clements and Fraser (1988) found that in rats, the capacity for the accumulation and storage of considerable quantities of vitamin D molecules in the fetus by this mechanism was large. This might also be true in pigs. Furthermore, it seems likely that the observed differences in serum 25-OH-D3 indicate a treatment effect on the extent of vitamin D accumulation in fetal tissues. The gradual exhaustion of those vitamin D stores during the course of the suckling period would further explain the decrease in serum 25-OH-D3 concentrations for piglets in the DL and DN treatments between d 21 and before weaning at d 33. Furthermore, piglets have been found to consume 60 to 80% of creep feed during the last week before weaning at 28 d of age (Fraser et al., 1994; Bruininx et al., 2002), which would explain the small differences in vitamin D status of the

### Table 4. Bone-breaking strength, bending moment, bone mineral content (BMC), and bone mineral density (BMD) in tibial bones of weanling pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations</td>
<td>DL</td>
<td>DN</td>
<td>HD</td>
</tr>
<tr>
<td>Length, mm</td>
<td>74.5</td>
<td>79.8</td>
<td>79.9</td>
</tr>
<tr>
<td>Fresh weight, g</td>
<td>9.88</td>
<td>13.70</td>
<td>12.94</td>
</tr>
<tr>
<td>Breaking strength, N</td>
<td>972</td>
<td>1.407</td>
<td>1.337</td>
</tr>
<tr>
<td>Bending moment, N × m</td>
<td>5.19</td>
<td>6.93</td>
<td>6.22</td>
</tr>
<tr>
<td>Total BMC, mg/mm</td>
<td>54.84</td>
<td>77.71</td>
<td>73.47</td>
</tr>
<tr>
<td>Total BMD, mg/mm²</td>
<td>506</td>
<td>611</td>
<td>620</td>
</tr>
<tr>
<td>Cortical BMC, mg/mm</td>
<td>47.3</td>
<td>69.8</td>
<td>65.8</td>
</tr>
<tr>
<td>Cortical BMD, mg/mm²</td>
<td>802</td>
<td>841</td>
<td>834</td>
</tr>
</tbody>
</table>

*Mean* *within* *a row* *with* *different* *superscripts* *differ* (P < 0.05).

1DL = low vitamin D₃ (5 μg/kg; NRC, 1998); DN = normal vitamin D₃ (50 μg/kg; practical amount; Rovimix D₃-500, DSM Nutritional Products, Basel, Switzerland); and HD = 25-hydroxycholecalciferol at the recommended quantity (50 μg/kg; Rovimix Hy-D 1.25%, DSM Nutritional Products).

### Table 5. Treatment response on various traits in metatarsal bones of weanling pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations</td>
<td>DL</td>
<td>DN</td>
<td>HD</td>
</tr>
<tr>
<td>Fresh weight, g</td>
<td>10.0</td>
<td>13.58</td>
<td>12.94</td>
</tr>
<tr>
<td>DM, %</td>
<td>40.5</td>
<td>42.5</td>
<td>42.1</td>
</tr>
<tr>
<td>Ash weight, g</td>
<td>2.63</td>
<td>3.67</td>
<td>3.45</td>
</tr>
<tr>
<td>Ash, % of DM</td>
<td>36.8</td>
<td>37.6</td>
<td>38.3</td>
</tr>
<tr>
<td>Total Ca, g</td>
<td>1.05</td>
<td>1.47</td>
<td>1.44</td>
</tr>
<tr>
<td>Ca, % of DM</td>
<td>14.6</td>
<td>14.7</td>
<td>15.5</td>
</tr>
<tr>
<td>Total P, g</td>
<td>0.50</td>
<td>0.70</td>
<td>0.68</td>
</tr>
<tr>
<td>P, % of DM</td>
<td>7.0</td>
<td>7.2</td>
<td>7.3</td>
</tr>
<tr>
<td>Ca/CaP</td>
<td>2.11</td>
<td>2.10</td>
<td>2.14</td>
</tr>
</tbody>
</table>

*Mean* *within* *a row* *with* *different* *superscripts* *differ* (P < 0.05).

1DL = low vitamin D₃ (5 μg/kg; NRC, 1998); DN = normal vitamin D₃ (50 μg/kg; practical amount; Rovimix D₃-500, DSM Nutritional Products, Basel, Switzerland); and HD = 25-hydroxycholecalciferol at the recommended quantity (50 μg/kg; Rovimix Hy-D 1.25%, DSM Nutritional Products).
animals among treatments before weaning. Moreover, because of the immaturity of the 25-hydroxylase, the efficiency of intestinal vitamin D absorption undergoes a postnatal age-dependent increase and therefore limits the efficacy of vitamin D supplementation in the newborn (Hollis et al., 1996). The same authors found that intestinal absorption of vitamin D in the term infant was influenced not only by their postnatal age, but also by the form of vitamin D administered. This would explain the increase found in 25-OH-D$_3$ concentrations between d 21 and 33 in piglets receiving 25-OH-D$_3$. After weaning, the switch to a diet consisting of exclusively solid feed in combination with endogenous vitamin D synthesis because of relocation to pens with a sun-shaded run caused a marked increase of serum 25-OH-D$_3$ by d 77.

Blood Ca and P were within the range of previously published reference values for pigs of comparable age (Ullrey et al., 1967; Tumbleson and Kalish, 1972; Friendship et al., 1984) at all sampling points. Serum Ca decreased in all treatment groups between d 21 and 33 and increased again in treatments DL and DN, but not in treatment HD until d 77, which led to differences among the groups. Ullrey et al. (1967) described a similar pattern in time among adequately nourished piglets of the same age. Phosphorus concentrations in serum have been reported to be least at birth, subsequently rise to a peak value at 2 wk postpartum, and gradually decline (Ullrey et al., 1967). This is consistent with the age-dependent changes in serum P reported here.

Markers of bone formation and resorption circulate at greater concentrations in children than in adults (Rauch and Schöna, 1997). This is in agreement with the values reported here, previously published data in reproducing sows (Lauridsen et al., 2010), and results obtained in growing small ruminants (Liesegang and Risteli., 2005). The reason for the delayed increase in OC concentrations for piglets in treatment DL is not clear. However, researchers have previously demonstrated that vitamin-D-deficient rats have reduced concentrations of OC, indicating that vitamin D is necessary for the increase in OC (Price et al., 1981; Lian et al., 1987). This might also hold true for sucking piglets, especially during the critical period around weaning, when fetal stores are exhausted and vitamin D status relies completely on intestinal absorption of the vitamin. Therefore, it seems likely that the reduced bone formation for piglets in treatment DL, which was probably the result of the smaller fetal vitamin D stores obtained across the placenta before birth, is attributable to an attempt to maintain Ca homeostasis in the later stage of the suckling period.

In infants, the greatest concentrations of the bone resorption marker CL occur in neonates during the first month of life. Afterward, concentrations decrease markedly between the age of 1 mo and 1 yr, and then increase again, with a peak at 11 to 13 yr (Crofton et al., 2002). Considering the shorter generation interval in pigs, the continuous increase in CL concentrations between d 21 and 77 observed for piglets in treatments DN and HD seems to correspond with the findings in humans. The plateau of concentrations in piglets in treatment DL between d 21 and 33 corresponds with the observed pattern of OC concentrations. This would confirm the hypothesis that further mineralization of the skeleton is downgraded for the benefit of Ca homeostasis.

The measurement and subsequent comparison of a variety of bone assessment variables indicated severe alterations in the bones of the piglets exposed to reduced dietary D$_3$ concentrations in the pre- and postnatal periods (treatment DL). In humans and rats, vitamin D deficiency during pregnancy is reflected in disturbed skeletal homeostasis in the newborn and, in extreme situations, with reduced bone mineralization (Miller et al., 1983; Pawley and Bishop, 2004). The compound 1,25-(OH)$_2$D$_3$ is regarded as one of the most potent factors affecting bone resorption. This compound markedly stimulates resorption activity (Raisz et al., 1980) and thereby promotes the differentiation of precursor cells into mature, bone-resorbing osteoclasts (Holtrop et al., 1981), the principal resorbing bone cells. However, the mechanism by which 1,25-(OH)$_2$D$_3$ alters the size of the osteoclast population, and thus the resorptive activity, is not well understood (Bar-Shavit et al., 1983).

Bone mineral content is known as the most sensitive index in vivo index of skeletal change (Prentice, 2003), and the greater precision of peripheral quantitative CT data measured at the tibia and femur of humans has been demonstrated (Groll et al., 1999). The applicability of this method to assess skeletal changes in other species has been confirmed in a variety of animals, such as pigs, dogs, sheep, and goats (Liesegang et al., 2002, 2006; Schneider et al., 2004). Indeed, the tibial bones of piglets reared at 5 μg of vitamin D$_3$ had decreased total and cortical BMC and BMD as well as decreased bone-breaking strength. The bone-breaking strength is also known to possess great sensitivity to mineral nutrition in the pig (Pointillart and Guégen, 1993; Pointillart et al., 1995). Consequently, regardless of the form provided, administration of vitamin D to gestating sows and their piglets at concentrations less than 50 μg/kg of feed clearly impaired bone health in the weanling pig with respect to bone mineralization and mechanical strength. In a study by Liesegang et al. (2002), tibial BMC and BMD were highly correlated with bone ash content. In addition, Hayes (1976) reported that the concentrations of Ca and P in bone ash do not change in response to extreme shifts in nutrient intake, but the total amount of ash accumulated varies with nutrient status. This was not true in the present study, although Ca and P contents in terms of percentage of DM and the total amount of P were not affected by treatment. In addition, the content of ash in terms of percentage of DM was similar among the groups. In turn, ash weight, DM content, and the total amount of Ca corresponded very well with the treatment effects found in the tibia bones. Similar results were reported by Pointillart et al. (1995), who concluded that bone tissue was prob-
ably normally mineralized but that less total bone was formed, as demonstrated by the decreased BMC, BMD, and bone ash weight. This could also hold true for the results of this study. Crenshaw et al. (1981b) reported that, depending on the age of the pigs, different bones are appropriate for identifying responses to dietary treatments. According to their suggestion, long bones should be used exclusively to determine bone traits in weaning pigs. This seems to explain the low correlation between the results in the tibial and metatarsal bones.

In conclusion, this study demonstrates that dietary administration of 50 μg of vitamin D₃ or 25-OH-D₃ to the suckling piglet, but particularly when provided to the pregnant and lactating sow, is necessary to maintain proper functioning of vitamin D metabolism to ensure normal skeletal mineralization and possibly support immunological health in the young pig. Offspring of sows provided 25-OH-D₃ obtained a markedly improved vitamin D status, and the longer maintenance of this neonatal vitamin D status supports the idea of actively enhancing fetal storage of 25-OH-D₃ via the maternal diet.

**LITERATURE CITED**


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