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 diets1,2

L. A. Rortvedt and T. D. Crenshaw3

Department of Animal Sciences, University of Wisconsin, Madison 53706

ABSTRACT: Kyphosis is an idiopathic disease characterized by abnormal, outward spinal curvature. A spontaneous outbreak and subsidence of kyphosis over a 4-mo period in the University of Wisconsin Swine Research and Teaching Center herd coincided with an accidental omission of vitamin D3 in 1 of 2 premixes used in sow diets. This controlled experiment was conducted to determine whether vitamin D deletion from premixes used in sow diets would induce kyphosis in their offspring. Crossbred (Landrace × Large White), multiparous sows (n = 8) were fed corn–soybean meal diets supplemented with either 325 IU vitamin D3/kg (+D) or 45 IU vitamin D3/kg (−D) diet from breeding through lactation. The vitamin D concentrations duplicated formulations of diets fed during the earlier spontaneous outbreak. At weaning (approximately 4 wk), pigs were fed diets devoid of supplemental vitamin D and formulated to supply either 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP) until wk 9. At wk 9, all pigs were fed the HCaP diet until wk 13. No evidence of kyphosis was observed in pigs at weaning. Pigs produced by –D sows and fed LCaP diets exhibited a 17% incidence (4/23 pigs) of kyphosis at wk 9. At wk 13, the incidence of kyphosis had increased to 32% (6/19 pigs). Unexpectedly at wk 13, pigs produced by +D sows and fed LCaP diets exhibited a 26% incidence (5/19 pigs) of kyphosis. None of the pigs fed HCaP diets from wk 4 to 13 displayed kyphosis, regardless of maternal diets. Evidence of kyphosis was detected at a younger age if pigs were produced by sows fed –D diets. Whole body and femur bone mineral content determined with dual energy X-ray absorptiometry were reduced (P < 0.05) in pigs fed LCaP vs. HCaP diets, but pigs produced by –D sows were more severely affected. Femur bending moments were reduced (P < 0.05) at wk 9 and 13 in pigs fed LCaP vs. HCaP diets. At wk 13, pigs produced by –D sows and fed LCaP diets had reduced (P < 0.05) bone mineral density and femur yield bending moment compared with pigs from +D sows fed LCaP diets. In conclusion, the 20 to 30% incidence of kyphosis induced by altering vitamin D, Ca, and P concentrations in maternal and nursery diets mimics the incidence observed in spontaneous outbreaks in afflicted herds. A reproducible vitamin D-induced kyphosis in young pigs offers a suitable model to study skeletal tissue characteristics, fetal skeletal tissue development, and potential treatments for pigs and human patients afflicted by this disease.

Key words: calcium, kyphosis, phosphorus, pigs, skeletal integrity, vitamin D

INTRODUCTION

Kyphosis is an idiopathic disease characterized by an abnormal, outward curvature of the spinal column.

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3Corresponding author: tdcrensh@wisc.edu

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Kyphosis occurs sporadically in swine herds with 20 to 30% of pigs displaying symptoms within afflicted herds (Straw et al., 2009). The lesions associated with kyphosis predominate in the 14th to 16th thoracic vertebra and are associated with dysfunctional ossification of the epiphyses (Nielsen et al., 2005).

In 2008, the University of Wisconsin Swine Research and Teaching Center (SRTC) herd experienced an outbreak and subsidence of kyphosis (approximately 20% incidence) during a 4-mo period. Symptoms, exemplified in Figure 1, were first observed in growing pigs assigned to a class project, which involved feeding diets with marginal Ca and P concentrations. Multiple factors initially evaluated to identify a cause...
of the outbreak included genetics, molds, mycotoxins, and diet mixing errors. No new genetic lines were introduced into the SRTC herd before or during the kyphosis outbreak; therefore, this factor was eliminated. Assays of corn and complete diets failed to detect mycotoxins. Diet mixing procedures were verified through quality control records. Individual feed ingredients were evaluated and, eventually, an accidental omission of supplemental vitamin D<sub>3</sub> was discovered in a custom-mixed vitamin premix used for routine diets fed to animals of all ages in the entire SRTC herd over the 4-mo period. The outbreak and subsidence of kyphosis coincided with the period during which the deficient premix was fed to gestating sows that produced the affected pigs.

Therefore, the current study was designed to determine whether a reduction in supplemental vitamin D<sub>3</sub> in maternal diets would induce kyphosis in young pigs fed diets without supplemental vitamin D and marginal Ca and P concentrations. The objective was to duplicate the omission of supplemental vitamin D<sub>3</sub> in 1 of the 2 premixes used in sow diets and assess the incidence of kyphosis and skeletal integrity in offspring.

**MATERIALS AND METHODS**

All animal procedures were approved by the College of Agricultural and Life Sciences Animal Care and Use Committee, University of Wisconsin-Madison.

The current experiment was designed to assess the consequences of a supplemental vitamin D<sub>3</sub> omission from a vitamin premix used in diets fed to sows and young pigs. An overview of the experimental design that involved gestation, lactation, nursery, and grower phases is outlined in Figure 2. Crossbred (Landrace × Large White), multiparous sows (n = 8) were bred via AI with semen collected from Line 19 (PIC Inc., Hendersonville, TN) boars. All sows and boars were housed at the SRTC throughout the trial. At breeding, sows were randomly assigned (n = 4 per treatment) to 1 of 2 dietary treatments that varied in vitamin D<sub>3</sub> supplementation.

**Maternal Diets**

Sows were fed gestation and lactation diets routinely used at the SRTC (Table 1). Routine SRTC diets are formulated to supply either 325 IU vitamin D<sub>3</sub>/kg (+D) or 45 IU vitamin D<sub>3</sub>/kg (−D) in complete diets fed for the entire gestation and lactation phases. Nursery and grower diets were formulated to supply 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP; NRC, 1998) for 5- to 10-kg [Phase (Ph) 1] or 10- to 20-kg (Ph 2) pigs. The HCaP Ph 2 diet was fed during the 28-d grower phase. Supplemental vitamin D was not added to any of the nursery or grower diets for pigs.
treatment groups, but they were switched to the +D or –D lactation diets (Table 1) for 26 d. Sows were allowed continuous access to feed throughout lactation. Sows were allowed continuous access to water throughout gestation and lactation periods.

Pig Diets and Housing

Pigs were weaned after a 26 ± 1 d lactation period (wk 4) and randomly assigned within litter and maternal diet groups to 1 of 2 nursery treatment groups (Figure 2). Nursery diets (Table 2) included the UW VTMM-G premix without supplemental vitamin D₃ (0 IU) that duplicated the conditions imposed in 2008. Nursery diets were formulated to supply either 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP; NRC, 1998) for 5- to 10-kg (Phase 1) or 10- to 20-kg (Phase 2) pigs. Pigs were fed their assigned Phase 1 diet for 12 d followed by the assigned Phase 2 diet until termination of the trial. After a 40-d nursery period (66 ± 1 d; 9 wk of age) 3 pigs in each dietary treatment group were selected for dual energy X-ray absorptiometry (DXA) scans. After DXA scans, pigs were euthanized and bone samples collected. Observations of poor mobility, mild tetany, and ataxia in pigs fed LCaP diets at wk 9 led to a decision to switch all pigs to the HCaP Phase 2 diet (Table 2) for the remainder of the trial (wk 9 to 13). The HCaP Phase 2 diet was fed to pigs for the additional 28-d period to allow observations on the incidence of kyphosis in the remaining pigs. Final observations of pigs were recorded at 94 ± 1 d of age (wk 13).

Pigs (n = 4/pen) were housed in nursery pens (1.4 m x 2.8 m x 1.0 m) and provided free access to feed and water during gestation and –D lactation diets. In combination with the UW VTMM-S premix (see footnote 4) that was formulated to supply 45 IU vitamin D₃/kg diet, the gestation and –D lactation diets were formulated to supply either 120% of the Ca and P requirements for 5- to 10-kg (Phase 1) or 10- to 20-kg (Phase 2) pigs. Pigs were fed their assigned Phase 1 diet for 12 d followed by the assigned Phase 2 diet until termination of the trial. After a 40-d nursery period (66 ± 1 d; 9 wk of age) 3 pigs in each dietary treatment group were selected for dual energy X-ray absorptiometry (DXA) scans. After DXA scans, pigs were euthanized and bone samples collected. Observations of poor mobility, mild tetany, and ataxia in pigs fed LCaP diets at wk 9 led to a decision to switch all pigs to the HCaP Phase 2 diet (Table 2) for the remainder of the trial (wk 9 to 13). The HCaP Phase 2 diet was fed to pigs for the additional 28-d period to allow observations on the incidence of kyphosis in the remaining pigs. Final observations of pigs were recorded at 94 ± 1 d of age (wk 13).

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Table 3. Number of observations, average BW, and growth rates of nursery pigs from birth to wk 13 of age

<table>
<thead>
<tr>
<th>Maternal dietary treatments¹</th>
<th>Nursery dietary treatments²</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+D</td>
<td>–D</td>
</tr>
<tr>
<td>Number of pigs³</td>
<td>HCaP</td>
<td>LCaP</td>
</tr>
<tr>
<td>Birth⁴</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>4⁴</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>9⁵</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Pig BW, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth⁴</td>
<td>1.76</td>
<td>1.65</td>
</tr>
<tr>
<td>4⁶</td>
<td>7.13</td>
<td>6.84</td>
</tr>
<tr>
<td>9⁷</td>
<td>26.20</td>
<td>20.88</td>
</tr>
<tr>
<td>13⁸,⁹</td>
<td>51.1</td>
<td>40.4</td>
</tr>
<tr>
<td>BW gain, kg/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to 9⁶,⁷</td>
<td>0.477</td>
<td>0.351</td>
</tr>
<tr>
<td>4 to 13⁸,⁹</td>
<td>0.649</td>
<td>0.480</td>
</tr>
</tbody>
</table>

¹Diets for sow gestation and lactation treatments are shown in Table 1. Sows (4/treatment) were fed diets formulated to supply either 325 IU vitamin D₃/kg (+D) or 45 IU vitamin D₃/kg (–D) supplemental vitamin D for the entire gestation and lactation phases.

²Diets for pig nursery treatments were formulated to supply either 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP; NRC, 1998) for 5- to 10-kg (Phase 1) or 10- to 20-kg (Phase 2) pigs. Supplemental vitamin D was not added to Phase 1 or Phase 2 diets for pigs. A total of 102 pigs were produced by 8 sows (52 for +D; 50 for –D). Pre-weaning mortality was distributed across maternal groups (9 for +D; 8 for –D). Weights of 4 pigs were not recorded at wk 4 and 9. At weaning, pigs were housed with 4 pigs per pen and fed assigned diets until wk 9 and then moved into grower pens (8 pigs/pen) and fed the Phase 2 HCaP diet until wk 13.

³Pig BW at birth were based on the average BW of the nursery group ultimately assigned to the individual pig.

⁴Pig numbers reflect the number and BW of pigs weaned and used in subsequent data analysis. Pig BW at birth were based on the average BW of the nursery group ultimately assigned to the individual pig.

⁵Interaction between maternal and nursery treatments, P < 0.05 and *P < 0.10.

⁶Difference due to maternal treatment, P < 0.05.

⁷Difference due to nursery treatment, P < 0.05.

by 2.4 m) for the duration of the 5-wk nursery trial and then housed in 1.97 by 3.94 m grower pens for a subsequent 4-wk growth period. Pigs were allowed ad libitum access to feed and water throughout the nursery and grower phases of the experiment. Pig BW were recorded at birth, weaning (wk 4), and weekly during the nursery and grower periods. The number of pigs at each period is defined in Table 3.

Serum Calcium and Phosphorus

Vacuum tubes (no anticoagulant) were used for collection of venous blood samples (10 mL) from the brachial region of pigs at wk 4, 9, and 13. Blood samples were allowed to clot at room temperature and then centrifuged (735 × g) for 20 min. Serum was separated and stored frozen (–15°C) until Ca and P analysis. Aliquots of serum were diluted with a lanthanum chloride solution (2,000 μg La/mL), and Ca concentrations were determined using atomic absorption spectrophotometry procedures (Perkin-Elmer Model 2280, Perkin-Elmer Corp., Norwalk, CT). A separate aliquot of serum was precipitated with tricarboxylic acid (10% wt/vol), and the P concentration in the supernatant was determined using a molybdovanadate, colorimetric procedure. Absorbance was measured at 660 nm using a spectrophotometer (Gilford Spectrophotometer 260, Gilford Instrument Laboratories, Inc., Oberlin, OH).

Kyphosis Incidence

Observations for gross, visual symptoms of kyphosis in pigs were recorded at wk 4, 9, and 13. A subjective score of 1, 2, or 3 was assigned to each pig based on visual evidence of spinal column curvature. A score of 1 represented no evidence of curvature, and a score of 3 represented clear evidence of abnormal spinal column curvature. The intermediate score 2 was assigned to pigs with marginal curvature. Scores were recorded by 2 observers at each period. The final assessments of the incidence of kyphosis at wk 9 and 13 were based on animals with a score of 3 (gross evidence of kyphosis).

Table 4. Whole body dual energy X-ray absorptiometry (DXA) scans of pigs at 4 (weaning), 9, and 13 wk of age¹

<table>
<thead>
<tr>
<th>Maternal dietary treatments²</th>
<th>Nursery dietary treatments²</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+D</td>
<td>–D</td>
</tr>
<tr>
<td>BMC, g</td>
<td>HCaP</td>
<td>LCaP</td>
</tr>
<tr>
<td>4</td>
<td>132</td>
<td>142</td>
</tr>
<tr>
<td>9⁴,⁵</td>
<td>461</td>
<td>232</td>
</tr>
<tr>
<td>13⁸,⁹</td>
<td>1,070</td>
<td>796</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.303</td>
<td>0.327</td>
</tr>
<tr>
<td>9⁴,⁵</td>
<td>0.708</td>
<td>0.614</td>
</tr>
<tr>
<td>13⁸,⁹*</td>
<td>0.968</td>
<td>0.824</td>
</tr>
</tbody>
</table>

¹DXA scans were analyzed to determine bone mineral content (BMC) and bone mineral density (BMD). Values are averages of DXA scans of 3 pigs per treatment at wk 4 (weaning) and 9, and averages of 4, 10, 4, and 7 pigs for respective treatments at 13 wk of age.

²Maternal dietary treatments were formulated to supply either 325 IU vitamin D₃/kg (+D) or 45 IU vitamin D₃/kg (–D) supplemental vitamin D for the entire gestation and lactation phases. Nursery dietary treatments were formulated to supply either 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP; NRC, 1998) for 5- to 10-kg (Phase 1) or 10- to 20-kg (Phase 2) pigs. Supplemental vitamin D was not added to Phase 1 or Phase 2 diets for pigs. Pig BW at birth were based on the average BW of the nursery group ultimately assigned to the individual pig.

³DXA scans were analyzed to determine bone mineral content (BMC) and bone mineral density (BMD). Values are averages of DXA scans of 3 pigs per treatment at wk 4 (weaning) and 9, and averages of 4, 10, 4, and 7 pigs for respective treatments at 13 wk of age.

⁴Difference due to maternal treatment, P < 0.05.

⁵Interaction between maternal and nursery treatments, P < 0.05 and *P < 0.10.

⁶Difference due to maternal treatment, P < 0.05.
Whole Pig and Femur Dual Energy X-Ray Absorptiometry Scans

At wk 4, 9, and 13, pigs (n = 3, 3, or at least 4 per treatment, respectively) were randomly selected for scans and tissue collections. Additional pigs (Table 4) were subjectively selected at wk 13 for DXA scans based on marginal, visual symptoms of kyphosis. Selected pigs were held without feed overnight before scans. Pigs were initially anesthetized with sevoflurane (7%) and maintained at a level to prevent movement using isoflurane (2 to 4%) for the duration of the scan (usually less than 10 min). Pigs were scanned in a ventral position using DXA (software version 12.20; GE Lunar Prodigy, Waukesha, WI). The selection of a DXA scan mode was based on previous methods developed in our laboratory to optimize accuracy (Schneider and Crenshaw, 2005). Three scan modes were used based on pig BW (<20 kg, small animal mode; 20 to 46 kg, adult standard mode; and >46 kg, adult thick mode) to determine bone mineral content (BMC). In preliminary studies in our laboratory, DXA scans of pigs (weights ranging from 1.8 to 60 kg), using the selected scan modes for DXA, were accurate predictors of dissected skeletal ash (BMC = 3.3 + 1.001 × dissected skeletal ash; R² = 0.999). Whole body BMC (grams of bone ash per pig) and bone mineral density (BMD; grams of bone ash per square centimeter of skeletal tissue) were analyzed from the scan data. As such, BMC values reflect the amount of ash in the entire skeleton and BMD values reflect an adjustment in BMC for the size (area) of skeletal tissue.

Immediately after the DXA scans, pigs were euthanized and both femurs were collected. Excised femurs were subsequently scanned using DXA (small animal scan mode) to determine BMC and BMD. Femurs were sealed in plastic bags and stored at –15°C until mechanical test procedures were completed.

Geometrical and Mechanical Test of Femurs

Femurs were allowed to thaw at room temperature and adherent tissue was removed by dissection before mechanical tests. Each femur was positioned against a 90° angle and the length along an axial plane was measured from the most extreme contact point of the distal condyle to the proximal, greater trochanter. The midshaft, external diameters parallel and perpendicular to the direction of force application during the mechanical test were measured with a digital caliper. A 4-point bending test (Aiyangar et al., 2010) was applied and the load-deformation curve recorded using an instrument (Instron Universal Testing Machine, Model 5566; Canton, MA) with a 1,000-kg load cell. Femurs were positioned on a 4-point support apparatus to allow application of load with the femur supported in an anterior–posterior anatomical orientation. The rate of deformation was 5 mm/min. The lengths between the upper and lower fulcrum supports were recorded and used to calculate the bending moment and strain at the yield point of the load-deformation curve as described earlier (Crenshaw et al., 1981a).

Statistical Analysis

Data were analyzed by regression using GLM procedures (SAS Inst. Inc., Cary, NC). Dietary treatments were in a 2 × 2 factorial arrangement with 2 maternal treatments (+D vs. –D) each with 2 nursery treatments (HCaP vs. LCaP). Even though maternal diets were fed to sows, the sow responses to diets were not evaluated in this experiment. Therefore, the individual pig was considered the experimental unit. Only response criteria measured on individual pigs were considered. Pigs were initially assigned to nursery pens based on weaning weight, so a block effect of BW was fit as a fixed effect in the regression model. Differences because of sex were not considered in the analysis.

Results are reported as the average of pigs within a treatment group. Inferences about differences among treatment groups were based on orthogonal contrasts used to identify the main effects because of variation among maternal diets (+D vs. –D), effects due to nursery diets (HCaP vs. LCaP), and the interaction effects between maternal diets and nursery diets.

RESULTS

Two pigs from +D litters failed to survive until weaning. However, no other unplanned deaths occurred throughout the trial. At wk 9, pigs from –D litters fed LCaP diets during the nursery period displayed poor mobility, mild tetany, and ataxia. Therefore, as shown in Figure 2, all pigs were switched to the HCaP Phase 2 diet (Table 2) for the remainder of the trial. The HCaP Phase 2 diet (devoid of vitamin D supplementation) was fed to pigs for the additional 28-d period to allow observations on the incidence of kyphosis in the remaining pigs.

Kyphosis Incidence

The primary objective of this experiment was to determine the effects of supplemental vitamin D deletion in maternal diets on the incidence of kyphosis in their offspring. Pigs were subjectively scored based on visual evidence of kyphosis. No visual evidence of kyphosis was present in pigs at weaning. Pigs produced by –D sows and fed LCaP diets during a 9-wk nursery period exhibited a 17% incidence (4/23 pigs) of kyphosis (Figure 3). None of the pigs from –D sows fed HCaP diets or
pigs from +D sows, regardless of nursery diet, displayed
gross symptoms of kyphosis at wk 9. By wk 13, the inci-
dence of kyphosis in pigs from –D sows fed LCaP diets
increased to 32% as a result of 2 additional pigs (6/19
pigs) displaying symptoms. Unexpectedly at wk 13, pigs
produced by +D sows and fed LCaP diets exhibited a
26% incidence (5/19 pigs) of kyphosis. However, none
of the pigs fed HCaP diets from weaning until wk 13
displayed kyphosis, regardless of maternal diets. There-
fore, the amount of maternal vitamin D supplementation
appeared to alter the period of time required for pigs
fed nursery diets devoid of supplemental vitamin D and
marginal Ca and P concentrations to display kyphosis.
To our knowledge, this is the first report to document
diet-induced kyphosis in pigs.

Additional pig traits (i.e., pig BW, growth, serum
Ca and P concentrations, whole body skeletal mineral
content, measurements of femur mineral content, and fe-
mur mechanical properties) were assessed in an attempt
to further characterize the responses of pigs to dietary
treatments, especially the treatment groups that induced
kyphosis. Because of the relatively small number of ob-
servations, attempts were not made to conduct post hoc
analyses of responses within treatment groups to com-
pare pigs that displayed gross evidence of kyphosis with
those with no visual evidence of kyphosis.

Growth Responses

Average pig BW and cumulative growth rates from
weaning to wk 9 or 13 were summarized in Table 3. Sur-
prisingly, maternal diets affected (P < 0.05) pig BW and
growth for the entire 13-wk period. Growth of pigs pro-
tuced by sows fed –D diets was reduced by more than
10% compared with pigs produced by sows fed +D diets.
Differences in growth responses because of nursery diets
were expected and can most likely be attributed to the re-
duction in dietary P (rather than Ca) concentrations of the
LCaP diets. Pooled across maternal dietary treatments,
pigs fed LCaP diets experienced more than a 20% reduc-
tion (P < 0.05) in growth at wk 9 and 13.

Given the interrelationships among vitamin D, Ca,
and P, an interaction between maternal diets and nurs-
ery diets was expected. However, differences in growth
because of an interaction between maternal and nursery
diets were not detected at wk 9 or 13. Pigs fed HCaP
diets had approximately 0.10 kg/d reduced growth rates
if produced by –D vs. +D sows, but growth rates of pigs
fed LCaP diets were almost identical regardless of the
maternal diet fed. All pigs were fed the same diet (HCaP)
during the 9- to 13-wk period. However, the increase in
dietary Ca and P concentrations apparently did not al-
low recovery in growth responses from wk 9 to 13 even
though pigs approximately doubled their BW over this
period. The results did not provide evidence that pigs
from +D sows differed in their ability to recover from
suppressed growth compared with pigs from –D sows.

Whole Body Dual Energy X-Ray
Absorptiometry Responses

Whole-body BMC responses (Table 4) reflect simi-
lar patterns across dietary treatment groups as observed
in pig growth responses. During the trial, pig BMC in-
creased approximately 1.5- to 3.5-fold between wk 4
and 9 with another 2.5- to 3-fold increase between wk
9 and 13. No differences were detected in BMC of pigs
produced by sows fed +D vs. –D diets. Absolute values
of BMC, pooled across nursery dietary treatments, were
approximately 8% less at wk 9 and 5% less at wk 13 in
pigs produced by sows fed –D vs. +D diets. Therefore,
maternal diets did not appear to contribute to differences
in pig BMC. Differences were detected (P < 0.05) in
BMC between pigs fed HCaP vs. LCaP diets; however,
these differences were dependent on the maternal diet
(interaction between maternal diet and nursery diets; P <
0.05). At wk 9, pigs produced by +D sows only accumu-
lated approximately 50% as much BMC if fed LCaP vs.
HCaP diets, but pigs from –D sows only accumulated
approximately 30% as much BMC if fed LCaP vs. HCaP
diets. Therefore, at wk 9, the extra Ca and P supplied in
HCaP diets did not allow pigs from –D sows to recover
BMC to the same extent as pigs fed HCaP diets from +D
sows. At wk 13, a reversed pattern was detected. Pigs
from –D sows accumulated approximately 45% less
BMC if fed LCaP vs. HCaP diets, but pigs from +D sows
fed LCaP diets only accumulated approximately 25% less
BMC than pigs fed HCaP diets. Whether the shift
in BMC responses between the 9- and 13-wk periods reflects carryover responses from maternal diets or simply an age-related maturity of skeletal tissue mineralization cannot be determined from the current experiment.

Differences in BMC were confounded by differences in BW. Another trait, BMD, calculated from DXA scan procedures was used to correct for differences in skeletal size because BMD is derived by dividing BMC by the skeletal tissue area. Differences in BMD are associated with differences in the mineral density of bone matrix although DXA BMD fails to measure bone volume. Therefore, BMD is not a measure of density (i.e., amount per unit volume). The DXA BMD trait is used routinely in clinical medicine as a predictor of bone density.

In the current study, use of BMD to correct for differences in pig size did not nullify responses to maternal and nursery treatments. The magnitude in BMD differences between treatment groups was less than differences observed in BMC responses; however, responses were detected. At wk 9, pigs produced by –D sows had approximately 5% less (P < 0.05) BMD than pigs from +D sows. By wk 13, the BMD differences due to maternal diets were only approximately 2% and not different. Pigs fed LCaP diets had the BMD differences due to maternal diets were only approximately 2% and not different. Pigs fed LCaP diets had a 10 to 20% reduction in BMD values compared with pigs fed HCaP diets regardless of maternal treatments. The trend (P < 0.10) for a greater difference because of nursery diets in pigs produced by sows fed –D vs. +D diets was consistent with BMC observations; therefore, responses were not simply a reflection of differences in BW.

**Femur Traits**

Individual bones have commonly been used as response criteria to assess Ca and P requirements. In the current experiment, femurs were collected to allow comparisons across dietary treatment groups. Similar responses to maternal and nursery diets were evident in the femur BMC and BMD values (Table 5) as presented for whole body BMC and BMD values (Table 4). Femurs of pigs fed LCaP diets were approximately 5 (wk 9) and 12% (wk 13) shorter (P < 0.05) than femurs from pigs fed HCaP diets. No differences because of maternal diets were detected in femur lengths. The shorter femur lengths were consistent with smaller BW of pigs fed LCaP diets. Femurs of pigs fed HCaP diets. However, the midshaft diameters of femurs from pigs at wk 9 fed the LCaP diets tended to be larger (P < 0.10) than diameters of femurs from pigs fed HCaP diets. The differences in femur diameters were more evident in pigs fed LCaP diets produced by +D sows.

Because of differences in femur lengths among treatment groups, direct comparisons of the force (load) required for failure at the yield point in mechanical tests were not appropriate. Therefore, corrections for the length over which the load was applied must be made.

The bending moment is a measure of force corrected for the span over which the load was applied. Dramatic differences were detected in the yield bending moments (Table 5) at both wk 9 and 13. Femur yield bending moments were approximately 2- to 4-fold larger in pigs fed HCaP vs. LCaP diets at wk 9 and 13. At wk 9, no differences were detected in yield bending moments of femurs because of maternal diets. However at wk 13, larger differences were detected in yield bending moments of femurs from pigs fed HCaP vs. LCaP diets if pigs were produced from –D vs. +D sows (interaction between maternal and nursery treatments, P < 0.05). Femurs from pigs fed LCaP vs. HCaP nursery diets withstood more (P < 0.05) strain (a measure of the amount of strain at yield point on the load deformation curve adjusted for test length (Crenshaw et al., 1981a).

### Table 5. Mineral content, mechanical, and geometrical properties of femurs from pigs at 4 (weaning), 9 and 13 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>Age, wk</th>
<th>Maternal dietary treatments</th>
<th>Nursery dietary treatments</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>HCaP</td>
<td>LCaP</td>
<td>HCaP</td>
</tr>
<tr>
<td>Femur DXA scan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC, g</td>
<td>9, 3, 4, 5, 6</td>
<td>18.9</td>
<td>10.6</td>
<td>15.1</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td>13, 4, 5</td>
<td>40.2</td>
<td>29.9</td>
<td>41.2</td>
</tr>
<tr>
<td>Femur geometry</td>
<td></td>
<td>0.603</td>
<td>0.364</td>
<td>0.514</td>
</tr>
<tr>
<td>Length, mm</td>
<td>13, 4</td>
<td>127.0</td>
<td>117.0</td>
<td>122.0</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>13</td>
<td>160.8</td>
<td>143.0</td>
<td>153.8</td>
</tr>
<tr>
<td>Strain, mm/mm</td>
<td>13, 4, 6*</td>
<td>17.4</td>
<td>18.6</td>
<td>16.5</td>
</tr>
<tr>
<td>BM, kg cm²</td>
<td>13, 4, 5</td>
<td>20.4</td>
<td>21.0</td>
<td>21.9</td>
</tr>
<tr>
<td>3Femur mechanical test</td>
<td></td>
<td>874</td>
<td>213</td>
<td>624</td>
</tr>
<tr>
<td>Strain, mm/mm</td>
<td>13, 4, 5</td>
<td>1.378</td>
<td>1.028</td>
<td>1.904</td>
</tr>
<tr>
<td>Strain, mm/mm</td>
<td>13, 4, 5</td>
<td>0.131</td>
<td>0.156</td>
<td>0.091</td>
</tr>
<tr>
<td>Strain, mm/mm</td>
<td>13, 4, 5</td>
<td>0.075</td>
<td>0.157</td>
<td>0.128</td>
</tr>
</tbody>
</table>

1Femur dual energy X-ray absorptiometry (DXA) scans were analyzed to determine femur bone mineral content (BMC) and femur bone mineral density (BMD). Values are averages of femurs from the same pigs submitted for DXA scans with 3 pigs per treatment at wk 9, and 4, 10, 4, and 7 pigs for respective treatments at 13 wk of age.

2Maternal dietary treatments were formulated to supply either 325 IU vitamin D₃/kg (+D) or 45 IU vitamin D₃/kg (–D) supplemental vitamin D for the entire gestation and lactation phases. Nursery dietary treatments were formulated to supply either 120% of the Ca and P requirements (HCaP) or 80% of the Ca and P requirements (LCaP; NRC, 1998) for 5- to 10-kg (Phase 1) or 10- to 20-kg (Phase 2) pigs. Supplemenal vitamin D was not added to Phase 1 or Phase 2 diets for pigs.

3Differences due to BW block, P < 0.05.

4Differences due to treatment, P < 0.05 and *P < 0.10.

5Interaction between maternal and nursery treatments, P < 0.05 and *P < 0.10.

6Differences due to maternal treatment, P < 0.10.

7Midshaft diameter measured in anterior–posterior direction (i.e., direction parallel to that of applied force).

8Bending moment (BM) calculated using force at the yield point on the load deformation curve adjusted for test length (Crenshaw et al., 1981a).
bending during the mechanical test). A larger strain indicated that the bones bent more during mechanical tests.

**Serum Calcium and Phosphorus Concentrations**

Serum Ca and P concentrations at wk 4, 9, and 13 are presented in Figures 4 and 5, respectively. Serum from blood samples collected at wk 4 reflected responses to lactation because nursery diets had not been fed before blood samples were collected. At wk 4, serum Ca concentrations were greater (P < 0.05) in pigs from +D vs. –D sows, but the magnitude of difference was small. A greater magnitude of difference due to maternal diets (P < 0.05) was observed in serum P concentrations at wk 4 compared with the differences noted in serum Ca at wk 4.

At wk 9 and 13, serum Ca concentrations approached the upper physiological range (14 mg/dL). Pigs from –D sows had slightly reduced serum Ca concentrations at wk 9 compared with pigs from +D sows (P < 0.05), but serum Ca concentrations were greater in pigs fed LCaP vs. HCaP diets (P < 0.05). At wk 13, no differences in serum Ca concentrations were detected among treatment groups. In contrast to the differences among dietary treatments in growth and bone mineral traits described before, serum Ca concentrations were consistent with a strict homeostatic regulation of Ca, implying that serum Ca does not reflect growth and bone mineralization responses to dietary inputs.

The serum P concentrations appeared to reflect growth and bone trait responses to dietary treatments. At wk 9, differences due to maternal diets were detected (P < 0.05) in serum P. Pigs from –D sows had approximately a 10% reduction in serum P concentrations compared with pigs from +D sows. Serum P concentrations in pigs fed LCaP diets were approximately half that of pigs fed HCaP diets (P < 0.05). At wk 9, serum P concentrations of pigs fed LCaP diets approached the lower physiological range (5 mg/dL). At wk 13, serum P concentrations of pigs from –D sows were reduced approximately 20% if fed LCaP vs. HCaP nursery diets even though pigs in the LCaP nursery treatment group were fed the HCaP diets from wk 9 to 13 (Figure 2). At wk 13, all serum P concentrations were approximately equal or approaching the upper physiological range (10 mg/dL).

**DISCUSSION**

The 2008 spontaneous outbreak and subsidence of kyphosis (approximately 20% incidence) during a 4-mo period in the SRTC herd was eventually attributed to an accidental omission of vitamin D3 from a vitamin premix fed to the entire herd. The outbreak and subsidence of kyphosis in young pigs coincided with the period, during which the deficient premix was fed to gestating sows that produced the affected pigs. The current, controlled study confirmed that deletion of vitamin D from the vitamin premix induced kyphosis in young pigs at approximately the same incidence as observed in 2008. The vitamin D concentrations in maternal diets affected the period of time required for pigs to display visible symptoms of kyphosis. The time effect was consistent with an involvement of vitamin D supplementation in maternal diets as a predisposing factor in the development of skeletal defects in young pigs.

A reduction in dietary Ca and P concentrations was also a factor in the induction of skeletal defects. However, based on earlier research in our laboratory (Crenshaw, 1986; Aiyangar et al., 2010) and other reports as summarized by NRC (1998), the marginal dietary Ca...
and P concentrations used to induce kyphosis in the current experiment were not dramatic deficiencies and have not previously been implicated with kyphosis. Marginal dietary Ca and P concentrations may have been exacerbated by limited supplemental vitamin D. Combs et al. (1966) fed weaned pigs diets with various amounts of Ca (0.48, 0.88, and 1.32% of the diet) and vitamin D (0, 220, and 880 IU vitamin D$_3$/kg or UV radiation). Negative consequences in structural integrity were not reported for these pigs. However, diets for the sows that produced the pigs were not provided. Based on previous studies, an induction of kyphosis was not expected in the current experiment. Either marginal deficiencies of all 3 nutrients exacerbated response symptoms or the life-cycle phases in which the deficiencies imposed were critical. As will be discussed, the vitamin D status of the SRTC sows used in the current experiment may have contributed to the observed responses.

The results from this experiment offer new insights into the role of vitamin D on skeletal tissue development and mineral homeostasis. The role of maternal vitamin D status in fetal and neonatal skeletal tissue development needs to be clarified. Observations of kyphosis in young pigs fed diets without supplemental vitamin D over relatively short trial intervals were not expected. The vitamin D status of the SRTC research herd may have contributed to the unexpected responses. Since 2004, the SRTC herd diets have been intentionally formulated to provide minimal amounts of minerals and vitamins that approximate the NRC (1998) recommendations rather than amounts typically added as a safety margin in commercial swine production diets. The minimal vitamin D concentrations recommended for sow diets (200 IU D$_3$/kg) were based on extrapolation of research with rats because no reports of work with sows were available when the swine requirements were published (NRC, 1998). The observations made in the 2008 spontaneous kyphosis outbreak and the results reported herein were recently confirmed in a subsequent experiment with preliminary results reported by Rortvedt et al. (2011). These experiments conducted over 3 years provide a foundation for a repeatable model with which to evaluate the effects of sow vitamin D status on subsequent skeletal tissue development in young pigs.

In the current experiment, pig structural integrity was compromised by the lack of supplemental vitamin D in maternal diets. These results offer evidence that maternal vitamin D supply plays a critical role in the development of fetal skeletal tissue. The mechanisms by which vitamin D affects fetal skeletal development are not well understood. Research with rats (Haddad et al., 1971; Weisman et al., 1976; Clements and Fraser, 1988) and pigs (Goff et al., 1984) have provided evidence that vitamin D$_3$ and hydroxylated metabolites of vitamin D readily cross the placenta. Placental transfer infers that maternal vitamin D status has the potential to influence fetal vitamin D stores, thus impacting neonatal development. However, evidence to support a direct role of vitamin D in the functional development of fetal skeletal tissue is limited. Johnson et al. (1996) were able to detect a differential display of the 1,25-dihydroxyvitamin D$_3$ receptors (VDR) in fetal rat bones. The VDR were displayed in fetal tissues before ossification and in patterns consistent with skeletal tissue differentiation. Receptors were present on gestational d 13 in the developing vertebral column and in developing limbs but were not present until d 17 in calvaria. The presence of VDR receptors before ossification is consistent with a functional role for vitamin D in proliferation and differentiation of chondrocytes in fetal skeletal tissues. Additional research is needed to determine whether a deficiency of vitamin D during fetal development directly affects vertebrae in the deformed regions of the spinal column of pigs displaying kyphosis. Continued research in this area will aid in the understanding of the maternal–fetal vitamin D relationship.

An ability to induce a skeletal defect in offspring due to maternal supplementation offers an important response criterion to help establish maternal requirements and perhaps prevent unexplained skeletal disorders. Limited research was available at the publication of the swine nutrient requirements (NRC, 1998). To our knowledge only 2 studies with vitamin D supplements for sows have been published since 1998, but both of the studies failed to establish a minimal amount of supplemental vitamin D required for skeletal tissue development because other objectives were explored.

One recent study involved an assessment of gestating sows fed various dietary supplements of vitamin D (Lauridsen et al., 2010). Sows were fed diets that varied (200, 800, 1,400, and 2,000 IU vitamin D$_3$/kg) in vitamin D concentrations. A strong justification to establish a minimal amount of supplemental vitamin D was not established. Negative consequences were not reported in the sows or pigs produced by sows fed diets with 200 IU/kg concentrations of vitamin D. The authors concluded that diets with 1,400 IU vitamin D$_3$ should be fed to gestating sows. Conclusions were based on serum 25-hydroxyvitamin D$_3$ concentrations with no other response criteria to support the recommendation. No evidence was provided that a reduction in serum 25-hydroxyvitamin D$_3$ compromised bone mineralization.

In another recent study (Witschi et al., 2011), sows and their respective offspring were fed diets that varied (560 and 2,000 IU vitamin D$_3$/kg) in vitamin D concentrations. Pig growth and skeletal integrity traits were reported. Even though differences due to vitamin D supplements were detected in tibia BMD and bone strength responses, no evidence of a failure in skeletal integrity was reported.

Neither of the 2 recent reports involving vitamin D supplements of sow diets established a negative control
to validate the response criteria used as a basis for dietary recommendations. The importance of a negative control was recently recognized in efforts to establish human requirements. Efforts to justify requirements based on upper levels of serum 25-hydroxyvitamin D3 were not endorsed (Ross et al., 2011), but justifications based on phenotypic evidence of insufficiency (such as ultrasound measurements of fetal bone) were more readily accepted (Hewison and Adams, 2010).

The current experiment is certainly not the first experiment that involved feeding marginal concentrations of Ca, P, and vitamin D. However, to our knowledge, evidence of kyphosis has not been previously directly linked to these nutrients. Earlier studies (Nielsen et al., 2005; Holl et al., 2008; Straw et al., 2009) have characterized the symptoms, incidence, and possible contributing factors involved in outbreaks of kyphosis in herds. Conclusions in these previous studies were based on observations made on pigs already affected by kyphosis. No attempts were made to induce kyphosis under controlled conditions.

In an attempt to establish estimated heritability of kyphosis in pigs, Holl et al. (2008) used a subjective scoring system of 0 (normal) to 3 (severe) to assess the vertebral column in split carcasses from 2 pig populations. Based on this system in which less than 2.5% of the population displayed a score of 3 (severe kyphosis), the estimated heritability was 0.30 for the parent population and 0.32 for the F2 population. However, if kyphosis was considered a binary trait, in which only severe cases are considered kyphotic, heritability estimates were only 0.02 and 0.03, respectively. Additional evidence for the complexity of a genetic basis for kyphosis was provided by the same researchers (Lindholm-Perry et al., 2010) who concluded that kyphosis is under polygenic control. These divergent estimates of heritability and the polygenic basis for kyphosis infer the potential for epigenetic factors (such as nutrients) in cases of severe kyphotic lesions. The active form of vitamin D functions in cell signaling at the level of gene expression and thus, offers a potential dietary input for an epigenetic control.

Severe kyphotic lesions (Figure 1) have not been observed in pigs at the SRTC since the herd was established approximately 12 yr ago, except during the period of accidental omission of vitamin D in 2008 and subsequent controlled experiments. The SRTC herd is maintained with minimal introduction of new litters as cesarean-derived pigs from the same parent herd. Semen used for AI is collected from boars produced and raised within the SRTC. Therefore, observations are made on all parent lines. These management procedures are imposed primarily to maintain herd health and to provide a consistent supply of animals for research projects. Even though a genetic predisposition might be present in the SRTC herd, relatively minor changes in nutrient concentrations were able to induce dramatic differences in kyphosis and associated skeletal mineral responses.

Given the limited evidence for a strong genetic component and the known genetic management of the SRTC herd, a simple genetic basis was discounted as the cause of the 2008 outbreak and subsidence of kyphosis. The current results support a role for a nutrient-induced kyphosis model.

An additional outcome of the current study involved the potential for the use of DXA scans as a noninvasive measurement of spinal curvature before visual symptoms of kyphosis were evident. A noninvasive measurement would allow early detection, a more quantitative assessment of kyphosis, and a method to monitor progression of kyphosis. In addition, the noninvasive method would allow assessment of the efficacy of treatments imposed to correct spinal column defects. A preliminary assessment of the use of DXA scans to measure spinal column curvature has recently been reported (Rortvedt et al., 2011). The results in the current study indicated that the femur provides a reliable prediction of whole body BMC, which is consistent with conclusions made in earlier studies (Crenshaw et al., 1981b) and more recent results (Crenshaw et al., 2009).

Factors that contribute to sporadic kyphosis outbreaks have not been thoroughly studied due to the lack of a suitable model that allows a controlled method to induce kyphosis with a predictable incidence and the lack of a noninvasive method to quantify responses. The current results offer potential for development of a model to study kyphosis and possible cures for the disease. Symptoms of kyphosis in pigs are comparable to a human form of the disease, Scheuermann’s Juvenile kyphosis, as described by Sorenson (1964). Similar to observations in swine, orthopedic surgeons continue to search for the cause and preventive treatments for patients with kyphosis. The swine kyphosis model also offers potential to gain additional insights into the role of vitamin D in signals that control bone and connective tissue integrity.

In conclusion, results from the current experiment provided evidence that nutritional status altered the expression of kyphosis in young pigs. The incidence of kyphosis mimicked the rate observed in spontaneous outbreaks in afflicted swine herds. Pigs fed diets with no supplemental vitamin D and marginal concentrations of Ca and P displayed kyphosis approximately 1 mo earlier if produced by sows fed diets with only 45 IU vitamin D3 vs. 325 IU vitamin D3/kg. The earlier evidence of kyphosis in pigs produced by sows fed vitamin D-deficient diets raised questions about implications of vitamin D in fetal development and subsequent skeletal growth. Relatively minor changes in dietary Ca and P concentrations (80 vs. 120% of requirements) altered responses in
skeletal integrity. Pigs fed diets that supplied only 80% of dietary Ca and P requirements displayed kyphosis and a reduction in skeletal mineral content. Diets that supplied 120% of Ca and P requirements prevented visible evidence of kyphosis in pigs regardless of maternal diet. However, effects of maternal vitamin D diets were still evident in skeletal mineral traits of pigs fed diets that supplied only 80% of Ca and P requirements. Vitamin D-induced kyphosis in young pigs offers a suitable model to study the skeletal tissue characteristics and developmental signals involved in the disorder. Additionally, the model offers a method to assess potential treatments and procedures designed to prevent or reverse the consequences of kyphosis in pigs and humans afflicted by this idiopathic disease.

LITERATURE CITED


