Effect of Dietary Flourine on Growth, Blood and Bone Characteristics of Growing-Finishing Pigs

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EFFECT OF DIETARY FLUORINE ON GROWTH, BLOOD AND BONE CHARACTERISTICS OF GROWING-FINISHING PIGS

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ABSTRACT

Three hundred eighty-four growing-finishing pigs were used in two experiments to determine the effect of dietary fluorine (F) on growth, blood and bone physical characteristics. Fourteen dietary treatments were formulated by supplementing F (as NaF) to a milo-soybean meal basal diet (7 ppm F) to provide levels of 7, 132, 257, 382, 507 and 632 ppm F for Exp. 1, and 7, 25, 43, 61, 79, 97, 115 and 133 ppm F for Exp. 2. Average daily gain (ADG) and average daily feed intake (ADFI) were not affected (P>.09) when F was fed at levels between 7 and approximately 132 ppm. Average daily gain and ADFI were reduced (P<.0001) for pigs consuming diets with F concentrations greater than 132 ppm (Exp. 1). Feed conversion was not affected (P>.17) by any level of F fed. Serum F and alkaline phosphatase concentrations increased (P<.01) with increasing dietary F levels. Serum and bone Ca and P concentrations were not affected (P>.13) by dietary F levels (Exp. 1). In Exp. 1 and 2, bone F increased (P<.0001) and metatarsal stress and modulus of elasticity decreased (P<.0001) as level of F increased in the diet. Bone thickness decreased quadratically (P<.02) in Exp. 1 and linearly (P<.0007) in Exp. 2 with increased dietary F levels. Scanning electron microscopy showed an increase in porosity of bones from pigs fed the higher levels of F. Growing-finishing pigs were able to tolerate approximately 132 ppm F for growth, but all of the F levels (>7 ppm) fed in these two experiments affected bone integrity.

(Key Words: Pigs, Fluorine, Bones.)

Introduction

Whether or not the therapeutic administration of fluorine (F) produces bones of greater strength and weight-bearing capacity is an important question. The overall incidence of senile osteoporosis has been reported to be less in persons ingesting low levels of F (Leone et al., 1955; Rich and Ensinck, 1961; Bernstein et al., 1966; Alffram et al., 1969). However, the ingestion of large amounts of F by cattle causes weakness of bones and intermittent lameness (Jones, 1972; Suttie et al., 1972). Similarly, a reduced breaking strength was found in bones of swine fed 650 to 970 ppm F, but breaking strength was not consistently affected when swine were fed 290 and 580 ppm F (Kick et al., 1933).

The use of the pig as a model for determining the effects of F on bone integrity has been minimal (Kick et al., 1933; Comar et al., 1953; Spencer et al., 1971; Forsyth, 1972; Speirs, 1979). The proposition that F can be beneficial for increasing bone breaking strength (without inhibiting growth or Ca:P balance) and, perhaps, preventing the occurrence or reducing the incidence of osteoporosis has not been thoroughly investigated. Therefore, two experiments were conducted with growing-finishing pigs to determine the effect of dietary F on weight gain, feed conversion, and blood and bone characteristics.

Experimental Procedures

Facilities and Animals. The pigs used in both experiments were housed in the same naturally ventilated, modified-open-front research facility. The building contained 24 pens (50% slatted and 50% solid floors), each measuring 1.8 m in width and 3.7 in length.

One hundred ninety-two crossbred pigs, with an average initial weight of 18.6 kg for Exp. 1 and 14.4 kg for Exp. 2, were allotted randomly...
within weight class groups to their respective dietary treatments with eight pigs per pen in each experiment. Only barrows were used in Exp. 1; sex was balanced within pen, within replication in Exp. 2. Experiment 1 was conducted for a period of 166 d; Exp. 2 for 133 d. Individual pig weights and pen feed consumption data were collected biweekly.

**Diets.** The composition of the basal diet for both experiments is shown in table 1. The milo-soybean meal diet was formulated to contain 15% crude protein, .65% Ca, .50% P and 7 ppm F. There were six dietary treatments in Exp. 1 and eight dietary treatments in Exp. 2. Fluorine (as NaF) was supplemented to the basal diet to provide levels of 7, 132, 257, 382, 507 and 632 ppm total F and 7, 25, 43, 61, 79, 97, 115 and 113 ppm total F for Exp. 1 and 2, respectively. Water consumed by the pigs analyzed .06 ppm F. Feed samples were taken biweekly and analyzed for crude protein, Ca, P and F (AOAC, 1980).

**Blood Collection and Analysis.** At the termination of the experiments, 40 ml of blood were drawn from each pig, using sterile collection tubes. Serum was separated by centrifugation and analyzed for F and alkaline phosphatase (AKP) concentrations by the procedures of Fuchs (1975) and Morgenstern et al. (1965), respectively. Serum AKP was determined on the day of bleeding to avoid possible loss of enzyme activity. Serum Ca and P concentrations were determined by the procedure of Kessler and Wolfman (1964).

**Bone Collection and Analysis.** Rear feet were collected from each pig (weighing at least 85 kg) at slaughter and stored at -18 C. The left rear foot was thawed at room temperature, and the third and fourth metatarsals were excised by manually removing the surrounding soft tissue. The bones were stored at -18 C in a moisture-proof plastic bag prior to being thawed at 22 C and subjected to physical measurements with an Instron Universal Testing Machine®. The same bones were then soaked in methanol for 24 h, extracted with anhydrous ethyl ether, dried at 105 C, weighed and the inside and outside diameters were measured. Finally, the bones were ashed at 700 C for 24 h. Concentrations of Ca and P in the bone ash were determined by the procedure of Frankel et al. (1970) and bone F concentration by the procedure of Singer and Armstrong (1968).

Maximum stress, modulus of elasticity and cortical bone thickness were calculated for each bone according to methods developed by Crenshaw et al. (1981). Average values for the third and fourth metatarsals were used for statistical analysis of the bone data.

The fourth metatarsal of the right rear foot was excised as described previously. This bone, from six pigs fed each of four dietary F levels (7, 132, 382 and 632 ppm; Exp. 1) and from six pigs fed each of eight dietary F levels (7, 25, 43, 61, 79, 97, 115 and 133 ppm; Exp. 2), was chosen randomly for histological evaluation. A 1-mm cross-section of bone from the midshaft of the fourth metatarsal was embedded in BioPlastic®, and, subsequently, a 50-μm tissue section was taken from this sample, stained with VonKossa stain (5% silver nitrate solution for 30 min, and 5% basic fuchsin solution for 10 min) and mounted on a glass microscope slide. The slides were observed under low power (4.2× magnification) on a dissecting microscope,

### TABLE 1. COMPOSITION OF BASAL DIET (EXP. 1 AND 2) 

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground milo</td>
<td>77.61</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>18.92</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.52</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
<td>.65</td>
</tr>
<tr>
<td>Salt</td>
<td>.25</td>
</tr>
<tr>
<td>Trace mineral premixb</td>
<td>.05</td>
</tr>
<tr>
<td>Vitamin premixcd</td>
<td>1.00</td>
</tr>
</tbody>
</table>

100.00

*aDiet formulated to contain 15% crude protein, .65% calcium, .50% phosphorus and 7.0 ppm fluorine.

bContributed the following in ppm: Zn, 100; Fe, 50; Mn, 27.5; Cu, 5; I, .75.

CContributed the following per kg of diet: vitamin A, 3,300 IU; vitamin D, 550 IU; vitamin E, 11 IU; riboflavin, 2.2 mg; d-pantothenic acid, 9.9 mg; niacin, 17.6 mg; choline chloride, 220 mg; vitamin B₁₂, .022 mg; ethoxyquin, 4.4 mg. Chlortetracycline, 55 g/metric ton.

NaF added to vitamin premix to provide F concentrations in the diets of 7, 132, 257, 282, 507 and 632 ppm for Exp. 1, and 7, 25, 43, 61, 79, 97, 115 and 133 for Exp. 2. (indicated in table 2).

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4 Model TM 1123, Instron Corp., Canton, MA 02021.
characterized by digitizing morphometry (image analysis)\textsuperscript{6}, and bone area and bone width were determined. Percentage of osteoid of the periosteal and endosteal surfaces were determined with a compound microscope (125× magnification).

The endosteal surface of bones from animals fed 7, 132, 382 and 632 ppm F (Exp. 1) were examined by scanning electron microscopy to determine general changes in bone integrity with levels of F.

\textit{Statistical Analysis.} Feed intake, weight gain, feed conversion efficiency, blood and macroscopic bone data were analyzed as a randomized complete block design. The main effect of F level was divided into single-degree-of-freedom orthogonal regression contrasts (Steel and Torrie, 1980). A general linear model procedure was used for computation (SAS, 1979).

\textbf{Results and Discussion}

\textit{Performance Data.} The effects of dietary F on weight gain, feed intake and feed conversion for Exp. 1 and 2 are summarized in table 2. Average daily gain and average daily feed intake were not affected when F was fed at levels up to approximately 132 ppm in Exp. 1 or 133 ppm in Exp. 2. However, weight gain and feed intake were significantly reduced (linear, \(P<.0001\); quadratic, \(P<.001\)) for pigs consuming F levels greater than 132 ppm (Exp. 1). None of the differences in feed-to-gain ratios was significant (\(P>.17\)) among treatments in either experiment. These results were consistent with those of Kick et al. (1933, 1935) and Comar et al. (1953), who reported that weight gain and feed intake of growing-finishing pigs decreased as level of dietary F increased from 200 to 970 ppm, regardless of F source. Fargo et al. (1938), however, suggested that 140 ppm F or less (fed as rock phosphate) was beneficial for growth and feed utilization of pigs.

Research with other species (NRC, 1974; 1980) has indicated that a common response noted with fluoride-treated animals is a consequent decline in growth rate from a decrease in feed consumption. Disturbances in carbohydrate metabolism may account for the depressed growth rate in the pigs fed the various levels of

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Criterion & Exp. 1 & & & & \multicolumn{2}{|c|}{Exp. 2} \\
\hline
 & 7 & 25 & 43 & 61 & 97 & 115 & 132 & 157 & 257 & 382 & 507 & 632 \\
\hline
Weight gained, kg/d & \textsuperscript{a} & .69 & .68 & .68 & .67 & .67 & .65 & .65 & .65 & .65 & .65 & .65 \\
Feed intake, kg/d & \textsuperscript{a} & 2.66 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 & 3.86 \\
Feed-to-gain ratio & & 3.69 & 5.48 & 4.87 & 4.87 & 4.87 & 4.87 & 4.87 & 4.87 & 4.87 & 4.87 & 4.87 \\
\hline
Weight gain, kg/d & \textsuperscript{a} & .69 & .70 & .70 & .69 & .69 & .69 & .69 & .69 & .69 & .69 & .69 \\
Feed intake, kg/d & \textsuperscript{a} & 2.34 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 & 2.36 \\
\hline
\textsuperscript{a}Mean of 5 pigs, \textsuperscript{b}Mean of 3 pigs, \textsuperscript{c}Mean of 2 pigs, \textsuperscript{d}Quadratic effect of F (\(P<.0001\)), \textsuperscript{e}Coefficient of variation (\%).
\end{tabular}
\caption{Effect of dietary fluoride on performance of growing-finishing pigs (Exp. 1 and 2).}
\end{table}

\textsuperscript{6}BIOQUANT, R and M Biometrics Corp., Nashville, TN 37209.
F, because the level of glucose-6-phosphate dehydrogenase is decreased and glycogen turnover is decreased in animals with fluorosis (Zebrowski et al., 1964; Carlson and Suttie, 1966).

**Blood Data.** The effects of dietary F on serum F, AKP, Ca and P concentrations, for Exp. 1 and 2, are reported in table 3. Serum F concentrations increased linearly (P<.0001) with increasing dietary F levels in both experiments. Serum AKP concentrations increased in Exp. 1 (linear, P<.0001; quadratic, P<.001) and in Exp. 2 (P<.01) as level of dietary F increased. Serum Ca and P concentrations were not affected (P>.13) by F treatment (Exp. 1). The increases in serum F concentrations observed in these experiments were consistent with the increases in serum or plasma F concentrations reported by other researchers (Kick et al., 1935; Armstrong et al., 1964, 1966; Carlson, 1966; Shearer and Suttie, 1967; Simon and Suttie, 1968; Singer and Armstrong, 1974; Hahn and Guenter, 1981; Richards et al., 1983). The data confirm that plasma concentrations of F increase in response to an increase in dietary F concentration.

The increases in serum AKP concentrations observed in the experiments reported herein are similar to increases reported by others (Phillips, 1932; Kick et al., 1935; Motzok and Branion, 1958; Miller et al., 1977; Farley et al., 1983), and may have indicated that proper bone mineralization did not occur when F was ingested at high dietary levels. In contrast, other researchers (Maurer and Day, 1957; Spencer et al., 1971; Jowsey et al., 1972; Ream, 1981) have reported that F ingestion does not affect serum AKP activity.

Research with various animal species, including humans, has shown no differences in serum Ca and P levels (Kick et al., 1935; Faccini, 1969; Spencer et al., 1971; Jowsey et al., 1972; Ream, 1981) or Ca:P ratio (Chan et al., 1973; Shupe et al., 1981) due to F treatment. Apparently, the body's homeostatic regulation of Ca and P is not disturbed even by ingestion of toxic levels of F.

**Bone Data.** The results of the macroscopic bone properties measured in Exp. 1 and 2 are summarized in table 4. Mean values are presented for bone data from pigs on all treatments of Exp. 1 and 2 but statistical significance is reported only for those animals consuming diets with F concentrations <382 ppm. The marked reduction in feed consumption, and
## Table 4. Effect of Dietary Fluorine on Bone Properties of Growing-Finishing Swine (Exp. 1 and 2)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Dietary F, ppm</th>
<th>CV^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Exp. 1bc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Ca, %</td>
<td>37.9</td>
<td>38.2</td>
</tr>
<tr>
<td>Bone P, %</td>
<td>18.8</td>
<td>18.8</td>
</tr>
<tr>
<td>Bone F^d, %</td>
<td>.02</td>
<td>.51</td>
</tr>
<tr>
<td>Bone ash^e, %</td>
<td>61.7</td>
<td>62.8</td>
</tr>
<tr>
<td>(dry, fat-free)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone wall thickness^f, cm</td>
<td>.22</td>
<td>.20</td>
</tr>
<tr>
<td>Metatarsal stress^g, kg/cm^3</td>
<td>683</td>
<td>547</td>
</tr>
<tr>
<td>Modulus of elasticity^h, kg/cm^2</td>
<td>790</td>
<td>709</td>
</tr>
<tr>
<td>Exp. 2^g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone, F^d, %</td>
<td>.02</td>
<td>.11</td>
</tr>
<tr>
<td>Bone ash^i, %</td>
<td>61.6</td>
<td>62.0</td>
</tr>
<tr>
<td>Bone wall thickness^j, cm</td>
<td>.21</td>
<td>.21</td>
</tr>
<tr>
<td>Metatarsal stress^k, kg/cm</td>
<td>821</td>
<td>738</td>
</tr>
<tr>
<td>Modulus of elasticity^l, kg/cm^2</td>
<td>1028</td>
<td>973</td>
</tr>
</tbody>
</table>

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^aCoefficient of variation(%).
^bOnly bone data from pigs fed diets with 0 to 375 ppm F were analyzed statistically because only 25% of the pigs fed diets containing ≥500 ppm F reached market wt.
^cData are the mean of values for the third and fourth metatarsal bones.
^dLinear effect of F (P<.0001).
^eQuadratic effect of F (P<.0001).
^fQuadratic effect of F (P<.02).
^gMean of 20 pigs fed 7 ppm F; 24 fed 25 ppm F; 18 fed 43 ppm F; 19 fed 61 and 79 ppm F and 21 fed 97, 115 and 133 ppm F.
^hLinear effect of F (P<.0003).
^iQuadratic effect of F (P<.003).
consequent decrease in growth rate of animals fed 507 and 632 ppm F, prevented 48 of the 64 pigs fed these two treatments from being slaughtered for bone collection at a constant average age and weight.

Bone F, as a percentage of the ash, increased linearly and quadratically (P<.0001; both experiments) as dietary F increased. In Exp. 1, percentage of bone ash responded quadratically (P<.0001) to F levels. That is, percentage of ash was the highest in bones from pigs fed 132 or 257 ppm F compared with bones from pigs fed 7 or 382 F. Similarly, in Exp. 2, percentage of bone ash increased (linear, P<.0001; quadratic, P<.003) with increasing dietary F levels. Bone Ca and P (Exp. 1) were not affected by dietary fluorine.

Bone wall thickness of pigs in Exp. 1 responded quadratically (P<.02) to increasing dietary F levels, but there was a significant linear decrease (P<.0003) in bone wall thickness of pigs fed the lower levels of F in Exp. 2 (table 4). When F was fed at levels up to approximately 132 ppm in the diet, cortical bone wall thickness decreased (both experiments). Metatarsal stress (force per unit of bone area) and modulus of elasticity (capacity of the bone to return to its original shape after it has been deformed by a force) were significantly reduced (P<.0007) in all pigs fed F levels greater than 7 ppm (Exp. 1 and 2).

Results of histological evaluation of bone sections by digitizing morphometry for the two experiments are reported in table 5. Histological evaluation of cortical bone properties provided additional evidence in support of the decreased (P<.0004) bone width (thickness) observed macroscopically. Cortical bone area, as determined by image analysis, had a tendency to increase (P<.09) in Exp. 1, but decreased (P<.01) in Exp. 2 with increasing dietary fluorine levels. The amount of osteoid present on the periosteal bone surface was numerically greater for pigs fed the higher F levels in both experiments. The larger percentage of osteoid on the periosteal surface of bones from those pigs fed the highest levels of F (Exp. 1) may explain the greater bone area observed. The percentage of osteoid on the endosteal bone surface did not change (P>.90) as a result of F treatment.

These data suggest that matrix formation continued at a normal (or accelerated) rate on the periosteal surface of bone from pigs fed supplemental F, and that calcification of bone was less than normal. As bone thickness and bone area decreased in pigs fed levels of F up to 132 ppm, it is possible that both matrix formation and calcification were inhibited or did not occur in balance with increased endosteal resorption. This hypothesis is supported by the increase (P<.0005) in marrow cavity diameter of bones from pigs on Exp. 2 and the increased serum AKP concentrations observed for both experiments.

In an attempt to characterize some of the differences in bone stress and modulus of elasticity, scanning electron microscopy was conducted on cross-sections of the metatarsals from pigs on Exp. 1. Generally, severity of bone lesions relates to the structure and function of bones and to the amount of stress and strain placed on specific areas in the bones. It would follow then, that because of functionality, the metatarsals should be susceptible to severe lesions. Fluorotic bones are usually chalky-white and have a roughened periosteal surface (NRC, 1974, 1980). These characteristics were evident in a large proportion of the bones from pigs in Exp. 1 (figures 1, 2 and 3). Although not measured, it appeared that the severity of bone lesions or exostoses was greater at the highest levels of F fed. No characteristic lesions were observed on the bones from pigs in Exp. 2 that had been fed lower levels of fluorine.

Porous areas with excessive bone resorption or accelerated remodeling occur when bone growth and remodeling fail to keep a proper balance between bone resorption and accretion. The electron micrographs (figures 1, 2 and 3) of bones from pigs on Exp. 1 show greater porosity and characteristics osteosclerotic changes as F consumption increased. Ream (1983), using a scanning electron microscope, made similar observations with rats.

According to Shupe (1980), the major bone changes associated with F occur on the periosteal surface. And, in some advanced cases of F toxicosis, endosteal proliferation may occur with encroachment of bone on the marrow cavity of some bones. One or more of the following conditions may occur: osteosclerosis, osteoporosis, osteophytosis and (or) osteomalacia.

The results of the experiments reported herein (table 5) showed an increase of periosteal osteoid formation and are consistent with the findings of Ream (1979, 1981) and Reid et al. (1984). Ream (1979) reported that periosteal osteoid production increased, while the rate of mineralization decreased in bones from rats fed
TABLE 5. HISTOLOGICAL EVALUATION OF CORTICAL BONE PROPERTIES (EXP. 1 AND 2).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>7</th>
<th>25</th>
<th>43</th>
<th>61</th>
<th>79</th>
<th>97</th>
<th>115</th>
<th>132</th>
<th>133</th>
<th>382</th>
<th>632</th>
<th>CV²</th>
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<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Bone width b, mm</td>
<td>2.04</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bone area b,c, mm²</td>
<td>89.4</td>
<td>84.6</td>
<td>92.0</td>
<td>100.8</td>
<td>14.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Periosteoid d, %</td>
<td>50.6</td>
<td>53.6</td>
<td>54.3</td>
<td>59.2</td>
<td>6.8</td>
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<tr>
<td>Endosteoid d, %</td>
<td>42.7</td>
<td>43.8</td>
<td>43.8</td>
<td>43.1</td>
<td>27.3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Bone width b,c</td>
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<td>1.89</td>
<td>1.97</td>
<td>1.89</td>
<td>2.01</td>
<td>1.77</td>
<td>1.78</td>
<td>1.71</td>
<td>8.5</td>
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<td>Bone area b,c, mm²</td>
<td>96.7</td>
<td>87.6</td>
<td>84.9</td>
<td>85.8</td>
<td>86.3</td>
<td>82.3</td>
<td>76.7</td>
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<tr>
<td>Periosteoid d</td>
<td>52.3</td>
<td>52.9</td>
<td>54.2</td>
<td>55.9</td>
<td>5.8</td>
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<tr>
<td>Endosteoid d</td>
<td>47.5</td>
<td>48.0</td>
<td>46.7</td>
<td>47.2</td>
<td>7.0</td>
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</tbody>
</table>

aCoefficient of variation (%).
bMean of values for six pigs per treatment.
cLinear effect of F (P<.09).
dMean of values for two pigs per treatment.
eLinear effect of F (P<.004).
fLinear effect of F (P<.01).
gOnly bones from F levels of 7, 25, 72, 133 ppm were evaluated.
Figure 1. Electron micrographs (magnified 18 to 19 times) of the endosteal surface of bones from pigs receiving various levels of F. From left to right (upper and lower rows, respectfully), dietary F levels were 7 (control), 132, 382 and 632 ppm. Note the greater porosity of the bones fed the higher levels of F.
Figure 2. Electron micrographs (magnified 150 times) of the endosteal surface of bones from pigs receiving various levels of F. From left to right (upper and lower rows, respectfully), dietary F levels were 7 (control), 132, 382 and 632 ppm. Note the greater array of disorganization of the bones fed the higher levels of F.
Figure 3. Electron micrographs (magnified 440 to 450 times) of the endosteal surface of bones from pigs receiving various levels of F. From left to right (upper and lower rows, respectively), dietary F levels were 7 (control), 132, 382 and 632 ppm. Note the rougher surface and chalky appearance of the bones fed the higher levels of F.
120 ppm F (as NaF) for 28 d. Additionally, he reported that since bone surface formation prevailed over intracortical resorption, total bone mass increased. This may explain the tendency for the metatarsal area and wall thickness to increase in pigs fed the higher levels of F in Exp. 1. However, since no changes were evident in endosteal osteroid formation or other bone rarefying diseases. Because bone integrity deteriorates at levels less than the F present in typical corn-soybean meal diets it may be advisable to consider widening the P:F ratio in mineral phosphate supplements from the current 100:1 recommendation (AAFCO, 1986).

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