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Osteopenic Disease in Growing Pigs: Diagnostic Methods Using Serum and Urine Calcium and Phosphorus Values, Parathormone Assay, and Bone Analysis

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Abstract

This research was performed to evaluate the utility of several serum and urine parameters as well as bone ash and plasma parathormone assay to diagnose and monitor diet-related osteopenia in growing pigs. Five diets were tested as follows: calcium-deficient, phosphorus-replete; moderate-deficiency of calcium and phosphorus; marked deficiency of calcium and phosphorus; calcium replete, phosphorus deficient; and vitamin D deficient. Parameters monitored included serum calcium and phosphorus as well as ratios of urine calcium to creatinine, phosphorus to creatinine, calcium to phosphorus, and percent fractional excretions of calcium and phosphorus. Plasma parathormone (PTH) levels were monitored in 2 of 3 experiments. Osteopenic bone differences at necropsy were evaluated by bone density, percent ash, ash per milliliter bone, calcium per milliliter bone, and phosphorus per milliliter bone. Marked change in urine mineral parameters, especially the calcium-to-phosphorus ratio, typically occurred within 1 to 2 days of treatment and preceded significant change in serum mineral or plasma PTH by 2 to 3 weeks. When monitored, plasma PTH levels were elevated following treatment, which confirms the hyperparathyroid state induced by the test diets. Significant differences in bone mineralization between control and treatment diets at necropsy were generally observed. The results of this study indicate that the analysis of urine minerals offers an early, noninvasive technique to investigate diet-associated osteopenic disease in growing pigs, which can be supported further by bone mineral analysis at postmortem using techniques herein described. Several urine mineral reference intervals for application to field investigations are included. Research into application of similar techniques to evaluate calcium and phosphorus homeostasis in pigs of all ages, including gestating and lactating gilts and sows, appears warranted.

Disciplines

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Osteopenic disease in growing pigs: diagnostic methods using serum and urine calcium and phosphorus values, parathormone assay, and bone analysis

Wayne A. Hagemoser, Jesse P. Goff, Thomas P. Sanderson, Joseph S. Haynes

Abstract. This research was performed to evaluate the utility of several serum and urine parameters as well as bone ash and plasma parathormone assay to diagnose and monitor diet-related osteopenia in growing pigs. Five diets were tested as follows: calcium-deficient, phosphorus-replete; moderate-deficiency of calcium and phosphorus; marked deficiency of calcium and phosphorus; calcium replete, phosphorus deficient; and vitamin D deficient. Parameters monitored included serum calcium and phosphorus as well as ratios of urine calcium to creatinine, phosphorus to creatinine, calcium to phosphorus, and percent fractional excretions of calcium and phosphorus. Plasma parathormone (PTH) levels were monitored in 2 of 3 experiments. Osteopenic bone differences at necropsy were evaluated by bone density, percent ash, ash per milliliter bone, calcium per milliliter bone, and phosphorus per milliliter bone. Marked change in urine mineral parameters, especially the calcium-to-phosphorus ratio, typically occurred within 1 to 2 days of treatment and preceded significant change in serum mineral or plasma PTH by 2 to 3 weeks. When monitored, plasma PTH levels were elevated following treatment, which confirms the hyperparathyroid state induced by the test diets. Significant differences in bone mineralization between control and treatment diets at necropsy were generally observed. The results of this study indicate that the analysis of urine minerals offers an early, noninvasive technique to investigate diet-associated osteopenic disease in growing pigs, which can be supported further by bone mineral analysis at postmortem using techniques herein described. Several urine mineral reference intervals for application to field investigations are included. Research into application of similar techniques to evaluate calcium and phosphorus homeostasis in pigs of all ages, including gestating and lactating gilts and sows, appears warranted.

Lameness in swine is often attributed to osteoporosis resulting from dietary calcium and phosphorus imbalance or deficiency or osteomalacia as a result of insufficient vitamin D. Analyzing the mineral and vitamin content of feed is one approach to investigating this problem; however, the actual amounts digested, absorbed, and retained are dependent on a number of factors. Source and particle size of calcium and phosphorus,^{8,14} meal-feeding versus direct calcium or phosphorus supplementation,^{11,12} gross excesses or deficiencies of either mineral,^{1,4} age and weight of the pigs,¹⁰ the presence of competing substances or ligands in the ration, and the activity of vitamin D are among the recognized variables. Changes in serum values generally lack sensitivity to detect problems with mineral imbalance because efficient homeostatic mechanisms regulating serum calcium and phosphorus allow these values to remain within reference intervals after skeletal demineralization and accompanying lameness or fractures have occurred.⁹ Calcium and phosphorus homeostasis is a complicated interaction of multiple fac-

tors including absorption from the gut, bone deposition and resorption, and renal excretion, which is controlled largely by circulating levels of parathormone (PTH).¹³ Parathormone increases calcium resorption and decreases phosphorus resorption from the renal tubules. Consequently, urine calcium and phosphorus theoretically could be used to monitor dietary excesses or deficiencies of these minerals because significant changes in urine concentration may precede alterations in the serum or bones.

Bone analysis is another avenue for investigation of lameness. The percent bone ash at necropsy is used to detect osteopenic disease, although methods are not standardized and reference values are not widely published. A bone biopsy technique has been described experimentally²; however, it is unlikely to be developed for field investigation. Recent work in poultry has shown that bone ash-to-bone weight ratios lack sensitivity to detect osteopenic change because bone weight decreases as bone mineralization decreases, leaving the ratio relatively unchanged.⁶ A bone ash-to-bone volume ratio has been superior and was included in these experiments.

Several diets replete and deficient in calcium and phosphorus in various combinations as well as dietary vitamin D deficiency were tested. Diets containing ex-

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Table 1. Source of calcium and phosphorus supplement for respective diets and percent composition in complete mixed feed.

	Added calcium source		Added phosphorus source	
Experiment 1				
Control 1-0	limestone (CaCO ₃)	(0.81%)*	monosodium phosphate (NaH ₂ PO ₄ ·7H ₂ O)	(0.68%)
Treatment 1-1 (Ca-deficient, P-replete)	none	(0.07%)	monosodium phosphate	(0.68%)
Experiment 2				
Control 2-0	limestone and dicalcium phosphate (DiCal) (CaHPO ₄)	(0.83%)	DiCal	(0.66%)
Treatment 2-1 (Mod Ca- & P-def)	DiCal	(0.34%)	DiCal	(0.55%)
Treatment 2-2 (Severe Ca- & P-def)	none	(0.09%)	none	(0.37%)
Experiment 3				
Control 3-0	limestone and DiCal	(0.83%)	DiCal	(0.67%)
Treatment 3-1 (Ca-replete, P-def)	limestone	(0.81%)	none	(0.42%)
Treatment 3-2 (Vit D-deficient)	same as control 3-0 less vitamin D		same as control 3-0 less vitamin D	

* Respective mineral content of each diet.

cesses of any of these substances were not included. This research examines the validity of using urine calcium and phosphorus to evaluate calcium and phosphorus homeostasis in growing pigs and also evaluates several additional techniques for the detection of porcine osteopenic bone disease.

Materials and methods

Experiment 1. Six female crossbred pigs weighing approximately 13.7 kg were assigned 3 each to a calcium- and phosphorus-replete control group (C1-0) or a calcium-deficient, phosphorus-replete treatment group (T1-1). Water was provided ad libitum, and pigs were hand fed mornings and evenings. Feed consumption was pen matched until consumption in the treated animals dropped significantly, at which time the control group was moderately feed-restricted to more closely equalize feed intake between the 2 groups. The base diet consisted of corn and soybean meal with appropriate trace minerals and vitamins to meet or exceed National Research Council requirements. Calcium and phosphorus were supplied as shown in Table 1. Both groups were fed the control diet for 10 days before the experiment began.

Urine was collected free catch 2 to 3 times weekly, and calcium, phosphorus, and creatinine were measured. In addition, calcium-to-creatinine, phosphorus-to-creatinine, and calcium-to-phosphorus ratios were calculated. The percent fractional excretion (%FE) of calcium and phosphorus in the urine was calculated according to the following formula:

$$\%FE = \frac{\text{urine calcium}}{\text{serum calcium}} \times \frac{\text{serum creatinine}}{\text{urine creatinine}} \times 100.$$

Venous blood was collected from the orbital sinus into clot and heparin vacutainer tubes on 0, 7, 21, 35, and 49 days posttreatment (DPT). Serum and urine chemistries for total calcium, phosphorus, and creatinine were performed

using an Abbott Spectrum^a chemical autoanalyzer. Heparinized plasma was analyzed for PTH activity by a commercially available immunoradiometric test kit (N-Tact PTH).^b

At the end of 7 wk, the right and left third metacarpal bones were removed at necropsy. The metacarpals were selected because the entire bone could be analyzed for ash and mineral content, thus minimizing the variability encountered when a portion of a larger bone such as the femur is selected.¹⁰ Bones were stripped to the periosteum, submerged in water for 4 hr under 625 mmHg vacuum and blotted dry with a paper towel before weighing. Bone volume was determined using weight in air minus weight under water according to Archimedes principle. Bones were ashed at 600 F overnight and dissolved in 50 ml of 3 N HCl, which was extended to a volume of 1,000 ml with distilled water before calcium and phosphorus analysis on an Abbott Spectrum autoanalyzer. Bone density (g bone/ml volume), percent ash (ash weight/preash weight × 100), as well as ash, calcium, and phosphorus per volume of bone were analyzed.

Experiment 2. Twelve female crossbred pigs weighing approximately 11.5 kg were assigned 4 each to a control group (C2-0), a moderately low calcium and phosphorus group (treatment 2-1), and a markedly calcium- and phosphorus-deficient group (treatment 2-2). The pigs were on self-feeders and water was provided ad libitum. The diet was a corn-soybean meal base with added trace minerals and vitamins. Calcium and phosphorus were supplied as shown in Table 1. All groups received the control diet for 10 days before the experiment began. Voided urine was collected 3 times weekly with measurements and calculations performed as described in experiment 1.

Venous blood was collected from the orbital sinus into clot and heparin vacutainer tubes on 0, 7, 15, 22, 29, 36, 42, and 50 days posttreatment except for treatment 2-2 pigs, which were sacrificed on DPT 36 for humane reasons. Hep-

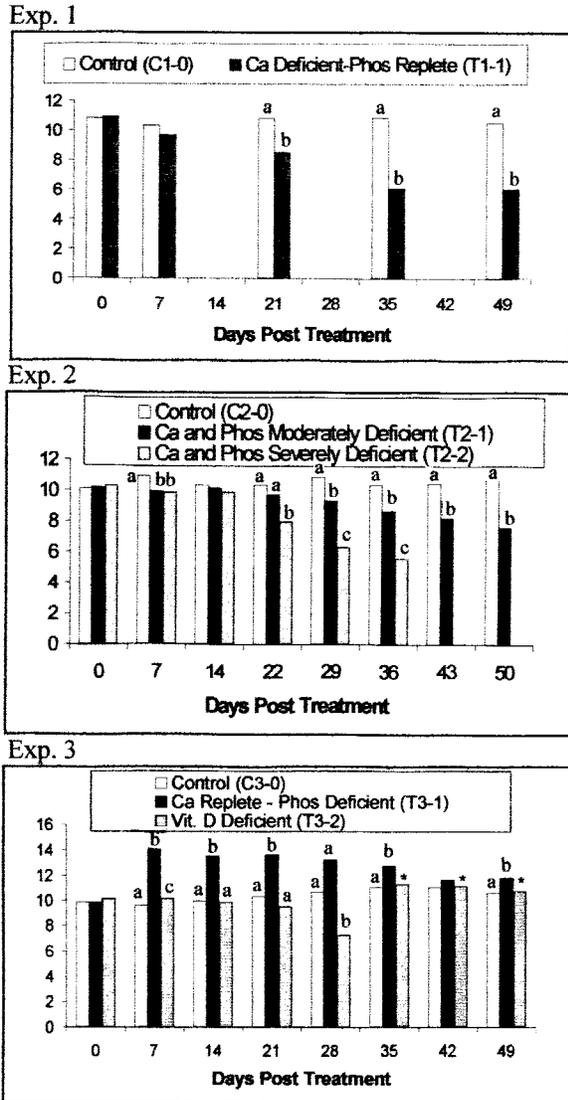


Figure 1. Mean serum calcium (mg/dl) of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

aritized plasma was analyzed for PTH activity by an immunoradiometric method as described in experiment 1. At the termination of the experiment, bones were harvested and processed as described in experiment 1.

Experiment 3. Twelve female crossbred pigs weighing approximately 11.5 kg were assigned 4 each to a control group (C3-0), a calcium-replete, phosphorus-deficient group (T3-1), and a vitamin D-deficient group (T3-2) that was housed in a sunlight-restricted environment throughout the experiment. Feed and water were provided in a manner similar to experiment 2. Calcium and phosphorus were supplied using limestone and dicalcium phosphate (Table 1). All groups were fed the control diet for 10 days preceding the experiment. Voided urine was collected and tested as in experiment 2.

Blood was collected in clot and heparin vacutainer tubes

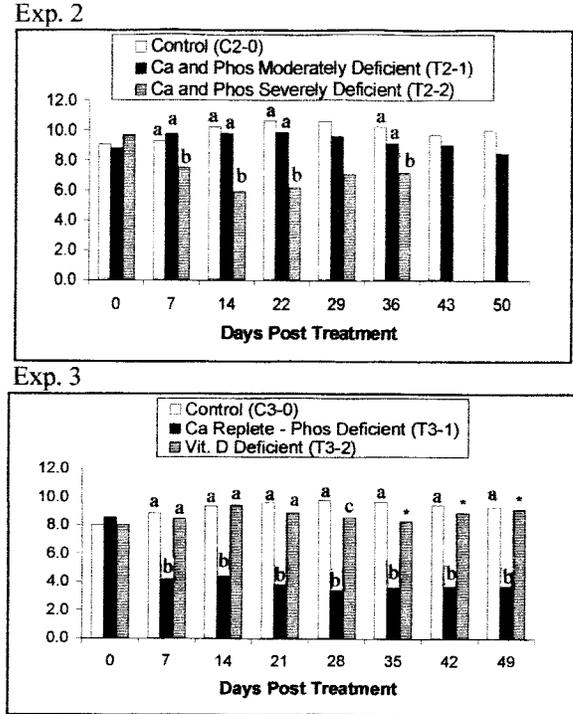


Figure 2. Mean serum phosphorus (mg/dl) of pigs on selected days from 2 experiments utilizing 2 separate within-experiment controls and 4 treatment diets. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

from the orbital sinus at weekly intervals from DPT 0 through 49 for controls and treatment group T3-1 and from DPT 0 through 28 for the vitamin D-restricted group (T3-2). Plasma parathormone values were not measured because an antibody substitution in the commercial kit in the interim made it unsuitable for assay of pig parathormone levels. Plasma levels of 2 metabolites of vitamin D [25OH₂D₃ and 1,25(OH)₂D₃] were monitored on DPT -7, 0, 21, 28, 35, 42, and 49. Right and left third metacarpal bones were harvested at necropsy and processed as described previously for experiment 1.

Statistical analysis. For all 3 experiments, differences among diets were evaluated with a repeated measures analysis of variance (ANOVA) using the SAS program.^c Differences between means were compared using Student's *t*-test.

Results

Experiment 1. Pigs in the calcium-deficient, phosphorus-replete group began to show signs of lameness and sporadic acute ambulatory pain by DPT 21 when lower serum calcium levels of treated pigs became evident (Fig. 1). Treatment did not affect serum phosphorus and creatinine. Significant differences in plasma PTH were noted at DPT 7 and persisted throughout, thus confirming the hyperparathyroid state of the treated pigs during the experimental course (Fig. 3). A rather sharp drop in urine calcium-to-creatinine ratio

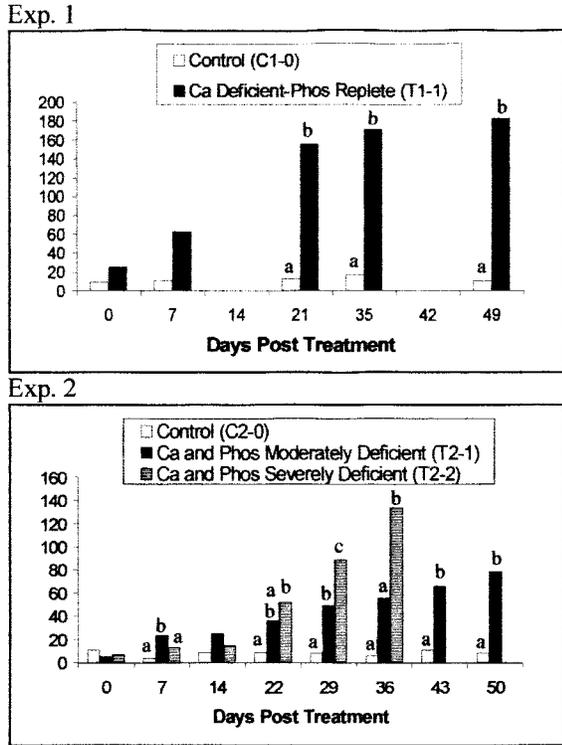


Figure 3. Mean plasma parathormone levels (pg/ml) of pigs on selected days from 2 experiment utilizing 2 separate within-experiment controls and 3 treatment diets. Treatment groups with different letters (a, b, c) differ significantly ($P < 0.05$).

occurred in control pigs between DPT 7 and 14, and a general trend for lower urinary calcium was evident for the rest of the 7-week experiment (Fig. 4). Mean urine phosphorus-to-creatinine ratios rose dramatically in the treated pigs by DPT 1, peaked at DPT 7, and remained high throughout the experiment (Fig. 5). The low urine calcium-to-phosphorus ratio demonstrates vividly the trend of low calcium and elevated phosphorus in the urine of treated pigs, where two separate scales are used in Fig. 6 to aid in demonstrating the marked difference between the groups. The overall (repeated measures) effect of diet to reduce the %FE of calcium in the urine was significant ($P = 0.027$); however, the difference between groups on individual days was significant ($P = 0.012$) on DPT 7 only (Fig. 7). The %FE of phosphorus for the treated group on all days measured after DPT 0 was significantly greater than for the controls (all P -values < 0.01) (Fig. 8). The overall (repeated measures) effect of diet on %FE of phosphorus was highly significant ($P = 0.002$). Simple t -tests of various bone analytes showed that the effect of diet was highly significant ($P < 0.01$) for decreased density and percent ash as well as decreased ash, calcium, and phosphorus per milliliter of bone (Table 2).

Experiment 2. A trend toward decreased serum cal-

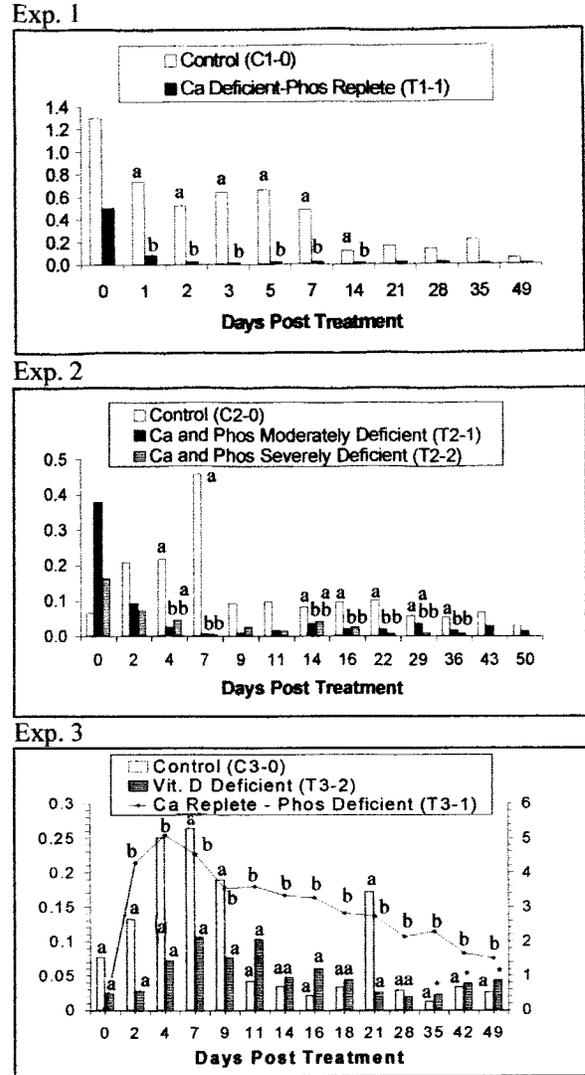


Figure 4. Mean urine calcium-to-creatinine ratio of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. In experiment 3, the scale for diet T3-1 is on the right. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

cium was evident in both treated groups by DPT 22 (Fig. 1). Serum phosphorus correlated closely with the amount of phosphorus supplied in the diet (Fig. 2). Plasma PTH values of the treated groups increased over the course of the experiment, and were somewhat proportional to the level of dietary calcium restrictions (Fig. 3). Mean urine calcium-to-creatinine ratios were reduced in both treatment groups (Fig. 4). While the mean phosphorus-to-creatinine ratio was reduced in the severely restricted group, it was increased in the moderately restricted group for the first 3 weeks, after which it tended to equate with control values (Fig. 5). The urine calcium-to-phosphorus ratio for the severely restricted group is similar to controls throughout the experiment (Fig. 6). However, the urine calcium-to-

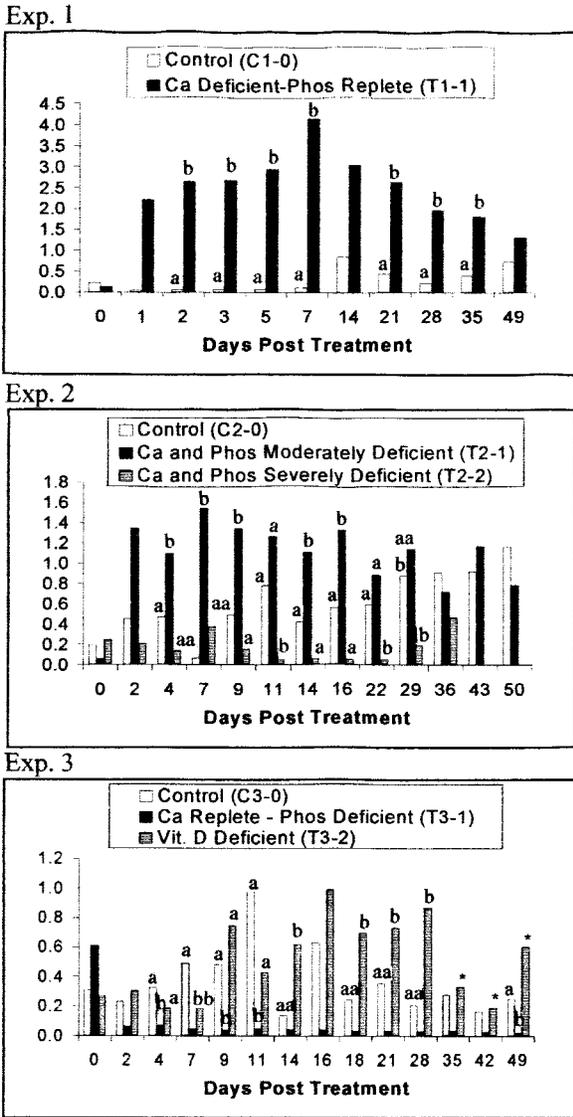


Figure 5. Mean urine phosphorus-to-creatinine ratio of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

phosphorus ratio of the moderately restricted group is consistently below the control ratio from DPT 2 through DPT 50 of the experiment. The %FE of calcium for the 2 calcium-restricted groups were significantly below the controls for the first 3 weeks. The control and moderately restricted group (T2-1) equalized in the last 4 weeks. The effects of the treatments on bone mineralization were similar to those for experiment 1 (Table 2).

Experiment 3. Serum calcium of the calcium-replete, phosphorus-deficient group (T3-1) rose dramatically by DPT 7 and remained high through DPT 28, after which rising control and decreasing treatment

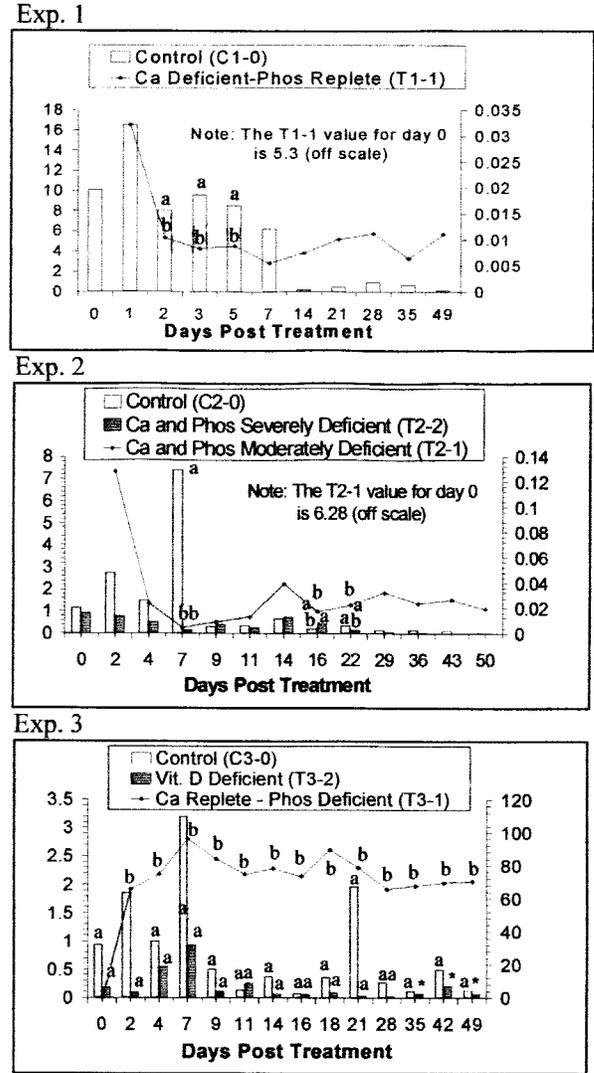


Figure 6. Mean urine calcium-to-phosphorus ratio of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. In experiments 1, 2, and 3, the scales for diets T1-1, T2-1, and T3-1, respectively, are on the right. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

values tended to equate (Fig. 1). Serum phosphorus in T3-1 dropped appreciably by DPT 7 and remained low through DPT 49 (Fig. 2). Three of the 4 pigs in the vitamin D-deficient group (T3-2) became severely lame clinically, showed sporadic clonic-tonic convulsions, and were sacrificed for humane reasons on DPT 28. The fourth pig in the group showed no clinical signs of illness, remained on the vitamin D-deficient diet, and was sacrificed on DPT 49. Values for that pig alone tended to be similar to the control pigs and are shown on the graphs for experiment 3 after 28 DPT. A trend toward lower serum calcium in the vitamin D-deficient group (T3-2) was evident by DPT 21 and became more marked by DPT 28 (Fig. 1). Se-

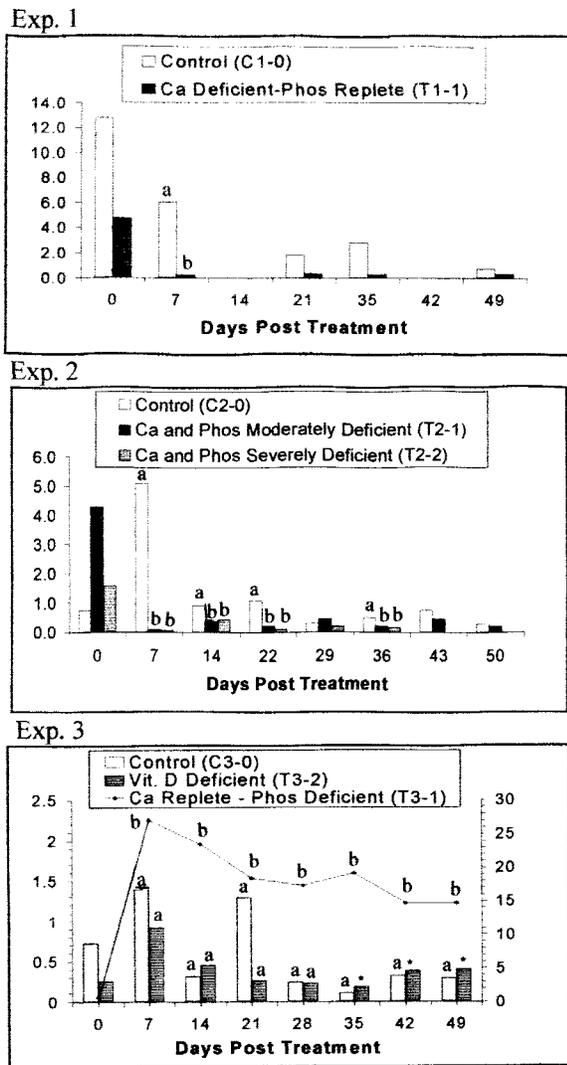


Figure 7. Mean urine fractional excretion of calcium of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. In experiment 3, the scale for diet T3-1 is on the right. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

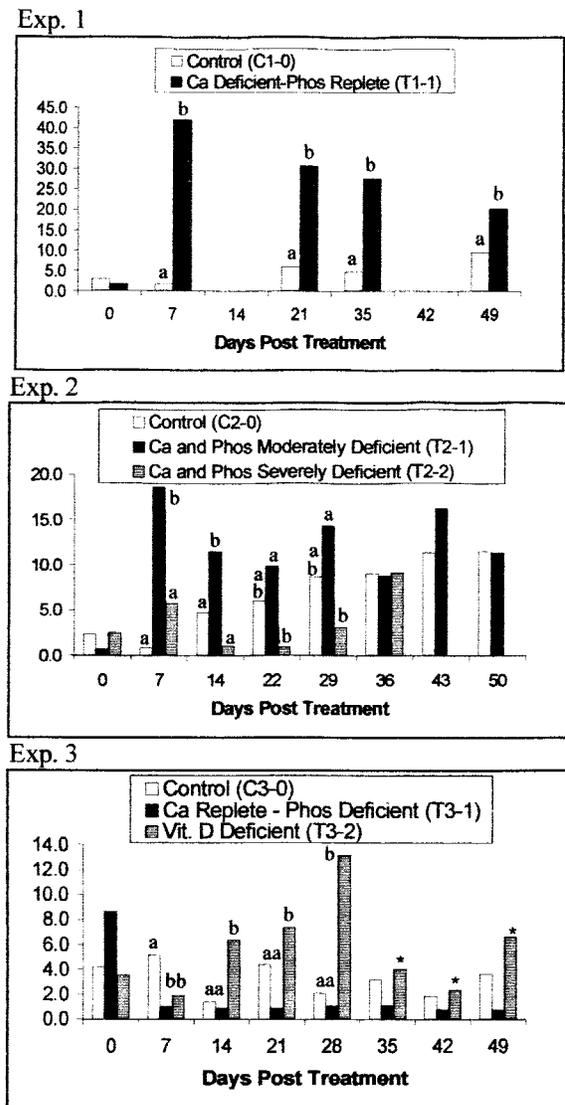


Figure 8. Mean urine fractional excretion of phosphorus of pigs on selected days from 3 experiments utilizing 3 separate within-experiment controls and 5 treatment diets. Treatment means with different letters (a, b, c) differ significantly ($P < 0.05$). * Value represents single remaining pig in group.

rum phosphorus of T3-2 paralleled controls through DPT 14, after which a possible trend toward lower values is noticeable for the following 2 weeks (Fig. 2). Plasma PTH values were not determined in experiment 3. The mean urine calcium-to-creatinine ratio of the phosphorus-restricted group greatly increased by DPT 4 and dropped continuously throughout the experiment; however, the ratio still remained significantly above control values through DPT 49. Values for the vitamin D-restricted group did not differ from controls throughout (Fig. 4). The mean urine phosphorus-to-creatinine values for the phosphorus-deficient group were very low throughout the experiment. Those for the vitamin D-deficient group were initially quite var-

iable but tended to be equal to or above controls from DPT 9 through 28, with the last 3 measurements being significantly higher than controls (Fig. 5). The urine calcium-to-phosphorus ratio for the phosphorus-restricted group was greatly increased from DPT 2 through 49, while the values for the vitamin D-restricted group tended to fall below control values (Fig. 6). The calcium-replete, phosphorus-restricted diet (T3-1) produced a very high %FE of calcium (range 15–25). Vitamin D deficiency had no significant effect on %FE of calcium (Fig. 7). The %FE of phosphorus for the calcium-replete, phosphorus-deficient diet was decreased by DPT 7 and remained constant through DPT 49. The value for the vitamin D-deficient diet was in-

Table 2. Measures of third metacarpal mineralization from experiments 1, 2, and 3.

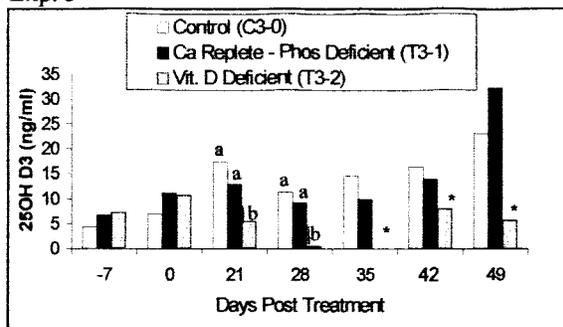
	Density	% ash	Grams ash per milliliter bone	Milligrams Ca per milliliter bone	Milligrams P per milliliter bone
Experiment 1					
Control mean (C1-0) (SD)	1.27 (0.03)	24.8 (2.0)	0.316 (0.033)	123.4 (12.6)	54.8 (5.8)
Treated (T1-1)	1.17 (0.02)	14.7 (1.6)	0.171 (0.021)	65.0 (8.7)	30.3 (4.0)
<i>P</i> -values*	(0.0078)	(0.0027)	(0.0036)	(0.0033)	(0.0043)
Experiment 2					
Control mean (C2-0) (SD)	1.25 (0.01)	23.25 (1.03)	0.29 (0.02)	98.73 (6.30)	44.84 (2.29)
Treated (T2-1)	1.20 (0.03)	16.17 (0.64)	0.19 (0.01)	74.53 (11.70)	36.36 (5.76)
Treated (T2-2)†	1.14 (0.01)	11.54 (0.52)	0.13 (0.01)	44.54 (2.46)	21.72 (1.14)
<i>P</i> -values*	(0.0102)	(0.0185)	(0.0206)	(0.0129)	(0.0435)
Experiment 3					
Control mean (C3-0) (SD)	1.22 (0.01)	21.82 (1.02)	0.27 (0.01)	82.87 (5.67)	35.98 (2.01)
Treated (T3-1)	1.15 (0.01)	14.75 (0.86)	0.17 (0.01)	51.21 (3.09)	23.73 (2.52)
Treated (T3-2)‡	1.19 (0.03)	14.60 (6.54)	0.20 (0.04)	60.25 (12.51)	30.87 (6.04)
<i>P</i> -values*	(0.0002)	(0.0002)	(0.0001)	(0.0003)	(0.0002)

* *P*-values are for differences among treatments using ANOVA.

† Means are those of 4 pigs killed at DPT 36.

‡ Means are those of 3 pigs killed at DPT 28.

Exp. 3



Exp. 3

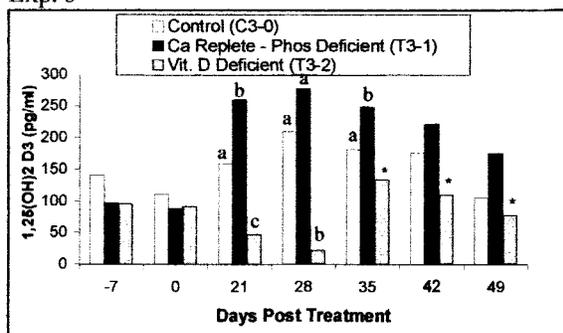


Figure 9. Mean plasma levels of 25OHD₃ and 1,25(OH)₂D₃ of pigs on selected days from controls and 2 treatment diets. Treatment means with different letters (a, b, c) differ significantly (*P* < 0.05). * Value represents single remaining pig in group.

creased by DPT 14 and continued through DPT 28, with significance shown on the last 3 samples. Plasma levels of vitamin D are shown in Fig. 9. In the vitamin D-restricted group, values for both 25OHD₃ and 1,25(OH)₂D₃ decreased considerably by DPT 28. In the phosphorus-restricted group, 25OHD₃ remained near control levels while 1,25(OH)₂D₃ increased above control levels by DPT 21 and 28. Mineral content of the third metacarpal bone in the treated groups was below control values when measured by several methods (Table 2). The low values reported for the vitamin D-deficient group (T3-2) are the means for the 3 pigs sacrificed at 28 DPT. Values for the fourth pig sacrificed at 49 DPT, although not reported, were as high or higher than controls and provided no support for bone hypomineralization.

Discussion

In those treatments where dietary calcium was restricted, serum calcium tended to decrease with time in proportion to the level of deficiency. Serum calcium of treated pigs rose above controls when phosphorus was restricted while calcium was replete (T3-1), which suggested that calcium is more freely absorbed from a phosphorus-restricted diet. In addition, the low phosphorus diet produced a decline in serum phosphorus, which has been shown in man and pigs to contribute

to elevated levels of plasma $1,25(\text{OH})_2\text{D}_3$ and associated enhanced calcium absorption from the gut.^{1,3,5} The serum calcium of the vitamin D-restricted group was significantly reduced by DPT 28. This corresponds to the time when plasma levels of 25OHD_3 and $1,25(\text{OH})_2\text{D}_3$ were very low, in which case, active calcium transport in the gut was likely restricted.

Serum phosphorus was similar to controls in those treated groups receiving normal dietary phosphorus or when phosphorus was moderately restricted. Groups T2-2 (Ca and P deficient) and T3-1 (Ca replete, P deficient) received no phosphorus supplementation. Although both diets provided similar levels of phosphorus, serum phosphorus levels dropped considerably lower in T3-1 (3.8 mg/dl), which had normal levels of calcium, than in T2-2 (6.8 mg/dl), where calcium was also restricted, suggesting that the replete dietary calcium supplied in T3-1 may have inhibited the absorption of the small amount of available phosphorus. In the vitamin D-deficient group (T3-2), serum phosphorus on DPT 21 and 28 suggests a lowering trend, although significance was not attained. Since vitamin D aids in the absorption of both calcium and phosphorus from the gut,⁷ lowered serum calcium and phosphorus would be expected.

Control PTH values remained constant, while PTH levels of calcium-restricted groups increased proportionately to the level of restriction. The trend toward hyperparathyroid activity was generally evident by DPT 7 to 21. Group T1-1, where calcium was restricted while phosphorus was replete, reached levels of 140 pg/ml or higher earlier than T2-2, where both calcium and phosphorus were restricted. This finding further supports the concept that calcium absorption from the gut is enhanced with low dietary phosphorus. In addition, the low serum phosphorus observed in the phosphorus-restricted group would stimulate $1,25(\text{OH})_2\text{D}_3$ production, which would increase calcium absorption from the gut and diminish the stimulus for PTH production.

In experiments 1 and 2, the urine calcium-to-creatinine ratio was reduced in those diets where calcium was restricted. In experiment 3, however, this ratio was significantly increased when phosphorus was restricted and calcium was replete. Both findings indicate that urinary calcium, when measured by the calcium-to-creatinine ratio, is influenced by dietary levels of both calcium and phosphorus. Vitamin D deficiency had no significant effect on the urine calcium-to-creatinine ratio.

The urine phosphorus-to-creatinine ratio was generally below 1.0 for all control groups except for C2-0 on DPT 50, when it was 1.16. In T1-1, where calcium was restricted and phosphorus was replete, values ranged from 1.3 to 3.0. However, in T2-1, where

calcium and phosphorus were both moderately restricted, the values were also mildly increased to a range of 0.7–1.5, with 9 of 12 values exceeding 1.0. This finding also supports an hypothesis of increased phosphorus absorption when calcium is restricted. In addition, the restricted dietary calcium results in increased levels of parathormone, which further enhances renal excretion of phosphorus. When dietary phosphorus was severely restricted (T2-2 and T3-1), the ratios were generally well below their respective controls. The consistently low values for T3-1, where calcium was replete and phosphorus was restricted, are to be expected for several reasons. Low dietary phosphorus, combined with replete dietary calcium, would decrease phosphorus absorption from the gut. In addition, adequate dietary calcium would provide no stimulus for parathormone production and hence no added stimulus for renal excretion of phosphorus. With vitamin D deficiency (T3-2), there was no notable effect on the phosphorus-to-creatinine ratio for the first 2 weeks of the experiment. From DPT 14 through 28, however, it was consistently above control values, although it remained below 1.0 throughout. These findings suggest that urine phosphorus-to-creatinine ratios of 1.0 or below indicate acceptable amounts and balance between calcium and phosphorus or that phosphorus is severely restricted in the diet. As demonstrated, however, values below 1.0 cannot rule out a dietary deficiency of vitamin D. Under the conditions of this study, ratios above 1.0 were found when dietary calcium was restricted and phosphorus was supplied in normal to moderately decreased amounts.

With control diets, urine calcium-to-phosphorus ratios were generally below 1.0. The ratio was extremely low (<0.035) with diet T1-1, in which calcium was restricted and phosphorus was replete, while it was extremely high (>64) with diet T3-1, where calcium was replete and phosphorus was restricted. Moderate restriction of both calcium and phosphorus (T2-1) also regularly produced ratios below 0.043, which was generally below those produced with control diets. In contrast, marked restriction of both calcium and phosphorus produced values similar to controls. These findings suggest that ratios above 1.0 and especially below 0.05 support a dietary mineral imbalance or abnormal calcium and phosphorus homeostasis. However, values falling in the normal range do not assure acceptable dietary mineral balance or normal calcium and phosphorus homeostasis.

The %FE of calcium or phosphorus is a measure of the percent of these minerals, respectively, that is excreted in the urine relative to the amount filtered at the glomerulus. The %FE of calcium is especially prone to error when total calcium in the blood, rather than ionized calcium, is measured since close to one half

Table 3. Bone mineralization parameters from all control groups combined.

	Density	% ash	Grams ash per milliliter bone	Milligrams Ca per milliliter bone	Milligrams P per milliliter bone
Mean (SD)	1.25 (0.024)	23.31 (1.87)	0.29 (0.03)	101.4 (17.7)	45.2 (8.0)
Range (mean \pm 2SD)	1.20–1.30	19.4–27.1	0.23–0.35	66–137	29–61
Coefficient of variation (%) (SD \div mean \times 100)	1.9	8.0	10.3	17.5	17.7

of the total calcium is bound to protein and, therefore, not subject to glomerular filtration. It does, however, provide a crude index of renal calcium and phosphorus excretion or conservation when treatment values are compared with control values calculated in a similar manner.

Although plasma PTH levels rose in the treated group throughout experiment 1, the %FE of phosphorus declined continuously from DPT 7 through 49 for reasons not apparent to the authors. In a similar manner, %FE of phosphorus with the moderately calcium- and phosphorus-restricted diet (T2-1) was highest on DPT 7 and tended to decrease toward controls by DPT 50. In contrast, values tended to rise with time in the severely calcium- and phosphorus-restricted (T2-2) and vitamin D-restricted (T3-2) diets. In the calcium-replete, phosphorus-restricted diet (T3-1), %FE of phosphorus was very low (<1%) by DPT 7 and remained low throughout. In general, %FE for calcium and phosphorus tended to parallel the urine calcium and phosphorus-to-creatinine ratio. Consequently, since additional cost and inconvenience are involved to obtain serum values in order to make this calculation, it will likely be performed only when serum chemistry values are readily available.

Bone analysis was performed for the group severely restricted on calcium and phosphorus (T2-2) and the vitamin D-restricted group (T3-2). However, several animals within these groups became severely lame clinically and, consequently, these groups were sacrificed before their respective experiments ended. Although bone analysis in these groups suggested significant osteopenic differences, statistical analysis was performed only in the groups for which age-matched controls were available. Differences in bone mineralization variables between control and treated groups were significant.

The marked departure from the expected by the fourth pig of the vitamin D-restricted group is interesting and problematic. Although the other 3 pen mates followed a course of deterioration leading to euthanasia at DPT 28, the fourth pig showed no clinical signs, was the largest of all the pigs sacrificed at DPT 49, and had fully mineralized bones at necropsy. Serum levels of the 2 forms of vitamin D measured in

this pig, however, were in a range comparable to its pen mates and reflected a state of severe vitamin D deficiency. Future studies involving vitamin D-restricted diets and growing pigs may shed light on this unexpected finding.

Calcium and phosphorus analysis of bone requires procedures and expense not required for the calculation of density or percent ash. Under the conditions of this study, procedures requiring calcium and phosphorus analysis provided no additional information and appear unwarranted. The mean and standard deviation of several variables used to evaluate bone mineralization have been compiled for the three control groups combined and are reported in Table 3. These values are offered as a suggested reference guideline for comparably processed bones from 40- to 55-kg pigs that have been fed a ration containing recommended levels of calcium, phosphorus, and vitamin D. Bone density, which is the simplest and least expensive of the bone analysis parameters tested here, was the most precise, having the lowest coefficient of variation (Table 3), and is recommended as a parameter for evaluating osteopenic disease.

Parathormone acts on renal tubules to promote calcium resorption and phosphorus excretion, leading, hypothetically, to lower urine calcium and higher phosphorus. Increased PTH activity was proven with 3 diets (T1-1, T2-1, and T2-2) by PTH assay and empirically implied in vitamin D deficiency (T3-2). All of these diets except T2-2, where dietary phosphorus was markedly restricted, produced a high urine phosphorus-to-creatinine ratio, a low urine calcium-to-phosphorus ratio, and a high %FE of phosphorus. A low urine calcium-to-creatinine ratio and reduced %FE of calcium proved less useful than the phosphorus counterparts to detect excess PTH activity. Urine values that suggest dietary imbalance leading to PTH excess is provided in Table 4 as a resource for field investigations. Results of this study support the use of urine chemistry to investigate diet-related problems of calcium-phosphorus homeostasis in growing pigs. When applied to field cases, a sample size of at least 10 is encouraged. If possible, 5–10 control samples from age-matched, unaffected pigs should be included. When postmortem examinations are feasible, a mean

Table 4. A menu of possible urine calcium and phosphorus results and suggested differentials.

Urine parameter	Suspicious values	Possible differentials
Ca : creatinine ratio	<0.025	calcium deficiency with or without a phosphorus deficiency (an untested premise may be excessive phosphorus)
Phosphorus : creatinine ratio	>0.25	phosphorus deficiency (an untested premise may be excessive calcium)
	>1.0	calcium deficiency with normal or moderate phosphorus deficiency (an untested premise may be excessive phosphorus)
Ca : phosphorus ratio	<1.0	normal; also compatible with severe phosphorus deficiency or vitamin D deficiency
	>1.0	severe phosphorus deficiency (an untested premise may be a moderate phosphorus deficiency alone or excessive calcium)
	<0.05	calcium deficiency with normal to moderately decreased phosphorus, and vitamin D deficiency (an untested premise may be excessive phosphorus)

density of the third metacarpal bone of 1.20 or below or a bone ash approaching 20% or below by the methods used here support a diagnosis of osteopenic disease.

Additional research will be needed to determine corresponding urine and bone values for market-weight pigs as well as gilts and sows in gestation and lactation. The investigation of diets containing excessive amounts of calcium, phosphorus, and vitamin D may also be warranted.

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Sources and manufacturers

- Abbott Laboratories Diagnostic Division, Irving, TX.
- Nichols Institute Diagnostics, San Juan Capistrano, CA.
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