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The effect of dietary phosphorus on bone development in dairy heifers

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ABSTRACT

Phosphorus requirements, as percent of dietary dry matter for heifers (0.20–0.35%) and endogenous levels of P in feeds (0.20–0.35% of dry matter) are similar, suggesting that supplementation of P in heifer diets may be infrequently required. Because long-term studies are unavailable, 183 Holstein heifers and 182 Holstein × Jersey crossbred heifers were fed diets with (0.39%) and without (0.29%) supplemental P from 4 to 21 mo of age in a replicated pen design. Two subpopulations of heifers were selected mid-trial for intensive measurement of bone development and metabolism. Thirty-two heifers at 628 d (±10.0 d) of age, balanced by breed and diet, were evaluated for bone development. External frame measurements included hip height, length, heart girth, hip width, cannon bone circumference, pelvic length, pelvic height, and pelvic width. Tails of heifers were surgically amputated with the 13 and 14th coccygeal vertebrae retained. After tissue removal, the 13th coccygeal vertebrae were scanned using peripheral quantitative computed tomography with cortical, trabecular, and total bone densities determined. A second subpopulation (n = 64) of heifers (375 d ± 33 d), balanced for breed and diet, were evaluated for serum pyridinoline and osteocalcin to assess systemic bone metabolism. Data were analyzed as a completely randomized design with breed, treatment, and their interaction in the model. External skeletal measurements revealed significant differences in hip height, hip width, heart girth, cannon bone circumference, and pelvic length between Holstein and crossbred heifers. Supplementing P had no effect on external frame measurements, bone density, or bone metabolism markers. Bone P content was lower (18.1 vs. 18.6%) in heifers fed no supplemental P. Data suggest P supplementation to heifers modestly increased bone P content but increased bone P was not reflected in frame growth, bone density, or bone metabolism.
Key words: phosphorus, bone, heifer

INTRODUCTION

The goal of a dairy replacement heifer management program is to rear heifers at a low economic and environmental cost without compromising future lactation performance. Dairy heifers require adequate amounts of dietary P to ensure normal soft and skeletal tissue development (NRC, 2001), but feeding excessive dietary P results in increased P excretion. Land application and runoff of manure containing high levels of P has been linked to environmental pollution of surface waters (Knowlton et al., 2004). The NRC (2001) P requirements for dairy heifers were assimilated from the Agriculture and Food Research Council (1991) and are the sum of absorbed P required for maintenance, growth, and pregnancy divided by the absorption coefficient(s) for dietary P. The dietary P requirement for dairy heifers (NRC, 2001) typically ranges from 0.20 to 0.35% of dietary DM and endogenous levels of P in feeds (0.20–0.35% of DM) are similar, suggesting supplemental P in heifer diets may be minimally required. Noller et al. (1977) demonstrated that supplementing P at 0.10 percentage units above the endogenous P content (0.22% of DM) in feedstuffs had no effect on dairy heifer growth or reproductive performance. Hurley et al. (1982) reported no negative effects of feeding 0.19 percent dietary P to dairy heifers on estrous behavior or endocrine function. Erickson et al. (1999, 2002) also concluded that supplemental P was not required for growing beef cattle to meet growth and meat production criteria when basal diets supplied 0.14 to 0.19% P.

Despite research evidence suggesting supplemental P is minimally required for dairy heifers, field research by Zygarlicke and Hoffman (2002) showed that dairy producers commonly feed diets containing >0.38% dietary P to dairy heifers, which is similar to dietary P requirements for lactating dairy cows (NRC, 2001). Reasons for disparity between on-farm P feeding levels (Zygarlicke and Hoffman, 2002) and P feeding requirements (NRC, 2001) are unclear but may be linked to the positive relationship observed between heifer frame size at first calving and first-lactation milk yield (Hoff-

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man and Funk, 1992). Because dietary P is integral to skeletal growth, and skeletal size at first calving is positively related to first-lactation milk yield, extra P supplementation may be occurring as a hedge to maximize skeletal growth of heifers. However, there are no long-term feeding studies available to confirm or reject the hypothesis that supplementing P to dairy heifers increases frame size at first calving and(or) future lactation milk yield. As a result, a long-term experiment was implemented at the Integrated Dairy Research Facility at the University of Wisconsin to explore this hypothesis. A subobjective of this project, represented in this manuscript, was to determine if supplementing P above endogenous levels of P in feedstuffs alters frame growth, bone composition, or bone metabolism in dairy heifers.

MATERIALS AND METHODS

All animal handling and experimental procedures were approved by the Research Animal Resource Committee at the University of Wisconsin-Madison.

Background

A long-term study was initiated at the Integrated Dairy Research Facility at the University of Wisconsin to investigate the effects of P feeding strategies on dairy heifers' nutrient utilization, extent of frame growth, reproductive efficiency, parturient metabolic disorders, and future milk yield. Holstein (n = 183) and Holstein × Jersey crossbred heifers (n = 182) were assigned to 1 of 2 dietary P treatments arranged as a 2 × 2 factorial with breed and dietary P as main effects. Heifers were fed diets containing no supplemental P or supplemented with monosodium phosphate to increase dietary P by 0.10% of DM. Dietary treatments for the long-term P feeding study were similar to Noller et al. (1977) but the study differed in 3 ways. First, treatments were initiated when heifers were 4 mo of age and treatment diets were fed for the entire growth period (±600 d) compared with 238- and 364-d feeding periods utilized by Noller et al. (1977). Second, large numbers of heifers (n = 365) were assigned to the study to provide statistical inference regarding reproductive efficiency, parturient metabolic disorders, and future milk production, which were not evaluated by Noller et al. (1977). Third, the extent of bone growth, bone composition, and bone metabolism of dairy heifers were evaluated, which have not been evaluated in other P feeding studies conducted with dairy heifers. Data presented in this manuscript detail the evaluation of bone growth extent, bone composition, and bone metabolism.

Heifers and Diets

To evaluate the extent of bone growth, bone composition, and bone metabolism, 2 subsets of dairy heifers were selected using a stratified random sampling procedure (Cochran, 1977) from the long-term trial. A subset of 32 heifers averaging 628 d (±10.0 d) old, stratified by breed (16 Holsteins and 16 crossbreds) and dietary treatment (16 heifers with and 16 without supplemental P) were randomly selected from 13 heifer pens at the termination of the heifer growth treatment period to intensively evaluate the extent of bone growth and bone composition. To evaluate bone metabolism, a second subset of 64 heifers (375 d ± 33 d) stratified by breed (32 Holsteins and 32 crossbreds) and dietary treatment (32 with and 32 without supplemental P) were randomly selected from 12 heifer pens for intensive bone metabolism evaluations. Younger heifers (375 d) were stratified and selected to evaluate bone metabolism because younger heifers have more active skeletal growth (NRC, 2001).

The dietary treatments fed in the long-term trial, which both subsets of heifers were fed, were executed as follows. Eight Holstein or crossbred heifers, at approximately 4.0 mo of age, were assigned to a 4.3- × 6.5-m pen bedded with wood shavings having access to 0.55 m of bunk space/heifer. In total, 48 pens containing 8 heifers per pen were assigned to the long-term pen-replicated trial. Holstein and crossbred heifers were not commingled but rather assigned to separate pens in groups of 8. Forages, grains, and protein supplements fed to heifers were sampled twice monthly and evaluated for DM by drying at 55°C for 48 h. Samples were then milled through an Udy mill (Udy Corp., Boulder, CO) fitted with a 1-mm screen and saved for nutrient analysis. Residual DM was determined on 1-mm-ground samples by drying at 105°C for 3 h. The CP, NDF, NDF-CP, and fat of forage and grain samples were determined by near-infrared reflectance spectroscopy (model 6500; FOSS-NIR System, Silver Spring, MD) by the University of Wisconsin Soil and Forage Analysis Laboratory (Marshfield, WI). For all feeds, Ca, K, and Mg were determined using atomic absorption spectroscopy and P was determined by colorimetry (Schulte et al., 1987). The total digestible nutrients (**TDN**), ME, NE_G, and NE_M of forages, grains, and protein supplements fed to heifers were estimated using summative equations (NRC, 2001).

After twice-monthly forage and grain analyses were completed, 3 rations were formulated for Holstein heifers weighing <275, 275 to 450, and >450 kg according to NRC (2001) requirements. Diets formulated for Holstein heifers were also fed to crossbred heifers but

different weight criteria (<250, 250 to 425, >425 kg) were used to account for differences in physiological maturity (NRC, 2001) of the crossbred heifers. Diet ingredients were fed separately to pens of heifers once daily at 0800 h. Heifers in pens were supplemented with a mineral-vitamin premix with or without monosodium phosphate.

The DM contents of forages were monitored weekly and diets were adjusted when forage DM content changed more than 4.0 percentage units. Consumption of feed was monitored daily using a bunk scoring system. Each day at 0730 h, before feeding, feed bunks were scored and classified as 0 = no feed remaining; 1 = few and scattered feed particles remaining; 2 = bottom of feed bunk covered in thin layer of feed; 3 = large amounts of feed remaining with the bottom of the feed bunk not visible. Orts were not weighed for heifer pens with bunk scores <2 because the amount of feed remaining was negligible (<1.0 kg). Orts were weighed for heifer pens with bunk scores of 2 and 3 with weight of orts subtracted from the daily feed offering. The DM content of the orts was not measured and the DM content of the diet was used as a surrogate orts DM. The bunk scoring system (0–3) was also used to manage daily pen feed allocations to feed heifers as close to exact DMI as possible. This objective was facilitated by systematically feeding heifers to achieve a postfeeding bunk score of 1 at all times.

Heifers were weighed at 60-d intervals using a cattle chute (Real Tuff, Clearbrook, MN) fitted with an electronic scale (Tru-Test Inc., Mineral Wells, TX).

Bone Density

To evaluate bone density and bone composition, the 13th and 14th coccygeal vertebrae of each heifer in the first subset (n = 32) were surgically removed. Vertebral bone was selected because it is abundant in trabecular bone, a site more sensitive to bone remodeling (Eklou-Kalonji et al., 1999). Hair on the tail of each heifer was clipped 10 cm above and below the surgical site. The site was made aseptic with an alternating scrub of Betadine and 70% alcohol-soaked gauze. An elastrator band was placed at the appropriate intercoccygeal vertebrae. Heifers were administered a caudal epidural block with an 18-gauge, 3.8-cm needle placed into the first intercoccygeal space where 5 mL of 2% lidocaine was injected. Tails were then amputated one vertebra below the band, approximately 10 cm below the ventral commisure of the vulva, at the site of the 13th and 14th coccygeal vertebrae. Following removal of the tail, the biopsy site was sprayed with iodine and fly spray. Immediately after tail removal, coccygeal bones were excised from docked tails. Tailbones were inserted into labeled Whirl-Pak (Nasco, Fort Atkinson, WI) bags, frozen at −20°C, and retained for bone density and mineral analysis.

The 13th coccygeal vertebrae were scanned for trabecular, cortical, and total bone densities using peripheral quantitative computed tomography (**pQCT**) at the University of Wisconsin School of Veterinary Medicine (Madison, WI). The pQCT scan assesses bone density by using multiple cross-sectional x-rays to reconstruct a volumetric model of the bone density distribution. The scanner used was a Stratec XCT 960A pQCT bone scanner (Norland Medical Systems, Fort Atkinson, WI). Each vertebra was placed in a holder orienting the bone longitudinally and perpendicular to the tomography tube in the scanner. After entering the scanner, each bone underwent an initial scan providing an overview of the bone and proper scanning orientation was identified. To provide scanning uniformity, a reference line on the proximal end of each vertebra was used where the clear demarcation between the soft tissue and bone occurred. Cross-sectional slices were scanned in 4 locations at 2-mm intervals starting from the reference line at the proximal end of the bone. Each 2-mm slice contained the analysis denoting total, trabecular, cortical, and subcortical bone densities expressed in milligrams per cubic centimeter. From the cross-sectional images, the 8-mm image was selected as the region of interest because it provided the highest sensitivity to trabecular and cortical bone densities. The default density threshold for bones was set at 280 mg/cm^3. Cortical and trabecular matrixes were separated by the area distribution of both bone structures. By default, 55% of the outer bone area was concentrically separated and defined as the cortical/subcortical region. The remaining 45% inner core was defined as trabecular bone.

Bone Composition

Following surgical removal of the 14th coccygeal vertebrae, bones were labeled, placed in Whirl-Pak bags, frozen at −20°C, and transferred to the University of Wisconsin Zoological Museum. Vertebrae were thawed and excess soft tissue was removed over a 3-wk period by flesh-eating beetles *(Dermestes* spp.). Following tissue removal, tailbones were defatted with ethyl ether for 72 h in a sidearm Soxhlet apparatus (AOAC, 1990). Bones were then dried at 100°C for 12 h to determine dry, fat-free weight. Bones were ashed at 700°C for 16 h in a muffle furnace and ground with mortar and pestle to a uniform white powder. Ash percentage was calculated as a percentage of the dry fat-free weight. Ash from crushed, defatted bones was assayed for P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, Al, and Na using inductively coupled plasma optical emission spectrometry by the

University of Wisconsin Soil and Plant Analysis Laboratory (Madison, WI).

Bone Growth

The subpopulation of heifers selected for the intensive bone growth evaluation were weighed in duplicate and external skeletal measurements were made to determine the effect of the experimental diets on extent of bone growth. External skeletal measurements included hip height, hip width, body length (point of the shoulders to the ischium), heart girth, and cannon bone circumference. Pelvic height, width, and calculated pelvic area were determined using a calibrated pelvic forceps (HCR, Wright City, MO).

Bone Metabolism

Blood was collected from the second subset of heifers (n = 64) selected for bone metabolism evaluation by jugular venipuncture in 10-mL silicone-coated Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) with 20-gauge, 2.54-cm needles. Blood collection occurred at 1300 h on the day of collection. Following collection, blood was immediately packed in ice, centrifuged at 2,000 × *g* for 20 min (Eppendorf 5416, Westbury, NY), and serum was poured off into polypropylene tubes and stored at −20°C. Serum was packed in dry ice and transferred to Michigan State University (East Lansing, MI) for bone metabolism marker analysis. Blood serum was frozen and stored for less than 60 d to avoid degradation of bone metabolism markers in storage. Serum osteocalcin concentrations (an indicator of systemic bone formation) were determined in duplicate using an ELISA test (Metra Osteocalcin, Quidel Corporation, San Diego, CA) according to procedures described in Peterson et al. (2005). Serum pyridinoline concentrations (an indicator of systemic bone resorption) were determined in duplicate using an ELISA test (Metra Serum Pyd, Quidel Corporation) by procedures described previously in Lang et al. (2001).

Statistics

A stratified random sampling procedure of a pen-based experiment was employed to intensively measure bone growth and bone metabolism in an effort to maintain power of statistical inference and minimize the number of animal surgeries and invasive procedures required, as prescribed by the Research Animal Resource Committee at the University of Wisconsin-Madison. As a result, randomly selected subjects (animal) of the stratified survey groups (pen) became the experimental unit (Cochran, 1977). Bone growth, density, composition, and bone metabolism data were analyzed as a completely randomized design using the GLM procedures of SAS (SAS Institute, 1999). The aforementioned data were determined by the model:

$$Y_{ij} = \mu + D_i + B_j + D_i \times B_j + e_{ij},$$

where μ = overall mean, D_i = effect of diet (i = 0.29 or 0.39% P), B_j = effect of breed (j = Holstein or crossbred) and $D_i \times B_j$ = effect of diet by breed interaction, and e_{ij} = random residual. Significance was declared for *P*-values ≤ 0.10, providing more liberal acceptance of the alternative hypothesis as opposed to the null hypothesis. More liberal acceptance of the alternative hypothesis was necessary to ensure potential negative affects associated with not supplementing P to dairy heifers were more liberally elicited.

RESULTS

The ingredient and nutrient composition of experimental diets containing 0.29 or 0.39% P fed to Holstein and crossbred heifers for bone metabolism and bone growth evaluations are presented in Table 1. Because different subpopulations of heifers were selected for bone metabolism and bone growth evaluations, ingredient and nutrient compositions were slightly different because of the longer feeding period of heifers selected to evaluate bone growth. Heifers selected for the bone metabolism evaluation were fed diets with similar ingredient and nutrient composition with the exception that heifers were fed diets supplemented or not supplemented with monosodium phosphate from 4 to 12 mo of age to achieve dietary P contents of 0.39 or 0.29%. Likewise, heifers selected for the bone growth evaluation were fed diets with similar ingredient and nutrient composition with the exception that heifers were fed diets supplemented or not supplemented with monosodium phosphate from 4 to 21 mo of age to achieve dietary P contents of 0.39 or 0.29%.

Skeletal measurements of Holstein and crossbred heifers fed diets containing 0.29 or 0.39% P from 4 to 21 mo of age are presented in Table 2. External skeletal measurements revealed significant differences ($P < 0.08$) in hip height, hip width, heart girth, cannon bone circumference, and pelvic length between Holstein and crossbred heifers. Supplementing heifers with P and correspondingly increasing dietary P from 0.29 to 0.39% had no effect on BW or any external skeletal measurement. Likewise, no significant ($P > 0.10$) breed × diet interaction was observed for BW or external skeletal measurements.

Table 1. Ingredient and nutrient composition of experimental diets containing 0.29 or 0.39% P fed to Holstein and crossbred heifers for bone metabolism and bone growth evaluations[1]

	Bone metabolism (4–12 mo of age)		Bone growth (4–21 mo of age)	
Item	0.29% P	0.39% P	0.29% P	0.39% P
Ingredient				
Legume silage	58.7	58.4	52.8	51.6
Corn silage	14.6	18.8	25.1	26.2
Grass silage	11.2	8.9	12.7	12.9
Concentrate[2]	14.8	13.1	8.6	8.5
P mineral-vitamin premix[3]	—	0.71	—	0.75
Mineral-vitamin premix[4]	0.69	—	0.74	—
Calcium carbonate	0.06	0.08	0.06	0.06
Nutrient composition				
DM, % (as fed)	52.0	50.6	46.9	46.4
CP	15.9	15.8	15.1	15.1
ADF	28.9	28.9	30.7	30.9
NDF	41.6	41.0	43.0	43.1
NFC	33.6	34.1	32.5	32.3
Fat	2.83	2.44	2.67	2.68
Ca	0.69	0.74	0.77	0.78
P	0.29	0.39	0.29	0.38
Mg	0.23	0.22	0.22	0.22
K	1.97	2.01	2.06	2.09
S	0.28	0.28	0.27	0.27
Ash	7.89	7.87	8.28	8.37
TDN[5]	65.4	65.8	64.3	64.2
NE_M, Mcal/kg	1.50	1.52	1.48	1.48
NE_G, Mcal/kg	0.91	0.93	0.90	0.90

[1]All values expressed as a percentage of DM unless otherwise indicated.

[2]Concentrate = compilation of a premix (16.1% CP, 5.0% ADF, 2.8% fat, 0.5% Ca, 0.2% P), oat hulls, shelled corn, and soybean meal.

[3]P mineral-vitamin premix = 1.2% Ca, 14.5% P, 0.52 Mg, 3.4% S, 2.1% Cl, 12.0% Na, 88,120 IU of vitamin A per kg, 29,640 IU of vitamin D per kg, 798 IU of vitamin E per kg.

[4]Mineral-vitamin premix = 1.2% Ca, 0.0% P, 0.0% Mg, 3.4% S, 10.1% Cl, 6.6% Na, 88,120 IU of vitamin A per kg, 29,640 IU of vitamin D per kg, 798 IU of vitamin E per kg.

[5]TDN = total digestible nutrients as calculated by NRC (2001).

Density and chemical composition of coccygeal vertebrae in Holstein and crossbred heifers fed diets containing 0.29 or 0.39% P from 4 to 21 mo of age are presented in Table 3. Increasing dietary P from 0.29 to 0.39% had no effect ($P > 0.10$) on density of trabecular or cortical bone in heifer coccygeal vertebrae. Because trabecular and cortical bone densities were unaffected by P supplementation, total bone density in coccygeal vertebrae was likewise unaffected ($P > 0.10$) by supplementing P to heifers. Holstein and crossbred heifers had

Table 2. Skeletal measurements of Holstein and crossbred heifers fed diets containing 0.29 or 0.39% P from 4 to 21 mo of age

	0.29% P		0.39% P			Effects (P-value)		
Item	Holstein	Crossbred	Holstein	Crossbred	SE	Diet	Breed	Diet × breed
BW, kg	621	573	638	587	28.2	0.25	0.001	0.91
Hip height, cm	146	138	146	139	0.4	0.89	<0.001	0.58
Hip width, cm	56	53	56	54	0.2	0.30	<0.001	0.30
Body length, cm	165	165	165	165	1.0	0.99	0.79	0.95
Heart girth, cm	206	200	206	203	0.9	0.64	0.08	0.59
Cannon bone, cm[1]	28	27	29	27	0.1	0.34	<0.001	0.34
Pelvic height, cm	17	17	17	17	0.4	0.87	0.74	0.41
Pelvic width, cm	16	16	16	16	0.3	0.21	0.40	0.40
Pelvic area, cm^2	211	205	214	210	6.9	0.54	0.51	0.91
Pelvic length, cm	56	54	57	55	0.3	0.60	0.02	0.92

[1]Values indicate cannon bone circumference.

similar trabecular, cortical, and total bone densities, and no breed × supplemental P interactions on any measure of bone density were observed.

Heifers fed diets without supplemental P had lower ($P < 0.07$) bone P content than heifers fed diets with supplemental P. Feeding heifers diets without supplemental P from 4 to 21 mo of age also resulted in lower bone Cu ($P < 0.01$) concentration. Bone K content was increased ($P < 0.05$) in heifers fed diets containing 0.29% P compared with heifers fed diets with supplemental P. Significant breed × diet interactions were also observed for bone K and Na content. Crossbred heifers fed diets containing 0.29% had higher K and Na content compared with Holstein heifers fed diets with supplemental P.

Bone metabolism as measured by serum pyridinoline or osteocalcin concentration of heifers fed diets containing 0.29 or 0.39% P are presented in Figure 1. Because no breed or breed × diet interactions were observed for serum pyridinoline or osteocalcin, only the main effects of dietary treatment are presented in Figure 1. The measurement of bone-specific collagen crosslinks, via pyridinoline, was used to monitor bone resorption metabolism (Liesegang, 2000). Feeding heifers diets with and without supplemental P had no effect on serum pyridinoline concentration, indicating bone resorption metabolism was not altered by P supplementation. Measurement of osteocalcin was used as a marker of bone formation. Osteocalcin is a noncollagenous protein found in bone, secreted by osteoblasts and functions as a hormone playing roles in bone mineralization and formation (Liesegang, 2000). As with pyridinoline, feeding heifers diets with and without supplemental P had no effect on serum osteocalcin concentration, indicating bone mineralization metabolism in dairy heifers was not altered by P supplementation. Although not specifically evaluated, variance associated with measuring serum osteocalcin in heifers was markedly larger compared with measurement of pyridinoline. Variance associated with measuring osteocalcin was largely an animal effect as opposed to a procedural effect because osteocalcin is more variable within growing animals compared with pyridinoline because osteocalcin is dependent on hormonal and cyclic growth status of the animal at the time of measurement (Shaw et al., 2006).

DISCUSSION

The NRC (2001) P requirements for dairy replacement heifers for the first and second year of growth are approximately 0.29 and 0.21% of dietary DM, respectively. The aforementioned P requirements are a generalization for discussion purposes as the actual P requirement for growing dairy heifers is dynamic and dependent on true BW, rate of growth, and bioavailability of P in the dietary ingredients (NRC, 2001). Using feed ingredients fed to heifers, the NRC (2001) mineral model and heifer DMI estimated by Hoffman et al. (2008), 300-kg heifers growing at 800 g/d require 6.6 and 6.2 g/d of P to meet maintenance and growth requirements, respectively, with a total absorbed P requirement of 12.7 g/d of P. The 0.29% P diet supplied 22.0 and 14.5 g of total and absorbable P per day exceeding total absorbable P requirements by 14.2%.

Table 3. Density and chemical composition of coccygeal vertebrae in Holstein and crossbred heifers fed diets containing 0.29 or 0.39% P from 4 to 21 mo of age

	0.29% P		0.39% P			Effects (P-value)		
Item	Holstein	Crossbred	Holstein	Crossbred	SE	Diet	Breed	Diet × breed
Bone density								
Trabecular bone density, mg/cm^3	467	439	408	457	24.0	0.39	0.66	0.13
Cortical bone density, mg/cm^3	573	589	628	563	33.2	0.67	0.46	0.23
Total bone density, mg/cm^3	525	522	529	515	14.7	0.92	0.54	0.72
Chemical composition[1]								
P, %	18.0	17.6	18.2	19.5	0.5	0.07	0.43	0.12
Ca, %	41.2	40.6	41.2	43.3	0.9	0.14	0.44	0.13
K, %	0.03	0.06	0.03	0.03	0.01	0.05	0.03	0.004
Mg, %	0.76	0.73	0.74	0.90	0.07	0.27	0.34	0.19
S, %	0.10	0.09	0.11	0.10	0.01	0.28	0.09	0.52
Na, %	0.73	0.83	0.79	0.71	0.03	0.34	0.63	0.01
Zn, mg/kg	126	119	127	123	3	0.45	0.14	0.63
Mn, mg/kg	0.54	0.45	0.53	0.56	0.05	0.29	0.46	0.22
Fe, mg/kg	8.0	8.6	9.0	8.8	0.7	0.40	0.83	0.55
Cu, mg/kg	11.2	5.8	18.4	12.3	2.5	0.01	0.02	0.88
Al, mg/kg	5.0	5.0	5.0	6.0	0.3	0.14	0.14	0.14
Ash, % of DM	58.2	58.3	58.4	58.0	0.5	0.87	0.80	0.67

[1]Chemical composition of bone presented as percentage or milligrams per kilogram of bone ash unless otherwise listed.

Similarly, a 500-kg pregnant heifer growing at 800 g/d requires 10.3 g/d and 5.7 g/d of P to meet maintenance, fetus, and growth requirements with a total absorbed P requirement of 15.9 g/d. The 0.29% P diet supplied 32.6 and 21.2 g/d of total and absorbable P, respectively, exceeding total absorbable P requirements by 33.3%.

The 0.29% P treatment was the minimum dietary P attainable because endogenous P in feedstuffs could not be removed and evaluated if long-term feeding of endogenous P results in any deficiencies in heifer frame development, bone density, bone composition, or bone metabolism. The 0.39% P treatment was implemented to assess luxury P feeding strategies, which are often implemented on commercial dairy operations (Zygarlicke and Hoffman, 2002).

In regard to heifer frame development, the bone density, bone composition, and bone metabolism data from this study do not support extra P supplementation to growing dairy heifers if dietary endogenous P is similar to NRC (2001) requirements for growing dairy heifers.

No alteration of heifer frame growth was observed when dietary P was increased from 0.29 to 0.39%. Heifers fed 0.29% P were similar in every measure of extent of frame growth compared with heifers fed 0.39% P, and heifers fed 0.29% P met or exceeded all frame growth guidelines for dairy heifers (MidWest Plan Service, 2003). These observations are supported by Erickson et al. (1999), who assessed effects of dietary P on finishing steer performance and carcass characteristics. Erickson et al. (1999) fed 386-kg crossbred steers diets containing 0.14, 0.19, 0.24, 0.29, or 0.34% dietary P and observed no effect of dietary P on steer growth, carcass composition, or skeletal maturity and concluded that supplementing P above levels supplied by basal feed ingredients was not necessary. Fradinho et al. (2006) fed developing foals 0.27 or 0.43% P from weaning to 1 yr of age and observed no differences in hip height, heart girth, or cannon bone circumference by feeding supplemental P. Data on the effects of dietary P on extent of frame growth of dairy heifers is unavailable, but Noller et al. (1977) fed Holstein dairy heifers 0.22 or 0.32% P from 5 until 17 mo of age and observed no difference in final BW or average daily gain, suggesting that increasing dietary P by 0.10 percentage units through supplementation had little effect on dairy heifer body growth.

Cortical, subcortical, and total bone density data support extent of frame growth data as no appreciable difference in bone densities were observed in coccygeal bones of heifers fed 0.29 or 0.39% P. Determination of bone density, which categorizes extent of bone mineralization, by pQCT is a relatively sensitive measurement technique. Shaw et al. (2006) was able to detect total bone density difference in pigs when trace minerals and two-thirds of the inorganic P were removed from the diet 28 d before slaughter. Specifically, the pQCT techniques used by Shaw et al. (2006) detected differences (373.1 vs. 332.6 mg/cm^3; $P < 0.01$) in total bone density as a result of increased bone resorption. Standard errors of measuring bone densities using pQCT in this study were similar to those of Shaw et al. (2006) but differences in cortical, subcortical, and total bone density were not observed in this study, suggesting that heifers fed 0.29% P had similar degrees of bone mineralization compared with heifers fed 0.39% P.

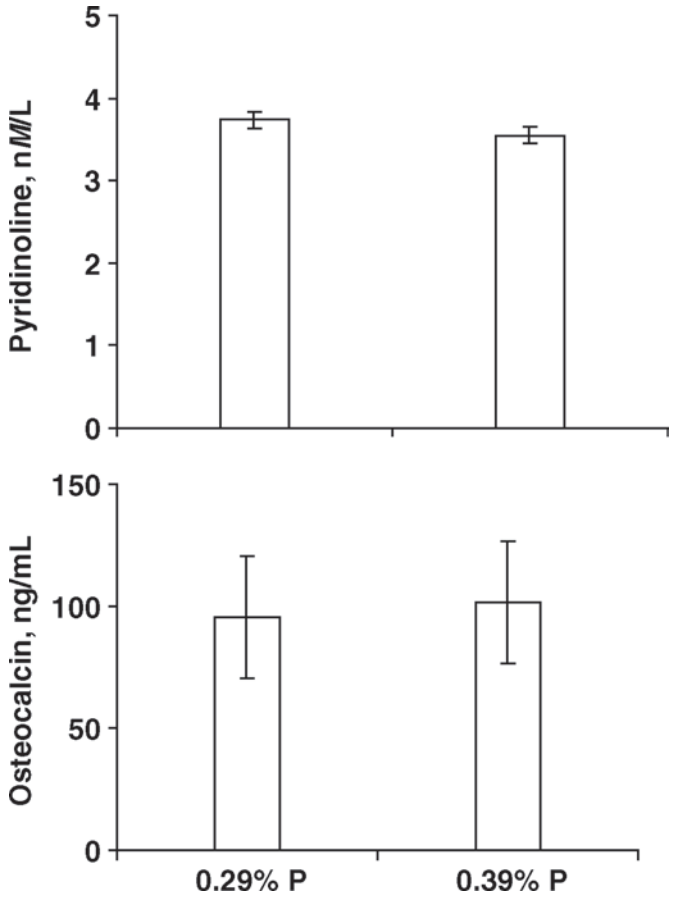

Figure 1. The effect of feeding dairy heifers 0.29 or 0.39% P from 4.0 to 12.5 mo of age on serum osteocalcin and pyridinoline levels.

Some nuances in bone mineral content were observed between heifers fed 0.29 and 0.39% P. Increasing dietary P from 0.29 to 0.39% increased ($P < 0.07$) bone P content from 17.8 to 18.9%, which is not an uncommon observation in dietary P experiments. Koch and Mahan (1985) fed growing swine 0.12, 0.31, or 0.50% P diets, and although P requirements were met at 0.31% of DM, bone P content increased linearly with increasing dietary P. Similar results have been observed in other studies (Hutcheson et al., 1992; Block et al., 2004) and

occur because bone P content is positively related to dietary P status (Williams et al., 1991) but not necessarily related to animal performance when animals are fed above P requirements (Koch and Mahan, 1985).

Dietary P levels fed to dairy heifers influenced bone K content, and a significant diet × breed interaction for bone Na was observed. Gillis (1948) reported that bone K content can be mediated by dietary P level but biological inference to altered bone K content in dairy heifers is unavailable. In bone, Na is in a nonexchangeable crystalline structure (Edelman et al., 1954), but the interactive effect of breed and dietary P content on bone Na metabolism and the corresponding effect on dairy heifer biology are unknown.

Markedly lower ($P < 0.02$) bone Cu contents were observed in crossbred heifers compared with Holstein heifers. Because crossbred heifers were Holstein-Jersey backcrosses (75% Holstein), increased bone Cu levels in crossbred heifers would be hypothesized because Cu absorption and utilization has been demonstrated to be more efficient in Jerseys compared with Holsteins (Du et al., 1996). The opposite effect was observed in this study, with bone Cu levels being lower in crossbred heifers with fractional Jersey genotypes. Data suggest crossbreeding Holstein and Jerseys may alter Cu utilization and further research is warranted.

Feeding heifers 0.39% P also increased ($P < 0.01$) bone Cu content compared with feeding heifers 0.29% P. Bovine bone contains approximately 10 μg of copper per g (Doyle, 1979) on an ash basis and copper plays an important role in collagen maturation linked to its function as a cofactor in lysyl oxidase (Turnlund, 2006). Increasing dietary P from nonphytin sources can adversely affect Cu bioavailability by increasing Cu excretion (Rama Rao et al., 2003), but that does not explain lower bone Cu content observed in this study when heifers were fed diets containing 0.29% P compared with feeding heifers 0.39% P. Prince et al. (1984) reported increased levels of liver Cu when pigs were fed high levels of dietary P (>1.00) but could not discount potential Cu contamination in P supplements used to alter dietary P. Likewise, in this study the need for intensive measurement of dietary Cu was not foreseen and thus not undertaken; therefore, dietary influences in regard to differences in bone Cu content observed in this study cannot be dismissed.

Increasing dietary P from 0.29% to 0.39% through supplementation of monosodium phosphate did not influence serum osteocalcin or pyridinoline levels. Dietary P deficiencies initiate bone resorption to meet acute metabolic P requirements. As a result, the bone's extracellular matrix is weakened and osteoblasts respond by stimulating osteocalcin synthesis in an effort to increase bone formation (Akesson et al., 1998). Thus, serum osteocalcin is inversely related to dietary P and Ca status (Eklou-Kalonji et al., 1999). Lack of an osteocalcin response in this study suggests that osteoblast numbers and activity were similar between heifers fed 0.29% P and heifers fed 0.39% P. Pyridinoline is a product of cross-linked collagen breakdown and has been successfully used as a marker of bone resorption by osteoclasts (Seibel et al., 1992). Liesegang et al. (2000) observed that lactating dairy cows had highly elevated pyridinoline levels 14 d postpartum, reflecting acute bone resorption in postpartum dairy cows in response to hypocalcemia. Lack of pyridinoline differences between heifers fed 0.29 and 0.39% P indicates that no appreciable difference in bone resorption was occurring.

CONCLUSIONS

Data from this study suggest that if endogenous P levels in feedstuffs are similar to the P requirements for growing dairy heifers, supplemental P is not required. These conclusions, however, can only be made in regard to extent of heifer frame growth, bone density, bone composition, and bone metabolism. Long-term production effects such as reproductive efficiency, postpartum metabolic disorders, and subsequent milk yield of heifers fed only endogenous P for the majority of the rearing period are not well defined. Intensive measurement of frame growth, bone composition, and bone metabolism in this study indicate that bone development, composition, or metabolism is unlikely to play a role in potential long-term production effects potentially associated with long heifer P feeding strategies during the rearing period.

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