Dietary calcium has little effect on mineral balance and bone mineral metabolism through twenty weeks of lactation in Holstein cows

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ABSTRACT

Calcium and P balance and mobilization from bone were evaluated through 20 wk of lactation to determine the timing and extent of net resorption of bone mineral and mineral balance in lactating dairy cows. Eighteen Holstein cows were blocked by parity and calving date and randomly assigned to 1 of 3 dietary treatments: high (1.03%, HI), medium (0.78%, MED), or low (0.52%, LOW) dietary Ca. Dietary P was 0.34% in all diets. Cows consumed treatment diets from calving to 140 DIM. Total collection of milk, urine, and feces was conducted 2 wk before expected calving and in wk 2, 5, 8, 11, and 20 of lactation. Blood samples were collected at 14 and 10 d before expected calving and 0, 1, 3, 5, 10, 14, 21, 28, 35, 56, 70, 84, 98, and 140 d after calving. Blood samples were analyzed for Ca, P, and parathyroid hormone concentration. Serum concentrations of osteocalcin (OC), a marker of bone formation, and deoxypyridinoline (DPD), a marker of bone resorption, were measured to assess bone mobilization. Rib bone biopsies were conducted within 10 d postcalving and during wk 11 and 20 of lactation. Dietary Ca concentration affected Ca balance, with cows consuming the HI Ca diet in positive Ca balance for all weeks with the exception of wk 11. Interestingly, all cows across all treatments had a negative Ca balance at wk 11, possibly the result of timed estrous synchronization that occurred during wk 11. At wk 20, Ca balances were 61.2, 29.9, and 8.1 g/d for the HI, MED, and LOW diets, respectively. Phosphorus balances across all treatments and weeks were negative. Bone Ca content on a fat-free ash weight basis was least in cows consuming the MED diet, but bone P was not different. Serum Ca and P were not affected by treatment. Dietary Ca concentration did not affect P balance in the weeks examined, but there was a clear effect of parity on balance, mark-

ers of bone metabolism, and bone P. Primiparous cows had greater serum OC and DPD concentrations than multiparous cows. Regardless of dietary treatment, serum OC concentration peaked around d 35 of lactation. Simultaneously, DPD concentration began to decrease, which may indicate a switch from net bone resorption to formation after d 35. However, this was not reflected in balance measures. This information may help refine dietary mineral recommendations for lactating dairy cows and suggests that dietary P requirements are independent of dietary Ca.

Key words: bone, calcium, phosphorus, cow

INTRODUCTION

Phosphorus metabolism of ruminants has been investigated by many (Braithwaite, 1983b; Morse et al., 1992; Wu et al., 2000; Wu and Satter, 2000; Knowlton and Herbein, 2002), yet estimates of bone resorption have not been fully described. The immediate Ca requirement for milk production at the time of parturition through the first weeks of lactation increases the demand for blood Ca by the mammary glands. This drain on the Ca pool is not preventable by feeding excess Ca (Horst et al., 1994), but is exacerbated when the diet is deficient in Ca (Benzie et al., 1955). A complex endocrine regulating system maintains blood Ca concentration within a narrow range (8 to 10 mg/dL, Goff et al., 1991). When this range is disturbed, an array of homeostatic mechanisms is activated, including resorption of bone.

Approximately 98% of total body Ca and 80% of body P are located in the skeleton. Bone Ca and P are stored as calcium phosphate $[\text{Ca}_2(\text{PO}_4)_2]$, which is amorphous, and as hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, which is a crystalline structure that provides binding locations for Ca and P. Mobilization of bone hydroxyapatite results in 10 ions of Ca and 6 ions of P being released into circulation (Goff, 2000) and is driven primarily by the concentration of blood Ca rather than blood P. The timing of bone resorption and formation are currently

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Table 1. Ingredient composition of diets

Item	Treatment ¹		
	LOW	MED	HI
Ingredient, % of diet DM			
Corn silage	39.7	39.7	39.7
Alfalfa silage	7.8	7.8	7.8
Cotton seed	2.9	2.9	2.9
High moisture corn	15.5	15.5	15.5
Concentrate mix	34.1	34.1	34.1
Concentrate mix, % of concentrate mix DM			
Soybean meal, 48% dehulled	58.3	59.1	59.9
Soy hulls, ground	35.8	32.0	28.3
Limestone	1.0	3.1	5.2
Tallow	1.5	2.1	2.8
Corn, fine ground	1.3	1.5	1.8
Salt	1.3	1.3	1.3
Sodium bicarbonate	0.6	0.6	0.6
Vitamin trace mineral mix ²	0.26	0.26	0.26

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively.

²Contained cobalt 135 mg/kg; copper 12,389 mg/kg; iodine 743 mg/kg; iron 61,946 mg/kg; manganese 49,556 mg/kg; selenium 334 mg/kg; zinc 49,556 mg/kg; vitamin A 394,685 IU/kg; vitamin D 264,819 IU/kg; vitamin E 5,460 IU/kg.

unknown in the dairy cow, but several blood markers of bone metabolism are available, allowing bone formation and resorption to be monitored noninvasively. However, these markers have yet to be validated in dairy cows with bone samples and mineral balance.

By accounting for drafts on bone with different dietary Ca concentrations, the P concentration of the diet could potentially be reduced to coincide with endogenous P release from bone during times of Ca-induced bone resorption. The amount of P excreted in the feces of dairy cows is directly proportional to excess dietary P (Morse et al., 1992). Phosphorus run-off is one of the leading causes of fresh water eutrophication and is a major focus of nutrient management for livestock producers (Knowlton et al., 2004). Field surveys demonstrate that P is typically fed at 20 to 40% above published recommendations for dairy cows (Knowlton et al., 2004). If these recommendations are excessive because of the lack of information on bone mineral resorption, the overfeeding problem is even worse than currently surmised. A more detailed understanding of bone mineral metabolism could help decrease excretion of excess P.

Our goal was to evaluate changes in body Ca and P during lactation based on Ca and P balance, serum markers of bone metabolism, and bone biopsy samples in cows fed 1 of 3 dietary concentrations of Ca. Our hypothesis was that feeding a low Ca diet will cause an increase in bone mobilization in early lactation that will result in an increase in the endogenous pool of P.

MATERIALS AND METHODS

Eighteen (10 first, 5 second, 2 third, and 1 fourth lactation) Holstein cows were assigned to 1 of 3 dietary treatments. The treatment diets were formulated to be isocaloric and isonitrogenous with Ca contents of 0.45 (**LOW**), 0.75 (**MED**), and 1.1% (**HI**) Ca on a DM basis (Table 1). Dietary energy and protein were maintained by substitution of corn, soybean meal, and tallow for soy hulls and limestone. Dietary P concentration was 0.34% in all diets. Before calving, all cows consumed the same dry cow diet (Ca 0.39% and P 0.38%). Treatment groups were balanced for parity and mature equivalent milk production. Treatments were applied from calving through 140 d of lactation. When cows were not in the metabolism stalls for total collection (described below) they were group-housed in a free stall barn and fed via a Calan gate system (American Calan, Northwood, NH). Cows were fed ad libitum once daily at 1530 h, feed offered and refused was recorded daily, and feed offered was increased daily if there were less than 10% refusals. Cows were milked twice daily at 0300 and 1500 h, and milk yield was recorded at each milking throughout the study. All procedures and protocols were approved by the Virginia Tech Animal Care and Use Committee.

Balance Periods

Total collection was conducted in 6, 4-d balance periods at 14 d before expected calving and at 14, 35,

56, 77, and 140 d of lactation. Cows were fitted with a urinary catheter (22 French, 75 mL; C.R. Bard Inc., Covington, GA) and moved into metabolism stalls on d 1 of each collection period for a 24-h adaptation to both the metabolism stall and the catheter. The urinary catheter was connected to tygon tubing that drained into a sealed clean plastic 12-L jug. Every 6 h, feces were removed from behind the cow and placed into sealed 130-L plastic containers, and the urine jugs were weighed and replaced with clean jugs. Urine was acidified (36 N H₂SO₄) to below pH 2 and then pooled into a larger sealed plastic jug until the end of the 24-h collection. Feces and urine were weighed, pooled by day, mixed thoroughly, and subsampled. Urine was stored frozen until analyzed for Ca and P via ICP (Thermo electron IRIS Intrepid II XSP; Thermo Fisher Scientific Inc., Waltham, MA). Fecal samples were dried at 60°C to a constant weight, ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and analyzed for Ca and P concentrations by a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) in accordance with AOAC (2006).

Cows were milked twice a day at 0530 and 1730 h while standing in the metabolism stalls. Milk was weighed and sampled at all milkings, and samples were analyzed for fat, protein, SCC, SNF, and lactose (Dairy Herd Improvement Association, Blacksburg, VA). A second milk sample was stored frozen at -20°C and later analyzed for Ca and P by the method of Walter et al. (1997; CEM Corporation, Matthews, NC).

In the metabolism barn, cows were fed at 0600 h; daily feed offered and refused was recorded during the balance periods. Feed refusals for each cow were sampled on d 3 of each balance period. Individual ration ingredients were sampled weekly throughout the study and pooled by month. Feed and feed refusal samples were dried at 60°C to a constant weight, ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas), and analyzed for DM, NDF, ADF, Ca, and P concentrations by Cumberland Valley Analytical Services in accordance with approved AOAC methods.

Blood Sampling and Analysis

Jugular blood samples were obtained at 14 and 10 d (averaged -12 and -8 d) before expected calving, at calving, and at 1, 3, 5, 10, 14, 21, 28, 35, 42, 56, 70, 84, 98, 119, and 140 d postcalving. Samples were collected and immediately placed on ice until centrifugation (2,200 × *g* for 20 min). Serum separator tubes (Fisher Scientific, Pittsburgh, PA) were utilized to facilitate serum separation during centrifugation. Serum was harvested and stored frozen at -20°C until analysis. Colorimetric

methods were used to analyze all serum samples for Ca (Aresenazo Reagent Set, Pointe Scientific, Canton, MI) and inorganic P (Inorganic Phosphorus Reagent Set, Pointe Scientific, Canton, MI). The interassay and intraassay coefficients of variation for serum Ca and serum P were 3.6 and 3.4%, respectively. Competitive immunoassays were used to quantify serum osteocalcin (OC; meta osteocalcin, Quidel Corporation, San Diego, CA) and deoxypyridinoline (DPD; total deoxypyridinoline, Quidel Corporation) in all serum samples. Serum OC had an interassay coefficient of variation of 2.8% and intraassay coefficient of variation of 2.7%, whereas interassay and intraassay coefficients of variation were 2.8 and 2.7%, respectively, for serum DPD. Parathyroid hormone (PTH) was analyzed in a pooled precalving sample (-14 and -10 d) and in the 1, 3, 5, and 10 d samples by a radioimmunoassay specific for the intact PTH peptide (N-tact PTH SP, DiaSorin, Saluggia, Italy). The PTH assay was completed in one run with an intraassay coefficient of variation of 9.1%.

Bone Biopsies

Rib bone samples were collected on d 8 ± 3.5 (left side, 11th rib) postcalving and on d 77 ± 5.6 (right side, 11th rib) and d 140 ± 3.7 (left side, 12th rib) using a modification of the procedure of Beighle et al. (1993). Briefly, biopsies were performed with the animal restrained in a standing head gate. The predesignated side for the bone sample was clipped ventrally for 25 cm beginning below the lumbar vertebrae and 20 cm caudal and cranial to the sample rib. The clipped area was cleaned and surgically prepared with alternate application of Betadine Surgical Scrub (Purdue Pharma L. P., Stamford, CT) and alcohol. The incision site was anesthetized with 2% lidocaine in an inverted L pattern. After a second surgical scrub and alcohol rinse, a 10-cm incision was made over the midline of the rib through the skin, fascia, and muscle. The periosteum was displaced from the biopsy site with a periosteal elevator (Jorgensen Laboratories, Loveland, CO), and the biopsy site was exposed with a Weitlaner retractor (Jorgensen Laboratories). A power drill and sterile drill bit were used to make a guide hole for the bone trephine (16 mm, Galt cranial trephine, Mercedes Medical, Tellevast, FL). Trephine depth was restricted to allow for sampling of the exposed medial and lateral bone to the depth of the medullary cavity. Bone samples weighed 700 to 1,000 mg. The circular piece of bone was removed and immediately stored in a sealed plastic bag and placed on ice. The periosteal flaps were replaced loosely without suturing followed by suturing of the muscle and stapling of the skin separately.

Table 2. Statistical model used to analyze all variables

Source	Collection variable (week)		Blood variable (day)		Error term
	df	Error df	df	Error df	
Treatment	2	12	2	12	Cow (treatment × parity)
Parity	1	12	1	12	Cow (treatment × parity)
Treatment × parity	2	12	2	12	Cow (treatment × parity)
Covariate	1 ¹	12	—	—	
Cow (treatment × parity)	11	—	12	—	
Time	4 ²	46	15 ³	174	Residual
Treatment × time	8	46	30	174	Residual
Parity × time	4	46	15	174	Residual
Treatment × parity × time	8	46	30	174	Residual
Residual	57		185		
Total	87		280		

¹The prepartum collection week data were used as a covariate for the feces and urine variables.

²Milk yield and DMI were evaluated in all 20 wk. Other collection variables were evaluated only during the 5 balance weeks. The time degrees of freedom for DMI and milk yield effects were 19.

³Serum parathyroid hormone (PTH) concentration was evaluated in the 1, 3, 5, and 10 d postpartum samples. The time degrees of freedom for PTH was 3.

Bone samples were extracted with ether using a modified Soxhlet procedure (AOAC, 2006) and subsequently ashed at 600°C in a muffle furnace. Ash samples were then analyzed for Ca and P using a microwave digestion procedure. Briefly, ashed samples were digested in 15.7 N nitric acid in a MARS 5 microwave (CEM Corporation, Matthews, NC) at 190°C and 14.1 kg/cm² for 10 min. Samples were further digested with 30% hydrogen peroxide for 30 min, diluted, and analyzed for Ca and P content colorimetrically.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (2003, SAS Institute Inc., Cary, NC) with the model defined in Table 2. Blood analyses included the −14 and −10 d samples which allowed for prepartum representation on the graphs. Two first-lactation cows consuming the MED diet did not have wk-2 balance data because of illness; one cow was treated for pneumonia, and another cow had a displaced abomasum at 10 d postpartum. Data from these 2 cows were removed from the analysis during times of illness (d 10 to 18). The wk −2 collection data (cows were consuming the same prepartum diet) were used as covariates for the feces and urine variables with the exception of one HI cow that calved 2 wk before her expected due date. The −2 wk data collected from the other HI cows were averaged and used as the covariate for that cow.

An autoregressive [AR(1)] covariance structure was used for DMI and milk yield data. The spatial power (SP POW) covariance structure was utilized for all other variables due to unequal spacing among sample intervals. The slice option was used when there was

a significant interaction. Treatments that were different in a particular week were further analyzed with pairwise Tukey tests. Orthogonal polynomial contrasts were used to test for linear and quadratic responses across time (day or week). The NESTED procedure was used to estimate correlations among OC, DPD, Ca balance, P balance, and bone Ca and P within a cow for each treatment. Significance was declared at $P < 0.05$ and trends at $P < 0.10$.

RESULTS AND DISCUSSION

DMI and Milk Yield

Actual Ca concentration of the treatment diets were 0.52, 0.78, and 1.03% for the LOW, MED, and HI diets, respectively (Table 3).

Dry matter intake was not affected by dietary Ca concentration (23.4 ± 0.6 kg/d; Table 4). Multiparous cows consumed more DM than did primiparous cows (25.4 vs. 21.4 ± 0.50 kg/d) and there was a signifi-

Table 3. Chemical composition (% of diet DM) of diets

Nutrient, %	Treatment ¹		
	LOW	MED	HI
CP	16.9	16.9	16.8
ADF	20.4	19.7	19.1
NDF	31.6	30.9	30.1
Ash	6.12	6.82	7.51
P	0.34	0.34	0.34
Ca	0.52	0.78	1.03

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively.

Table 4. Effect of dietary Ca concentration on DMI, milk yield, and milk component yields of 18 lactating Holstein cows

Item	Treatment ¹					Parity				Week
	LOW	MED ²	HI	SEM	<i>P</i> <	First	Multi	SEM	<i>P</i> <	<i>P</i> <
DMI, kg/d	24.1	23.4	22.7	0.6	0.26	21.4	25.4	0.5	<0.01	<0.01
Milk, ³ kg/d	32.4	34.2	31.9	1.0	0.28	29.5	36.3	0.85	<0.01	<0.01
Milk, ⁴ kg/d	39.8	42.1	42.0	2.1	0.69	35.5	47.1	1.8	<0.01	<0.01
Milk fat, %	3.87	3.72	3.35	0.19	0.20	3.65	3.63	0.17	0.91	<0.01
Milk fat, kg/d	1.55	1.53	1.41	0.11	0.66	1.27	1.71	0.09	<0.01	<0.01
Milk protein, %	2.94	2.85	2.81	0.06	0.43	2.88	2.84	0.05	0.62	<0.01
Milk protein, ⁵ kg/d	1.17	1.17	1.16	0.07	0.99	1.00	1.33	0.06	<0.01	<0.01
Milk lactose, %	5.01	4.94	4.89	0.05	0.22	5.04	4.94	0.04	<0.03	0.22
Milk lactose, ⁶ kg/d	2.00	2.00	2.03	0.10	0.98	1.74	2.28	0.09	<0.01	<0.01
Milk SNF, %	8.91	8.72	8.68	0.10	0.26	8.88	8.66	0.08	0.18	0.15
Milk SNF, ⁷ kg/d	3.54	3.56	3.69	0.20	0.98	3.06	4.05	0.18	<0.01	<0.01

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively. Data are presented as least squares means.

²Data from 2 cows consuming the MED diet were removed during d 10 to 18 due to illness.

³Actual daily milk yield for the entire 20 wk of the study.

⁴Data for the collection weeks only.

⁵Treatment × week, *P* < 0.003.

⁶Treatment × week, *P* < 0.006.

⁷Treatment × week, *P* < 0.002.

cant parity × week interaction. Dry matter intake responded both linearly (*P* < 0.0001) and quadratically (*P* < 0.0001) over time; however, the multiparous cows had a more rapid increase in DMI for the first 4 wk of lactation. Intake by primiparous cows peaked around wk 14, whereas multiparous cows peaked during wk 11. Dry matter intake was different across week, with cows consuming an average of 11 kg/d DM during wk 1, 27.4 kg/d at peak, and 27.2 kg/d DM at the conclusion of the study.

Milk yield was not affected by dietary Ca concentration (Table 4). As expected, multiparous cows had greater daily milk yield as compared with primiparous cows (36.3 vs. 29.5 ± 0.86 kg/d). Diet had no effect on milk fat yield, but there was a significant interaction of parity and time. Multiparous cows had greater milk fat yield because of greater production, with the exception of wk 8 and 20 in which the 2 parity groups were not different. There was a significant treatment × week and parity × week interaction for milk protein yield. Milk P content averaged 0.88 g/kg, and milk Ca content averaged 1.13 g/kg; treatment had no effect on these measures (data not shown).

Serum Minerals

Serum Ca was not affected by dietary Ca concentration (Table 5; Figure 1). At calving cows were within or just below the normal serum Ca range of 8 to 10 mg/dL (Goff, 2000) with no clinical signs of hypocalcemia. Similar to our findings, dietary Ca concentration had no effect on plasma Ca concentration in sheep fed 0.47

or 0.82% dietary Ca (Takagi and Block, 1991) or in postpartum cows fed 0.99 or 1.5% dietary Ca prepartum (Chan et al., 2006). There was a linear response in serum Ca over time (Table 5; Figure 1). First lactation cows tended to have greater serum Ca concentration than the multiparous group (*P* < 0.08; 11.45 vs. 10.76 ± 0.27 mg/dL). Chan et al. (2006) also observed that primiparous cows had greater serum Ca than multiparous cows.

Dietary Ca concentration did not affect serum P (Figure 1). Chan et al. (2006) also reported no difference in postpartum serum P concentration when cows were fed dietary Ca at 0.99 or 1.50% of diet in the prepartum period. In the present study, serum P concentration was greater in first lactation cows compared with multiparous cows (5.7 vs. 4.9 ± 0.13 mg/dL). However, there was a significant 3-way interaction of treatment, parity, and day for serum P (*P* < 0.04) concentration, making main effects and 2-way interactions difficult to interpret on their own.

Serum Markers of Bone Metabolism

The interaction of treatment × parity was significant for serum OC concentration (Figure 2). Within the primiparous group, the MED cows had less OC concentration as compared with the other dietary treatments, whereas OC concentration was not affected by treatment in multiparous cows. Osteocalcin concentration reflects formation of the protein matrix; the biological explanation for this observation is not apparent.

Figure 1. Effect of dietary Ca concentration (LOW 0.52% ●, MED 0.78% ■, or HI 1.03% ◆) on serum Ca (closed symbols) and P (open symbols) concentration from –14 d prepartum to 140 d of lactation (A: SEM = 1.61; $P < 0.72$ for treatment $\times$ time interaction and B: SEM = 0.62; $P < 0.27$ for treatment $\times$ time interaction).

Figure 2. Effect of dietary Ca concentration (LOW 0.52%, MED 0.78%, or HI 1.03%) on osteocalcin (OC) in primiparous and multiparous lactating Holstein cows from –14 d prepartum until 140 d of lactation ($P < 0.05$ for treatment $\times$ parity interaction).

Serum concentrations of both DPD and OC were greater in primiparous cows than in multiparous cows across 140 d of lactation (Table 5; Figure 3A and B). These results are similar to a study by Kamiya et al. (2005) that examined the effect of prepartum dietary Ca concentration (0.46 or 0.86%) on bone turnover from 13 d prepartum to 3 d postpartum in primiparous and multiparous Holstein cows. In that study, there was no effect of dietary Ca concentration on plasma OC or urinary DPD, but primiparous cows had greater concentrations of both as compared with multiparous cows (Kamiya et al., 2005). This is likely because the younger cows were still growing and had a greater rate of bone turnover than the older cows.

In the present study there was a parity $\times$ time interaction (Figure 3 A) for serum DPD concentrations. Both parity groups had a negative linear response in DPD over time. Primiparous cows had greater ($P < 0.05$) serum DPD concentration than multiparous cows

from –14 d prepartum to d 3, at d 10, and again at d 35 and 77. Otherwise the 2 groups were not different. Kamiya et al. (2005) found no effect of parity over time on urinary DPD concentration, but that study concluded at 3 d postpartum.

Both serum OC and DPD concentrations varied with time (Table 5). In early lactation serum OC was low in both primi- and multiparous cows suggesting relatively low formation of bone protein matrix. Osteocalcin then increased until 35 DIM where both groups reached a plateau. There was a linear decrease in serum DPD concentration over time (2.8 ng/mL at calving; 1.5 ng/mL at d 140), suggesting reduced bone formation immediately after calving. Liesegang et al. (2006) reported similar results for the marker of bone resorption they used, cross-linked carboxyterminal telopeptides of Type-I collagen (CTx), with an increase in serum concentration after parturition until 9 d postpartum. It was anticipated that serum DPD would be high around

Table 5. Effect of dietary Ca concentration on serum minerals and markers of bone metabolism in 18 lactating Holstein cows

Variable	Treatment ¹					Parity				Day	Linear	Quadratic
	LOW	MED ²	HI	SEM	$P <$	First	Multi	SEM	$P <$	$P <$	$P <$	$P <$
Cows (n)	6	6	6			10	8					
Ca, mg/dL	10.9	11.2	11.2	0.33	0.81	11.5	10.8	0.27	0.08	<0.01	<0.01	<0.01
P, mg/dL	5.29	5.12	5.42	0.15	0.40	5.65	4.90	0.12	<0.01	<0.01	<0.01	0.05
OC, ng/mL	56.8	50.8	56.3	3.16	0.31	68.1	41.2	2.59	<0.01	<0.01	<0.01	<0.01
DPD, ng/mL	2.19	2.21	2.33	0.08	0.40	2.56	1.94	0.07	<0.01	<0.01	<0.01	0.08

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively. Data are presented as least squares means.
²Data from 2 cows consuming the MED diet were removed during d 10 to 18 due to illness.
³Treatment $\times$ parity $\times$ day, $P < 0.04$.
⁴OC = osteocalcin; treatment $\times$ parity, $P < 0.05$.
⁵DPD = deoxypyridinoline; parity $\times$ day, $P < 0.01$.

calving when the cows would likely be resorbing bone, but the lack of effect of dietary Ca was counter to our hypothesis.

Ekelund et al. (2006) found an interaction of treatment and time for plasma OC from cows consuming normal vs. low to normal dietary P concentrations (0.43 vs. 0.32% for the first 4 mo of lactation then 0.43% for the remainder). These authors concluded there was net bone formation in mid lactation (wk 17 to 24) as indicated by greater concentrations of OC during those weeks. However, there was not a corresponding decrease in concentration of CTx. Also, they saw no change in P retention as measured by total collection.

Peterson et al. (2005) observed no effect of prepartum dietary P on blood osteocalcin or deoxypyridinoline. Naito et al. (1990) observed that plasma osteocalcin was positively correlated with both plasma Ca and plasma inorganic P in periparturient cows. Those relationships were not observed in the current study. Plasma Ca was in the normal range prepartum, and whereas dietary Ca was less than is common in the field, Ca intake (~54 g/d during total collection 14 d prepartum) is in excess of the NRC requirement (35 to 45 g of Ca/d) and well above the intake thought to stimulate early bone resorption (10 g/d of Ca; Goings et al., 1974).

Bone is a dynamic tissue constantly being resorbed and formed; the relative rates of resorption and formation determine net bone mass. Parity clearly has an effect on bone metabolism, as does stage of lactation. When serum concentration of markers of formation and resorption over time are compared in the present study, the graphs appear to be mirror images (Figure 3 A and B). Serum OC concentration peaked around d 35 of lactation; simultaneously, DPD concentration began to decrease. The change in direction of serum DPD and OC may provide an indicator of a net change from bone resorption to bone formation, but closer evaluation suggests that this relationship only holds in cows fed adequate Ca. In cows fed the MED and HI diets, the correlation between OC and DPD was strong and negative (-0.41 and -0.71 , respectively). In cows fed the LOW diet, however, there was no correlation between OC and DPD.

Serum Parathyroid Hormone

There was no effect of dietary Ca treatment on serum PTH concentration (9.9 ± 2.9 pg/mL). Typically, serum PTH increases near calving, activating bone resorption and renal tubular reabsorption to increase blood Ca (Goff, 2000). In the present study, this was not observed in the days examined (1, 3, 5, and 10 d postpartum). Kamiya et al. (2005) also observed no effect of dietary Ca on serum PTH. However, the researchers did re-

Figure 3. Effect of lactation on markers of bone metabolism from -14 d prepartum until 140 d of lactation in primiparous (◆) and multiparous (□) cows. A: DPD = deoxypyridinoline, marker of bone resorption (SEM = 0.13; $P < 0.006$ for parity $\times$ time interaction) and B: OC = osteocalcin, marker of bone formation (SEM = 5.1; $P < 0.23$ for parity $\times$ time interaction).

port an effect of parity, with PTH concentration less in primiparous cows as compared with multiparous cows. This was not observed in the present study (8.3 ± 1.9 vs. 11.4 ± 2.3 pg/mL; primiparous and multiparous, respectively).

Bone Mineral Content

Bone data are presented in Table 6. Data are expressed as grams of Ca or P/sample in addition to percentage of wet weight, percentage of dry weight, and percentage of fat free ash weight. As bone resorption occurs through reduction of bone density (increased porosity) or bone wall thickness, loss in bone mineral mass will be reflected in a loss of mineral per unit of surface area. Thus, taking samples of fixed surface area and assessing for mineral mass in the total sample allows detection of loss of bone mineral from either reduction in density or thinning of the cross-sectional area.

Table 6. Effect of dietary Ca concentration on bone Ca, P, and ash in 18 lactating Holstein cows

Item	Treatment ¹			SEM	<i>P</i> <	Parity		SEM	<i>P</i> <	Week
	LOW	MED	HI			First	Multi			
Calcium										
Ca, g/sample	0.21	0.19	0.20	0.008	0.11	0.20	0.20	0.007	0.48	<0.01
Ca, % wet weight	33.4	30.9	32.2	0.70	0.08	32.2	32.2	0.60	0.99	<0.01
Ca, % dry weight	36.3	31.5	33.1	1.61	0.13	34.4	32.9	1.40	0.41	0.59
Ca, % ash weight	54.5 ^a	49.5 ^b	53.3 ^{ab}	1.24	0.03	52.4	52.5	1.06	0.96	0.02
Phosphorus										
P, g/sample	0.06	0.06	0.06	0.003	0.98	0.06	0.07	0.003	0.24	<0.01
P, % wet weight	10.2	10.5	10.0	0.27	0.45	9.7	10.8	0.23	0.003	0.10
P, % dry weight	11.1	10.7	10.3	0.47	0.51	10.4	11.0	0.40	0.25	0.32
P, % ash weight	16.6	16.8	16.5	0.25	0.74	15.7	17.5	0.21	<0.01	0.80
Ash										
Ash, g/sample	0.39	0.38	0.38	0.01	0.94	0.39	0.38	0.015	0.49	<0.01
Ash, % wet weight	61.3	62.7	60.8	1.3	0.57	61.7	61.5	1.1	0.88	0.05
Ash, % dry weight	66.7	63.7	62.3	2.8	0.54	65.8	62.7	2.4	0.35	0.40

^{a,b}Values within the same row with different superscripts are significantly different ($P < 0.05$).

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively. Data are presented as least squares means.

Bone P content was not affected by dietary Ca concentration regardless of how results were expressed (Table 6). Dietary Ca concentration had no effect on bone Ca content on a mass or dry weight basis, but when expressed on a percentage of ash weight basis, the MED cows had lesser bone Ca content as compared with the LOW cows (Table 6). Bone Ca of cows consuming the HI diet was not different from the LOW or MED cows. The wk-11 bone sample for one cow on the LOW diet was identified as an outlier and removed from all bone analyses. Several covariates were tested to account for any possible difference in starting bone Ca concentration in the cows on the MED diet, including the 8 d sample, the preliminary OC and DPD concentrations, or the ratio of OC to DPD, but they were all nonsignificant. The SAS profile analysis was used to examine the difference between the samples. There were no significant differences for treatment with the profile analysis.

An unexpected result was the finding of greater bone Ca content for cows fed the LOW diet than for those fed the MED diet; nonetheless, the correlation of bone Ca and Ca balance was stronger for cows consuming the LOW diet ($r = 0.56$ vs. 0.37 MED and -0.06 HI). For the LOW cows, the strong correlation indicates that increased bone Ca was associated with a corresponding increase in Ca retention. However, for cows consuming the HI diet, Ca content in bone was not correlated to Ca retention. This is likely because they were consuming Ca in excess of their requirement. Excess Ca was excreted rather than used to build bone (Table 7).

It is puzzling that there were diet effects on bone Ca and not bone P because bone mineral exists primarily in the form of calcium phosphate or hydroxyapatite. We

postulate that diet may have affected the bone mineral compounds formed. Cohen (1973) observed that bone Ca was unrelated to pasture Ca concentration in yearling steers given no supplement or an oral drench of 35 or 70 g of P (sodium dihydrogen orthophosphate) each week. There was a negative relationship between bone Ca and P content in both studies. Cohen (1973) suggested that calcium carbonate was being removed and deposited without phosphate, resulting in an increase in the proportion of Ca in the bone. Most minerals that are contained in the body contain carbonate substitutions that are described as type A (OH^- substituted by CO_3^{2-}) and type B (PO_4^{3-} substituted by CO_3^{2-}). Type B is most prevalent (Rey et al., 1989; Penel et al., 1998). In the present study it appears cows were forming bone matrix after d 35 according to the serum markers of bone metabolism. It is reasonable to assume that cows were mineralizing the bone matrix with Ca and carbonate as indicated by the increase in bone Ca content with no change in bone P by treatment. However it is unclear why this appears to be favored in the MED treatment and not in the LOW and HI treatments.

Parity had no effect on bone Ca content but multiparous cows had greater bone P compared with primiparous cows on a percentage of wet and ash weight basis (Table 6). Wu et al. (2001) reported no differences in bone P as a percentage of ash (17.6% of ash) when cows were fed dietary P at 0.31, 0.39, or 0.47% for 2 or 3 yr. There was a linear increase in bone Ca content on a fat free ash basis across week (49.6 , 52.8 , and $54.8 \pm 1.8\%$ of fat free ash, for 8 d and 11 and 20 wk, respectively). However, there was a quadratic response in bone Ca grams/sample (0.23 , 0.17 , and 0.20 ± 0.007 for d 8

Table 7. Effect of week of lactation and dietary Ca concentration on Ca intake and partitioning in 18 lactating Holstein cows

Item	Week of lactation					SEM ²	Trt	Contrasts (week)			Trt × Wk
	2	5	8	11	20			Week	Linear	Quadratic	
Ca intake, g/d							<0.01	<0.01	<0.01	0.18	0.01
LOW ¹	114.7	120.4	125.3	123.7	128.6	11.2					
MED	129.5	181.8	191.4	192.0	228.5	14.8					
HI	205.7	246.5	248.3	248.2	299.7	11.9					
Fecal Ca, g/d							<0.01	<0.01	<0.01	<0.01	0.08
LOW	78.2	87.1	88.1	105.8	79.1	8.92					
MED	84.6	124.8	138.9	148.2	151.6	12.3					
HI	148.3	161.7	168.2	199.2	195.4	9.45					
Apparent Ca digestibility, %							0.91	<0.01	0.60	<0.01	0.72
LOW	32.1	27.6	29.6	13.5	38.2	4.8					
MED	36.2	30.3	26.5	22.5	32.5	6.8					
HI	26.0	34.2	30.8	19.0	34.6	5.1					
Urinary Ca, g/d							0.43	0.06	0.80	0.54	0.73
LOW	1.00	0.19	0.64	1.26	0.50	0.26					
MED	0.96	0.51	0.51	0.50	0.51	0.26					
HI	1.10	0.44	0.91	0.92	0.76	0.27					
Milk Ca, g/d							0.80	<0.01	0.05	<0.01	0.44
LOW	46.2	47.7	47.8	45.6	40.6	3.09					
MED	45.2	47.7	48.6	48.7	45.1	3.60					
HI	45.9	52.5	49.3	50.3	42.0	3.28					
Ca balance, g/d							<0.01	<0.01	<0.01	0.02	0.85
LOW	−10.7	−14.5	−11.2	−28.8	8.10	10.9					
MED	−7.48	7.42	1.96	−6.77	29.9	15.4					
HI	10.3	31.7	29.8	−2.32	61.3	11.5					

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively. Data are presented as least squares means.

²Unequal n (wk 2) on the MED diet. Largest SEM reported n = 4.

and wk 11 and 20, respectively). A quadratic response across time was also observed for grams/sample of fat free bone ash and P with the 8-d sample being greatest for both. The interactions of treatment $\times$ time and treatment $\times$ parity were not significant for bone Ca or P in grams/sample, or concentration on a wet, dry, or ash basis.

Ca Partitioning and Balance

There was a linear effect of dietary Ca treatment on fecal Ca excretion (Table 7); cows consuming more dietary Ca excreted more fecal Ca. Fecal Ca was different by week with linear and quadratic responses over time for all dietary treatments. There was a linear increase in fecal Ca excretion from wk 2 through the wk 11 balance. Week 11 had the greatest fecal Ca excretion across all treatments. This could be caused by a decrease in Ca binding protein (**CaBP**) potentially associated with estrus synchronization (discussed below; Inpanbutr et al., 1994). The decline in fecal Ca excretion at wk 20 caused the quadratic response. Multiparous cows excreted more fecal Ca as compared with primiparous cows (142 vs. 119 ± 4.3 g/d). This is likely the result of increased intake and is similar to the observations of Knowlton et al. (2001).

Urinary Ca excretion was not affected by diet (Table 7). Irrespective of treatment, all cows excreted the least urinary Ca during the wk-5 balance period, but urinary Ca was less than 1 g/d throughout. Primiparous cows excreted more Ca in their urine as compared with multiparous cows (0.90 vs. 0.57 g/d). Chan et al. (2006) reported no effect of prepartum dietary Ca (0.99 vs. 1.50%) on urinary Ca excretion and no effect of parity.

Milk Ca secretion was not influenced by dietary Ca treatment (Table 7). Multiparous cows had greater daily milk yield; therefore, multiparous cows also had greater daily milk Ca secretion as compared with primiparous cows (54.2 vs. 39.5 ± 2.2 g/d). The parity $\times$ time interaction was significant with multiparous cows decreasing in milk Ca secretion from wk 8 to 20, whereas primiparous cows did not change. This is the result of a significant decrease in milk yield in multiparous cows from wk 8 to 20; milk yield of primiparous cows remained constant in this time period. Weller et al. (2006) found that primiparous cows peaked in milk yield lower and later but had greater persistency of milk production as compared with multiparous cows. The interactions of treatment $\times$ week and treatment $\times$ parity were not significant.

As expected, Ca balance was affected by dietary Ca concentration (Table 7; Figure 4A). Cows consuming the LOW diet were in negative Ca balance for the en-

Figure 4. Effect of dietary Ca concentration (LOW 0.52% ●, MED 0.78% ■, or HI 1.03% ♦) on Ca balance (A) and P balance (B) in lactating Holstein cows at 2, 5, 8, 11, and 20 wk of lactation (A: SEM = 11.5, treatment $P < 0.001$ and B: SEM = 4.5, treatment $P < 0.89$).

tire 20 wk observed. During wk 2, cows consuming the MED diet had a negative Ca balance, but Ca balance was positive by wk 5. Knowlton and Herbein (2002) observed that all cows were in positive Ca balance by wk 5 with dietary Ca concentration at 0.72 to 0.77% of the diet DM. Cows consuming the HI diet had a positive Ca balance in most weeks except for wk 11, where all cows had a negative Ca balance due to an increase in fecal Ca excretion.

The decrease at wk 11 is not the effect of one specific calendar week because cows had a staggered start. Instead, the effect may be an unexpected side effect of the university herd's standard reproductive protocol. All cows in this study were enrolled in a first service synchronization program, Ovsynch (described in Cornwall et al., 2006). The Ovsynch protocol was intended to align all cows to be inseminated between 75 and 80 d postpartum. In this study, all cows were inseminated in their respective metabolism stall during the wk-11 balance period. This synchronization may have influenced Ca metabolism. Expression of calbindin-D9k (**CBP**), a calcium binding protein, and CBP mRNA in the bovine

uterus has been shown to be 3-fold greater during the luteal phase (progesterone dominant) than during the follicular phase (estrogen dominant; Inpanbutr et al., 1994). During the wk-11 balance period, all cows would have been in an estrogen dominant phase for ~24 to 48 h of the 120-h period, potentially explaining the dramatic drop in Ca balance observed during wk 11. The observation made in uterine tissue could also affect intestinal CBP. With less CBP in the intestines to bind Ca, there would be less Ca retained in the animal and therefore a lesser Ca balance. This could explain the greater fecal Ca excretion during wk 11 as compared with all other weeks. Cows were likely cycling throughout the other balance weeks, but it was during wk 11 that all cows were synchronized and bred for the first time.

The positive Ca balance throughout the study in cows fed HI diets contradicts earlier work. In sheep fed a plentiful Ca diet, Ca balance was negative until 63 to 70 d of lactation (Braithwaite, 1983a). Others have concluded that cows are unable to absorb enough Ca to meet their demands in early lactation regardless of dietary Ca concentration (Braithwaite, 1983a; Horst et al., 1994). Our data at 2 wk postpartum does not support this. Cows consuming the HI diet with 1.03% dietary Ca apparently absorbed enough dietary Ca to meet their needs. However, our first balance period was not until 2 wk postcalving; Ca balance may well have been negative in all diets immediately postpartum.

The interaction of treatment $\times$ time was not significant for Ca balance (Table 7). The main effect of week was different with a positive linear and quadratic response. The quadratic response to dietary treatment may be explained by the dramatic drop at wk 11 followed by the increase at wk 20.

Phosphorus Partitioning and Balance

There was no effect of dietary Ca on fecal P excretion (47.5 ± 2.4 g/d), and the interactions of treatment $\times$ parity, treatment $\times$ week, and parity $\times$ week were not significant (Table 8). There was, however, both a linear and quadratic effect of week. Fecal P was least at 2 wk postpartum and greatest at wk 11 and 20 for all treatments. Apparent P digestibility was different over time with the greatest value of 37.3% occurring during wk 8 and 11 (quadratic response).

Phosphorus was included in the diet at 0.34%, which is in the range of the current NRC (2001) P recommendations. It is suggested that dairy cows have the ability to absorb enough P to meet their needs when dietary P supplied is adequate and available (Morse et al., 1992). The present study had lesser apparent P digestibility ($35.7\% \pm 3.2$) than observed elsewhere (Wu et al., 2000; Knowlton et al., 2001; Ekelund et

al., 2006), and all cows were in negative P balance for the entirety of the study. This unexpected result may be because the P in the diet was not entirely available because P was incompletely recovered in the collection system, or because of error inherent with the analytical methods of P analysis. Also, it is possible that the stress of moving cows from the free-stall facility to the metabolism stalls induced stress sufficient to disrupt normal homeostatic mechanisms, perhaps artificially increasing fecal output during collection weeks. The relatively normal apparent DM digestibility (66.2%, no effect of diet) argues against this possibility.

Most likely, however, the negative P balance was simply because these cows produced more milk than expected without concomitant increase in DMI. Diets were formulated for a cow consuming 23.2 kg of DM and producing 36.3 kg of milk per day, but the cows actually consumed 23.9 kg of DM and produced 41.0 kg of milk per day. The P requirement was calculated for these cows using their actual DMI and milk yield during each of the balance weeks and averaged 0.39, 0.41, and 0.42% of the diet for the LOW, MED, and HI, respectively. The difference in milk yield increased the requirement for absorbed P by 4.2 g/d.

Multiparous cows had greater fecal P excretion as compared with primiparous cows (53.6 vs. 41.1 ± 2.0 g/d) due to greater daily P intakes. These results are similar to Knowlton et al. (2001) where primiparous cows had lesser P intakes, reduced fecal P, and lesser milk P secretion. In contrast to the present study, Knowlton et al. (2001) observed an effect of parity on apparent P digestibility with primiparous cows having greater apparent P digestibility than multiparous cows.

Urinary P excretion was not affected by treatment or parity, and interactions of treatment $\times$ parity and treatment $\times$ week did not occur. The main effect of week was significant with a quadratic response for urinary P excretion (Table 8). The greatest urinary P occurred during the 2-wk balance (1.6 ± 0.3 g/d) followed by a decrease until wk 20 (1.2 ± 0.2 g/d). It is well accepted that P absorption, and in turn excretion, are directly related to P intake but that the kidneys are a minor route of P excretion (Hibbs and Conrad, 1983; Horst, 1986; Morse et al., 1992; Knowlton and Herbein, 2002). However, when bone mineral is resorbed in support of blood Ca concentrations, the urinary excretion of P can increase to maintain P homeostasis (Todd et al., 1962) if it is not needed. Wu et al. (2001) suggested that spilling of P into urine greater than 1 g/d per cow is a reliable sign that the animal has adequate P. The present study suggests otherwise because urinary P exceeded this threshold and all cows remained in a negative P balance (data below).

Table 8. Effect of week of lactation and dietary Ca concentration on P intake and partitioning in 18 lactating Holstein cows

Item	Week of lactation					SEM ²	Trt	Contrasts (week)			
	2	5	8	11	20			Week	Linear	Quadratic	Trt × Wk
P intake, g/d							0.61	<0.01	<0.01	<0.01	0.20
LOW ¹	61.3	70.9	82.5	85.9	79.9	4.43					
MED	47.9	72.7	80.0	84.6	79.2	5.42					
HI	58.0	64.9	78.0	81.8	73.5	4.70					
Fecal P, g/d							0.22	<0.01	<0.01	<0.01	<0.01
LOW	40.3	47.8	50.3	59.2	56.7	3.16					
MED	28.8	42.9	49.6	49.4	55.5	3.99					
HI	36.8	41.3	49.9	50.8	53.5	3.41					
Apparent P digestibility, %							0.39	<0.01	<0.01	0.24	0.16
LOW	34.1	32.4	38.6	31.2	26.2	4.1					
MED	46.6	39.6	36.3	41.0	28.7	5.1					
HI	39.0	37.5	37.0	39.6	28.0	4.3					
Urinary P, g/d							0.18	0.03	0.92	0.04	0.28
LOW	0.74	0.41	0.65	0.94	1.34	0.40					
MED	2.95	0.62	0.91	1.07	1.46	0.55					
HI	1.04	0.63	0.81	0.94	0.86	0.43					
Milk P, g/d							0.99	<0.01	0.46	<0.01	0.19
LOW	35.5	37.5	37.0	35.0	32.9	2.46					
MED	29.0	36.7	39.4	38.5	36.4	2.95					
HI	34.7	39.1	37.0	37.5	30.9	2.61					
P balance, g/d							0.89	0.08	0.55	0.01	0.44
LOW	−15.6	−15.2	−5.87	−9.58	−11.3	3.19					
MED	−12.6	−8.59	−11.0	−5.41	−15.3	4.45					
HI	−12.9	−14.7	−8.15	−6.03	−10.3	3.39					

¹LOW, MED, and HI were dietary treatments with 0.52, 0.78, or 1.03% Ca as a percentage of diet DM, respectively. Data are presented as least squares means.

²Unequal n (wk 2) on the MED diet. Largest SEM reported.

Dietary Ca concentration had no effect on milk P secretion (Table 8). Multiparous cows secreted more daily P in milk as compared with primiparous cows across all balance weeks with the exception of wk 20 where the 2 lactation groups were not different (parity $\times$ time). Milk P concentration, like milk Ca, is thought to remain relatively constant throughout lactation in ruminants (Gallego et al., 2006), so this is likely due to the decrease in milk yield in the multiparous cows during wk 20.

Phosphorus balance was not affected by dietary Ca concentration (Table 8). This does not support the original hypothesis. It was postulated that dietary Ca concentration would directly affect P balance in the lactating dairy cow when dietary P was held constant.

Whereas no effect of dietary Ca was observed, parity had a clear effect on these variables. Multiparous cows consumed more P, excreted more fecal P, and secreted more milk P, which resulted in a more negative P balance compared with primiparous cows (-13.69 vs. -7.98 ± 1.5 g/d).

Cows were in negative P balance regardless of dietary Ca concentration for the first 20 wk of lactation (Figure 4B). There was a quadratic effect of time with wk 11 approaching a 0 balance. This contrasts with the Ca balance data where the wk 11 decrease in Ca retention is the result of increased fecal Ca excretion. Knowlton and Herbein (2002) observed negative P balance until wk 7 when cows were fed a diet containing 0.34% P. However, over the entire 11-wk study the P retention was never above 5 g/d (Knowlton and Herbein, 2002).

Ekelund et al. (2006) attempted to force cows to utilize mobilized bone P with low dietary P (0.31%) in early lactation as compared with normal dietary P (0.43%). In that study, however, bone resorption and overall P retention were not different between the low and normal P dietary treatment groups; low dietary P did not induce increased bone resorption. Ultimately there was no change in P excretion between the 2 dietary groups. Similarly, Braithwaite (1983b) reported that in sheep, bone is mobilized in response to the animal's needs for Ca and is not affected by dietary P content. The present study complements the study by Ekelund et al. (2006) in that varying dietary Ca concentration also did not affect P retention, bone resorption, or bone formation during the weeks examined.

Implications for P Feeding Recommendations

This study is unique in its examination of P requirements based on dietary Ca concentration to account for mobilized bone minerals. Bone that is resorbed is an available source of Ca and P that has not been entirely accounted for in current recommendations. There is

not an environmental concern for Ca, but excess P in the diet can increase P in the manure. Therefore, if P requirements can closely match the animal's needs and account for useable P coming from bone, the amount of P being excreted in manure and ultimately into the environment may be reduced. However, net times of bone resorption and formation need to be determined over the course of a lactation to ensure adequate mineral in the diet for when the animal switches from net resorption to formation of bone.

Regardless of treatment, data collected during balance weeks suggest that net bone formation occurred after the wk 5 balance based on ratios of OC to DPD across time. Irrespective of treatment, OC concentration was least during the wk-2 balance period and DPD concentration was greatest during wk 2 and 5. These relationships were inverted by the wk-8 balance. These changes may be indicative of a switch from net resorption to net formation of bone.

The changes in bone metabolism suggested by serum markers were not reflected in Ca and P balance. Calcium balance was greatest at wk 20 with values nearly double what was observed during all other balance weeks. Phosphorus balance was not different across time and was in fact negative during all weeks measured. Negative P balance cannot support net bone formation unless cows deposited bone mineral primarily as Ca, such as calcium carbonate. Our data support this hypothesis; Ca, but not P, content of bone samples increased over time and was greatest at wk 20 on a mass, wet weight, and ash weight basis. Also, bone Ca and P were not correlated, which further suggests that the bone mineral was something other than hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$.

Bone biopsy data are useful in shedding light on changes in mineral metabolism, but the choice of sampling site influences conclusions. Benzie et al. (1955) examined which bones were the most sensitive to resorption in sheep consuming diets with differing Ca concentration and determined that bone resorption is not uniform within a bone and throughout the skeleton. The relative sensitivity of bones to resorption in descending order was vertebrae > pelvis > skull > sacrum > mandible > ribs > proximal end of tibia > scapula.

In the present study, the ribs were chosen for biopsy because of their accessibility and success of previous work utilizing such an approach to assess bone mineral status (Little, 1972; Beighle et al., 1993). However, it appears in the present study that Ca and P were being mobilized from bones other than the ribs, especially in the LOW Ca cows. From parturition until after wk 11, cows on the LOW diet were mobilizing between 10 and 30 g/d of Ca based on balance data. Through wk 20

they mobilized between 5 and 15 g/d of P. If cows were not severely deficient, the drafts on other bones were able to meet the demand for the minerals before the cow began resorbing the ribs. In contrast, Little (1972) reported when yearling cattle were fed a P deficient (P at 0.08% of diet) diet for 6 wk, rib bone had a greater bone P content at wk 1 than the 6-wk samples. Cattle were reported to be in negative P balance (1 to 2 g/d) over the entire study, but the methods for determining balance were not reported.

One of our objectives was to validate the use of OC and DPD as markers of bone metabolism with total collection and bone biopsies. Total collection is often used as the standard to determine mineral requirements but it is not error-free. There is an inherent compounding of error that occurs when calculating mineral balance from intake, feces, urine, and milk each with error that may lead to an increased likelihood of committing a type I error. Variability could potentially mask the physiological activity that is implied by the serum marker data. Alternatively, the markers of bone metabolism that were utilized are simply that: markers. Alone, they may not be a perfect method for determining Ca and P metabolism either. Based on our data, validation of these bone markers of metabolism with total collection and bone biopsy is conditional. During the wk-20 balance, there was greater Ca retention, bone formation (i.e., lesser DPD and greater OC), and greater bone Ca content as compared with earlier collections. This indicates that the markers of bone metabolism can be used as a noninvasive indicator of dietary effects on bone activity when the cow is in a state of metabolic stability (i.e., mid to late lactation).

CONCLUSIONS

Dietary Ca concentration had no effect on serum minerals, serum markers of bone metabolism, or P balance from wk 1 to 20 of lactation. However, parity and time had clear effects on these variables. Relationship of the serum markers of bone metabolism with total collection and biopsy appears to be conditional and limited to times of metabolic steady-state. Ultimately, dietary Ca did not affect P retention in the weeks examined. Therefore diets can be formulated for P independent of Ca concentration in the diet.

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