Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows

V. R. Moreira,^{*1} L. K. Zeringue,^{*} C. C. Williams,[†] C. Leonardi,[‡] and M. E. McCormick^{*}

^{*}Louisiana State University AgCenter Southeast Research Station, Franklinton 70438

[†]Louisiana State University AgCenter School of Animal Sciences, and

[‡]Louisiana State University Department of Experimental Statistics, Baton Rouge 70803

ABSTRACT

A study was carried out to verify the effect of Ca and P levels on production, digestibility, and serum bone metabolism biomarkers in dairy cows. Fifty-two nonlactating multiparous cows (≥ 3 lactations) were confined in a free-stall barn approximately 20 d before calving. A standard close-up diet was fed to cows once daily until d 2 postpartum. Cows were randomly assigned to 1 of 4 dietary treatments arranged in a 2×2 factorial approach averaging 0.64% Ca for high Ca (HCa), 0.46% Ca for low Ca (LCa), 0.47% P for high P (HP), and 0.38% P for low P (LP) on a dry matter basis. Experimental diets were fed twice daily from 3 d in milk (DIM) until 31 DIM. Intake and milk yield were recorded daily. Milk samples were collected on d 28, 29, and 30 postpartum for components analyses. Blood samples were drawn 10 d before expected calving, at calving, and at 15 and 30 DIM for serum analyses of osteocalcin, a biomarker of bone accretion, and pyridinoline, a biomarker of bone resorption. Total fecal collection was conducted when cows in a block averaged 20 DIM. Intake and production traits were not significantly affected by any of the dietary treatments. Cows averaged nearly 21 kg/d dry matter intake and 44 kg/d milk yield from 6 to 31 DIM. There were no significant differences across treatments in body weight or body condition score loss. Phosphorus intake, P fecal output, P digestibility, and P apparent absorption were affected by dietary P content. Calcium intake was higher with HCa, but Ca fecal output, digestibility, and apparent absorption showed an interaction between dietary Ca and dietary P. Calcium fecal output was 100.6 g/d for cows fed HCaHP, intermediate for cows on the HCaLP diet (89 g/d), and similar among cows fed the 2 LCa diets (70 g/d with LCaHP and 75 with LCaLP). There was no significant effect of Ca or P on osteocalcin measurements. Pyridinoline concentrations

were affected by dietary Ca levels and tended to have a significant dietary Ca $\times$ dietary P interaction. Phosphorus apparent digestibility occurred independently of dietary Ca levels. Results of this study suggest that more bone was mobilized in cows fed LCa diets, but excess dietary P caused greater and prolonged bone mobilization regardless of dietary Ca content.

Key words: cow, bone, calcium, phosphorus

INTRODUCTION

Daily dietary mineral requirements for lactating dairy cows have been more precisely defined since the development of the factorial approach to estimating nutrient requirements in the mid-20th century. A factorial approach considers the sum of maintenance, lactation, pregnancy, and growth requirements corrected for absorption coefficients (NRC, 2001). More recently, uncertainty associated with measurements of maintenance requirements (Spiekens et al., 1993) and absorption coefficients (Martz et al., 1990; Knowlton and Herbein, 2002), and fear of poor productive (Kincaid et al., 1981; Valk and Šebek, 1999), reproductive (Lopez et al., 2004a,b), and health (Wu et al., 2000, 2001) performances justified several feeding trials evaluating dietary P recommendations for high-producing dairy cows. Long-term studies indicate that cows fed dietary P at a flat rate of 0.32% throughout the lactation can support 9,000 kg of milk production or more (Kincaid et al., 1981; Valk and Šebek, 1999; Knowlton and Herbein, 2002). In a few other trials, nonetheless, cows fed dietary P concentrations as low as 0.28 and 0.31% were able to maintain high productive performance (Valk and Šebek, 1999; Wu et al., 2001). It should be noted, however, that lactation studies including marginal concentrations of dietary P invariably assume that cows can cope with deficiency during early stages of lactation.

Hormonal control of P homeostasis is not completely understood but is thought to be closely related and mostly secondary to Ca homeostasis (Horst, 1986; Breves and Schröder, 1991). Calcium metabolism in dairy cows is tightly regulated by parathyroid hormone

Received April 10, 2009.

Accepted June 13, 2009.

¹Corresponding author: vmoreira@agcenter.lsu.edu

(PTH) and vitamin D metabolites stimulating bone resorption of Ca and P in response to hypocalcemia. Intestinal absorption of Ca and P occurs primarily by passive diffusion when cows are fed diets containing sufficient amounts of those minerals. Hypophosphatemia and hypocalcemia along with PTH can independently induce 25-hydroxycholecalciferol renal hydroxylation to 1,25-dihydroxycholecalciferol [**1,25-(OH)₂D₃**]. Vitamin D metabolites promote active intestinal transport of Ca and P across the epithelial cells (Breves and Schröder, 1991; Horst et al., 1994; Goff, 2006).

It has been suggested that 0.8 to 1.3 kg of Ca can be mobilized from the bones of adult cows to cope with absorption deficiencies (dietary or physiological) and with high milk production in early lactation (Liesegang et al., 2000; NRC, 2001). Most cows are likely in negative balance during the first 10 d of lactation because of slow adaptation of Ca absorption mechanisms in response to the sudden increase in Ca demand for milk production after calving. Positive balance should be reestablished by 45 to 60 DIM (NRC, 2001). Most body P is stored as hydroxyapatite and calcium phosphate in the bones at a 1.67:1 ratio of Ca: P (Ternouth, 1990; Goff, 2000). Assuming P is mobilized along with Ca from bones at a similar rate, it can be estimated that 0.5 to 0.8 kg of P could be made available from bone resorption in the first few weeks of lactation, which is equivalent to saying that P resorption can supply P secreted into 26 to 42 kg of milk/d during the first 21 d of lactation. The requirement models described in NRC (2001) did not credit Ca or P mobilization from bones.

Few studies evaluated the effect of P on bone metabolism in ruminants. Braithwaite (1983a,b) fed ewes 2 diets containing Ca and P either above or below dietary requirements and injected Ca and P trace isotopes intravenously for a kinetics study. Although bone resorption was not measured, the author suggested that Ca bone resorption occurred at a constant rate because of limited intestinal absorption during late pregnancy and early lactation. Ekelund et al. (2006) fed a group of cows a diet containing 0.64% Ca and either 0.32% P for the first 4 mo of lactation followed by 0.43% dietary P for the remainder of the lactation period or 0.43% dietary P throughout the lactation. Changes in bone metabolism indicators in blood [osteocalcin (**OC**) and cross-linked carboxyterminal telopeptides of type-I collagen (**CTX**)] showed a similar pattern of mobilization during early lactation, but higher osteocalcin indicated more bone accretion with 0.43% dietary P between 14 and 24 wk of lactation. Kichura et al. (1982) fed 9.5 or 86 g of Ca and 10 or 82 g of P to cows for 20 d before calving. The authors concluded that gastrointestinal absorption of Ca and P was more efficient in cows fed 10 g of P, possibly as a result of greater numbers or higher

efficiency rate of intestinal receptors for 1,25-(OH)₂D₃. Results from the studies discussed above suggest that P homeostasis is secondary to Ca metabolism control. It is frequently noted in P feeding trials that P metabolism in bones is subject to Ca supply in early lactation. However, studies concurrently investigating the effect of dietary Ca and P fed to early-lactation cows on animal performance and on markers of bone metabolism could not be found in the literature.

It was hypothesized that the effect of dietary P levels on bone metabolism in early-lactation dairy cows depends on dietary Ca content, warranting attention to both minerals when preparing diets for cows in the transition period. The objective of this study was to evaluate the influence of dietary Ca and P levels on performance and bone metabolism markers of cows during the first month of lactation.

MATERIALS AND METHODS

This experiment was conducted at the Louisiana State University Agricultural Center Southeast Research Station, located in Franklinton, Louisiana, after the protocol was approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee.

Animals and Management

Fifty-two multiparous cows (3 lactations or more) were utilized in a randomized block design. Cows were grouped by expected calving date in 13 blocks of 4 cows each. Six cows were removed from all data sets before statistical analyses because of issues unrelated to treatments. Three cows were removed before treatments were applied because of calving too early, mastitis/pneumonia, and postpartum paralysis. Another cow was removed within a week after calving because of coliform mastitis. Two cows completed the study but were later removed for poor performance (deemed outlier) and a displaced abomasum.

The study was carried out between October and May of 2004–2005 and 2005–2006. Animals were housed in a free-stall barn fitted with electronic gates (American Calan Inc., Northwood, NH) for individual feeding. Each block of cows was brought to the barn when cows were at least 20 d before expected calving date and remained in the study until the last cow in each block reached 31 DIM. For calving comfort, dry cows had free access to a grassed area, which was mowed as needed to limit consumption of fresh herbage. Cows were randomly assigned to 1 of 4 treatments before calving. Treatment feeding began 3 d after calving. Blocks of cows remained in the barn on average for 65

Table 1. Mean chemical composition ($\pm$ SD, % of DM) of the forages

Item	Alfalfa hay	Bermuda hay	Corn silage
CP	19.9 $\pm$ 1.23	14.5 $\pm$ 0.83	8.70 $\pm$ 1.05
NDF	43.7 $\pm$ 3.25	70.7 $\pm$ 1.75	46.3 $\pm$ 4.08
ADF	34.2 $\pm$ 3.19	35.1 $\pm$ 1.01	27.1 $\pm$ 2.98
Calcium	1.11 $\pm$ 0.22	0.38 $\pm$ 0.05	0.15 $\pm$ 0.04
Phosphorus	0.25 $\pm$ 0.03	0.19 $\pm$ 0.01	0.26 $\pm$ 0.02

$\pm$ 5 d, from when dry pregnant cows were brought in the barn until the last cow completed 31 DIM. Animals produced $12,130 \pm 2,090$ kg of milk over the 305 d of their previous lactation. Dry cows were fed once daily, whereas lactating cows were fed (0800 and 1400h) and milked (0600 and 1700h) twice daily.

Diets

Diets were fed as TMR (Tables 1 and 2). Nutrient contents of feeds changed in the course of the study. Each block of cows was offered feeds from a single batch or harvest from the beginning to the end of their experimental period to limit changes in nutrient composition. Diets were reformulated using the same feedstuffs for each block of cows entering the study to maintain similar dietary Ca and P concentrations across the entire experiment. Treatment diets were mixed twice daily using a Data Ranger (American Calan Inc.) and offered in approximately equal portions. All cows were fed a standard close-up diet from arrival to the barn until the second day after calving. Treatment diets were offered from the third day after calving until cows left the experiment. A basal diet was formulated for mineral content below NRC (2001) recommendations. Four premixes were prepared with ground corn, calcium carbonate, and monosodium phosphate as supplements to the basal diet to obtain treatments arranged as a 2×2 factorial giving high Ca–high P (**HCaHP**), high Ca–low P (**HCaLP**), low Ca–high P (**LCaHP**), and low Ca–low P (**LCaLP**), where high and low indicate nutrient contents above and below predicted requirements, respectively. Average nutrient contents calculated using actual ingredient analyses were (on a DM basis) 0.64% Ca in HCa and 0.46% Ca in LCa diets, and 0.47% P in HP and 0.38% P in LP diets (Table 2).

Sampling, Laboratory Analyses, and Calculations

Daily DMI was determined from the beginning of the pretrial period until the end of the experimental period for each block of cows. Samples of TMR, feed refusals, and corn silage were collected daily, stored frozen, and subsampled weekly. Concentrates and hays were sampled weekly. Feed samples were dried at 60°C for 48 h every week. Dried samples were ground through a

2-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and then passed through a 1-mm screen in a Cyclotec 1093 Sample Mill (Foss Tecator, Höganäs, Sweden). Ground samples were composited by month. Feed and fecal samples were analyzed for Kjeldahl N and P in an automated colorimetric assay adapted for flow-injection analyzer (QuickChem 8000 FIA, Lachat Instruments, Milwaukee, WI). Fibers were analyzed according to the sequential NDF/ADF analysis utilizing heat-stable amylase and sodium sulfite (Van Soest et al., 1991) modified for the Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Fairport, NY). Calcium, Mg, and K in feed samples and Ca in fecal samples were determined by flame atomic absorption spectrophotometry (Perkin-Elmer Analyst 300, Norwalk, CT) after dry-ashing at 500°C overnight in porcelain crucibles. Ashed samples were dissolved over heat in 5 mL of 20% HCl, transferred to 100-mL volumetric flasks, and diluted to 100 mL with deionized water.

Milk production was measured in AfiFlo milk meters (SAE Afikim, Kibbutz Afikim, Israel) and recorded daily from each milking for statistical analyses by Afimilk software (Afirm version 3.01A, SAE Afikim). Milk was sampled from 6 consecutive milkings when cows in a block averaged 28 DIM. Milk samples were analyzed by the Louisiana DHIA Laboratory (Baton Rouge) for fat and true protein by near-infrared spectroscopy (Bentley 2000, Bentley Instruments, Chaska, MN), and for somatic cells in a flow cytometer (Bentley Somacount 300). Milk components were weighted based on a.m. and p.m. milk production on sampling dates.

Blood samples were drawn at 0700 h from coccygeal vessel into vacuum tubes (BD Vacutainer Serum, Becton Dickinson, Franklin Lakes, NJ) on d 10 before expected calving and d 1 (calving date), 15, and 30 postpartum. Whole blood samples were allowed to clot for at least 20 min, and then centrifuged at $2,200 \times g$ for 30 min at 4°C (Marathon 22 KBR, Fisher Scientific, Pittsburgh, PA). Serum samples were stored frozen for later analyses of serum bone metabolism markers (Liesegang et al., 2000; Allen, 2003). Enzyme immunoassay for the quantitation of pyridinoline (**PYD**, Metra Serum PYD; Quidel, San Diego, CA) and OC (Metra Osteocalcin; Quidel, San Diego, CA) were performed at an optical density of 405 nm in a BioTek Powerwave XS microplate spectrophotometer using Gen5 Data

Table 2. Ingredient and analyzed chemical contents of diets (mean $\pm$ SD) containing different Ca and P concentrations

Item	Diet ¹				
	Prepartum	HCaHP	HCaLP	LCaHP	LCaLP
Ingredients, % of DM					
Alfalfa hay	15.4	10.1 $\pm$ 0.2	10.1 $\pm$ 0.2	10.2 $\pm$ 0.2	10.2 $\pm$ 0.2
Bermudagrass hay	11.7	5.10 $\pm$ 0.08	5.16 $\pm$ 0.09	5.16 $\pm$ 0.09	5.17 $\pm$ 0.09
Corn silage	45.6	37.3 $\pm$ 2.2	37.4 $\pm$ 2.2	37.4 $\pm$ 2.2	37.4 $\pm$ 2.2
Whole cottonseed	3.91	0.0	0.0	0.0	0.0
Corn grain, ground	13.3	24.9 $\pm$ 1.2	25.0 $\pm$ 1.1	25.0 $\pm$ 1.1	25.0 $\pm$ 1.1
Soybean meal (48% CP)	9.66	18.0 $\pm$ 1.8	18.1 $\pm$ 1.7	18.1 $\pm$ 1.7	18.1 $\pm$ 1.8
Treatment premix ²	0.00	2.80 $\pm$ 0.19	2.31 $\pm$ 0.16	2.29 $\pm$ 0.18	2.30 $\pm$ 0.55
SE Sta. Exp. Mineral ³	0.43	0.51 $\pm$ 0.01	0.51 $\pm$ 0.01	0.51 $\pm$ 0.02	0.51 $\pm$ 0.01
Calcium carbonate	0.00	0.69 $\pm$ 0.06	0.70 $\pm$ 0.06	0.70 $\pm$ 0.06	0.70 $\pm$ 0.07
Monosodium phosphate	0.00	0.03 $\pm$ 0.03	0.03 $\pm$ 0.03	0.03 $\pm$ 0.03	0.03 $\pm$ 0.03
Salt	0.00	0.66 $\pm$ 0.15	0.67 $\pm$ 0.15	0.67 $\pm$ 0.15	0.67 $\pm$ 0.15
Nutrient content ⁴					
DM, % as fed	48.8 $\pm$ 4.7	50.6 $\pm$ 3.4	50.6 $\pm$ 3.5	51.0 $\pm$ 3.7	51.2 $\pm$ 3.6
CP, % of DM	15.4 $\pm$ 0.6	17.4 $\pm$ 0.7	17.5 $\pm$ 0.7	17.5 $\pm$ 0.7	17.5 $\pm$ 0.7
NDF, % of DM	39.9 $\pm$ 1.8	29.1 $\pm$ 0.8	29.2 $\pm$ 0.8	29.2 $\pm$ 0.8	29.2 $\pm$ 0.8
ADF, % of DM	24.1 $\pm$ 1.3	17.1 $\pm$ 0.7	17.1 $\pm$ 0.7	17.1 $\pm$ 0.7	17.1 $\pm$ 0.6
Ca, % of DM	0.34 $\pm$ 0.04	0.63 $\pm$ 0.02	0.65 $\pm$ 0.02	0.46 $\pm$ 0.03	0.45 $\pm$ 0.02
P, % of DM	0.33 $\pm$ 0.01	0.47 $\pm$ 0.02	0.37 $\pm$ 0.01	0.47 $\pm$ 0.02	0.38 $\pm$ 0.01
Mg, % of DM	0.27 $\pm$ 0.05	0.31 $\pm$ 0.05	0.31 $\pm$ 0.05	0.31 $\pm$ 0.05	0.31 $\pm$ 0.05
K, % of DM	1.49 $\pm$ 0.53	1.36 $\pm$ 0.50	1.37 $\pm$ 0.50	1.37 $\pm$ 0.50	1.37 $\pm$ 0.51
RUP, % of DM	4.60	6.50	6.50	6.60	6.50
NE _L , Mcal/kg of DM	1.62	1.59	1.60	1.58	1.59
NFC, % of DM	36.9	42.6	42.9	42.9	43.3
DCAD, mEq/kg	182.7	229.0	197.0	229.0	196.0

¹HCa and HP indicate dietary concentration of Ca and P above NRC (2001) predicted requirement; LCa and LP indicate dietary concentration of Ca and P below NRC (2001) predicted requirement.

²Treatment mixes contained ground corn, calcium carbonate, and monosodium phosphate.

³Southeast Experiment Mineral Concentrate contained Na, 18.2%; Cl, 27.3%; Mg, 13.9%; S, 5%; K, 3.47%; Ca, <1.5%; Zn, 12,700 mg/kg; Mn, 4,139 mg/kg; Cu, 1,937 mg/kg; I, 182 mg/kg; Co, 96 mg/kg; Se, 55 mg/kg; vitamin A, 1,146 kIU/kg; vitamin D, 231 kIU/kg; and vitamin E, 3.81 kIU/kg; manufactured for Kentwood Co-op (Kentwood, LA).

⁴RUP, NE_L, NFC, and DCAD as estimated by NRC (2001).

Analysis Software (BioTek Instruments, Winooski, VT). Intra- and interassay coefficients of variation were, respectively, 9.35 and 18.4% for PYD and 10.2 and 16.3% for OC.

Feces were collected during 3 consecutive days when cows in a block averaged 20 ± 4 DIM. Blocks of 4 cows were corralled within the single-alley free stall to allow access between their respective automatic gates and 5 stalls. Cows were followed by an individual with 4 dedicated buckets attached to 150-cm wooden poles to collect feces as they were voided. Two individuals accompanied the cows during milking. Feces were stored in 70-L plastic tubs. Weights were recorded and feces were blended using a mud-mixer attached to an electric drill before representative samples were taken 4 times a day at 6, 12, 18, and 24 h after the morning TMR was fed. Apparent digestibility was calculated based on the average DMI measured during the 7 d ending with the fecal collection. Ten blocks (38 cows) out of the 13 blocks of cows were used for total fecal collection in the digestion trial because of labor and time constraints. Nutrient digestibility was calculated using the following equation (Church, 1988):

$$\frac{(\text{DM consumed}) \times (\% \text{ nutrient in feed DM}) - (\text{fecal DM}) \times (\% \text{ nutrient in feces DM})}{(\text{DM consumed}) \times (\% \text{ nutrient in feed DM})} \times 100.$$

Apparent nutrient absorption was the difference between nutrient ingested and nutrient fecal excretion. Total absorbable requirement (**TAR**) was calculated for every cow used in the digestion study using the NRC (2001) model adjusted for BW and milk yield.

Body condition score (1 = thin to 5 = fat) was determined at 10 d before expected calving and at 30 DIM by 3 different evaluators (Wildman et al., 1982). Cows were weighed for 2 consecutive days after the a.m. milking, 10 d before expected calving, after calving, and 29 DIM. Weights and scores were averaged for the consecutive days and among scorers before statistical analyses.

Statistical Analyses

All the response variables were analyzed using the MIXED procedure of SAS (Littell et al., 1996; SAS Institute, 2003). Milk yield and DMI were analyzed

including fixed effect of Ca, P, day (from 6 to 31 DIM), and their 2- and 3-way interactions in the model. Block was included in the model as random effect. Daily values were analyzed as repeated measures using a Toeplitz covariance structure. Block $\times$ Ca $\times$ P was the subject of the repeated statement. The covariance structure was selected by choosing the best fitting model according to the Akaike information criterion. Average intake during the third week before calving was used as covariate for postpartum DMI. Milk production in the previous lactation was used as covariate for milk yield.

Body weight loss and BCS loss were analyzed including in the model Ca, P, and their interaction as fixed effects. Block was included in the model as a random term. The PYD and OC concentrations were analyzed including fixed effect of Ca, P, day (15 and 30 DIM), and their 2- and 3-way interactions in the model. Block and block $\times$ Ca $\times$ P were included as random effects in the model. The OC and PYD measured at calving day were included in the model as a covariate. The statistical analysis of OC was conducted on the natural logarithm-transformed variable. Milk composition, digestibility data, and production traits measured during the digestibility portion of the trial were analyzed including Ca, P, and their interaction as fixed effects in the model. Block, block $\times$ Ca $\times$ P, and day (3 sampling days) were included in the model as random terms.

The Kenward-Roger denominator degrees of freedom adjustment was used in all the analyses. Values reported are least squares means. Significance was declared at $P \leq 0.05$ and a trend was reported if $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Diets

Ingredient composition, nutrient contents, and their respective variation (standard deviations) for diets fed during the dry period (standardization diet) and postcalving (treatment diets) are presented in Table 2. Dietary P content in the basal diet was higher and Ca content was lower than originally planned, particularly because of nutrient content in corn silage (0.15% Ca and 0.26% P, DM basis, Table 1). High Ca and P diets were 7 and 21% above NRC (2001) estimated requirements, and low Ca and P diets were 25 and 9% below NRC (2001) estimated requirements, respectively (Table 2). Calcium-to-phosphorus ratios in treatment rations ranged between 1:1 and 1.7:1, in accordance with the recommended range (NRC, 2001).

Typical feeds used to prepare the basal diet (LCaLP) in this study supplied almost all P requirements for early-lactation dairy cows, except for 3 blocks in which a small proportion of monosodium phosphate (0.03%,

DM basis) was used. More severe P deficiency could not be achieved without risking the use of feeds that could potentially limit animal performance because of dietary protein or energy deficiencies.

Production Performance

Pregnant cows averaged 13.9 kg of DMI during the last 3 wk of gestation and reached lowest levels (7.6 kg/cow) during the day immediately before calving and on the calving date. Multiparous cows used in this study achieved high levels of milk production during their first month of lactation, averaging more than 44 kg/cow per day. There was no significant ($P \geq 0.05$) effect of Ca and P levels on feed consumption, milk yield, and yield of milk components (Table 3). Milk protein content decreased from 2.90 to 2.76% with higher dietary Ca, but milk protein yield changed less than 2% (30 g) with dietary Ca. There was a tendency of lower fat yield for LCa diets compared with HCa diets ($P \leq 0.07$). Somatic cell score tended ($P \leq 0.10$) to increase with the extreme diets—both HCaHP and LCaLP (Ca $\times$ P interaction)—but the physiologic significance of this finding is unclear. Cows in all treatments lost 2 to 2.5 kg of BW daily from calving to 30 DIM. Body condition score averaged 0.60 units lost between d 10 before expected calving to 30 DIM. Cows are expected to lose as much as 0.5 to 1.0 unit of BCS within the first 60 d of lactation (NRC, 2001). Given the body of research published in the last few decades, significant changes in production performance were not expected from early-lactation, high-producing cows fed diets containing 0.46% Ca and 0.38% P because bone mobilization is expected to supply sufficient mineral to correct for the dietary deficiency (Valk and Šebek, 1999; Wu et al., 2001; Taylor et al., 2009).

Ca and P Apparent Digestibility

Intake and milk yield during fecal collection were similar to the means obtained for the entire experimental period for all treatments (21.3 vs. 21.4 kg/d, and 44.4 vs. 43.9 kg/d, respectively). Dry matter digestibility was not significantly affected by mineral content in the diets (Table 4).

Calcium intake was 32 g greater with HCa than with LCa diets but fecal excretion was only 22 g higher for HCa, suggesting that LCa diets and HCa diets absorbed Ca similarly. Fecal Ca excretion was higher with HCaHP, decreased with HCaLP, but tended to remain similar between LCa diets (Ca $\times$ P interaction; $P \leq 0.10$). Calcium $\times$ phosphorus interaction was significant for Ca apparent digestibility and Ca apparent absorption (ingested Ca minus fecal Ca). Dietary Ca

Table 3. Performance of early lactation cows fed diets containing Ca and P above or below NRC (2001) estimated requirements in a factorial arrangement

Item	Treatment ¹				SEM	Effect ² ($P > F$)		
	HCaHP	HCaLP	LCaHP	LCaLP		Ca	P	Ca × P
DMI, ³ kg/d	21.0	20.7	21.9	21.4	0.57	0.26	0.53	0.90
Milk yield, ³ kg/d	44.4	44.5	44.0	42.9	1.46	0.45	0.74	0.68
Milk fat, %	3.57	3.64	3.63	3.27	0.22	0.41	0.44	0.25
Fat yield, kg/d	1.74	1.80	1.69	1.53	0.10	0.07	0.56	0.21
Milk protein, %	2.70	2.81	2.92	2.88	0.08	0.02	0.51	0.20
Protein yield, kg/d	1.33	1.38	1.40	1.36	0.05	0.56	0.93	0.29
SCS	3.51	2.29	2.56	3.24	0.51	0.06	0.35	0.10
BW lost, ⁴ kg/d	2.16	2.51	2.11	2.13	0.66	0.42	0.50	0.53
BCS lost ⁵	0.66	0.56	0.62	0.56	0.08	0.85	0.25	0.38

¹Treatment mineral contents in the diet DM: HCaHP = 0.63% Ca and 0.47% P; HCaLP = 0.65% Ca and 0.37% P; LCaHP = 0.46% Ca and 0.47% P; and LCaLP = 0.45% Ca and 0.38% P.

²Ca = main effect of dietary calcium; P = main effect of dietary phosphorus; Ca × P = interaction between dietary Ca and P.

³From DIM 6 to 31.

⁴From calving to 30 DIM.

⁵From BCS measured at 10 d before expected calving date to BCS measured at 30 d after calving.

digestibility averaged 21.4% with HCaHP and LCaLP, whereas treatments HCaLP and LCaHP resulted in higher Ca digestibility (32.8%). Low digestibility was expected with mature cows in all treatments, particularly those on HCaHP treatment (NRC, 2001). Somewhat unexpected was the low digestibility with LCaLP treatment. Braithwaite (1983a) estimated Ca digestibility in ewes at 13% for the “plentiful” Ca and P treatment

diet between 14 and 21 d in milk. Calcium digestibility was 48% with ewes fed recommended Ca and P levels (Braithwaite, 1983a). Calcium digestibility in cows (primiparous and multiparous) fed 0.52% dietary Ca and 0.34% dietary P was 32.1% during the second week and 27.6% during the fifth week of lactation (Taylor et al., 2009). Calcium and P deficiencies are thought to independently influence 1,25-(OH)₂D₃ hydroxylation,

Table 4. Digestion trials of 39 early-lactation cows fed diets containing Ca and P above or below NRC (2001) estimated requirements in a factorial arrangement

Item	Treatment ¹				SEM	Effect ² ($P > F$)		
	HCaHP	HCaLP	LCaHP	LCaLP		Ca	P	Ca × P
Production traits								
DMI, kg/d	21.0	20.9	22.8	21.1	0.99	0.31	0.34	0.41
Milk yield, kg/d	44.2	44.4	45.5	43.5	1.97	0.91	0.62	0.55
DM digestibility, %	62.3	66.6	66.3	66.4	1.45	0.16	0.11	0.13
Calcium								
Intake, g/d	131.3	133.8	104.3	95.8	4.67	<0.001	0.48	0.20
Fecal, % of DM	1.31	1.31	0.93	1.07	0.08	<0.001	0.20	0.19
Fecal, g/d	100.6	89.2	70.2	75.4	6.63	0.001	0.53	0.10
Apparent digestibility, %	22.2	33.1	32.5	20.6	6.11	0.81	0.92	0.02
Apparently absorbed, g/d	31.1	44.7	33.7	20.4	7.43	0.06	0.97	0.02
TAR, ³ g/d	75.6	77.3	77.7	74.3	2.64	0.85	0.74	0.29
Apparently absorbed, % of TAR	41.0	57.7	44.3	27.0	10.0	0.08	0.97	0.03
Phosphorus								
Intake, g/d	99.2	78.3	106.5	79.2	3.79	0.25	<0.001	0.37
Fecal, % of DM	0.75	0.59	0.84	0.62	0.03	0.08	<0.001	0.30
Fecal, g/d	58.0	40.8	64.2	43.9	3.13	0.09	<0.001	0.56
Apparent digestibility, %	40.5	47.7	39.6	44.8	3.60	0.52	0.04	0.74
Apparently absorbed, g/d	41.2	37.4	42.5	35.2	3.96	0.89	0.12	0.62
TAR, g/d	63.0	63.7	65.9	61.8	2.34	0.82	0.42	0.27
Apparently absorbed, % of TAR	65.2	59.1	65.4	57.0	6.03	0.85	0.16	0.83

¹Treatment mineral contents in the diet DM: HCaHP = 0.63% Ca and 0.47% P; HCaLP = 0.65% Ca and 0.37% P; LCaHP = 0.46% Ca and 0.47% P; and LCaLP = 0.45% Ca and 0.38% P.

²Ca = main effect of dietary calcium; P = main effect of dietary phosphorus; Ca × P = interaction between dietary Ca and P.

³TAR = total absorbed requirements, calculated using the NRC (2001) model adjusted for BW and milk yield (assuming 1.22 g of Ca and 0.90 g of P/kg of milk) of every cow used in digestion trials.

but it has been suggested that HP suppresses kidney α -hydroxylase function (Horst et al., 1994). Calcium digestibility and Ca apparent absorption in the current study appeared to be negatively influenced by P when dietary Ca was plentiful (HCa treatments). Phosphorus influence on Ca digestibility and apparent absorption was positive in LCa diets. Very low dietary Ca levels (0.46% Ca) fed in this trial may have limited Ca excretion to inevitable losses and nonabsorbed dietary Ca, which may be high in early lactation. In this case, low Ca digestibility and low Ca apparent absorption observed with cows fed LCaLP could have been the combination of 8.5 g/d lower Ca intake, 5.8 g/d higher fecal Ca output with LCaLP compared with LCaHP, and low Ca absorption efficiency in early-lactation cows across all treatments. High dietary P may have limited Ca absorption in HCa diets because HCaLP had similar Ca intake but significantly lower fecal Ca output than HCaHP. However, HP had little influence on fecal Ca excretion in LCa diets. In fact, cows in LCaHP appeared to absorb Ca even more efficiently than LCaLP. Absorption was probably at its maximum capacity in LCa diets. In that case, priority was given to Ca absorption, whereas with HCa diets some active absorption may occur. Active absorption may have been limited by diminished kidney α -hydroxylase function in HCaHP because of P negative feedback.

Phosphorus intake, fecal output (g/d and % of fecal DM), and digestibility were significantly affected by P content in the diets. A tendency ($P \leq 0.09$) for lower fecal P output (g/d and % of fecal DM) with HCa diets (4.67 g/d) was likely the result of cows ingesting 4.12 g more P in LCa diets than HCa diets, as a consequence of P intake in LCaHP diet (Table 4). Phosphorus apparent absorption was not affected by Ca or P.

In the present study, treatment diets appeared to supply insufficient apparently absorbed Ca and P. Total absorbable requirements averaged about 76 g of absorbed Ca per day and 64 g of absorbed P per day (Table 4). Treatment diets supplied between 27 and 58% Ca TAR and between 57 and 65% P TAR. Apparent digestibility represents the least possible absorption coefficient because of the contribution of endogenous sources of Ca and P. True absorption is most likely higher than apparent absorption, suggesting that at most 33 to 54 g of Ca and 22 to 27 g of P needed to be supplied daily by endogenous sources as secreted minerals reabsorbed from the intestines or bone mineral resorption. Assuming that apparent absorption measured in this study was the average for the experimental period, we can estimate the upper limit for bone resorption to be between 990 and 1,620 g of Ca and from 660 to 810 g of P for the diets fed in the present study.

Phosphorus intake increased protein output in feces ($P \leq 0.04$). Cows fed HP diets excreted 124 g more CP in feces than cows fed LP diets, regardless of dietary Ca. Higher CP fecal excretion was not a result of higher CP intake or lower milk protein yield. Phosphorus supplementation has been shown to increase the efficiency of microbial protein synthesis from nonprotein nitrogen (Komisarczuk-Bony and Durand, 1991). In the present study, fecal microbial protein was not measured, but it could represent as much as a 10% decrease in potentially labile N if the increase in fecal N output occurred because of microbial growth in the large intestine at the expense of urinary N.

Bone Metabolism Markers

Serum markers can replace more invasive techniques to establish patterns of bone turnover. Osteocalcin is a metabolite of protein synthesis by osteoblasts, which can be used as a marker for bone formation. Pyridinoline is a biomarker of bone resorption that can also be found in collagen type I from skin but can be safely detected in serum (Allen, 2003). For the purposes of this study, we specifically selected mature cows to minimize the effect of increased growth and remodeling of bone and skin tissues, conditions more likely to be observed in growing animals such as primiparous and second-lactation cows (Allen, 2003). Dietary Ca and P concentrations were not expected to significantly affect collagen metabolism in the skin of multiparous cows (≥ 3 lactations), thus serum PYD concentration changes were attributed to bone resorption.

Osteocalcin. Serum OC was not affected by dietary Ca or P levels (Table 5) but changed over time (Figure 1). Serum concentration of OC reached a nadir immediately after calving, returned to precalving levels (~ 30 ng/mL) at 15 d postpartum, and continued to increase through the end of the study at 30 DIM. The decrease in plasma OC concentration could be a result of increased PTH in the bloodstream around parturition (Naito et al., 1990). The pattern of change in serum OC concentration was similar to that in other studies (Naito et al., 1990; Liesegang et al., 2000; Taylor et al., 2009). Liesegang et al. (2000) reported serum OC concentrations below 1 ng/mL throughout the lactation of Brown Swiss and crosses of Brown Swiss cows with significantly higher concentrations for cows producing more milk. Peterson et al. (2005) observed OC content in serum similar for multiparous Holstein cows fed different P contents in the prepartum. Serum OC means were similar to those found in our study during the dry period, but concentrations were lower ranging between 19.7 and 21.4 ng/mL in lactating cows from calving

Table 5. Natural logarithm of serum osteocalcin (OC) concentration and pyridinoline (PYD) concentration of early-lactation cows fed diets containing Ca and P above or below NRC (2001) estimated requirements in a factorial arrangement¹

Item	Treatment ²				SEM	Effect ³		
	HCaHP	HCaLP	LCaHP	LCaLP		Ca	P	Ca × P
OC, ln ng/mL	3.52	3.62	3.43	3.60	0.21	0.76	0.45	0.85
PYD, nM	5.58	5.94	7.25	6.23	0.54	0.01	0.42	0.08

¹Concentrations represent means of samples collected at 15 and 30 DIM.

²Treatment mineral contents in the diet DM: HCaHP = 0.63% Ca and 0.47% P; HCaLP = 0.65% Ca and 0.37% P; LCaHP = 0.46% Ca and 0.47% P; and LCaLP = 0.45% Ca and 0.38% P.

³Ca = main effect of dietary calcium; P = main effect of dietary phosphorus; Ca × P = interaction between dietary Ca and P.

to 28 DIM. Serum OC contents in the present study were within the range observed in a recent study in which multiparous Holstein cows were fed 0.52, 0.78, and 1.03% Ca and 0.34% P on a DM basis (Taylor et al., 2009).

Serum OC reached a peak around 60 DIM in 2 studies (Liesegang et al., 2000; Taylor et al., 2009). Peak serum OC was achieved between 20 and 30 wk of lactation in Swedish Red and White cows (Ekelund et al., 2006). Ekelund et al. (2006) observed a treatment by time interaction with higher serum OC concentration between 17 and 20 wk of lactation for cows fed the lower P content diet compared with adequate P content diet. The difference in serum OC concentrations was narrow during the dry period and early lactation among cows fed diets containing either 0.31% or 0.41% P and 0.64% Ca, DM basis (Ekelund et al., 2006). Another study compared serum OC from cows fed different dietary P levels prepartum (Peterson et al., 2005). A significant treatment by time interaction was observed in serum OC measured on d 0, 1, 2, 3, and 14 postpartum, but the difference was considered small (Peterson et al.,

2005). Concentrations of OC in serum indicated that osteoblastic activity was increasing for all treatments in the present study. Serum OC concentrations were numerically higher and the gap increased over time for cows fed HCa and LP diets compared with LCa and HP diets in our study, but those differences were not significant.

Despite seemingly contradictory results for serum OC concentrations among published studies, it is reasonable to expect that bone accretion rates, and thus serum OC, changes with Ca and P dietary contents. However, the demands for Ca and P by early-lactation cows can supplant their ability to absorb from intestines or mobilize from the bones. Peterson et al. (2005) suggested that a P deficiency could elicit a reduction in bone formation to aid in the maintenance of adequate phosphatemia and calcemia. Because it appears that all treatments resulted in considerable mobilization, significant differences in serum OC should be expected only after bone stores of Ca and P are depleted or demand for milk production over intestinal absorption supply decreases. Serum OC differences should be expected after milk yield peak and as DMI reaches its maximum, thus later in the lactation cycle. Our study may have been too short to detect significant differences in serum OC induced by dietary Ca or P.

Pyridinoline. Serum PYD concentration in multiparous cows fed HCa was 1 nM lower ($P \leq 0.01$) than in cows fed LCa (Table 5). Cows in HCaHP treatment had the lowest serum PYD concentration among all treatments. The interaction between Ca and P tended to be significant ($P \leq 0.08$), suggesting that P levels may affect bone mobilization differently with HCa versus LCa. Phosphorus levels had little effect between HCa diets. Pyridinoline concentration was highest in the serum of cows fed LCaHP. Furthermore, serum PYD concentration was similar at 15 DIM for cows fed HP or LP. Cows fed HP treatments tended to show a slower rate of decline of serum PYD ($P \leq 0.09$) compared with LP diets from 15 to 30 DIM (24 vs. 40%, respectively), but no interaction over time was observed for Ca levels

Figure 1. Natural logarithm of serum osteocalcin (◆) and serum pyridinoline (□) content of mature cows from 10 d before expected calving date to 30 DIM. Dashed lines represent the 95% confidence interval.

in the diets (Figure 2). These observations suggest that HP could delay the return of bone mobilization to basal levels when LCa was fed to multiparous cows.

Serum PYD concentration was significantly affected by time (Figure 1). Lowest PYD concentration was observed on calving day and reached highest levels when cows were 15 DIM. Serum PYD of cows at 30 DIM returned to concentrations similar to those observed in cows 10 d before expected calving. Other studies also noted that lactating cows adequately fed Ca and P peaked in deoxypyridinoline (Liesegang et al., 2000; Taylor et al., 2009) and CTx (Liesegang et al., 2000; Ekelund et al., 2006) serum concentrations around 15 d postpartum. Serum deoxypyridinoline in mature cows also returned to precalving period levels after approximately 30 DIM (Taylor et al., 2009). Concentrations of PYD observed by Liesegang et al. (2000) were not comparable to those obtained in the current study because PYD was determined in urine samples in that study. The authors observed similar peak PYD concentration in the urine of cows around 14 DIM, but values reached prepartum levels only around 150 DIM. A reason for that discrepancy with serum PYD concentration pattern over time may have been because the dry cow diet was limited in Ca (0.34% DM) and P (0.33% DM) in the present study, whereas Liesegang et al. (2000) fed diets containing very high levels of Ca (0.58% DM) and P (0.56% DM) in the prepartum period. Bone resorption was probably stimulated shortly after late-gestation cows were offered the standard nonlactating cow TMR in this study, thus releasing larger quantities of PYD in the bloodstream. An alternative explanation could be that Brown Swiss cows (used in Liesegang et al., 2000) respond differently to Ca demands with longer periods of bone mobilization throughout lactation compared with Holstein cows used in our study.

Reconciling Digestibility and Bone Metabolism Markers Results

Calcium and P metabolism are inextricably associated in lactating cows. High dietary Ca should increase the supply of Ca available for milk production, decreasing the need for bone mobilization in early-lactation dairy cows. Hypocalcemia and very low phosphatemia can independently stimulate kidneys to produce 1,25-(OH)₂D₃ or enhance intestinal receptor binding affinity to 1,25-(OH)₂D₃, thus changing intestinal absorption of both Ca and P (Horst, 1986; Breves and Schröder, 1991). In fact, Kichura et al. (1982) showed a tendency for greater 1,25-(OH)₂D₃ when pregnant, nonlactating Jersey cows were fed 86 g of Ca and 10 g of P. On the other hand, it has been suggested that intestinal absorption of Ca can be limited when an excessive sup-

Figure 2. Dietary P by time interaction for serum pyridinoline (PYD) concentration in mature cows at 15 and 30 DIM ($P \leq 0.09$). Treatments contained 0.47% P (HP: x) and 0.38% P (LP: ■) in the diet DM.

ply of P suppresses α -hydroxylase activity, the enzyme responsible for most 1,25-(OH)₂D₃ production in the kidneys, rendering active gastrointestinal absorption of P and Ca less efficient (Horst et al., 1994; NRC, 2001). In summary, dietary P may indirectly influence Ca absorption in the intestines by affecting 1,25-(OH)₂D₃ hydroxylation in the kidneys or by changing intestinal 1,25-(OH)₂D₃ receptor binding affinity (Kichura et al., 1982; Horst, 1986; Goff, 2006).

In our study, Ca apparent absorption was higher and serum PYD lower in cows fed HCa diets. Higher absorption was observed in cows ingesting HCaLP diet, but PYD was similar between dietary P levels in HCa diets. These results seem to be in agreement with the observations of Kichura et al. (1982), suggesting higher Ca absorption in low P diets. Serum PYD of cows fed HP diets suggested an extended period of bone resorption, which also appears to agree with lower Ca absorption with high P diets. However, cows fed low dietary Ca had higher Ca apparent absorption when P level was similar (LCaHP). Although Ca apparent absorption was similar between HCaHP and LCaHP (31 and 34 g/d), PYD treatment means were, respectively, 5.58 and 7.25 nM, the lowest and highest among the 4 treatments. These results seem to suggest that cows in deficiency of Ca can override a negative P feedback; however, Ca absorption in LCaHP was ineffective in reducing bone resorption. It appears that dietary Ca and P levels can both independently influence bone resorption in early lactation dairy cows.

CONCLUSIONS

Dietary Ca and P contents had no significant effect on feed intake, milk yield, or milk components yield

during the first month of lactation. Calcium intake negatively affected concentrations of the bone resorption biomarker pyridinoline. Cows fed 100 g of P/d had higher bone resorption ($\text{Ca} \times \text{P}$ interaction) shown as an extended period of high serum pyridinoline levels ($\text{P} \times \text{time}$ interaction). Calcium content in the diets did not influence P digestion. Results of this study suggest that P not only could but should be reduced in early-lactation, high-producing cows to allow for more efficient dietary Ca utilization.

ACKNOWLEDGMENTS

The authors thank the Louisiana State University AgCenter Southeast Research Station's dairy crew for feeding, milking, and caring of the cows. Assistance provided by Bill Barber, Regina Barnett, Catherine Cox, Tara Doughty, Justin Jones, Douglas McKean, Jerry Simmons, and Randy Walz during feed and fecal collections and analyses is sincerely appreciated. The authors are also grateful to Ashley Howard and Jennifer Laborde at LSU AgCenter School of Animal Sciences for their skillful technical assistance with the analyses of serum osteocalcin and pyridinoline.

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