Timothy Hay with a Low Dietary Cation-Anion Difference Improves Calcium Homeostasis in Periparturient Holstein Cows

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ABSTRACT

The current study was undertaken to evaluate the effects of feeding timothy (*Phleum pratense* L.) hay differing in dietary cation-anion difference (DCAD) on the capability of cows to maintain calcium homeostasis around parturition. We hypothesized that feeding low-DCAD timothy hay during the prepartum period would induce a mild metabolic acidosis prepartum and improve calcium homeostasis postpartum with no effect on dry matter intake. Forty-one dry pregnant Holstein cows entering their second lactation or greater were used in a randomized complete block design. Timothy hay was obtained from an established timothy stand under a pivot irrigation system. Low-DCAD timothy hay was produced by fertilizing the area between the second and third pivot towers at a rate of 224 kg of CaCl_2/ha , and control timothy hay (high DCAD) was grown on the area between the fourth and fifth pivot towers of the same field. The chloride concentration was 1.07 and 0.15% on a dry matter (DM) basis, and the DCAD was 1.2 and 21.6 mEq/100 g of DM for the low- and high-DCAD timothy hay, respectively. Experimental diets, containing timothy hay at 63% of dietary DM, were fed ad libitum starting 30 d before the expected calving date. The DCAD values were 1.6 vs. 14.5 mEq/100 g of DM for the low- and high-DCAD timothy-based diets, respectively. At the beginning of the study, urine pH and blood bicarbonate concentration averaged 8.22 ± 0.06 and 28.5 ± 0.3 mM, respectively. The low-DCAD timothy diet decreased urine pH compared with the high-DCAD timothy diet on d 21 (7.75 vs. 8.31), d 14 (7.69 vs. 8.22), and d 7 (7.50 vs. 8.19) before calving, and it also decreased the prepartum blood bicarbonate concentration by 2 mM. In addition, cows fed the low-DCAD timothy diet had greater blood ionized calcium concentration prepartum (1.22 vs. 1.19 mM), greater blood ionized calcium concentration at 0 and 8 h after

calving, and similar prepartum dry matter intake. These results indicate that timothy hay differing in DCAD affects the acid-base balance of periparturient dairy cows, and that low-DCAD timothy hay improves calcium homeostasis postpartum with no negative effect on dry matter intake.

Key words: dairy cow, dietary cation-anion difference, calcium homeostasis, hypocalcemia

INTRODUCTION

Parturient paresis is a metabolic disorder caused by the inability of a cow to meet the calcium demands of colostrum production via dietary calcium absorption, bone calcium mobilization, and renal calcium resorption. The resulting hypocalcemic condition affects the health and profitability of transition dairy cows. In a controlled research setting, prevalence rates for clinical hypocalcemia ranged between 13 and 20%, with an additional 20 and 53% experiencing subclinical hypocalcemia, for cows fed prepartum diets containing a DCAD of -7 and $+30$ mEq/100 g of DM, respectively (Joyce et al., 1997). Block (1984) conducted a 2-yr crossover study in which cows were fed a prepartum diet containing DCAD of either 3.3 mEq/100 g or -1.3 mEq/100 g of DM and reported no incidence of milk fever for cows fed the low-DCAD diet, whereas feeding the positive DCAD diet resulted in an incidence of milk fever of nearly 50%. Further, cows fed the prepartum diet containing a DCAD of -1.3 mEq/100 g produced nearly 500 kg more milk per lactation than cows receiving the diet containing a DCAD of 3.3 mEq/100 g. As such, reducing the prevalence of hypocalcemia has the potential to improve both animal health and productivity.

Lowering the DCAD of prepartum diets has been shown to improve calcium homeostasis during the periparturient period. Diets containing anionic salts or an acidifying agent cause a compensated metabolic acidosis improving parathyroid hormone (PTH) sensitivity, increasing 1,25-dihydroxyvitamin D [$1,25\text{-(OH)}_2\text{D}$] concentration per unit of PTH (Goff et al., 1991; Block, 1994), and improving renal resorption, osteoclastic mo-

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Table 1. Nutrient composition of the low- and high-DCAD timothy hays

Nutrient	Timothy hay			
	Low DCAD	SD	High DCAD	SD
DM, %	89.7	1.5	89.7	1.6
CP, % of DM	7.1	0.5	7.7	0.8
ADF, % of DM	43.4	0.8	41.7	1.0
NDF, % of DM	69.0	1.7	65.6	1.9
Ash, % of DM	5.5	0.3	5.6	0.6
Ca, % of DM	0.32	0.06	0.30	0.04
P, % of DM	0.13	0.01	0.14	0.01
Na, % of DM	<0.01	0.00	<0.01	0.01
K, % of DM	1.48	0.05	1.28	0.08
Mg, % of DM	0.12	0.01	0.12	0.02
S, % of DM	0.11	0.01	0.12	0.01
Cl, % of DM	1.07	0.06	0.15	0.03
Fe, mg/kg	44.3	9.7	112.0	86.6
Mn, mg/kg	22.5	2.6	30.5	2.5
Zn, mg/kg	24.8	12.9	24.5	2.6
Cu, mg/kg	7.3	1.0	7.3	1.0
DCAD, ¹ mEq/100 g of DM	1.2	2.3	21.6	1.8

¹Calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ (Ender et al., 1971).

bilization, and intestinal absorption of calcium (Horst et al., 1997). Selection of forage species and fertilization with chloride may be a possible alternative to decrease the DCAD. Recently, Tremblay et al. (2006) evaluated the DCAD of 5 cool-season grasses grown on various soils and reported that timothy hay had the lowest DCAD due to low potassium concentration. Subsequent studies have consistently demonstrated that the DCAD of timothy hay can be further reduced when it is fertilized with various sources of chloride (Oba et al., 2007; Pelletier et al., 2007, 2008). However, data are limiting on whether timothy hay with a low DCAD can improve the cow's ability to maintain calcium homeostasis around parturition.

Feeding anionic salts or acidifying agents often decreases DMI predisposing animals to other disorders such as displaced abomasum and ketosis. Charbonneau et al. (2006a) conducted a meta-analysis encompassing 22 published studies and reported a positive linear relationship between the DCAD calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.6 \text{S}^{2-})$ and DMI ($P < 0.01$; $R^2 = 0.66$). However, a recent study (Charbonneau et al., 2008) comparing a control diet (DCAD = 29.6 mEq/100 g of DM), a diet containing timothy hay with a low DCAD (−2.4 mEq/100 g of DM), and the control diet supplemented with HCl (−1.9 mEq/100 g of DM) suggests that the methods to decrease the DCAD (hay vs. HCl supplement) may have an impact on DMI. In that study, feeding cows with the low-DCAD timothy hay diet did not decrease DMI relative to the control; however, feeding cows with the HCl-supplemented diet tended to decrease DMI relative to the low-DCAD timothy hay diet. Thus, further research is warranted to investigate the effect of low-DCAD forages on DMI.

The objective of the current study was to evaluate the effects of feeding timothy (*Phleum pratense* L.) hay differing in DCAD on DMI and the capability of cows to maintain calcium homeostasis around parturition.

MATERIALS AND METHODS

This experiment was conducted between October 2006 and February 2007 at the University of Alberta Dairy Research and Technology Centre (Edmonton, Alberta, Canada). All procedures were approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and were performed according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Experimental Design and Dietary Treatments

Timothy hay was obtained from an established timothy stand under a pivot irrigation system near Lethbridge (Alberta, Canada). Soil K content of the field was 486 kg/ha. The field was fertilized with 112 kg of N/ha as urea, and 39 kg of P/ha as monoammonium phosphate on April 15, 2006. In addition, anhydrous calcium chloride was applied using a fertilizer broadcaster to the area between the second and third pivot towers at a rate of 224 kg/ha (143 kg of Cl/ha) on April 15 to produce the low-DCAD timothy hay. Control timothy hay (high-DCAD) was grown on the area between the fourth and fifth pivot towers of the same field. The hay was harvested on July 8 as small rectangular bales weighing approximately 27 kg each, transported to the University of Alberta (Edmonton, Alberta, Canada), and stored in a covered shelter. The nutrient and min-

Table 2. The ingredient and nutrient composition of the low- and high-DCAD diets

Ingredient, % of DM	Dietary treatment	
	Low DCAD	High DCAD
Low-DCAD timothy hay	63.3	—
High-DCAD timothy hay	—	63.3
Concentrate mix ^{1,2}	36.7	36.7
Nutrient		
CP, % of DM	12.5	12.9
ADF, % of DM	30.6	29.5
NDF, % of DM	49.6	47.4
Ash, % of DM	7.0	7.1
Ca, % of DM	0.74	0.72
P, % of DM	0.35	0.35
Na, % of DM	0.04	0.04
K, % of DM	1.31	1.18
Mg, % of DM	0.35	0.35
S, % of DM	0.20	0.21
Cl, % of DM	0.75	0.17
Fe, mg/kg	427	470
Mn, mg/kg	105	110
Zn, mg/kg	184	183
Cu, mg/kg	30	30.3
DCAD, ³ mEq/100 g of DM	1.6	14.5

¹Contained 24.0% ground corn grain, 20.0% rolled corn grain, 20.0% soybean meal, 15.0% dehydrated beet pulp, 4.0% corn gluten meal, 4.0% SoyPlus (West Central Coop., Ralston, IA), 3.0% rolled barley, 2.8% mineral and vitamin mix, 2.5% molasses, 2.0% canola meal, 1.8% limestone, and 1.0% canola oil on an as fed basis.

²Nutrient composition on a DM basis, 21.8% CP, 8.6% ADF, 16.1% NDF, 9.6% ash, 1.46% Ca, 0.73% P, 0.10% Na, 1.03% K, 0.74% Mg, 0.37% S, 0.20% Cl, 1,087 ppm of Fe, 248 ppm of Mn, 458 ppm of Zn, 70 ppm of Cu, 25,000 IU/kg of vitamin A, 6,000 IU/kg of vitamin D, 360 IU/kg of vitamin E, and 2.14 mEq/100 g of DCAD.

³Calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ (Ender et al., 1971).

eral composition of the low-DCAD and high-DCAD timothy hay fed during the animal study ($n = 4$; samples were collected twice weekly and composited monthly) are listed in Table 1.

Forty-one Holstein cows entering their second or greater parity (mean parity $\pm$ standard deviation; 2.95 ± 1.14) were blocked by parity and then randomly assigned to 1 of the 2 experimental diets containing 63% forage (low- or high-DCAD timothy) and 37% concentrate (Table 2) at 30 d before the expected calving date. Diets were formulated to meet or exceed the nutrient requirements for cows in the close-up period (NRC, 2001). Cows were component fed timothy hay and concentrate at the same time, and equal portions of the diets were fed twice daily at 0900 and 1700 h. Cows were housed in tie-stalls bedded with wood shavings and had free access to water. Following parturition, cows were fed a typical lactation diet formulated to contain adequate metabolizable protein and energy for a cow producing 35 kg/d of 3.5% FCM.

Data and Sample Collection

The amount of feed offered and refused was recorded daily and adjusted to minimize refusals without re-

stricting intake (5% refusal). Forage samples were collected twice weekly and a concentrate sample was collected once weekly. Samples were oven-dried at 55°C for 48 h for determination of DM concentration. Dried feed samples were composited by month and analyzed by Cumberland Valley Analytical Service (Hagerstown, MD). The nitrogen concentration was determined by flash combustion (Leco FP-528 Nitrogen Combustion Analyzer, Leco, St. Joseph, MI; AOAC, 2000; method 990.03) and was multiplied by 6.25 for calculation of CP. The ADF concentration was determined according to AOAC (2000; method 973.18). The NDF concentration was determined according to the procedure of Goering and Van Soest (1970) using α -amylase and sodium sulfite. The ash concentration was determined by subjecting samples to 535°C according to the AOAC (2000; method 942.05). The Ca, P, Mg, K, Na, Fe, Mn, Zn, and Cu concentrations were determined according to AOAC (2000; method 985.01). Samples were digested in nitric acid and analyzed using an optical emission spectrometer (Perkin Elmer 3300 XL ICP, Perkin Elmer, Shelton, CT). The S concentration was determined using Leco S-144DR Sulfur Combustion Analyzer (Leco). Samples were extracted with 1% nitric acid and determined for chloride ion by potentiometric measurement on a Corning 925 Chloride Analyzer (Ciba Corning Diagnostics, Medfield, MA). The DCAD (mEq/100 g of DM) was calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ (Ender et al., 1971). The DMI was calculated as the difference between the amount of DM offered and the amount of DM refused. Orts were assumed to have the same DM concentration as the original feed offered. Data for prepartum DMI were summarized as weekly means.

Body weight and BCS were determined at the start of the study for each cow. An initial spot sample of urine was collected on the enrollment day and thereafter urine samples were collected twice weekly at 1100 h via manual stimulation or voluntary urination. Frequent sample collection was designed to obtain samples collected on d -21, -14, and -7 relative to parturition. Urinary pH was determined immediately using a portable pH meter (Accumet AP61, Fisher Scientific, Pittsburgh, PA). The pH meter was calibrated daily using standard buffers 4 and 7.

Blood was collected on the enrollment day and thereafter twice weekly (Tuesday and Friday) corresponding to urine collection. Cows nearing parturition were observed hourly to collect blood samples immediately following parturition (0 h), and at 4, 8, 12, 16, 20, 24, 48, and 72 h following parturition. Blood from coccygeal vessels was drawn into 2 vacutainer tubes; one containing Li heparin (Fisher Scientific Co., Nepean, Ontario, Canada) and the other containing Na heparin (Fisher Scientific Co.). Whole blood in tubes containing

Li heparin was immediately analyzed for pH and concentrations of Na, K, ionized Ca (**iCa**), and bicarbonate using an auto analyzer (GEM Premier 3000, Instrumentation Laboratory Ltd., Richmond Hill, Ontario, Canada). The blood in Na heparin tubes was immediately placed on ice and centrifuged within 30 min at $3,000 \times g$ and 4°C for 20 min. Following centrifugation, the plasma was harvested and stored at -20°C . Plasma samples collected on the enrollment day, and at h 0, 8, 16, 24, and 72 following parturition were analyzed for PTH and $1,25\text{-(OH)}_2\text{D}$ concentrations by the Diagnostic Centre for Population Animal Health (Lansing, MI). The $1,25\text{-(OH)}_2\text{D}$ concentration in plasma samples were analyzed using a DiaSorin $1,25\text{-(OH)}_2\text{D}$ RIA Kit (DiaSorin, Stillwater, MN). Briefly, $1,25\text{-(OH)}_2\text{D}$ was extracted and purified from plasma using C18OH and "Extra Clean" cartridges (DiaSorin). Following extraction, the sample was assayed using a polyclonal antibody specific for $1,25\text{-(OH)}_2\text{D}_2$ and $1,25\text{-(OH)}_2\text{D}_3$, which avoids cross contamination with other vitamin D metabolites. The sample, antibody, and tracer were incubated for 2 h at 20°C followed by incubation with a second antibody to form a precipitating complex. After centrifugation and decantation, the bound fraction remaining in the pellet was counted in a gamma counter. Values were calculated directly from a standard curve of known concentrations. Intact PTH was measured using a 2-site immunoradiometric assay (Diagnostic Systems Laboratory, Webster, TX) that has been previously validated (Diagnostic Center for Population and Animal Health, Lansing, MI).

Statistical Analysis

Statistical analysis was conducted independently for prepartum and postpartum data. Preliminary statistical analysis determined that parity differences were not detectable between cows entering their third or greater lactation. Thus, for statistical analysis, cows were classed under 2 categories; 1) cows entering their second lactation, and 2) cows entering their third or greater lactation. The parity classification was used to group cows with similar susceptibility to hypocalcemia. Furthermore, data were analyzed to determine if initial values for DMI, urine pH, blood pH, blood **iCa**, plasma $1,25\text{-(OH)}_2\text{D}$, and plasma PTH were different between treatments. Statistical analysis revealed that initial treatment means for the previously mentioned variables were not different and therefore covariates were not used in subsequent analyses.

All data were analyzed using the PROC MIXED procedure of SAS (version 9.1.3; SAS Institute Inc., Cary, NC) accounting for repeated measures with the random effect of cow nested in the parity class and the fixed

effects of treatment and parity class. The prepartum repeated measures were week and day relative to parturition for DMI and blood parameters, respectively, and the postpartum repeated measure was hour relative to parturition. Various covariance error structures were tested for each dependent variable and the one that yielded the lowest Akaike's and Bayesian information criterion values was used. All data were analyzed for the main effects of treatment, parity class, time, and their interactions.

Postpartum data were also analyzed using the PROC MIXED procedure of SAS (version 9.1.3, SAS Institute Inc.) with hour relative to parturition as a regression variable to further characterize the response to timothy hay differing in DCAD. Linear effects for treatment, hour, parity, and their interactions and quadratic effects for hour, and the quadratic interactions between hour $\times$ treatment, hour $\times$ parity class, and hour $\times$ treatment $\times$ parity class were tested.

The change in blood **iCa** concentration following parturition was evaluated by comparing the nadir concentration to the prepartum baseline concentration. For this calculation, the nadir value observed postpartum was used and the baseline value was calculated by averaging the blood **iCa** concentrations for d -21, -14, and -7. Days -21, -14, and -7 relative to parturition were used for the baseline calculation because they did not differ over time. These data were analyzed using the PROC MIXED procedure of SAS (version 9.1.3; SAS Institute Inc.) with the fixed effects of treatment and parity class and the random effect of cow nested in parity class. Data were analyzed for the main effects of treatment, parity class, and the interaction between treatment and parity class.

For all statistical analyses mentioned above, differences were considered significant when $P < 0.05$ and trends are discussed when $P < 0.10$.

RESULTS

Low- and High-DCAD Timothy Hay

Timothy hay fertilized with CaCl_2 had a greater K concentration (nearly 16%) than timothy hay not fertilized with CaCl_2 (Table 1), but this slightly greater K concentration was offset by a much greater Cl concentration (86% increase). Thus, the marginal increase in K concentration and the large increase in Cl concentration for timothy hay fertilized with CaCl_2 resulted in a lower DCAD than timothy hay not fertilized with CaCl_2 (1.2 vs. 21.6 mEq/100 g of DM).

Prepartum

The actual days for prepartum blood and urine collection were (mean $\pm$ SD) -29.1 ± 0.7 , -20.5 ± 0.2 , -13.7

Table 3. The BW, BCS, DMI, prepartum urine pH, blood pH, blood bicarbonate, and blood ionized calcium concentrations of cows fed the low- or high-DCAD timothy diet during the 30-d prepartum period

Item	Treatment		SEM	P-value		
	Low DCAD	High DCAD		Treatment	Day	Parity class ¹
BW, kg ²	740	707	21.8	0.28	—	0.45
BCS ²	3.32	3.22	0.08	0.39	—	0.54
DMI, kg/d	11.0	10.2	0.6	0.37	<0.01	0.15
Urine pH ³	7.77	8.25	0.06	<0.01	<0.01	0.46
Blood pH ⁴	7.44	7.44	0.01	0.34	0.11	0.90
Blood bicarbonate, mM	27.2	29.4	0.42	<0.01	0.94	0.86
Blood ionized Ca, mM	1.22	1.19	0.01	0.01	<0.01	0.13

¹Cows entering their second lactation and their third or greater lactation were assigned to different parity classes.

²Body weight and BCS measured on the study enrollment day (-29 ± 0.7 relative to parturition). Body condition score was measured using a 5-point scale according to Wildman et al. (1982).

³Significant treatment $\times$ day interaction ($P < 0.01$).

⁴Significant day $\times$ parity class interaction ($P < 0.05$).

± 0.2 , and -6.6 ± 0.2 d relative to parturition. These collection days were close to the targeted days of -30 , -21 , -14 , and -7 d relative to parturition, respectively.

The prepartum BW and BCS of cows fed the low-DCAD and high-DCAD treatments were not different and averaged 724 kg and 3.27 BCS respectively (Table 3). Prepartum DMI was not affected by dietary treatment ($P = 0.37$; Table 3) but it was affected by week relative to parturition ($P < 0.01$; Figure 1). Average DMI across treatments was 11.3, 11.2, and 9.2 kg/d for wk -3 , -2 , and -1 relative to parturition, respectively; for both treatment groups of cows, DMI decreased by an average of 18% during the week before calving. Mean urine pH over the entire prepartum period was nearly 0.5 pH units lower for cows fed low-DCAD timothy than cows fed high-DCAD timothy hay (Table 3). Furthermore, there was a treatment $\times$ day interaction for urine pH. Feeding low-DCAD timothy hay decreased urinary pH from 8.16 ± 0.08 on d -30 to 7.50 ± 0.08 on d -7

relative to parturition; however, feeding high-DCAD timothy hay had no effect on urinary pH with values of 8.27 ± 0.08 and 8.19 ± 0.09 on d -30 and -7 , respectively (Figure 2). Blood pH was not affected by treatment, day relative to parturition, or parity class, although an interaction between parity class and day relative to parturition was detected ($P < 0.05$). The interaction was a result of cows entering their third or greater lactation having a decrease in blood pH from trial initiation to d -7 , whereas cows entering their second lactation had no change in their blood pH values during the same time (data not shown). Blood bicarbonate concentration was 7% lower for cows fed low-DCAD timothy hay than for cows fed high-DCAD timothy hay (Table 3). Furthermore, there was a tendency ($P = 0.07$) for a treatment $\times$ day interaction for blood bicarbonate concentration; the low-DCAD treatment tended to reduce blood bicarbonate concentrations as the calving date approached,

Figure 1. The DMI of cows fed the low- or high-DCAD timothy diet for 30 d before the expected calving date. Weeks with uncommon letters are significantly different ($P < 0.05$).

Figure 2. The treatment $\times$ day interaction for prepartum urine pH in cows fed the low- or the high-DCAD timothy diet for 30 d before their expected calving date. All data points with uncommon letters are significantly different ($P < 0.05$).

Table 4. Percentage of cows experiencing disorders during the first 72 h postpartum after they were fed the low- or high-DCAD timothy diet for 30 d before their expected calving date

Item	Treatment	
	Low DCAD	High DCAD
Number of cows per treatment	20	21
Retained placenta, %	5.0	19.0
Displaced abomasums, %	5.0	9.5
Subclinical hypocalcemia, ¹ %	35.0	66.7

¹Ionized calcium concentration in blood <1 mM.

whereas the high-DCAD treatment did not affect blood bicarbonate concentrations (data not shown). The prepartum concentration of blood iCa was greater for cows fed low-DCAD timothy diet when compared with cows fed high-DCAD timothy diet (1.22 vs. 1.19 mM, respectively; $P = 0.01$). Blood iCa concentration increased slightly from the initial concentration of 1.18 mM on d -30 to 1.21 mM for d -21 and reached a plateau thereafter (week; $P < 0.01$, data not shown). An interaction between parity class and day relative to parturition was detected for blood iCa concentration where cows entering their third or greater lactation initially had lower blood iCa concentration than cows entering their second lactation (1.16 vs. 1.20 mM, respectively) but had similar blood iCa concentrations on d -21, -14, and -7 relative to parturition (data not shown).

Postpartum

Metabolic Disorders. The occurrence of metabolic disorders is summarized in Table 4, but statistical analysis was not conducted. Although no visual signs of clinical hypocalcemia were observed, 35% of the cows fed the low-DCAD diet and 67% of the cows fed the high-DCAD diet had blood iCa concentrations below 1 mM, a threshold used to define subclinical hypocalcemia (Joyce et al., 1997). There was also a numerically greater percentage of retained placenta and displaced abomasum in cows fed the high-DCAD timothy diet.

Treatment Effects. Prepartum treatment did not affect postpartum blood pH ($P > 0.10$; Table 5). A treatment $\times$ postpartum hour interaction was detected for blood pH ($P = 0.02$; data not shown). Cows fed the low-DCAD timothy diet had numerically lower blood pH at the time of calving (0 h), numerically greater blood pH at 12 h postpartum, but similar blood pH 72 h postpartum when compared with cows fed the high-DCAD timothy diet. The overall effect of this interaction was a reduction in the difference for blood pH over time between the low-DCAD and high-DCAD treatments. Feeding low-DCAD timothy diet tended to increase ($P =$

0.10) the concentration of iCa in blood postpartum when compared with high-DCAD timothy diet (1.09 vs. 1.06 mM). Because there was no difference in prepartum blood iCa concentrations for d -21, -14, and -7, values were averaged for each cow individually to determine the baseline concentration. The baseline concentration was then compared with the nadir value observed postpartum for each cow. The nadir value for blood iCa concentration was nearly 6% greater ($P = 0.05$) for cows fed the low-DCAD diet compared with the high-DCAD timothy diet. However, the nadir value for blood iCa concentration as a proportion of the baseline was not different between dietary treatments, and averaged 81.5%. Plasma concentrations of 1,25-(OH)₂ D and PTH were not affected by dietary treatments.

Hour Effects. We further examined the response of postpartum blood parameters over time (Table 6). There was a quadratic relationship ($P < 0.01$) for blood pH over time with pH increasing from the time of calving until 16 h after calving but reaching a plateau thereafter. A significant quadratic hour $\times$ treatment interaction ($P = 0.04$; data not shown) was detected for blood bicarbonate concentration. This interaction was a result of cows on the low-DCAD treatment having lower blood bicarbonate concentrations than cows on the high-DCAD treatment at h 0 (27.7 vs. 30.6 mM) and 4 (28.8 and 31.5 mM) after parturition respectively. But similar concentrations of blood bicarbonate were observed thereafter, averaging 31.5 and 32.8 mM for the low-DCAD and high-DCAD treatments, respectively. Blood iCa concentration responded linearly ($P < 0.01$); however, there was a quadratic hour $\times$ parity class interaction ($P < 0.01$; data not shown) and a quadratic hour $\times$ treatment interaction ($P < 0.01$; Figure 3). Cows entering their third or greater parity had lower blood iCa at 0 and 4 h following parturition but greater iCa after 48 h (data not shown). The quadratic hour $\times$ treatment interaction was a result of cows fed the low-DCAD timothy diet having greater blood iCa at 0 and 8 h after parturition and numerically greater iCa for the first 20 h following parturition than cows fed the high-DCAD timothy diet. However, iCa concentration was similar between treatments at h 24, 48, and 72 (Figure 3). A linear effect of postpartum time was detected for plasma PTH (Table 6) with PTH concentrations remaining high for more than 24 h following parturition, but it decreased by 15% at h 72. Plasma 1,25-(OH)₂ D was lowest immediately following parturition (0 h) and increased 8 and 16 h after calving with no further increase thereafter (quadratic; $P < 0.01$, Table 6).

DISCUSSION

Periparturient Ca Homeostasis

This is the first study known to the authors that has investigated the effect of forages differing in DCAD

Table 5. Postpartum blood pH, blood concentrations of bicarbonate and ionized Ca (iCa), and plasma concentrations of parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D [1,25-(OH)₂ D] in cows fed the low- or high-DCAD timothy diet for 30 d before their expected calving date

Variable	Treatment			P-value		
	Low DCAD	High DCAD	SEM	Treatment	Hour	Parity class
Blood						
pH ¹	7.45	7.46	0.01	0.15	<0.01	0.87
Bicarbonate, mM	30.8	32.4	0.78	0.14	<0.01	0.82
iCa, ² mM	1.09	1.06	0.01	0.10	<0.01	0.68
Baseline iCa, ³ mM	1.23	1.20	0.01	0.01	NA ⁵	0.54
Nadir iCa, ⁴ mM	1.02	0.96	0.02	0.05	NA	0.44
Nadir iCa, ⁴ % of baseline	82.9	80.2	1.47	0.20	NA	0.52
Plasma						
PTH, pM	5.05	6.68	1.12	0.27	<0.01	0.76
1,25(OH) ₂ D, pM	267	320	27.1	0.13	<0.01	0.75

¹Significant treatment × hour interaction ($P = 0.02$).²Significant hour × parity class interaction ($P < 0.01$).³The mean blood iCa concentration for d -21, -14, and -7 relative to parturition calculated for each cow.⁴The lowest blood iCa concentration observed for each cow postpartum.⁵NA = not available.

on calcium homeostasis in transition dairy cows. To produce timothy hays with contrasting DCAD, we fertilized the hay with 0 or 224 kg of CaCl₂/ha. Our results confirm those of previous studies that demonstrated that Cl fertilization decreases the DCAD of timothy hay (Oba et al., 2007; Pelletier et al., 2007, 2008) or alfalfa (Goff et al., 2007).

In the current study, feeding the low-DCAD timothy diet reduced the metabolic alkalosis prepartum as indicated by decreased urinary pH and blood bicarbonate concentration when compared with feeding the high-DCAD timothy diet. Previous studies also reported that low or negative DCAD diets induce metabolic acidosis as indicated by reduced urine pH and blood bicarbonate

concentration (Goff and Horst, 1998; Moore et al., 2000; Roche et al., 2003). However, the degree of metabolic acidosis in the previously mentioned studies was greater than in the current study. Metabolic acidosis increases the tissue responsiveness to PTH, increasing the synthesis of 1,25-(OH)₂ D, and thus improving the ability to increase plasma Ca through mobilization, resorption, and absorption (Horst et al., 1997). Correspondingly, we observed a greater concentration of iCa prepartum for cows fed the low-DCAD timothy diet when compared with cows fed the high-DCAD timothy diet. Greater blood iCa concentration has been consistently reported when cows are fed diets containing a low or negative DCAD (Tucker et al., 1992; Moore et al.,

Table 6. The effect of time after parturition on blood pH, bicarbonate, ionized Ca (iCa), parathyroid hormone (PTH), and 1,25 dihydroxyvitamin D [1,25-(OH)₂ D] when cows were fed the low- or high-DCAD timothy diet for 30 d before their expected calving date (average of the 2 dietary treatment groups)

Variable	Hour relative to parturition									P-value	
	0	4	8	12	16	20	24	48	72	Linear	Quadratic
Blood pH	7.42 ^e	7.43 ^{de}	7.44 ^{cd}	7.46 ^{bc}	7.46 ^{bc}	7.47 ^{ab}	7.47 ^{ab}	7.47 ^{ab}	7.48 ^a	<0.01	<0.01
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
Bicarbonate, ¹ mM	29.2 ^e	30.2 ^{de}	30.5 ^d	31.2 ^{cd}	32.1 ^{bc}	32.6 ^{ab}	32.7 ^{ab}	33.5 ^a	32.4 ^b	<0.01	<0.01
SEM	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65		
iCa, ² mM	1.06 ^{cd}	1.05 ^d	1.07 ^{bcd}	1.07 ^{bc}	1.06 ^d	1.05 ^d	1.06 ^{cd}	1.11 ^{ab}	1.12 ^a	<0.01	0.90
SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
PTH, pM	5.40 ^a	—	5.15 ^a	—	5.42 ^a	—	5.58 ^a	—	4.56 ^b	<0.01	0.08
SEM	0.82	—	0.75	—	0.82	—	0.92	—	0.70		
1,25-(OH) ₂ D, pM	235 ^c	—	279 ^b	—	321 ^a	—	338 ^a	—	293 ^{ab}	0.32	<0.01
SEM	18.5	—	18.6	—	20.0	—	21.2	—	18.6		

^{a-d}Means within a row with uncommon superscripts differ significantly ($P < 0.05$).¹Significant quadratic hour × treatment interaction ($P = 0.04$).²Significant quadratic hour × parity class interaction ($P < 0.01$).

Figure 3. The quadratic hour $\times$ treatment interaction for postpartum blood ionized calcium (iCa) concentration in cows fed the low- or high-DCAD timothy diet for 30 d before the expected calving date. All data points with uncommon letters are significantly different ($P < 0.05$).

2000). In the current study, the blood iCa concentration following parturition also tended to be greater for cows fed the low-DCAD timothy hay than cows fed the high-DCAD timothy hay as indicated by a quadratic hour $\times$ treatment interaction. These data indicate improved iCa homeostasis immediately after parturition for cows fed low-DCAD over cows fed high-DCAD timothy diets.

Recently, there has been interest in the potential of using diets with a low but positive DCAD to improve Ca homeostasis around parturition. Kurosaki et al. (2007) reported that multiparous cows fed a diet containing 1.2 mEq/100 g of DM had lower urinary pH prepartum, greater serum Ca during the first 3 d following parturition, and required less treatment for hypocalcemia compared with multiparous cows fed diets containing 14.6 or 15.3 mEq/100 g of DM. As such, multiparous cows fed the 1.2 mEq/100 g of DM diet had 50% less hypocalcemia (serum Ca < 5 mg/dL) than multiparous cows fed high-DCAD diets. Similarly, in the current study, we observed decreased urinary pH and blood bicarbonate concentration prepartum, improved iCa concentration postpartum, and a 48% reduction in the prevalence of subclinical hypocalcemia for cows fed the low-DCAD (1.6 mEq/100 g of DM) compared with cows fed the high-DCAD (14.5 mEq/100 g of DM) diet.

Although we saw a drastic reduction in the prevalence of subclinical hypocalcemia, 35% of the cows fed the low-DCAD timothy diet were still diagnosed with this disorder. Reported prevalence rates for hypocalcemia in cows fed low but positive DCAD diets have

ranged from 11% for cows fed a diet with a DCAD of 1.2 (Kurosaki et al., 2007; serum Ca < 5 mg/dL) to 50% for cows fed a diet with a DCAD value of 0 mEq/100 g of DM (Moore et al., 2000; plasma Ca < 4 mg/dL). Furthermore, Moore et al. (2000) reported improvements in Ca homeostasis as the DCAD value of the diet was reduced from +15 to 0 and from 0 to -15 mEq/100 g of DM. Although diets containing a low but positive DCAD may reduce the prevalence of hypocalcemia, the prevalence could be reduced to a greater extent by reducing the DCAD further. Further research is warranted to determine the optimal DCAD that accounts for both reducing the prevalence of hypocalcemia and minimizing depression in DMI.

Much attention has been directed toward the effects of PTH and $1,25\text{-(OH)}_2\text{D}$ on calcium homeostasis in dairy cows. In the current study, plasma concentrations of PTH and $1,25\text{-(OH)}_2\text{D}$ did not differ between the 2 dietary treatments. The plasma concentration of PTH remained high for 24 h after calving but decreased by h 72. Further, it took approximately 16 h before plasma $1,25\text{-(OH)}_2\text{D}$ reached its peak concentration. The slow response for an increase in plasma $1,25\text{-(OH)}_2\text{D}$ concentration may be due in part to PTH resistance (Martig and Mayer, 1973). Supporting the notion of PTH resistance, upon simple correlation analysis, there was no relationship between plasma PTH and $1,25\text{-(OH)}_2\text{D}$ concentrations, nor between plasma PTH and iCa concentrations. Consistent with previous findings (Horst et al., 1997), we observed that plasma $1,25\text{-(OH)}_2\text{D}$

increased when iCa concentration was low but decreased from h 24 to 72 corresponding to an increase in iCa concentration. This corresponded to a significant but weak negative correlation between plasma 1,25-(OH)₂ D and blood iCa concentration ($r^2 = 0.18$, $P < 0.01$). These results suggest that cows in the current study were responsive to circulating 1,25-(OH)₂ D in plasma but may have experienced tissue resistance to PTH.

In the current study, feeding a low-DCAD timothy diet increased the baseline blood iCa concentration and the nadir iCa concentration relative to the high-DCAD timothy diet. However, there was no difference between treatments for the extent of reduction in blood iCa concentration following parturition. We speculate that differences in tissue sensitivity to PTH between the prepartum and postpartum periods may explain why cows fed the low-DCAD timothy diet in the current study had greater prepartum blood iCa concentration but a similar decrease in postpartum blood iCa concentration. Martig and Mayer (1973) injected PTH intramuscularly before and after parturition and observed that PTH resistance was greater before parturition than following parturition. In our study, feeding a low-DCAD timothy diet decreased the prepartum blood bicarbonate concentration, an indicator of mild metabolic acidosis, but following parturition there was no difference in blood bicarbonate concentration between dietary treatments. Thus, carry-over effects of feeding the low-DCAD timothy diet on metabolic acidosis may have been minimal. The dietary treatment effect on prepartum blood iCa concentration observed in our study was in agreement with those reported by Kurosaki et al. (2007). Furthermore, in the study of Moore et al. (2000), blood samples were collected within 4 h of calving and they reported that the postpartum iCa concentration in blood decreased to a similar extent (83 and 82% of the prepartum iCa) for cows fed diets with DCAD values of +15 and 0 mEq/100 g of DM. Based on results of the current and previous studies, the postpartum improvement in blood iCa concentration for diets having a low but positive DCAD may be a response of greater prepartum blood iCa concentration rather than a greater ability to prevent a reduction in postpartum iCa blood concentration, whereas diets with a negative DCAD value seem to reduce the extent of blood iCa postpartum depression in addition to increased prepartum iCa blood concentration (Moore et al., 2000; Kurosaki et al., 2007).

DMI

It is well documented that feeding close-up diets of a negative DCAD value improves Ca homeostasis around parturition (Oetzel, 1991; Horst et al., 1997; Lean et

al., 2006), but a negative DCAD ration often decreases DMI especially if a great amount of anionic salts are used (Tucker et al., 1992; Joyce et al., 1997; Vagnoni and Oetzel, 1998). Charbonneau et al. (2006a) recently conducted a meta-analysis encompassing 22 studies and found a positive linear relationship between the DCAD and DMI. In the current study, we did not observe a reduction in DMI with the low-DCAD relative to the high-DCAD timothy diet. Although the low-DCAD timothy hay provided a lower DCAD than the high-DCAD timothy hay, the diet based on the low-DCAD timothy hay still provided a positive DCAD (cations > anions). As a result, the metabolic acidosis in the current study for cows fed the low-DCAD timothy diet was not as severe as in previous studies using anionic supplements (Tucker et al., 1992; Goff and Horst, 1998; Vagnoni and Oetzel, 1998). It is therefore possible that the low-DCAD timothy diet did not induce metabolic acidosis severe enough to reduce DMI.

Alternatively, it is possible that the form of the DCAD supplement may influence the depression in DMI. Charbonneau et al. (2008) compared 3 diets consisting of 1) a low-DCAD diet (-2.4 mEq/100 g of DM) using only low-DCAD timothy hay, 2) a low-DCAD diet (-1.9 mEq/100 g of DM) made with a combination of low- and high-DCAD timothy hay and additional HCl, and 3) a high-DCAD diet (29.6 mEq/100 g of DM) made with the same combination of low- and high-DCAD timothy hay. These diets were fed to nonpregnant and nonlactating cows, and cows fed the low-DCAD diet had similar DMI to those fed the high-DCAD timothy diet. Cows fed the diet supplemented with HCl, however, tended ($P = 0.08$) to have lower DMI (9.8 kg/d) than cows fed the low-DCAD timothy hay diet (11.5 kg/d) even though the DCAD of the low-DCAD timothy diet was slightly lower than that of the HCl-supplemented diet. However, not all studies using HCl have resulted in reduced DMI (Goff and Horst, 1998). The results of the current study and those of Charbonneau et al. (2008) suggest that the form of DCAD supplement, rather than the DCAD value itself, may have an impact on DMI. However, in another study, feeding timothy silage with a low DCAD value (-2.1 mEq/100 g of DM) tended to reduce DMI when compared with the control treatment (23 mEq/100 g of DM; Charbonneau et al., 2006b). More data are needed to elucidate the relationship between decreased DMI and the severity of the metabolic acidosis while using different anionic supplements.

CONCLUSIONS

Feeding a diet based on a low-DCAD timothy hay during the prepartum period had no effect on DMI and induced a mild but compensated metabolic acidosis,

improving the ability of cows to maintain calcium homeostasis around parturition compared with feeding a diet based on a high-DCAD timothy hay. These results indicate that feeding timothy hay with a low DCAD may be one strategy to reduce hypocalcemia in periparturient dairy cows.

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