Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows

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ABSTRACT

Feeding rations with low dietary cation-anion difference (DCAD) to dairy cows during late gestation is a common strategy to prevent periparturient hypocalcemia. Although the efficacy of low-DCAD rations in reducing the incidence of clinical hypocalcemia is well documented, potentially deleterious effects have not been explored in detail. The objective of the study presented here was to determine the effect of fully compensated metabolic acidosis on calcium and phosphorus homeostasis, insulin responsiveness, and insulin sensitivity as well as on protein metabolism. Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date. Blood and urine samples were obtained periodically between 14 d before to 14 d after calving. Intravenous glucose tolerance tests and 24-h volumetric urine collection were conducted before calving as well as 7 and 14 d postpartum. Cows fed the low-DCAD ration had lower urine pH and higher net acid excretion, but unchanged blood pH and bicarbonate concentration before calving. Protein-corrected plasma Ca concentration 1 d postpartum was higher in cows on the low-DCAD diet when compared with control animals. Urinary Ca and P excretion was positively associated with urine net acid excretion and negatively associated with urine pH. Whereas metabolic acidosis resulted in a 6-fold increase in urinary Ca excretion, the effect on renal P excretion was negligible. A more pronounced decline of plasma protein and globulin concentration in the periparturient period was observed in cows on the low-DCAD diets resulting in significantly lower total protein and globulin concentrations after calving in cows on low-DCAD diets. Intravenous glu-

cose tolerance tests conducted before and after calving did not reveal group differences in insulin response or insulin sensitivity. Our results indicate that fully compensated metabolic acidosis increased the Ca flux resulting in increased urinary calcium excretion before calving and increased plasma Ca concentration on the day after calving, whereas the effect on P homeostasis was unlikely to be clinically relevant. The clinical relevance of the effect of metabolic acidosis on the plasma protein and globulin concentration is unclear but warrants further investigation.

Key words: endocrinology, lactation

INTRODUCTION

Periparturient hypocalcemia or milk fever is a common condition of dairy cows with an annual incidence of 5 to 8% (USDA, 2007a). Feeding rations with low DCAD to dairy cows for 2 to 3 wk before calving is used in 27% of dairy operations and 45% of dairy cows in the United States to decrease the incidence of periparturient hypocalcemia (USDA, 2007b). The mechanism by which diets with low DCAD prevent clinical hypocalcemia in the periparturient period is not completely understood but appears to be associated with metabolic acidosis and increased Ca flux due to increased Ca absorption from the gastrointestinal tract and mobilization of Ca from bone, which combine to increase Ca excretion in urine (Fredeen et al., 1988; Schonewille et al., 1994; Constable, 1999; Goff and Horst, 2003). The efficacy of low-DCAD rations as a strategy to prevent periparturient hypocalcemia is well documented (Ender et al., 1971; Dishington, 1975; Block, 1984; Oetzel et al., 1988). However, concerns have been raised about the safety of low-DCAD rations in that acidogenic rations decrease DMI in late gestation (Charbonneau et al., 2006; Constable et al., 2009), thereby exacerbating the metabolic effects of negative energy balance in early lactation.

Chronic acidemia due to persistent metabolic acidosis has been associated with several deleterious metabolic

Received April 4, 2010.

Accepted September 20, 2010.

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Table 1. Ingredients and composition of close-up ration of treatment and control group

Item	Treatment	
	Control	Low DCAD
Ingredients (% DM)		
Grass hay	13.9	13.9
Corn silage	39.4	39.4
Alfalfa haylage	6.9	6.9
Cottonseed hulls	6.2	6.2
Soybean hulls	7.14	7.14
Ground corn grain	6.0	6.0
Soybean meal	6.0	2.0
Biochlor ¹	0.0	4.0
ProvAAI Basic ²	0.6	0.6
Calcium carbonate	0.5	0.5
Calcium phosphate	0.2	0.2
Magnesium oxide	0.1	0.1
Sodium chloride	0.5	0.5
TMV ³	0.9	0.9
Yeast	0.3	0.3
Magnesium sulfate	0.2	0.2
Vitamin E, 20,000 IU	0.2	0.2
Rumensin 80 ⁴	0.2	0.2
Omnigen AF ⁵	0.2	0.2
Megalac ¹	0.5	0.5
Chemical analysis		
DM (% as fed)	50.7	50.7
NE _L (Mcal/kg)	1.43	1.42
NDF (%)	40.1	40.5
NFC (%)	34.6	34.3
Starch (%)	19.8	20.1
CP (%)	15.3	15.1
Lignin (%)	4.2	4.4
Ca (%)	0.97	0.98
K (%)	1.21	1.20
Cl (%)	0.30	0.98
P (%)	0.33	0.33
Mg (%)	0.38	0.38
S (%)	0.25	0.37
Na (%)	0.08	0.16
DCAD	+15	-13

¹Arm and Hammer Animal Nutrition, Princeton, NJ.²Venture Milling, Salisbury, MD.³Trace mineral pack; KNS Inc., Lawrenceburg, KY.⁴Rumensin 80; Elanco Animal Health, Indianapolis, IN.⁵Omnigen AF; Phibro Animal Health Corp., Ridgefield Park, NJ.

effects in humans and domestic animals, including decreased feed intake, decreased insulin responsiveness and insulin sensitivity, as well as increased protein catabolism (MacKler et al., 1951; Ballmer and Imoberdorf, 1995; Bigner et al., 1996; Hu et al., 2007). To our knowledge, some of the deleterious effects of sustained acidemia and metabolic acidosis have been minimally investigated in dairy cows fed rations with a low DCAD in late gestation, and it is not clear whether fully compensated metabolic acidosis (i.e., pH remaining within the reference range) is associated with deleterious effects in dairy cattle. Accordingly, the purpose of the study reported here was to determine the effect of fully compensated metabolic acidosis on feed intake, acid–

base balance, calcium and phosphorus homeostasis, insulin responsiveness, and insulin sensitivity as assessed by the intravenous glucose tolerance test, and plasma concentrations of different protein fractions in high-yielding dairy cows.

MATERIALS AND METHODS

All methods were approved by the Purdue University Institutional Animal Care and Use Committee.

Animals, Housing, and Feeding

Twenty pregnant, nonlactating multiparous Holstein-Friesian cows were dried off 8 wk before the predicted calving date. Cows were moved from a dry lot into a temperature-controlled building 25 to 21 d before the predicted calving date and housed in individual tiestalls bedded with rubber mats covered with sawdust. Cows were healthy on the basis of a physical examination. Feed was offered as TMR based on grass hay, alfalfa haylage, and corn silage formulated to meet the requirements of dry cows based on the recommendations of the National Research Council (NRC, 2001, Table 1). Cows were acclimatized to their diet for 7 to 11 d before being studied from 14 d before the anticipated calving date to 14 d after calving.

Cows were randomly assigned to 1 of 2 groups fed rations with different DCAD from at least 21 d before the predicted calving date until calving. Cows assigned to the low-DCAD (L) group were fed a balanced, close-up, dry-cow ration to which a commercial chlorine-based anionic salt mixture (Biochlor, Arm and Hammer Animal Nutrition, Princeton, NJ; Table 1) was added to obtain a target DCAD of -12 mEq/100 g of DM, where $\text{DCAD (in mEq/100 g)} = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{S}^{2-}])$ (Lean et al., 2006). Cows assigned to the control (C) group received a similar ration to which an equivalent mass of soybean meal was added instead of the anionic salt mixture to obtain a target DCAD of approximately $+15$ mEq/100 g of DM. A DCAD of $+15$ mEq/100 g was chosen for the control ration as this value could be easily obtained when formulating a balanced dry-cow ration using feed components with a low K concentration without further supplementation with anionic salts (Lean et al., 2006; Ramos-Nieves et al., 2009).

After calving, cows in both groups were fed the same balanced TMR that met the requirements of early lactating cows as recommended by the National Research Council (NRC, 2001). This ration was based on corn silage, alfalfa haylage, and high-moisture corn (CP, 17.3%; ADF, 20.1%; NDF, 30.2%; Ca, 0.97%; P, 0.37%; Mg, 0.34%; K, 1.42% in DM with an energy density of 1.56 MCal/kg of NE_L). Cows were milked twice daily

after calving between 0530 and 0800 and between 1900 and 2000 in a milking parlor with the exception of the evenings of d 7 and d 14 postpartum where cows were milked in the tiestalls to allow for volumetric urine collection as described below. Milk weights were recorded for every milking.

Feed samples were obtained twice weekly from the feed bunk for each group immediately after mixing and were analyzed for Na, K, S (nitric acid digestion), and Cl (potentiometry with ion selective electrode) content to calculate the DCAD as described previously (Ag-Source, Bonduel, WI). Dry matter was determined by atmospheric oven drying at 100°C to constant weight.

Cows were fed once daily between 0800 and 1000 h. Amounts of TMR fed and refused were recorded daily during the whole study period. Cows that showed signs of preparation for parturition were moved to individual box stalls to calve. Dam and calf were separated within a few hours of calving and cows were returned to tie stalls by d 5 postpartum. Cows were weighed using a calibrated digital large animal scale in the evening before each intravenous glucose tolerance test (IVGTT).

Experimental Study

Cows in both groups entered the study period on d -14 relative to the predicted calving date following at least 7 d of acclimatization. Blood samples were obtained from the jugular vein on d -14, -9, -5, and -3 relative to expected calving as well as on d 1, 3, 5, 7, and 14 postpartum between 0600 and 0900 and before the morning feeding. All blood samples were obtained by venipuncture with the exception of d -5, -3, 7, and 14 where blood was obtained from a jugular venous catheter used for the IVGTT described in the following section. Blood for blood gas analysis was collected anaerobically into prechilled, heparinized 3-mL plastic syringes to measure blood gas tensions and pH. Collected samples were placed on ice and analyzed within 30 min using an automated blood gas analyzer (Ciba-Corning 248, Bayer Diagnostics, Tarrytown, NY). Rectal temperature was measured at the same time as blood sampling for blood gas analysis. Blood for plasma biochemical analysis was collected into prechilled evacuated tubes containing sodium heparin. Tubes were immediately placed on ice, centrifuged within 30 min at $1,000 \times g$ for 15 min at room temperature, and the plasma harvested and stored at -21°C until analyzed as described under "Plasma Biochemical Analysis."

Urine was collected by perineal stimulation within 1 h of blood collection. If urine collection was successful, samples of spontaneously voided urine were collected in 15-mL vials that were completely filled with urine and immediately closed to minimize exposure to air.

Samples were then placed in a water bath at 37°C and the urine pH measured within 15 min of collection. Thereafter urine samples were stored at -21°C until further analyzed as described under "Urine Biochemical Analysis."

Intravenous Glucose Tolerance Test

All cows underwent four 24-h urine collection episodes, of which 3 were combined with an IVGTT and 1 was combined with a sham-IVGTT. From each group, 6 cows were selected at random; blood and urine samples obtained during the 3 IVGTT as well as the sham IVGTT were analyzed as described below. The purpose of the sham-IVGTT was to serve as a control treatment to the IVGTT to determine the effect of parenteral dextrose infusion on renal electrolyte excretion. At least 12 h before each IVGTT or sham-IVGTT a 16-gauge, 83-mm catheter (Angiocath, Becton Dickinson, Franklin Lakes, NJ) was placed into the left or right jugular vein approximately 10 cm below the mandible and an extension set attached for blood collection. A 14-g, 83 mm catheter (Angiocath, Becton Dickinson) was placed approximately 15 cm below the 16-g catheter and an extension set attached for IV dextrose administration. Both extension sets were filled with heparinized saline (40 IU of sodium heparin/mL of 0.9% NaCl) and sealed with injection caps, and the catheters were secured to the skin. Approximately 1 h before the start of each IVGTT or sham-IVGTT, a Foley catheter (28 French, 30-mL balloon) was placed aseptically in the bladder and fixed in position by inflating the balloon with sterile saline. Tubing was attached to the Foley catheter and routed to a 4-L collection jar containing mineral oil for anaerobic urine collection.

During each IVGTT, cows received an intravenous bolus infusion of 500 mL of 50% dextrose solution (sterile 50% dextrose solution, Baxter Co., Columbus OH), approximating a dextrose dose of 0.3 to 0.4 g/kg of BW, through the preplaced 14-g catheter at 55 to 100 mL/min. Periodic blood samples were obtained beginning immediately before the start of infusion over 24 h. For the sham-IVGTT, cows were not infused but instrumented and sampled in a manner similar to that for IVGTT. One IVGTT and 1 sham-IVGTT were performed 5 and 3 d before the predicted calving date in randomized order with the start of the IVGTT 48 h apart. The remaining 2 IVGTT were performed on d 7 and 14 postpartum.

Blood was collected from the 16-gauge catheter immediately before beginning the dextrose infusion and 10, 20, 30, 45, 60, 90, 120, 240, 360, 480, 720, 960, 1,200 and 1,440 min after the onset of infusion. At each sampling time, the catheter was flushed with 3 mL of

heparinized saline (40 IU of sodium heparin/mL of 0.9% NaCl) and 5 mL of blood was withdrawn through the catheter. Additional blood was then aspirated via syringe and immediately transferred to prechilled Na-heparin tubes. After obtaining the blood sample, the 5 mL of blood withdrawn before sample collection were reinjected and the catheter was flushed with 10 mL of heparinized 0.9% NaCl solution. Blood collection tubes were placed on ice immediately after collection and centrifuged within 30 min at $1,000 \times g$ for 15 min. Harvested plasma was stored at -80°C (for determination of immunoreactive insulin concentration) and at -21°C (for all other serum biochemical parameters) until analyzed.

Urine was collected from the start of the IVGTT at 2-h intervals for the first 4 h and thereafter at 4-h intervals for a total duration of 24 h. The bladder was emptied before the start of the IVGTT by manipulating the Foley catheter and thereby stimulating urination. At each sampling time, the total volume of urine was determined and a urine sample was obtained anaerobically from the collection jar. The sample was then transferred into two 5-mL vials that were completely filled and immediately capped to minimize exposure to air. Urine samples were placed in a water bath at 37°C and the pH was measured within 15 min before the sample was stored at -21°C until analyzed.

Acid-Base Homeostasis

Measured values for blood pH, Pco_2 , and Po_2 were corrected for rectal temperature using standard equations (Burnett et al., 1995). Anion gap (**AG**; mEq/L) was calculated from the calculated value for $[\text{HCO}_3^-]$ (Constable, 1999) as $\text{AG} = ([\text{Na}] + [\text{K}]) - ([\text{Cl}] + [\text{HCO}_3^-])$. Strong ion gap (**SIGtp**; in mEq/l) was calculated as (Constable et al., 2005) $\text{SIGtp} = [\text{total protein}] \times [3.43/(1 + 10^{7.08 - \text{pH}})] - \text{AG}$, where [total protein] was measured in grams per deciliter.

Urinary net acid excretion (**NAE**) and ammonium concentration were determined by titration as described elsewhere (Chan, 1972; Constable, 2007; Constable et al., 2009). Briefly, previously frozen urine was thawed at room temperature, acidified by addition of a fixed volume of 1 *N* HCl, and heated to a slow boil for at least 2 min to expel CO_2 . Urine was then back-titrated with 0.1 *N* NaOH to the pH of 7.4. Eight percent formaldehyde was then added and the volume of 0.1 NaOH needed to back-titrate the urine sample to a pH of 7.4 was determined.

Plasma Biochemical Analysis

Plasma concentrations of inorganic phosphate (Pi, ammonium molybdate), total Ca (cresolphthalein), Mg

(arsenazo dye binding), albumin (bromocresol green), total protein (TP, biuret), creatinine (picric acid), NEFA (ACS-ACOD method), BHBA (3-hydroxybutyrate dehydrogenase), and glucose (hexokinase) were determined spectrophotometrically (Hitachi 911, Roche Diagnostics, Basel, Switzerland). Plasma concentrations of Na, K, and Cl were determined using ion-selective electrodes (Hitachi 911). Although it may have been preferable to have measured ionized Ca concentration instead of total Ca concentration, 85% of the variation in ionized Ca concentration in dairy cattle can be explained by variation in the total Ca concentration (Blum et al., 1972), with the remainder of the variation most likely due to changes in plasma total protein concentration. Plasma Ca concentration was therefore corrected for the plasma TP concentration using an equation validated for lactating dairy cattle (Seifi et al., 2005): $\text{Ca}_{\text{corr}} = [(\text{Ca} \times 0.2495) - (0.015 \times \text{TP} \times 10) + 1.2]/0.2495$, where Ca is the plasma total Ca concentration in milligrams per deciliter and TP is in grams per deciliter. Plasma globulin concentration was calculated as the difference between plasma TP and albumin concentrations.

Analysis of Intravenous Glucose Tolerance Test

The glucose-concentration time curve after IV administration of 50% dextrose was modeled as an exponential decline with nonzero asymptote such that $[\text{glucose}] = (A_o - A_{\text{asym}}) \times e^{-kt} + A_{\text{asym}}$, with t = time after the start of intravenous glucose administration; k = elimination rate constant for glucose; A_o = the extrapolated plasma glucose concentration assuming instantaneous mixing when $t = 0$; A_{asym} = asymptotic plasma glucose concentration when time $\gg 0$ min. The modeled curve was used to calculate the plasma half life (**T₅₀**) for glucose such that $T_{50} = \ln(2)/k$. The apparent volume of distribution for glucose (**V_d**) in liters was calculated from the modeled curve for each IVGTT by dividing the amount of glucose infused (250 g) by the difference between A_o and A_{asym} (in mg/dL) using the equation $V_d = (250 \times 1,000)/[(A_o - A_{\text{asym}}) \times 10]$. The apparent volume of distribution for glucose was calculated to provide an estimate for the extracellular fluid volume because V_d should reflect the instantaneous distribution volume for glucose and thereby assist in interpretations of changes in TP and albumin over time.

An aliquot of plasma was assayed for immunoreactive insulin (**IRI**) using a radioimmunoassay (insulin, Diagnostic Systems Laboratories, Inc., Webster TX). Inter- and intraassay coefficients of variation for IRI were 10 and 7%. The IRI-concentration time curve after IV administration of 50% dextrose was modeled for every IVGTT as an exponential decline with nonzero

asymptote such that $[IRI] = (I_o - I_{asym}) \times e^{-kt} + I_{asym}$, with t = time after the start of intravenous glucose administration; k = elimination rate constant for IRI; I_o = the extrapolated plasma IRI concentration assuming instantaneous mixing when $t = 0$; I_{asym} = asymptotic plasma IRI concentration when time $\gg 0$ min.

Insulin responsiveness (insulin response to glucose) was evaluated by calculating the peak plasma [IRI], the increment in plasma [IRI] for each IVGTT by subtracting the time = 0 min (basal) [IRI] from the peak plasma [IRI], by calculating the area under the plasma [IRI] curve from 0 to 90 min after the start of the dextrose infusion (AUC_{IRI-90}) and by calculating the ratio of baseline plasma [IRI] to baseline plasma [glucose] (I/G ratio, Muniyappa et al., 2008).

Insulin sensitivity (tissue responsiveness to insulin) was evaluated by calculating the reciprocal of plasma [IRI] ($1/[IRI]$) and the glucose to IRI ratio ($[glucose\text{ (mg/dL)}]/[IRI\text{ (}\mu\text{U/mL)}]$) from the basal plasma [glucose] and [IRI] values obtained before the IVGTT. Insulin sensitivity also was evaluated by calculating the quantitative insulin sensitivity check index (QUICKI) and the revised quantitative insulin sensitivity check index (RQUICKI) which are empirically derived mathematical transformations of basal [glucose], [IRI], and [NEFA] such that $QUICKI = 1/\{\log_{10}(\text{basal } [IRI]) + \log_{10}(\text{basal } [glucose])\}$ and $RQUICKI = 1/\{\log_{10}(\text{basal } [IRI]) + \log_{10}(\text{basal } [glucose]) + \log_{10}(\text{basal } [NEFA])\}$, with [IRI], [glucose], and [NEFA] being measured in $\mu\text{U/mL}$, mg/dL , and mmol/L respectively (Katz et al., 2000, Perseghin et al., 2001, Muniyappa et al., 2008). Values for $1/[IRI]$ and QUICKI provide simple, robust, and accurate indirect methods for evaluating insulin sensitivity (Muniyappa et al., 2008) and RQUICKI is hypothesized to provide an index of insulin sensitivity in dairy cows (Holtenius and Holtenius, 2007).

Insulin sensitivity was also evaluated by examining the plasma phosphorus concentration-time relationship during the IVGTT. Insulin induces a compartmental shift of Pi into cells of insulin responsive tissue in cattle as in other species (Knochel, 1977; Grünberg et al., 2006), presumably by upregulating the Na/Pi co-transporter gene expression (Li et al., 1996). A change in insulin sensitivity might therefore be associated with a change in the plasma [Pi]-time relationship. The maximal decrease in plasma [Pi] was calculated by subtracting the lowest [Pi] measured within 2 h after dextrose infusion from the baseline plasma [Pi]. The area under the plasma glucose concentration curve ($AUC_{Gluc-120}$) and the area under the plasma Pi concentration curve (AUC_{Pi-120}) from 0 to 120 min after the start of dextrose infusion were calculated using the trapezoidal rule.

Urine Biochemical Analysis

Urine pH was measured immediately after collection using a glass electrode with a rapid response (M3 internal reference glass pH electrode, Medical Instruments Corp., Solothurn, Switzerland). Stored urine samples were thawed at room temperature and vortexed for 10 s immediately before biochemical analysis. Urine concentrations of P, Ca, Na, K, Mg, creatinine, and glucose were determined as described for plasma using appropriate dilutions. Urine Cl concentration was determined spectrophotometrically (mercuric nitrate) using a commercial test kit (QuantiChrom chloride assay kit, Bioassay Systems, Hayward, CA). Total renal excretion was calculated for Na, K, Ca, P, Cl, Mg, and glucose by multiplying urine concentrations by the volume of urine produced per collection time interval. The total amount of glucose excreted in urine in the 12 h following the dextrose administration was calculated by adding the amounts of glucose excreted in urine determined for each collection interval.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation or as geometric mean and range. A $P < 0.05$ was considered significant. Values were log-transformed when necessary to achieve a normal distribution. Repeated measures ANOVA was used to detect differences in measured parameters between treatment groups and over time using PROC MIXED (SAS 9.1, SAS Inst. Inc., Cary, NC). Terms in the used model were treatment, time and the interaction of treatment and time. Bonferroni-adjusted P -values were used to assess differences within and between treatment groups whenever the F -test was significant. The mean actual day before calving was calculated for every sampling time point before calving that was scheduled relative to the predicted calving. A Student's t -test was conducted to compare differences in sampling times relative to actual calving between groups.

The associations between several determined parameters and measured urine pH and NAE, as well as the associations between plasma TP, albumin, and globulin concentrations and the V_d for glucose were tested using multivariate linear regression analysis. This analysis used dummy variable coding for each cow, which accounted for between-cow variability, thereby increasing the precision with which slope and intercept coefficients for the regression line could be estimated (Glantz and Slinker, 1990). This analytical approach enforces a uniform slope but different intercept value for each cow;

Figure 1. Mean $\pm$ SD daily DMI (lower curves) in group C (closed circles, solid line) and group L (open circles, dashed line) and average milk yields (upper curves) in group C (closed circles, dashed-dotted line) and group L (open circles, dotted line) during the study period. The vertical dashed line marks parturition. Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date.

this approach is appropriate whenever the slopes are similar as in this study. Dummy variables (C_1 to C_n) were defined as follows: $C_1 = 1$ if cow i ($i < n$), -1 if cow = n , and 0 otherwise. A statistical software package was used for analysis (SAS 9.1, SAS Inst., Inc.).

RESULTS

Animals, Feed Intake, and Milk Production

The mean body weight of cows 6 d antepartum was 761 ± 51 kg in group C and 779 ± 62 kg in group L. Six days postpartum, cows in group C weighed 670 ± 62 kg, and cows in group L weighed 675 ± 61 kg. The average age was 5.1 ± 1.5 yr and 4.5 ± 1.2 yr in groups C and L, respectively. Differences between groups in BW or age were not significant. Cows on trial received the experimental dry cow ration for 23 ± 3 d in group C and for 24 ± 4 d in group L. The time to calving was underestimated by 2 ± 3 d in group C and 3 ± 4 d in group L, therefore blood and samples obtained 14, 9, 5 and 3 d before predicted calving were equivalent to -16 ± 3 , -12 ± 3 , -7 ± 3 , and -5 ± 3 d relative to the actual calving time in group C and -17 ± 4 , -12 ± 4 , -8 ± 4 , and -6 ± 4 d in group L, respectively. The difference between groups was not significant. The mean DCAD of the ration fed was -8.7 ± 2.8 mEq/100 g in group L (target was -12 mEq/L) and $+11.3 \pm 2.1$ mEq/100 g in group C (target was $+15$ mEq/L).

All 20 cows completed the study. One cow in group C developed pneumonia shortly after calving and was treated with antimicrobial agents and a nonsteroidal antiinflammatory drug. Data obtained from this cow after calving were excluded from statistical analysis. One cow in group L was diagnosed with a left displaced abomasum 8 d postpartum but recovered uneventfully after surgery. Data obtained from the IVGTT performed on d 7 postpartum of this cow were not included in the statistical analysis. Clinical cases of periparturient hypocalcemia were not observed in either group.

Feed intakes and milk yields did not differ between groups but were numerically lower in group L (Figure 1).

Acid-Base Homeostasis

No treatment effects were observed on blood pH, $p\text{CO}_2$, $p\text{O}_2$, base excess (BE), or $[\text{HCO}_3^-]$ (Table 2). A decline in the BE and HCO_3^- between d -14 and 1 d postpartum was observed in both groups but was statistically significant only in group C. Base excess and HCO_3^- increased in both groups after calving by d 5 postpartum, whereas only a numerical increase of the blood pH was noticed during the same time period. The AG ranged between 15.9 ± 3.0 mEq/L and 18.7 ± 3.4 mEq/L antepartum and between 16.8 ± 2.7 mEq/L and 18.6 ± 1.8 mEq/L postpartum in group C. In group L, mean values for the different sampling times ranged from 15.6 ± 1.6 mEq/L and 17.6 ± 3.7 mEq/L antepartum and 16.4 ± 3.0 and 19.8 ± 7.9 mEq/L postpartum. Neither group nor time effects on AG were significant. The SIGtp ranged between -2.4 ± 3.2 and $+1.7 \pm 3.3$ mEq/L in group C and between -4.2 ± 4.4 and 0.2 ± 3.8 mEq/L in group L. Values were numerically lower in group L than in group C, but neither group nor time effects were significant.

Despite the lack of change in blood gas values, a metabolic acidosis was present in group L as indicated by a higher mean urinary NAE and lower urine pH than in group C from d -14 to d -3 relative to calving (Figure 2). Considerable cow-to-cow variation was observed in urine pH in group L before calving; in contrast, variability in urinary NAE was similar in both groups. After parturition, the mean urinary pH in group L increased immediately (by d 1 postpartum) to approximate the values in group C. Differences in urine pH and NAE over time were significant in group L but not in group C (Figure 2). Mean urine ammonium concentrations were similar for both groups and did not change over time, with mean values remaining below 10 mmol/L. Elevated urine ammonium concentrations were observed only when urine pH was below 6.30.

Table 2. Mean $\pm$ SD blood pH, partial pressure of CO₂ (pCO₂), partial pressure of O₂ (pO₂), base excess (BE), and HCO₃⁻ stratified by sampling time and group

Time relative to calving (d)	Blood pH		Blood pCO ₂ (mmHg)		Blood pO ₂ (mmHg)		BE (mEq/L)		Plasma HCO ₃ ⁻ (mEq/L)	
	Low		Low		Low		Low		Low	
	Control	DCAD	Control	DCAD	Control	DCAD	Control	DCAD	Control	DCAD
-14	7.384 $\pm$ 0.046	7.382 $\pm$ 0.047	45.0 $\pm$ 3.2 ^a	42.3 $\pm$ 3.0	28.3 $\pm$ 3.6 ^a	30.0 $\pm$ 2.6 ^a	3.3 $\pm$ 4.1 ^a	1.6 $\pm$ 4.4 ^{ab}	28.0 $\pm$ 3.4 ^a	26.2 $\pm$ 3.7
-9	7.353 $\pm$ 0.053	7.357 $\pm$ 0.052	43.7 $\pm$ 5.2 ^a	41.2 $\pm$ 3.7	29.1 $\pm$ 3.7 ^a	29.9 $\pm$ 3.0 ^a	-0.2 $\pm$ 2.9 ^b	-1.0 $\pm$ 4.2 ^a	25.0 $\pm$ 2.3 ^a	24.0 $\pm$ 3.5
-5	7.364 $\pm$ 0.041	7.364 $\pm$ 0.035	39.9 $\pm$ 2.4 ^{bc}	40.1 $\pm$ 2.9	38.4 $\pm$ 5.6 ^b	38.7 $\pm$ 6.8 ^b	-1.2 $\pm$ 3.2 ^b	-1.1 $\pm$ 2.3 ^a	23.8 $\pm$ 2.6 ^b	23.8 $\pm$ 2.3
-3	7.365 $\pm$ 0.020	7.369 $\pm$ 0.013	41.6 $\pm$ 3.8 ^{ac}	40.5 $\pm$ 2.8	38.7 $\pm$ 6.1 ^b	37.6 $\pm$ 6.8 ^b	0.5 $\pm$ 3.4 ^{ab}	-0.1 $\pm$ 1.4 ^{ab}	24.9 $\pm$ 3.1 ^{ab}	24.8 $\pm$ 1.4
1	7.352 $\pm$ 0.028	7.366 $\pm$ 0.031	43.0 $\pm$ 4.7 ^{ac}	40.1 $\pm$ 2.3	32.3 $\pm$ 6.8 ^a	32.1 $\pm$ 1.3 ^{ab}	-0.3 $\pm$ 3 ^b	-0.6 $\pm$ 2.4 ^a	24.8 $\pm$ 2.8 ^{ab}	24.2 $\pm$ 2.0
3	7.363 $\pm$ 0.014	7.373 $\pm$ 0.037	45.5 $\pm$ 1.5 ^a	42.3 $\pm$ 3.5	31.9 $\pm$ 1.6 ^a	34.2 $\pm$ 2.2 ^{ab}	2.1 $\pm$ 0.8 ^{ab}	1.2 $\pm$ 2.1 ^{ab}	27.1 $\pm$ 0.6 ^a	25.9 $\pm$ 1.8
5	7.368 $\pm$ 0.022	7.395 $\pm$ 0.043	43.3 $\pm$ 3.6 ^{ac}	43.3 $\pm$ 3.8	32.6 $\pm$ 3.1 ^{ab}	35.0 $\pm$ 5.5 ^{ab}	1.2 $\pm$ 2.4 ^{ab}	3.5 $\pm$ 3.7 ^b	26.4 $\pm$ 2.7 ^a	27.9 $\pm$ 3.1
7	7.373 $\pm$ 0.032	7.383 $\pm$ 0.023	44.8 $\pm$ 3.7 ^{ac*}	41.9 $\pm$ 3.1	35.3 $\pm$ 7.8 ^{ab}	40.6 $\pm$ 3.7 ^b	2.6 $\pm$ 2.7 ^a	1.5 $\pm$ 1.6 ^{ab}	27.3 $\pm$ 2.6 ^a	26.1 $\pm$ 1.5
14	7.362 $\pm$ 0.013	7.371 $\pm$ 0.029	40.7 $\pm$ 3.8 ^{bc}	42.4 $\pm$ 2.6	24.1 $\pm$ 2.5 ^{ab}	36.0 $\pm$ 5.7 ^b	-0.8 $\pm$ 2.5 ^{ab}	0.9 $\pm$ 2.1 ^{ab}	24.1 $\pm$ 2.5 ^b	25.7 $\pm$ 1.8

^a Values with different letters within a column are significantly different.

*Values within a row are significantly different between groups ($P < 0.05$, Bonferroni adjusted).

Figure 2. Mean $\pm$ SD urine pH for group C (closed circles, solid line) and group L (open circles, dashed line) and urine net acid excretion (NAE) for group C (closed circles, dash-dotted line) and group L (open circles, dotted line). Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = +11 mEq/100 g, group C) for the last 3 wk before the expected calving date. *Values are significantly different between groups ($P < 0.05$); time points with different letters within one group are significantly different ($P < 0.05$, Bonferroni corrected).

Regression analysis using only values obtained before calving, when cows in group L were on the low DCAD ration, revealed a negative linear association between daily feed intake and urinary NAE (adjusted $R^2 = 0.68$, $P = 0.015$), as well as negative linear associations between urinary NAE and BE (adjusted $R^2 = 0.71$, $P = 0.0097$), plasma [HCO₃⁻] (adjusted $R^2 = 0.72$, $P = 0.0095$), and blood pCO₂ (adjusted $R^2 = 0.72$, $P = 0.0092$). The association between daily feed intake and urine pH before calving was not significant.

Regression analysis using all time points (obtained antepartum and postpartum) revealed that feed intake was negatively associated with NAE ($R^2 = 0.33$, $P = 0.028$) and positively associated with urine pH (adjusted $R^2 = 0.40$, $P = 0.0092$).

Plasma Biochemical Analysis

Mean TP corrected plasma [Ca] on d 1 postpartum was lower than that determined at any other time point in both groups and was lower in group C than in group L (Figure 3). Three cows in group C and 2 cows in group L had protein-corrected plasma [Ca] below 8.0 mg/dL on d 1 postpartum.

Figure 3. Mean $\pm$ SD plasma Ca concentration corrected for plasma protein and plasma Pi concentration (upper panel), plasma Na and Cl concentration (middle panel), and plasma K and Mg concentration (lower panel). Plasma Ca, Na, and K concentration for group C are represented as solid lines with closed circles and for group L as dashed lines with open circles. Plasma Pi, Cl, and Mg concentration for group C are represented as dashed-dotted lines with closed circles and for group L as dotted lines with open circles. Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date. Time points with different capital letters differ significantly within group C; time points with different lowercase letters differ significantly within group L. *Values differ significantly between groups ($P < 0.05$, Bonferroni adjusted). The vertical dashed line marks parturition.

Mean plasma [Pi] decreased after calving in both groups and remained below mean preparturient values throughout the remainder of the study period. A treatment effect on plasma [Pi] was not identified (Figure 3). Regression analysis using values obtained before calving revealed that the plasma [Pi] was positively associated with the urine NAE (adjusted $R^2 = 0.64$, $P < 0.0001$) and negatively associated with the urine pH (adjusted $R^2 = 0.54$, $P < 0.0001$). Regression analysis using all time points (obtained antepartum and postpartum) revealed a positive association of plasma [Pi] with NAE (adjusted $R^2 = 0.32$, $P < 0.0001$) and a negative association with urine pH (adjusted $R^2 = 0.42$, $P < 0.0001$).

Plasma concentrations of Na, K, Cl, and Mg are presented in Figure 3. Mean plasma [Na] and [Cl] significantly decreased in both groups after calving with concentrations remaining below preparturient values until the end of the study period; a treatment effect was not identified for any these electrolytes.

Changes in mean plasma albumin ([alb]) and [TP] concentrations over time are presented in Figure 4. Significant time effects were observed for [alb] ($P < 0.0001$), globulin ([glob]) ($P < 0.0001$) and [TP] ($P = 0.0002$), with plasma [alb] being decreased from d 3 postpartum until the end of the study in group L, but not in group C. Significant treatment effects as well as treatment $\times$ time interactions were present for [glob] and [TP]. Decreases in plasma [glob] ($P < 0.0001$) and [TP] ($P = 0.0005$) were present in both groups from 14 d antepartum to 9 d antepartum, whereas a decrease in plasma [alb] was only apparent between d 1 and 3 postpartum ($P = 0.0003$). Treatment effects were determined for plasma [glob] ($P = 0.019$) and [TP] ($P = 0.027$) but not for plasma [alb]. Plasma [glob] from 5 d postpartum until the end of the study period, and plasma [TP] from 3 d postpartum until the end of the study period, were lower in group L than in group C (Figure 4).

Energy Metabolism

A significant group effect for plasma [glucose], [NEFA], and [BHBA] was not present; however, all 3 indicators of metabolic status changed over time (Figure 5).

Intravenous Glucose Tolerance Test

Plasma glucose, IRI, and Pi concentrations during the IVGTT were determined in 6 cows selected at random from each group. Changes in the plasma concentrations of glucose, IRI, and Pi over time during the 3 IVGTT conducted antepartum 7 and 14 d postpartum are reported in Figure 6.

Figure 4. Mean $\pm$ SD plasma total protein concentration for group C (solid line) and group L (dashed line) and mean $\pm$ SD plasma albumin concentration at different time points for group C (dashed-dotted line) and group L (dotted line) over time. Time points with different letters differ significantly within groups. Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date. *Values are significantly different between groups ($P < 0.05$, Bonferroni adjusted). The vertical dashed line indicates parturition. Time points with different letters within one group differ significantly ($P < 0.05$, Bonferroni adjusted).

Mean area under the glucose concentration time curve from 0 to 120 min after intravenous dextrose infusion (AUC_{gluc}) and T_{50} following intravenous dextrose infusion were similar for both groups at all 3 IVGTT (Table 3). By contrast, basal [IRI], peak [IRI], and IRI increment after intravenous dextrose infusion were higher antepartum than on d 7 and 14 postpartum, with no difference between groups. The reciprocal of baseline [IRI] ($1/IRI$), the mean G/I ratio, the QUICKI and RQUICKI were higher and the I/G ratio was lower in both groups after calving than before calving but a group effect was not identified. No difference between the values determined 7 d postpartum and 14 d postpartum were determined (Table 3).

The total amount of glucose excreted in urine in the 12 h following dextrose infusion in group C was 16.9 ± 6.0 g, 5.1 ± 6.6 g and 6.9 ± 5.7 g antepartum and 7 and 14 d postpartum, respectively. This corresponds to an average 6.8, 2.0, and 2.8% of the administered glucose load for the respective treatment times. In group L, 10.8 ± 4.9 , 10.6 ± 14.2 , and 6.8 ± 3.0 g of glucose were excreted in urine following dextrose infusion antepartum and 7 and 14 d postpartum, respectively. These amounts were equivalent to a mean 4.3%, 4.2% and 2.7% of the administered amount of dextrose on treatment times antepartum and 7 d and 14 d postpartum, respectively. Differences in the amount of glucose ex-

Figure 5. Mean $\pm$ SD plasma glucose concentration (upper panel), plasma NEFA concentration (middle panel), and plasma BHBA concentration (lower panel) for group C (solid line, closed circles) and group L (dashed line, open circles). Time points with different letters differ significantly within groups ($P < 0.05$, Bonferroni adjusted). The vertical dashed line marks parturition. Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date.

Figure 6. Mean $\pm$ SD plasma glucose concentrations (upper row), plasma immunoreactive insulin (IRI) concentrations (middle row), and plasma Pi concentration (lower row) during intravenous glucose tolerance test conducted antepartum (left column), on d 7 postpartum (middle column), and on d 14 postpartum (right column) in 6 randomly selected cows for group C (solid line) and group L (dashed line). Twenty multiparous Holstein-Friesian dairy cows were assigned to 1 of 2 treatment groups and fed a low-DCAD ration (DCAD = -9 mEq/100 g, group L) or a control ration (DCAD = $+11$ mEq/100 g, group C) for the last 3 wk before the expected calving date. *Values within 1 group are significantly different from values determined at T0 (immediately before start of infusion, $P < 0.05$, Bonferroni corrected). Values with + are significantly different between groups ($P < 0.05$).

creted in urine between groups or over time were not significant.

The absolute decline in plasma [Pi] within 60 min after dextrose infusion was similar before and after calving and between groups (ranging from 1.1 ± 0.4 mg/dL to 1.4 ± 0.6 mg/dL). Plasma [Pi] was numerically higher in group C than group L on all 3 treatment days at baseline and in the 12 h following dextrose infusion but the group effect was not significant. Neither a group nor time effect on the decline of plasma [Pi] was significant. The AUC_{PI-120} after dextrose infusion was lower on d 7 (339.8 ± 101.5 mg·min/dL and 251.4 ± 62.3 mg·min/dL in group C and L, respectively) than antepartum (451.8 ± 76.8 mg·min/dL and 446.9 ± 82.9 g·min/dL in group C and L, respectively). No group differences were found on any of the treatment days.

Regression analysis using V_d as an independent variable and [TP], [alb], and [glob] as dependent variables indicated a negative linear association between V_d and plasma [alb] (adjusted $R^2 = 0.39$, $P = 0.020$) but not with [TP] or [glob]. This result indicated that a low albumin concentration was linearly associated with a larger V_d for glucose, suggesting that the decrease in [alb] was due, in part, to extracellular fluid volume expansion.

Urine Biochemical Analysis

Spontaneously voided urine samples were obtained within 1 h of blood sampling from 12 cows on d -14 (6 group C, 6 group L), 17 cows on d -9 (8 group C, 9 group L), 9 cows on d 1 (5 group C, 4 group L), 11 cows on d 3 (6 group C, 5 group L), and 11 cows on d 5 relative to calving (6 group C, 5 group L). Urine samples were obtained from all cows on the days (-5, -3, 7, 14 postpartum) where urine was collected volumetrically. The number of urine samples obtained at these time points (overall percentage, 60%) was not different between groups at any time.

Cows in group C produced a mean urine volume of 11.9 ± 1.6 L, 13.1 ± 1.9 L, 21.2 ± 4.1 L and 19.2 ± 5.4 L over 24 h following the sham-IVGTT antepartum, the IVGTT antepartum and the IVGTT on d 7 d and 14 d postpartum, respectively. In group L, measured urine volumes were 15.0 ± 2.9 L, 13.2 ± 2.5 L, 16.7 ± 5.0 L, and 17.9 ± 5.7 L following the sham-IVGTT antepartum, and the IVGTT conducted antepartum, and on 7 d and 14 d postpartum, respectively. The volumes of urine produced over 24 h before and after parturition were different in group C ($P = 0.0063$), but not in group L. No group effect on urine volume or the difference in urine volume after IVGTT antepartum and the sham-IVGTT antepartum was detected.

Table 3. Mean $\pm$ SD or geometric mean and range of peak [immunoreactive insulin (IRI)] after intravenous dextrose infusion, area under the IRI concentration time curve from 0 to 90 min after intravenous dextrose infusion (AUC_{IRI}), and the insulin:glucose ratio (I:G ratio) as parameters for insulin response, and baseline [IRI], area under the glucose concentration time curve from 0 to 120 min after intravenous dextrose infusion (AUC_{gluc}), the plasma half life for glucose following intravenous dextrose infusion ($T_{50_{gluc}}$), the reciprocal of [IRI] (1/IRI), the glucose:IRI ratio (G:I ratio), the quantitative insulin sensitivity check index (QUICKI), and the revised quantitative insulin sensitivity check index (RQUICKI) stratified by treatment time and group, as parameters used to assess insulin sensitivity

Item	Antepartum			7 d postpartum			14 d postpartum		
	Control	Low DCAD	Low DCAD	Control	Low DCAD	Low DCAD	Control	Low DCAD	Low DCAD
Insulin response									
Peak [IRI] (pmol/L)	529 $\pm$ 130	533 $\pm$ 174	533 $\pm$ 174	216 $\pm$ 99	192 $\pm$ 46	208 $\pm$ 107	200 $\pm$ 53	208 $\pm$ 107	208 $\pm$ 107
AUC_{IRI} (pmol·min/L)	19,154 $\pm$ 4,652	16,519 $\pm$ 8,898	16,519 $\pm$ 8,898	6,928 $\pm$ 2,801	7,189 $\pm$ 2,908	6,656 $\pm$ 3,526	6,755 $\pm$ 1,634	6,656 $\pm$ 3,526	6,656 $\pm$ 3,526
I:G ratio	0.61 [0.37–1.09]	0.55 [0.21–1.03]	0.55 [0.21–1.03]	0.14 [0.02–1.2]	0.12 [0.02–0.46]	0.09 [0.02–0.49]	0.05 [0.02–1.00]	0.09 [0.02–0.49]	0.09 [0.02–0.49]
Insulin sensitivity									
Baseline [Glucose] (mg/dL)	70.5 $\pm$ 4.8	71.2 $\pm$ 5.4	71.2 $\pm$ 5.4	56.0 $\pm$ 12.6	56.7 $\pm$ 21.2	56.9 $\pm$ 8.4	55.2 $\pm$ 8.2	56.9 $\pm$ 8.4	56.9 $\pm$ 8.4
Baseline [IRI] (pmol/L)	38.4 [10–60]	30.6 [17–69]	30.6 [17–69]	5.6 [1–14]	6.7 [1–48]	4.8 [1–34]	2.7 [1–6]	4.8 [1–34]	4.8 [1–34]
AUC_{gluc} (mg·min/dL)	13,758 $\pm$ 1,490	13,692 $\pm$ 1,855	13,692 $\pm$ 1,855	12,550 $\pm$ 673	12,519 $\pm$ 2,006	11,700 $\pm$ 1,549	12,283 $\pm$ 2,133	11,700 $\pm$ 1,549	11,700 $\pm$ 1,549
$T_{50_{gluc}}$ (min)	21.3 $\pm$ 4.4	23.0 $\pm$ 4.8	23.0 $\pm$ 4.8	26.5 $\pm$ 5.2	26.6 $\pm$ 10.0	23.4 $\pm$ 8.4	24.8 $\pm$ 3.1	23.4 $\pm$ 8.4	23.4 $\pm$ 8.4
1/IRI	0.02 [0.01–0.04]	0.03 [0.01–0.07]	0.03 [0.01–0.07]	0.18 [0.07–1.00]	0.15 [0.02–1.00]	0.31 [0.04–1.00]	0.38 [0.18–1.00]	0.31 [0.04–1.00]	0.31 [0.04–1.00]
G:I ratio	1.58 [0.92–2.29]	1.96 [0.97–5.00]	1.96 [0.97–5.00]	9.69 [4.78–54.00]	8.73 [2.17–56.00]	11.62 [2.23–60.00]	20.25 [10.40–56.00]	11.62 [2.23–60.00]	11.62 [2.23–60.00]
QUICKI	0.28 [0.26–0.30]	0.29 [0.27–0.33]	0.29 [0.27–0.33]	0.41 [0.34–0.58]	0.40 [0.27–0.57]	0.624 [0.33–2.97]	0.47 [0.39–0.60]	0.624 [0.33–2.97]	0.624 [0.33–2.97]
RQUICKI	0.330 $\pm$ 0.028	0.348 $\pm$ 0.053	0.348 $\pm$ 0.053	0.420 $\pm$ 0.089	0.376 $\pm$ 0.102	0.431 $\pm$ 0.134	0.458 $\pm$ 0.052	0.431 $\pm$ 0.134	0.431 $\pm$ 0.134

Table 4. Mean $\pm$ SD or geometric mean and range of urinary excretion of Na, K, Ca, Cl, P, and Mg in grams per 24 h stratified by treatment, time, and group

Electrolyte	Treatment	Time (d) relative to calving			
		-5	-3	7	14
Na (g/24 h)	Control	8.2 $\pm$ 4.8 ^a	5.2 $\pm$ 2.3 ^a	33.7 $\pm$ 19.2 ^b	29.9 $\pm$ 11.8 ^b
	Low DCAD	18.7 $\pm$ 4.0	14.4 $\pm$ 7.2	27.5 $\pm$ 15.8	24.3 $\pm$ 11.4
K (g/24 h)	Control	151.0 $\pm$ 22.2	127.9 $\pm$ 28.7	141.7 $\pm$ 51.4	155.7 $\pm$ 39.7
	Low DCAD	121.0 $\pm$ 26.4 ^{ab}	91.8 $\pm$ 23.8 ^a	126.2 $\pm$ 28.1 ^{ab}	151.6 $\pm$ 12.8 ^b
Ca (g/24 h)	Control	0.5 $\pm$ 0.1*	0.4 $\pm$ 0.2*	0.9 $\pm$ 0.6	1.2 $\pm$ 1.0
	Low DCAD	3.4 $\pm$ 1.3 ^a	4.1 $\pm$ 0.9 ^a	0.7 $\pm$ 0.2 ^b	0.6 $\pm$ 0.2 ^b
P (mg/24 h)	Control	45.8 [12.7–100.1]	37.1 [12.0–86.2]	44.2 [15.9–127.1]	45.0 [17.1–92.8]
	Low DCAD	70.0 [11.9–132.0]	45.4 [13.5–110.6]	56.7 [16.3–128.2]	61.0 [33.9–100.0]
Cl (g/24 h)	Control	15.6 $\pm$ 6.2*	12.0 $\pm$ 3.3*	26.5 $\pm$ 15.1	31.5 $\pm$ 26.2
	Low DCAD	74.4 $\pm$ 16.5 ^a	64.3 $\pm$ 24.0 ^a	33.6 $\pm$ 13.2 ^b	30.6 $\pm$ 17.6 ^b
Mg (g/24 h)	Control	5.3 $\pm$ 2.8 ^{ab}	4.5 $\pm$ 1.5 ^a	6.2 $\pm$ 1.9 ^{ab}	8.4 $\pm$ 3.2 ^b
	Low DCAD	4.1 $\pm$ 2.2	3.2 $\pm$ 1.7	4.8 $\pm$ 2.2	6.9 $\pm$ 0.6

^{a,b}Values with different letters within a row are significantly different.

*Values differ significantly between groups ($P < 0.05$, Bonferroni adjusted).

The amount of Na, K, Ca, Pi, Cl, and Mg excreted in urine over 24 h following dextrose infusion stratified by treatment and time is summarized in Table 4. Whereas treatment effects on total urinary electrolyte excretion were observed for Ca and Cl before calving, with significantly higher amounts of Ca and Cl excreted in cows on the low-DCAD diet compared with control cows, significant time effects were observed for all analyzed parameters (Table 4). The amount of Ca excreted in urine over 24 h before calving was more than 6-fold higher ($P < 0.0001$) in group L than group C and more than 4 times higher before than after calving ($P < 0.0001$), indicating marked hypercalciuria in cows fed a low DCAD diet. Differences between sham-IVGTT antepartum and IVGTT antepartum in renal electrolyte excretion over 24 h were not observed. The amount of P excreted in the urine was very low but showed broad between-animal variability (Table 4). A group or time effect on P excretion was not identified.

Urine pH was related to urine NAE in a nonlinear manner (Figure 7, top panel). Multivariable regression analysis revealed a nonlinear positive association between urine [Ca]/[creatinine] and NAE (adjusted $R^2 = 0.49$, $P < 0.0001$; Figure 7, middle panel). Multivariable regression analysis also revealed a linear negative association with urine [Ca]/[creatinine] and urine pH (adjusted $R^2 = 0.67$, $P < 0.0001$; Figure 7, bottom panel) whereby urine [Ca]/[creatinine] = $1.13 - 0.135 \times \text{pH}$. In other words, urinary calcium excretion increased nonlinearly with an increase in NAE and increased linearly with a decrease in urine pH. Regression analyses revealed a linear association between urine [Pi] and NAE (adjusted $R^2 = 0.67$, $P < 0.0001$) and urine [Pi] and urinary pH (adjusted $R^2 = 0.70$, $P < 0.0001$).

DISCUSSION

The main objective of the study presented here was to explore potential effects of feeding a dry cow ration of moderately low DCAD on acid–base, calcium, and phosphorus homeostasis as well as glucose and protein metabolism during the periparturient period in high-yielding dairy cows. Optimal target values for urine pH to decrease effectively the incidence of milk fever in dairy herds have not been identified, and recommendations for optimal urine pH vary widely (Horst et al., 1997; Roche et al., 2003; Charbonneau et al., 2006; Constable et al., 2009). A recent meta-analysis suggested that decreasing urine pH from 7.0 to 6.0 or lower led to a modest decrease in the incidence of milk fever but markedly increased the risk of decreased DMI in the prepartum period (Charbonneau et al., 2006). In the study presented here, cows ingesting a low DCAD ration had mean urine pH values of 7.2 and a 6-fold increase in renal Ca excretion without affecting blood pH or plasma [Ca] before calving. This indicated that the DCAD diet in group L effectively challenged acid–base and calcium homeostasis and markedly increased calcium flux without overwhelming compensatory mechanisms or decreasing DMI.

Comparison of parameters in blood and urine characterizing acid–base homeostasis showed strong linear associations between urinary NAE and plasma $[\text{HCO}_3^-]$ and BE; this finding is consistent with the consensus view that urinary NAE, and to a lesser extent urine pH, are the most sensitive indicators of deregulations in acid–base homeostasis (Jørgensen, 1957; Kutas, 1965; Chan, 1972; Charbonneau et al., 2006; Hu et al., 2007; Constable et al., 2009). The nonlinear relationship

Figure 7. Relationship between urine pH, net acid excretion (NAE), and ratio of urine calcium to creatinine concentration determined on urine samples obtained from dairy cows ($n = 20$) between 14 d antepartum and 14 d postpartum. Cows were fed a low-DCAD diet ($n = 10$) or a normal DCAD diet ($n = 10$) antepartum. A nonlinear relationship exists between urine pH and NAE, such that $\text{pH} = a + \log_{10}(-\text{NAE} + b)$ (Constable et al., 2009). The dashed lines in the top panel indicate that urine NAE approximates zero when urine pH approximates 7.4. A nonlinear relationship also exists between urine $[\text{Ca}]/[\text{creatinine}]$ and urine NAE (middle panel). In contrast, a linear relationship exists between urine $[\text{Ca}]/[\text{creatinine}]$ and urine pH (bottom panel), whereby urine $[\text{Ca}]/[\text{creatinine}] = 1.13 - 0.135 \times \text{pH}$.

between pH and NAE in urine (Figure 7) is consistent with that recently determined for bovine urine, whereby urine $\text{pH} = 6.12 + \log_{10}(-\text{NAE} + [\text{NH}_4^+] + 2.6)$ (Constable et al. 2009). In other words, changes in NAE reflect mild deregulations in acid–base homeosta-

sis, whereas alterations in urine pH reflect more severe acidogenic effects.

The most likely reason that low-DCAD diets prevent periparturient hypocalcemia is that ingestion of low-DCAD diets increases Ca flux. The increase in Ca flux

is due to an increase in the rate of Ca entry into the exchangeable Ca pool that approximates the rate of Ca exit from the exchangeable Ca pool (Vagg and Payne, 1970; Fredeen et al., 1988). Ingestion of a low DCAD diet increases Ca entry into the exchangeable Ca pool by 3 main mechanisms: enhanced intestinal absorption (Lomba et al., 1978; Schonewille et al., 1994; Roche et al., 2007), increased bone resorption (Block, 1984), and decreased bone accretion (van Mosel et al., 1994), with the latter 2 mechanisms appearing to be active only in the presence of acidemia and metabolic acidosis. Ingestion of a low DCAD diet also increases Ca exit from the exchangeable Ca pool by decreasing renal tubular Ca reabsorption (Stacy and Wilson, 1970; Fredeen et al., 1988), manifest as hypercalciuria. An increase in Ca flux allows the periparturient dairy cow to more readily cope with the marked perturbation in Ca homeostasis that occurs at the onset of lactation by shifting Ca exit from the exchangeable Ca pool from the kidney to the mammary gland. The net result is a marked reduction in urine calcium excretion and increased plasma total calcium concentration immediately after calving, as observed in this study (Table 4).

The results of plasma and urine biochemical analyses in our study indicated that ingestion of a low-DCAD diet did not affect the exchangeable Ca pool size, as characterized by the plasma Ca concentration, except for the first day postpartum, where TP-corrected plasma [Ca] was higher in group L than group C. This finding was consistent with results from other studies that acidogenic diets only affect plasma Ca concentration during times of marked disturbance in Ca homeostasis, such as at the onset of lactation (Vagnoni and Oetzel, 1998; Gasperlin et al., 2002; Ramos-Nieves et al., 2009). Ingestion of diets with more acidifying potential (lower DCAD than used in our study) appear to cause a greater increase in Ca flux before calving because such diets cause more than twice the amount of Ca to be excreted in urine than that observed in our study (Goff and Horst, 1998; Vagnoni and Oetzel, 1998; Moore et al., 2000). The proposed association between low-DCAD diets and increased Ca flux is supported by our finding that urine Ca concentration is positively associated with NAE in a nonlinear manner and negatively associated with urine pH in a linear manner (Figure 7), indicating that metabolic acidosis severe enough to decrease urine pH markedly is more effective in increasing Ca flux than milder metabolic acidosis that increases NAE with minor decreases in urine pH (Figure 7). Of considerable interest is that our finding of a nonlinear positive relationship between urine Ca excretion and NAE is remarkably similar to that observed in humans (Lemann et al., 2003), consistent with the presence of fundamental relationships between

ingestion of an acidogenic diet and acid-base physiology and renal physiology. Assuming that increased Ca flux is the most important method for decreasing the incidence and severity of hypocalcemia at calving and that urine Ca concentration provides a clinically useful insight into Ca flux in the periparturient cow, it appears that measurement of urine Ca concentration will provide the best method for evaluating the risk of periparturient hypocalcemia. However, because of the linear negative association between urine [Ca]/[creatinine] and urine pH (Figure 7), measurement of urine pH provides a practical on farm method for evaluating calcium flux in cows before parturition.

An important finding of this study was that renal Ca loss decreased markedly in the low DCAD group from a mean 3.4 g/24 h in late gestation to 0.7 g/24 h after calving, whereas renal Ca excretion was not altered in cows on the control diet. The markedly higher daily renal Ca loss during late gestation in cows fed a low DCAD diet has been reported in other studies (Joyce et al., 1997; Vagnoni and Oetzel, 1998; Spanghero, 2002). Assuming an extracellular distribution volume for Ca of 176 L in a 675-kg cow (26% of the BW, Payne et al., 1967) the difference in renal Ca loss between ante- and postpartum in cows fed a low-DCAD diet of 2.7 g/d provided a potential increase in mean extracellular total Ca concentration of approximately 1.5 mg/dL; this calculated value was slightly higher than the difference of 0.8 mg/dL observed in plasma total Ca concentration between the 2 groups on the day after calving (Figure 3).

Our results do not support the hypothesis that acidemia-induced increases in parathyroid hormone (PTH) receptor sensitivity, presumably by structural alteration of the receptor due to changes in pH, play an important role in the mechanism by which ingestion of an acidogenic diet decreases the incidence and severity of periparturient hypocalcemia (Goff, 2008). We did not observe an effect of diet on blood pH, yet plasma protein-corrected total calcium concentration was higher on the day after calving in cows consuming a low-DCAD diet, indicating that mechanisms other than decreased PTH receptor sensitivity were responsible.

Chronic acidemia and metabolic acidosis in humans results in negative phosphorus balance and a small decrease in serum phosphorus concentration due to increased urinary phosphorus loss (Lemann et al. 1966). Other studies investigating the effect of mild metabolic acidosis on phosphorus homeostasis in cows during late gestation suggest that mild to moderate acidemia and metabolic acidosis does not alter plasma [Pi] or Pi balance (Fredeen et al., 1988; Joyce et al., 1997; Gasperlin et al., 2002), although a weak association between decreasing plasma [Pi] and decreasing DCAD has been

reported (Borucki Castro et al., 2004). These reports are in contrast with results of the study presented here where although a group effect on plasma [Pi] was not identified; the plasma [Pi] was found to be negatively associated with urine pH and positively associated with NAE in urine. Another recent study reported higher plasma P concentrations in the first week of lactation in cows fed a low-DCAD diet during late gestation (Ramos-Nieves et al., 2009). Our finding that urinary [Pi] was associated with urine pH and NAE is similar to findings in monogastric animals where Pi is an important buffer in urine and contributes considerably to the increase in urine titratable acidity during acidemia. A time effect on plasma [Pi] was identified; the small decrease in plasma [Pi] in late gestation paralleled the decrease in DMI, whereas the decrease in plasma [Pi] after calving was most likely due to the increased loss of P in milk.

In the present study the renal excretion of Ca and Cl in late gestation were higher in cows in group L compared with group C. Acidogenic diets have consistently produced a hypercalciuric effect through decreased renal reabsorption of filtered calcium (Lemann et al., 1966; Fredeen et al., 1988; Constable, 2007). A potential mechanism through which aciduria is likely to affect renal tubular Ca reabsorption is through altered expression of the epithelial Ca channel ECaC1 in the connecting tubule. The significant dependence of this Ca channel on luminal pH was demonstrated in vitro in rabbits whereby transcellular Ca transport was decreased by approximately 75% when luminal pH was decreased from 7.4 to 6.2 (Müller et al. 2001). The higher Cl content in the low DCAD ration is the probable cause for the higher renal Cl excretion in group L, and the decrease in renal Cl excretion after calving in group L is likely caused by the difference in chloride content between the close-up ration and the ration fed during early lactation.

The major purpose of conducting IVGTT in cows on diets with low and high DCAD was to identify a possible effect of acidogenic diets on insulin responsiveness and sensitivity. Severe and moderate metabolic acidosis and acidemia decrease insulin responsiveness in humans and dogs (DeFronzo and Beckles, 1979; MacKler et al., 1951). Feeding an extremely low DCAD (-40.5 mEq/100 g) ration to nonlactating nonpregnant dairy cows induced acidemia (blood pH, 7.32), metabolic acidosis (plasma $[\text{HCO}_3^-]$, 17.8 mmol/L; urine pH, 5.4), and decreased insulin responsiveness, as assessed by a lower peak [IRI] and higher peak plasma [glucose] in response to an IVGTT (Bigner et al., 1996). From the results of the study presented here, we conclude that feeding a DCAD diet that induces a mild fully compensated metabolic acidosis (blood pH and $[\text{HCO}_3^-]$ within

the reference range, urine pH approximately 7.2) does not affect the insulin response to an intravenous glucose load in dairy cows close to parturition, as a difference in by peak plasma [IRI], peak plasma [glucose], or I:G ratio between groups was not determined. Differences in insulin sensitivity between groups were assessed by comparing the baseline plasma [IRI], the reciprocal of baseline [IRI], the glucose/insulin ratio (G:I ratio), the QUICKI as well as the RQUICKI parameters that have been proposed as a surrogate indexes for insulin sensitivity (Katz et al., 2000; Perseghin et al., 2001; Holtenius and Holtenius, 2007; Muniyappa et al., 2008). A group effect was not observed for any of these indices. The differences in baseline plasma [glucose], [IRI], $1/\text{IRI}$, G:I ratio, QUICKI, and RQUICKI, as well as $C_{\text{max}} \text{IRI}$ obtained before and after calving indicate that large changes in insulin responsiveness and tissue sensitivity to insulin occur around parturition in dairy cattle, which is in agreement with the results of earlier studies (Hart et al., 1978; Giesecke et al., 1987; Sano et al., 1993). An unchanged glucose disappearance curve, despite marked changes in insulin responsiveness, suggests large redundant capacity of the homeostatic mechanisms regulating the glucose homeostasis to compensate for the reduced insulin dependent glucose uptake. In dry cows shortly before calving, glucose clearance from plasma is achieved through glucose uptake by insulin responsive organs, glucose uptake by organs unresponsive to insulin (mainly the fetus), and by renal glucose excretion (Bell, 1995). A change in insulin-dependent glucose uptake must affect renal glucose excretion and fetal glucose uptake by the same proportion because neither is an active process (Stacey et al., 1978). The similar but numerically lower amounts of glucose excreted in urine in group L suggest that renal glucose excretion and thus probably fetal glucose uptake are not increased, implying that insulin-dependent glucose uptake might not be different between groups.

An insulin-dependent decline in the plasma [Pi] as a result of an intracellular shift following an intravenous glucose load has been reported in several species, including cattle (MacKler et al., 1951; Rasmussen et al., 1988; Grünberg et al., 2006), an effect that is likely due to an insulin dependent upregulation of Na/Pi cotransporter gene expression (Li et al., 1996). Therefore, in the study presented here, we elected to determine the hypophosphatemic effect of insulin as a tool to identify possible differences in tissue responsiveness to insulin between groups. Neither the plasma [Pi], the decline in plasma [Pi] following dextrose infusion, the AUC_{PI} nor the amount of Pi excreted in urine was different between groups. These findings further imply that cows fed the low-DCAD diet were similarly sensitive to insulin as cows on the control diet. Differences in the AUC_{PI}

between before and after parturition are likely associated with the lower baseline plasma [Pi] after calving in both groups.

A potential concern of feeding acidogenic rations is that poor "palatability" of these supplements may affect DMI in late gestation and thereby exacerbate the negative energy balance that is present in all high-yielding cows during early lactation, although it should be noted that feeding acidogenic rations antepartum can lead to increased postpartum feed intake (Joyce et al., 1997; Goff and Horst, 1998). Feed intake in the study presented here was numerically (but not significantly) lower in cows fed the low DCAD ration in the 2 wk before as well as in the 2 wk after calving. Even though a group effect on antepartum feed intake was not evident, multivariable regression analyses revealed a significant negative association between antepartum intake and urinary NAE on feed intake. A negative effect of low-DCAD diets on feed intake antepartum and postpartum has been reported in many studies and attributed to either poor palatability or deregulation of acid-base homeostasis (Oetzel and Barmore, 1993; Joyce et al., 1997; Vagnoni and Oetzel, 1998; Hu and Murphy, 2004; Ramos-Nieves et al., 2009). The negative effect of a low DCAD on feed intake antepartum was estimated in a meta-analysis to be around 11% for a DCAD reduction of 30 mEq/100g (Charbonneau et al., 2006); however, Goff and Horst (1998) reported a positive effect on prepartum feed intake when dairy cows were fed a low DCAD ration during the last weeks of gestation. Positive effects of close-up diets low in DCAD on feed intake after calving have been reported in some (Joyce et al., 1997; Goff and Horst, 1998), but not all, studies (Moore et al., 2000; Ramos-Nieves et al., 2009). A positive effect of low-DCAD diets during the last weeks of gestation on feed intakes during early lactation may be due to improved Ca homeostasis and thus better motility of the gastrointestinal tract (Goff and Horst, 1998). Parameters reflecting energy balance, such as plasma NEFA or BHB concentration, did not reveal a significant group effect in our study. These results are in agreement with a recent study that was unable to identify negative effects on energy balance or liver triglyceride content in early lactating multiparous cows fed a low-DCAD ration during the close-up period (Ramos-Nieves et al., 2009). Deleterious effects of low-DCAD rations on the energy balance during early lactation have only been reported in heifers fed a close-up ration with low DCAD (-15 mEq/100 g, Moore et al., 2000).

Results of our study indicate that ingesting a low-DCAD ration before calving results in lower plasma albumin, globulin, and total protein concentrations af-

ter calving. A marked periparturient decline in plasma total protein concentration in dairy cows is associated with the active translocation of immunoglobulins into the mammary gland during colostrogenesis (Larson and Kendall, 1957; McLennan and Willough, 1973). The results of a 1957 study indicated that the periparturient decline in plasma total protein concentration was largely due to a decreased globulin fraction whereas changes in plasma [alb] were marginal (Larson and Kendall, 1957). The results of a subsequent study in 1974 that used radiolabeled albumin suggested that plasma [alb] decreases rapidly postpartum as a result of decreased albumin synthesis in the liver and an increased extracellular fluid volume (Little, 1974); decreased albumin synthesis in early lactation can result, in part, from hepatic lipidosis as indicated by the strong correlation between serum [alb] and liver fat percentage ($r = -0.80$, $P < 0.001$, $n = 54$; Sevinc et al., 2003). The results of our study indicated that plasma [alb] was negatively associated with the apparent volume of distribution for glucose, indicating that part of the decrease in plasma [alb] was due to plasma volume expansion. As expected, the periparturient decrease in plasma total protein concentration was largely driven by a decrease in the globulin fraction. The decrease in plasma [globulin] was present before calving which is consistent with increased uptake of immunoglobulin G₁ by the mammary gland, whereas the plasma [alb] decreased further after calving, when extracellular fluid volume increases in response to the demands of milk production (Larson and Kendall, 1957; McLennan and Willough, 1973) and liver fat percentage increases due to negative energy balance that is present in early lactation (Moore et al., 2000; Sevinc et al., 2003; Kessel et al., 2008).

An effect of ingesting a low-DCAD ration on plasma globulin concentrations in early-lactation dairy cows does not appear to have been reported previously. Chronic acidemia and metabolic acidosis decreases plasma protein concentration in humans and domestic animals by enhancing protein catabolism with a minimal increase in the rate of protein synthesis (Ballmer and Imoberdorf, 1995; Safránek et al., 2003). Nonetheless, we are hesitant to suggest that ingestion of a low-DCAD ration increases protein catabolism because studies in humans indicating that ingestion of an acidogenic diet perturbs protein metabolism and homeostasis report significant decreases in blood pH, BE, and bicarbonate concentration, suggesting that acidemia due to a partially uncompensated metabolic acidosis is needed to trigger this response (Boirie et al., 2000; Hannaford et al., 1982). Furthermore, acidemia-induced protein catabolism also has been reported to decrease plasma

albumin synthesis (Ballmer et al., 1995), whereas our findings suggest that the plasma globulin fraction, but not the albumin fraction, was decreased by low-DCAD diet. On the other hand, when considering plasma globulins as the proinflammatory protein fraction, a lower plasma globulin concentration after calving in cows fed a low-DCAD diet during the close-up period could be indicative of a lower occurrence of inflammatory phenomena during early lactation in this group (Bertoni et al., 2008). The approximately 16% decline in plasma globulin concentration from d 14 antepartum to 1 d postpartum was similar in both groups suggesting that translocation of IgG₁ into the mammary gland was similar for both groups but that the major difference was in the postparturient increase in plasma [glob]. The clinical relevance of this finding is not clear.

Despite standardized housing and feeding conditions, considerable interanimal variability was encountered for several parameters reflecting metabolic activity in these transition cows, including indices characterizing insulin responsiveness and sensitivity, urine pH and NAE, urine electrolyte excretion, and plasma NEFA and BHBA concentrations. Variability in the response to changing homeorhetic needs in dairy cows at the onset of lactation has been attributed to genetic factors (Kessel et al., 2008). These genetic factors are considered to strongly influence the individual metabolic activity potentially explaining the variability in the capacity to cope with metabolic stress in the transition period.

In conclusion, our results indicate that feeding a moderately acidogenic ration (DCAD, -9 mEq/100 g of DM) to late gestation Holstein-Friesian cows decreased urine pH to approximately 7.0 to 7.2, markedly increased Ca flux as assessed by increased urine Ca concentration, urine [Ca]/[creatinine], and 24-h Ca excretion, and increased plasma Ca concentration on the day after calving, but did not affect insulin responsiveness and sensitivity. The effects of this moderately acidogenic diet on P homeostasis were small and unlikely to be of clinical relevance. A negative association between daily feed intake and urine NAE before calving was consistent with findings in other studies that low DCAD diets decrease feed intake before calving. Whereas parameters characterizing the energy balance of transition cows did not reveal a significant effect of diet, a low-DCAD diet was associated with a lower plasma total protein and globulin concentration after calving. Further investigation of the hypoproteinemic and hypoglobulinemic effect reported here is warranted because low-DCAD rations used in practice frequently target a urine pH of approximately 6.0 to 6.5 (Constable et al., 2009) and are therefore much more acidogenic than the ration tested in the present study.

ACKNOWLEDGMENTS

The authors thank the staff of the Purdue Dairy Center, Dept. of Animal Sciences, at Purdue University and E. Wieck from the Clinical Discovery Laboratory, School of Veterinary Medicine at Purdue University, for their valuable technical assistance.

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