Dietary Cation-Anion Difference Effects on Performance and Acid-Base Status of Dairy Cows Postpartum¹

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ABSTRACT

Our objective was to examine the effect of dietary cation-anion difference (DCAD) on performance and acid-base status of cows postpartum. Sixteen Holstein and 8 Jersey multiparous cows were used immediately after calving to compare 2 DCAD [22 or 47 milliequivalents (Na + K – Cl – S)/100 g of dry matter (DM)] in a completely randomized design. The corn silage-based diets were formulated to contain 19.0% crude protein, 25.4% neutral detergent fiber, 15.0% acid detergent fiber, and 1.69 Mcal of net energy for lactation per kilogram (on a DM basis). An additional 2.3 kg of alfalfa hay was fed during the first 5 d postpartum, and then milk, blood, and urine samples were collected weekly for 6 wk. Repeated-measures (with an extra between-cow effect) mixed model analysis indicated that DCAD did not affect DM intake (18.2 and 18.3 kg/d), milk production (33.5 and 33.3 kg/d), milk composition (3.96 and 4.11% fat, 3.11 and 3.00% protein, and 8.95 and 8.83% solids-not-fat), jugular venous blood pH (7.395 and 7.400), HCO₃[–] concentration (27.3 and 27.6 mEq/L), or partial pressure of CO₂ (46.7 and 46.5 mmHg). Elevated coccygeal venous plasma branched-chain AA (431 and 558 μ M) and ratio of essential AA to total AA (0.390 and 0.434) in cows with DCAD of 22 vs. 47 mEq/100 g of DM indicated that N metabolism in the rumen was affected, probably resulting in more microbial protein flowing to the small intestine. Urinary pH tended to increase with DCAD (8.12 vs. 8.20). Higher net acid excretion in cows with DCAD of 22 vs. 47 mEq/100 g of DM (–24

and –41 mM:mM) suggested that net acid excretion was much more indicative of acid load than blood acid-base parameters in cows postpartum. Intake of DM and performance of cows postpartum were not improved when DCAD increased from 22 to 47 mEq/100 g of DM, likely because cows immediately after calving respond more variably to dietary treatments and that makes treatment effects difficult to detect.

Key words: dietary cation-anion difference, performance, acid-base status, dairy cow

INTRODUCTION

Tucker et al. (1988) were the first to evaluate DCAD in lactating dairy cows and reported that milk yield was 9% higher when a diet with DCAD of 20 vs. –10 mEq (Na + K – Cl)/100 g of DM was fed. Significant influences of DCAD on lactating cows were also found in subsequent studies (West et al., 1991, 1992; Delaquis and Block, 1995a,b). A recent meta-analysis of previous research (Hu and Murphy, 2004) indicated that DCAD altered acid-base status and affected performance of lactating dairy cows.

In transition from pregnancy to lactation, dairy cows require dramatic increases in nutrient intake to support milk production. An increased proportion of concentrate in the ration is a routine practice to help high-producing dairy cows meet their net energy requirements. Excess ingestion of feeds rich in readily available carbohydrates may result in a substantial acid load. Manipulating DCAD might benefit lactating dairy cows immediately after calving to about 50 d postpartum. However, there was only a little information available about DCAD effects on those lactating cows. Chan et al. (2005) reported that increasing DCAD from 20 to 50 mEq/100 g of DM had no effects on DMI and milk production in cows from 0 to 42 d postpartum. Further efforts need to be made to examine the effect of DCAD on milk performance, acid-base status, and N and mineral metabolism in cows postpartum.

Received August 7, 2006.

Accepted March 20, 2007.

¹Supported by Illinois Experiment Station NE-132 “Environmental and Economic Impacts of Nutrient Management on Dairy Forage Systems” and Church & Dwight Co. Inc., Arm & Hammer Animal Nutrition Group, Princeton, NJ.

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Table 1. Ingredients and nutrient composition in experimental diets (DM basis) in 2 breeds fed diets with 2 levels of DCAD

	DCAD ¹	
	22	47
Ingredient (%)		
Corn silage ²	50.0	50.0
Corn grain, cracked	18.5	17.0
Soybean meal, 48%	27.7	28.1
NaCl	0.34	0.34
Dicalcium phosphate	0.99	0.64
MgO	0.09	0.09
Limestone	1.52	1.06
NaHCO ₃	0.42	1.35
K ₂ CO ₃	0.23	1.21
Mineral and vitamin premix ³	0.19	0.19
Composition		
CP, %	19.4	19.9
NE _L , Mcal/kg	1.69	1.64
ADF, %	14.7	15.2
NDF, %	26.3	27.1
NFC ⁴ , %	49.5	46.3
Ca, %	1.23	1.00
P, %	0.59	0.56
Mg, %	0.28	0.28
K, %	1.28	1.85
Na, %	0.29	0.63
Cl, %	0.41	0.39
S, %	0.20	0.20
DCAD, mEq/100 g of DM	21.8	51.1

¹DCAD in milliequivalents of (Na + K – Cl – S)/100 g of DM.

²Contained 37.1% DM, 44.0% NDF, 25.0% ADF, and 6.0% CP on a DM basis.

³Contained 5.00% Mg, 10.00% S, 7.50% K, 2.00% Fe, 3.00% Zn, 3.00% Mn, 5,000.0 mg/kg Cu, 250.0 mg/kg I, 40.0 mg/kg Co, 150.0 mg/kg Se, 2,205,000 IU/kg vitamin A, 661,500 IU/kg vitamin D₃, and 22,050 IU/kg vitamin E.

⁴NFC = 100 – CP – fat – NDF – ash.

MATERIALS AND METHODS

Experimental Design and Animal Care

Twenty-four multiparous cows (16 Holsteins and 8 Jerseys) were divided into 2 groups (12 cows per group). Because not all cows could be obtained at once, cows entered the experiment as pairs based on breed, parity, and previous milk yield; members of each pair were assigned randomly to DCAD of either 22 or 47 mEq (Na + K – Cl – S)/100 g of DM diets.

The diets were composed of 50% concentrate mix of mainly cracked corn-soybean meal and 50% conventional corn silage on a DM basis. The DCAD was varied by using NaHCO₃ and K₂CO₃ in the concentrate mix (Table 1).

Cows were housed in tie stalls indoors except during milking and during the exercise period on a dirt lot between the a.m. milking and feeding. Feed offered was adjusted daily and 110% of consumption the previous day (as-fed basis) was provided at 1100 and 1630 h.

All cows were offered experimental diets immediately after calving to 47 DIM; an additional 2.3 kg/d of alfalfa hay was fed during the first 5 d postpartum. Water was available for ad libitum consumption. Cows were milked twice daily at approximately 0600 and 1500 h.

Sample Collection and Analysis

Drinking Water. Drinking water was collected in 1,000-mL containers weekly, the water samples were stored at –15°C until the end of the experiment, and then composited and pooled for mineral analysis (Dairy One Forage Laboratory, Ithaca, NY). Drinking water analysis indicated (per kg) 41.8 mg of Na, <0.1 mg of K, 12.0 mg of Cl, 0.0 mg of S, 17.1 mg of Ca, 0.1 mg of P, and 13.3 mg of Mg, which suggested that the mineral intake from drinking water had little effect on total mineral intake.

Feeds and Orts. Feed intake of each cow was recorded during the experimental period from 6 to 47 DIM; samples of feed and Orts were collected weekly. Orts were measured daily before the a.m. feeding and scored visually for DM content. Ort scores (integers from 1 to 4) were related to their actual DM content by drying all Orts samples in a forced-air oven at 55°C weekly (Shah et al., 2004). Weekly samples of corn silage, concentrate mix, and the TMR were stored at –15°C until the end of the experiment, and then composited and pooled for later analysis. Nutrient contents of corn silage, concentrate, and the TMR were analyzed by wet chemistry for DM, CP, ADF, NDF (Dairy One Forage Laboratory). Also, energy concentration was calculated (Dairy One Forage Laboratory). The nutrient composition presented in Table 1 was based on calculation from nutrient content analysis of corn silage and concentrate.

Urine. Urine was sampled weekly from 6 to 47 DIM. Cows were manually stimulated to urinate at 0900 h, and a sample of midstream urine was collected in 50-mL plastic containers. Urine pH was measured immediately, and 30 mL of urine was stored at –15°C for further analysis. Urine concentrations of Na⁺, K⁺, and Cl[–] were determined using an ion-selective electrode; urine Ca, urea N, and creatinine were measured spectrophotometrically. All these assays were performed on the Hitachi 917 analyzer (Roche, Indianapolis, IN) using Roche diagnostic reagents.

Urine titratable acidity (TA) and ammonium concentrations were determined by titration of urine samples with 0.1 N NaOH, which was standardized by potassium biphthalate (Chan, 1972). Net acid excretion (NAE) was the sum of urine TA measured and ammonium; the urine TA measured was actually the

amount of urinary TA minus HCO_3^- (Chan, 1972). Urinary mineral excretions (Na^+ , K^+ , Cl^- , and Ca) were expressed as minerals to creatinine concentration to overcome variations in urine volume among animals.

Blood. Blood was sampled weekly from 6 to 47 DIM. Immediately before the a.m. feeding, 5 mL of jugular venous blood was collected anaerobically with a plastic syringe containing lithium heparin, capped, placed on crushed ice, and analyzed for pH, partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), HCO_3^- , and base excess in a blood gas analyzer (Rapidlab 850 System, Bayer Diagnostics, Tarrytown, NY) within 2 h. Simultaneously, Na^+ , K^+ , Cl^- , and Ca^{2+} were determined using ion-selective electrode, and anion gap was calculated in the blood gas analyzer (Rapidlab 850 system, Bayer Diagnostics). Values of pH, pCO_2 , and pO_2 were corrected for rectal temperature. Coccygeal venous blood (there was slight chance for artery blood to be included in the blood sample, but venous blood is referred to herein) was also collected in Vacutainers (Becton Dickinson, Franklin Lakes, NJ) containing lithium heparin, placed on crushed ice, and immediately centrifuged at $1,500 \times g$ for 15 min. Plasma was then retrieved, transferred to 5-mL plastic tubes, and frozen at -15°C for further analysis.

Coccygeal venous plasma samples were prepared for AA determination; individual AA and ammonia were then separated by ion-exchange chromatography (Beckman model 6300 amino acid analyzer, Beckman Instruments Inc., Palo Alto, CA).

Coccygeal venous plasma samples were analyzed for Na^+ , K^+ , Cl^- , Ca, urea N, and creatinine using the same analytical methods as for the urine. Also, plasma glucose, BHBA, and NEFA were measured spectrophotometrically. All of these assays were performed on the Hitachi 917 analyzer (Roche) using Roche diagnostic reagents.

Milk Production and BW. Milk production was measured at 0600 and 1500 h daily. Milk samples were collected weekly from 6 to 47 DIM. Samples from consecutive p.m. and a.m. milkings were composited based on production and then analyzed for milk fat, true protein, lactose, SNF, SCC, and urea N by an infrared method using a Milkoscan System 4000 (Foss North American, Eden Prairie, MN; Dairy Lab Services, Dubuque, IA). Milk samples were collected from a.m. milking, refrigerated at 4°C , and measured for pH within 2 h. The BW was determined weekly from 6 to 47 DIM.

Statistical Analysis

Data on daily DMI and milk yield were reduced to weekly means for each cow. Jugular venous blood,

urine and milk pH were converted to free H^+ concentration ($[\text{H}^+]$, assuming an activity coefficient of 1) and subjected to statistical analysis (Murphy, 1982). The resulting mean $[\text{H}^+]$ could still be transformed for convenience and reported as pH. Therefore, each mean was presented as both $[\text{H}^+]$ and pH; because of the asymmetric standard error of pH resulted from transformation, the larger number was presented as the standard error of pH (Murphy, 1982).

Weekly data were analyzed using the MIXED procedure (SAS Institute, 2001) with a repeated-measures model. Cow was treated as a random variable, and breed (i.e., Holstein and Jersey) was included as an extra between-cow effect. The first-order autoregressive structure type was selected as the appropriate covariance structure based on the goodness-of-fit criteria (Littell et al., 1998). The model was

$$Y_{ijk} = \mu + B_i + W_j + T_k + (B \times W)_{ij} + (B \times T)_{ik} + (W \times T)_{jk} + (B \times W \times T)_{ijk} + e_{ijk}$$

where μ = overall mean; B_i = effect of breed i ($i = 1, 2$); W_j = effect of week j ($j = 1, 2, 3, 4, 5, 6$); T_k = effect of treatment k ($k = 1, 2$); $(B \times W)_{ij}$ = effect of interaction between breed i with week j ; $(B \times T)_{ik}$ = effect of interaction between breed i with treatment k ; $(W \times T)_{jk}$ = effect of interaction between week j with treatment k ; $(B \times W \times T)_{ijk}$ = effect of interaction among breed i , week j , and treatment k ; and e_{ijk} = error term.

Significance was defined as $P \leq 0.05$; whereas $0.05 < P \leq 0.10$ was considered to indicate a trend toward a significant effect.

RESULTS AND DISCUSSION

Diets

Ingredient and chemical composition of experimental diets are shown in Table 1. Corn silage was fed to dairy cows as the sole forage fiber source, and dietary ADF and NDF average contents of the 2 experimental diets were 15.0 and 26.7%, respectively. An analysis of TMR particle size by dry sieving (Murphy and Zhu, 1997) found that 13.4% was >6.3 mm, 20.8% >4.75 mm, 30.6% >3.35 mm, 38.5% >2.36 mm, 50.3% >1.7 mm, and 68.0% >1.18 mm; therefore, average particle size was 1.8 mm and the $\log_{10}$ standard deviation was 0.49.

Dietary CP concentrations were $<1\%$ higher than formulated (19%). A higher than expected Na content of the grain mix resulted in a higher DCAD of 51.1 than the formulated 47 mEq/100 g of DM. To balance the P contents or other nutrients in both experimental diets, dicalcium phosphate was added, resulting in

Table 2. Least squares means of DMI, milk yield, 4% FCM, and milk composition in 2 breeds fed diets with 2 levels of DCAD

Variable	DCAD ¹		Breed		SE	Effect (<i>P</i>)	
	22	47	Holstein	Jersey		DCAD	Breed
DMI, kg/d	18.2	18.3	19.9	16.7	2.2	NS ²	<0.01
DMI, kg/kg of BW ^{0.75}	0.163	0.164	0.349	0.372	0.019	NS	NS
BW, kg	554	540	636	458	27	NS	<0.01
Milk							
Yield, kg/d	33.5	33.3	38.2	28.6	3.0	NS	<0.01
4% FCM, kg/d	32.8	33.3	35.6	30.5	3.9	NS	0.01
Fat, %	3.96	4.11	3.58	4.49	0.65	NS	<0.01
Fat, kg/d	1.29	1.33	1.35	1.27	0.23	NS	NS
Protein, %	3.11	3.00	2.74	3.38	0.23	NS	<0.01
Protein, kg/d	1.03	0.98	1.04	0.96	0.14	NS	NS
Lactose, %	4.88	4.92	4.93	4.87	0.12	NS	NS
Lactose, kg/d	1.64	1.64	1.88	1.40	0.21	NS	<0.01
SNF, %	8.95	8.83	8.57	9.22	0.27	NS	<0.01
SNF, kg/d	2.99	2.91	3.26	2.64	0.34	NS	<0.01
Urea, mg of N/dL	21.8	23.6	21.8	23.5	4.3	NS	NS
SCC, ×1,000/mL	152	254	236	171	366	NS	NS

¹DCAD in milliequivalents of (Na + K – Cl – S)/100 g of DM.

²NS = *P* > 0.10.

higher dietary P contents (average of 0.58%) than required for dairy cows (NRC, 2001).

DMI and BW

Table 2 presents DMI and DMI per unit of metabolic body size for cows fed DCAD of 22 or 47 mEq/100 g of DM postpartum. As expected, both DMI (*P* < 0.01) and DMI per unit of metabolic body size (*P* < 0.01) increased with week of lactation. However, DMI expressed as kilograms per day or kilograms per unit of metabolic body size were not affected by treatment. No treatment effect on BW was observed.

Milk Yield and Composition

Milk yield and 4% FCM were similar for postpartum cows fed diets with DCAD of 22 or 47 mEq/100 g of DM (Table 2). Likewise, milk fat, true protein, lactose, and SNF percentages and yields; urea N; and SCC were similar between treatments. These results suggested that increasing DCAD from 22 to 47 mEq/100 g of DM did not affect performance. West et al. (1992) reported similar results; milk yield and milk fat and protein percentage did not differ in heat-stressed cows fed diets with varying DCAD of 12 to 46 mEq (Na + K – Cl)/100 g of DM. Tucker et al. (1988) found that cows yielded 9% more milk when fed DCAD of +20 vs. –10 mEq (Na + K – Cl)/100 g of DM, but milk fat concentration and fat yield were unaffected. Milk fat concentration and fat yield, in contrast, increased with increasing DCAD of 23 to 88 mEq/100 g of DM in pasture-based dairy cows in early lactation (Roche et

al., 2005). It was shown that different ranges of DCAD were used in those different experiments; the range of DCAD could impact experimental results.

Milk pH and Jugular Venous Blood Acid-Base Status

A change of DCAD from 22 to 47 mEq/100 g of DM did not affect milk pH, jugular venous blood pH, HCO₃[–], pCO₂, pO₂, or base excess (Table 3). However, increasing DCAD would be expected to improve acid-base status in lactating cows, indicated by increased blood pH and HCO₃[–] (Hu and Murphy, 2004). Anion gap represents the difference between the concentration of unmeasured anions and the concentration of unmeasured cations in serum and can be expressed as a concentration of K⁺ + Na⁺ – Cl[–] – HCO₃[–] (Constable, 1999, 2000). Its usefulness in evaluating acid-base status in lactating dairy cows is unclear. However, jugular venous blood anion gap tended to have a higher concentration for the diet with DCAD of 47 vs. 22 mEq/100 g of DM (*P* = 0.06); anion gap differed (*P* = 0.04) between the Holstein and Jersey cows (Table 3). It implied that the diet might be less acidogenic with a DCAD of 47 vs. 22 mEq/100 g of DM, and that less blood acidity might exist in Jersey vs. Holstein cows postpartum. Minerals (Na, K, Cl, and Ca) in both whole blood collected from the jugular vein and blood plasma collected from the coccygeal vein were determined in the present experiment. There was no treatment effect on mineral concentrations of the 2 blood samples except for jugular venous blood Cl[–]; jugular venous blood Cl[–] concentration tended to decrease (*P* =

Table 3. Least squares means of milk pH, jugular venous blood acid-base measures and mineral concentrations in 2 breeds fed diets with 2 levels of DCAD

Variable	DCAD ¹		Breed		SE	Effect (<i>P</i>)	
	22	47	Holstein	Jersey		DCAD	Breed
Milk							
H ⁺ concentration, nM	230	225	217	238	43.3	NS ²	NS
pH	6.64	6.65	6.66	6.62	0.09		
Blood							
H ⁺ concentration, nM	40.3	39.8	39.7	40.4	1.94	NS	NS
pH	7.395	7.400	7.401	7.394	0.022		
pCO ₂ , mmHg	46.7	46.5	46.9	46.4	3.9	NS	NS
pO ₂ , mmHg	25.9	26.1	25.5	26.5	2.7	NS	NS
HCO ₃ ⁻ , mEq/L	27.3	27.6	27.8	27.2	2.8	NS	NS
Base excess, mM	2.53	2.97	3.16	2.34	2.58	NS	NS
Na ⁺ , mEq/L	132.9	132.5	132.7	132.6	1.7	NS	NS
K ⁺ , mEq/L	3.99	3.93	4.01	3.91	0.27	NS	NS
Cl ⁻ , mEq/L	98.8	97.6	98.7	97.7	2.0	0.06	NS
Ca ²⁺ , mg/dL	4.62	4.68	4.73	4.57	0.39	NS	0.09
BCAD, ³ mEq/L	38.1	38.9	38.1	39.0	2.2	NS	NS
Anion gap, ⁴ mEq/L	10.4	11.4	10.3	11.4	2.0	0.06	0.04

¹DCAD in milliequivalents of (Na + K - Cl - S)/100 g of DM.²NS = *P* > 0.10.³BCAD = jugular venous blood cation-anion difference (Na + K - Cl).⁴Anion gap = jugular venous blood Na⁺ + K⁺ - Cl⁻ - HCO₃⁻.

0.06) as DCAD increased from 22 to 47 mEq/100 g of DM. In addition, a tendency of jugular venous blood Ca²⁺ to be lower (*P* = 0.09) was observed in Jersey vs. Holstein cows.

The lack of responses in milk performance and blood acid-base status to DCAD might be attributed to the stage of lactation. The transition from the pregnant, nonlactating state to the nonpregnant, lactating state imposes enormous stress on dairy cows, greatly increasing susceptibility to metabolic disorders (Goff and Horst, 1997). The tremendous physiological challenges to the homeostatic mechanisms of the cows during this stage contribute to the large variation in milk yield, DMI, or other responses to dietary treatments (Drackley, 1999). A calculation was performed to estimate how large a difference of treatment effects would likely be detectable in a similar future experiment (Ott and Longnecker, 2001). Given 12 dairy cows in each treatment group and standard errors of milk yield (3.0 kg/d) and DMI (2.2 kg/d; Table 2), if a 95% confidence level and 80% statistical power were specified, the detectable differences of milk yield and DMI between the 2 treatments were 3.4 and 2.5 kg/d, respectively.

Coccygeal Venous Plasma Metabolites

Effects of experiment diets on coccygeal venous plasma metabolites are presented in Table 4. There were no effects of treatments or interactions involving treatment on coccygeal venous plasma concentrations

of urea N, ammonia, creatinine, glucose, BHBA, and NEFA, except for an interaction between treatment and breed for creatinine (*P* = 0.03). Creatinine concentrations of Holstein and Jersey cows were 0.72 and 0.55 mg/dL for DCAD of 22 mEq/100 g of DM, and 0.69 and 0.65 mg/dL for DCAD of 47 mEq/100 g of DM, respectively. Interestingly, higher NEFA (*P* = 0.01), and lower glucose (*P* = 0.01) and creatinine (*P* < 0.01) in coccygeal venous plasma were also observed in Jersey vs. Holstein cows.

Coccygeal Venous Plasma AA

Higher concentrations of His (*P* = 0.02), Ile (*P* = 0.01), Leu (*P* < 0.01), Lys (*P* = 0.02), Phe (*P* = 0.02), Val (*P* = 0.01), and total branched-chain AA (BCAA; *P* < 0.01) were observed for cows with DCAD of 47 vs. 22 mEq/100 g of DM (Table 5). For the nonessential AA (NEAA), only Glu was affected by the experimental diets (*P* < 0.01). There was no effect of interactions involving treatments on coccygeal venous plasma AA, except for an interaction of treatment by breed on Glu (*P* < 0.01). Coccygeal venous plasma Glu concentrations of Holstein and Jersey cows were 58.9 and 52.8 μM for DCAD of 22 mEq/100 g of DM, and 59.4 and 66.8 μM for DCAD of 47 mEq/100 g of DM, respectively. Because of higher essential AA (EAA; *P* < 0.01) in cows with DCAD of 47 vs. 22 mEq/100 g of DM, greater ratios of EAA to NEAA (*P* = 0.01) and of EAA to total AA (TAA; *P* = 0.01) were observed in cows with DCAD of 47 vs. 22 mEq/100 g of DM.

Table 4. Least squares means of coccygeal venous plasma metabolites and mineral concentrations in 2 breeds fed diets with 2 levels of DCAD

Variable	DCAD ¹		Breed		SE	Effect (<i>P</i>)	
	22	47	Holstein	Jersey		DCAD	Breed
Urea N, mg/dL	22.2	22.5	21.1	23.6	3.2	NS ²	0.09
Ammonia, mM	0.545	0.549	0.548	0.545	0.073	NS	NS
Creatinine, mg/dL	0.64	0.67	0.71	0.60	0.08	NS	<0.01
Glucose, mg/dL	56.5	54.4	57.6	53.2	5.3	NS	0.01
BHBA, mM	0.64	0.66	0.67	0.63	0.43	NS	NS
NEFA, mEq/L	0.55	0.56	0.39	0.73	0.33	NS	0.01
Na ⁺ , mEq/L	138.8	138.2	138.6	138.4	2.8	NS	NS
K ⁺ , mEq/L	4.14	4.18	4.23	4.09	0.26	NS	NS
Cl ⁻ , mEq/L	98.5	97.4	98.5	97.4	2.3	NS	NS
Ca, mg/dL	9.49	9.59	9.46	9.62	0.65	NS	NS
PCAD, ³ mEq/L	44.2	44.8	44.1	44.9	2.4	NS	NS

¹DCAD in milliequivalents of (Na + K - Cl - S)/100 g of DM.²NS = *P* > 0.10.³PCAD = coccygeal venous plasma cation-anion difference (Na + K - Cl).

Protein flowing to the small intestine depends on the amount of dietary protein escaping ruminal degradation, microbial protein synthesis, and the abomasal

emptying rate. The BCAA, relative to other AA, are less degraded by the liver and serve as indicators of AA supply to the small intestine in dairy cows (Lobley,

Table 5. Least squares mean concentrations of AA in coccygeal venous plasma (μmol/L) in 2 breeds fed diets with 2 levels of DCAD

Variable	DCAD ¹		Breed		SE	Effect (<i>P</i>)	
	22	47	Holstein	Jersey		DCAD	Breed
Essential AA (EAA)							
Arg	76	82	83	75	16	NS ²	0.05
His	44.3	47.9	42.1	50.0	6.3	0.02	<0.01
Ile	99	127	115	110	31	0.01	NS
Leu	118	156	137	138	33	<0.01	NS
Lys	84	97	87	93	18	0.02	NS
Met	16.9	17.5	18.0	16.3	4.3	NS	NS
Phe	42	48	46	44	8	0.02	NS
Thr	87	95	96	87	17	NS	NS
Trp	48	50	45	52	20	NS	NS
Val	214	276	240	250	65	0.01	NS
Nonessential AA (NEAA)							
Ala	201	214	207	207	32	NS	NS
Asp	18.5	21.2	18.8	20.9	3.2	NS	NS
Asn	101	106	117	91	36	NS	NS
Glu	56	63	59	60	7	<0.01	NS
Gln	269	243	296	216	70	NS	0.01
Gly	432	415	409	438	86	NS	NS
Pro	75	83	77	81	14	NS	NS
Ser	113	109	113	109	24	NS	NS
Tyr	38	41	42	36	10	NS	0.06
Other AA							
Citrulline	101	105	103	102	21	NS	NS
Hydroxyproline	25	24	27	21	10	NS	0.02
Ornithine	47	50	50	48	10	NS	NS
Branched-chain AA	431	558	492	497	122	<0.01	NS
EAA	829	995	908	916	164	<0.01	NS
NEAA	1,303	1,292	1,339	1,257	175	NS	NS
Total AA (EAA + NEAA)	2,131	2,286	2,245	2,172	287	0.06	NS
Urea cycle AA	225	236	236	225	35	NS	NS
EAA:NEAA	0.650	0.776	0.687	0.738	0.122	0.01	NS
EAA:total AA	0.390	0.434	0.402	0.421	0.041	0.01	NS

¹DCAD in milliequivalents of (Na + K - Cl - S)/100 g of DM.²NS = *P* > 0.10.

Table 6. Least squares means of urine pH and urine component concentrations in 2 breeds fed diets with 2 levels of DCAD

Variable	DCAD ¹		Breed		SE	Effect (<i>P</i>)	
	22	47	Holstein	Jersey		DCAD	Breed
H ⁺ concentration, nM	7.66	6.25	6.88	7.03	2.30	0.08	NS ²
pH	8.12	8.20	8.16	8.15	0.18		
Creatinine, mg/dL	58	44	57	45	17	0.01	0.02
TA, ³ mM	-116	-158	-135	-138	41	0.02	NS
Ammonium, mM	9.5	8.7	8.2	10.0	4.4	NS	NS
NAE, ⁴ mM	-107	-149	-127	-128	41	0.01	NS
TA:creatinine, mM:mM	-26	-43	-31	-38	13	<0.01	0.06
Ammonium:creatinine, mM:mM	1.99	2.17	1.68	2.48	0.92	NS	<0.01
NAE:creatinine, mM:mM	-24	-41	-29	-35	13	<0.01	0.10
Urea N:creatinine, mg:mg	16.4	17.3	14.5	19.2	2.7	NS	<0.01
Na ⁺ :creatinine, mg:mg	2.43	3.76	2.85	3.34	1.76	<0.01	NS
K ⁺ :creatinine, mg:mg	10.22	14.18	10.39	14.00	3.06	<0.01	0.01
Cl ⁻ :creatinine, mg:mg	1.56	1.78	1.28	2.09	0.94	NS	<0.01
Ca:creatinine, mg:mg	0.39	0.43	0.26	0.56	0.29	NS	<0.01

¹DCAD in milliequivalents of (Na + K - Cl - S)/100 g of DM.

²NS = *P* > 0.10.

³TA = titratable acidity; the amount of NaOH required to titrate HCl acidified and boiled urine to pH 7.4.

⁴NAE = net acid excretion; measured TA plus ammonium.

1992; Dhiman and Satter, 1997). In the present experiment, DMI was not affected by diet treatment; coccygeal venous plasma concentrations of Ile, Leu, and Val, and of BCAA (Ile + Leu + Val), plasma ratios of EAA to NEAA and of EAA to TAA were higher in the diet with a DCAD of 47 vs. 22 mEq/100 g of DM. Therefore, more protein apparently reached the small intestine for absorption with DCAD of 47 vs. 22 mEq/100 g of DM. Because microbial protein synthesized in the rumen supplies the majority of AA flowing to the small intestine of dairy cows (Bach et al., 2005), elevated coccygeal venous plasma BCAA and EAA concentrations with DCAD of 47 vs. 22 mEq/100 g of DM probably resulted from increased microbial protein synthesis.

Addition of buffers such as NaHCO₃ increases DCAD. The DCAD may have similar effects on ruminal N metabolism as buffers. Buffers are expected to increase protein solubility in the rumen by raising ruminal pH and, consequently, to increase protein degradability (Trenkle, 1979). Okeke et al. (1983) reported an increased rate of N disappearance of soybean meal from nylon bags in the rumen of steers supplemented with 2.5 or 5% NaHCO₃. However, increased ruminal pH and dilution rate by buffer addition results in higher microbial growth rates, which could offset increased protein degradability. Mees et al. (1985) observed increased bacterial N flow at the duodenum and efficiency of bacteria protein synthesis in sheep with addition of NaHCO₃ to the diet. Further research is warranted to elucidate the role of DCAD in

manipulating ruminal N metabolism and potentially increasing protein flow to the small intestine.

Urine pH, TA, and Minerals

Because excretion of creatinine is relatively constant (De Groot and Aafjes, 1960; Albin and Clanton, 1966; Asai et al., 2005), urine creatinine concentration was used as an index to estimate excretion of metabolites and minerals in urine. Urine creatinine was affected by DCAD (*P* = 0.01); higher creatinine concentration in cows with DCAD of 22 mEq/100 g of DM (Table 6) suggested that more concentrated urine was excreted and, consequently, total daily urine volumes were less than those for cows with DCAD of 47 mEq/100 g of DM.

Urine pH tended to be higher (*P* = 0.08, based on [H⁺]) for cows with DCAD of 47 vs. 22 mEq/100 g of DM (Table 6). Urine pH is very sensitive to the supplementation of acidogenic salts in prepartum cows (Vagnoni and Oetzel, 1998; Charbonneau et al., 2006), but changing DCAD from 22 to 47 mEq/100 g of DM had a relatively small effect on urine pH. The normal pH of bovine urine, like that of all herbivores, is greater than 8 (Oetzel, 2002). Therefore, cows fed with DCAD of 22 mEq/100 g of DM or higher maintained a normal urine pH (i.e., >8.0).

Most excreted urine H⁺ are associated with buffers or ammonia, in addition to free H⁺ excreted in the urine. Decreased TA:creatinine (*P* < 0.01) and unchanged ammonium:creatinine was noted for cows

with DCAD of 47 vs. 22 mEq/100 g of DM. Consequently, NAE:creatinine decreased ($P < 0.01$) in cows with DCAD of 47 vs. 22 mEq/100 g of DM. The NAE result indicated that acid-base status differed for cows with DCAD of 22 vs. 47 mEq/100 g of DM and confirmed that urinary NAE could be a much more sensitive indicator of metabolic acid load in dairy cows than blood acid-base parameters (Erdman, 1988).

The DCAD was manipulated by NaHCO_3 and K_2CO_3 addition. Addition of NaHCO_3 and K_2CO_3 would increase urinary Na and K excretion. In the present experiment (Table 6), higher Na^+ excretion, as Na^+ :creatinine ($P < 0.01$) and higher K^+ excretion, as K^+ :creatinine ($P < 0.01$) were observed in cows with DCAD of 47 vs. 22 mEq/100 g of DM, reflecting diet contents (Table 1). Urinary mineral excretions were much more responsive than plasma mineral concentrations to dietary mineral contents. In addition, urinary Ca excretion, as Ca:creatinine, did not differ with DCAD of 22 vs. 47 mEq/100 g of DM, but differed ($P < 0.01$) between the Holstein and Jersey cows (Table 6). Jersey cows are more susceptible to parturient paresis, likely because of their high milk production in relation to their body size. Nonetheless, tending to have lower coccygeal blood plasma Ca^{2+} , together with higher Ca excretion in Jersey vs. Holstein cows postpartum as discussed above, might have implications in susceptibility of parturient paresis.

CONCLUSIONS

The DMI and performance of dairy cows immediately postpartum were not improved when DCAD increased from 22 to 47 mEq/100 g of DM. Cows at this stage of lactation respond more variably to dietary treatments, making treatment effects difficult to detect. Jugular venous blood pH and HCO_3^- remained similar, whereas blood Cl^- concentration tended to decrease as DCAD increased from 22 to 47 mEq/100 g of DM. Higher NAE with DCAD of 22 vs. 47 mEq/100 g of DM suggested that NAE was a much more sensitive indicator of acid load than blood acid-base parameters in cows postpartum. Elevated coccygeal venous plasma BCAA and ratio of EAA to TAA in cows with DCAD of 47 vs. 22 mEq/100 g of DM indicated that N metabolism in the rumen was affected, probably resulting in more microbial protein flowing to the small intestine.

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