Effects of Dietary Cation-Anion Difference on Intake, Milk Yield, and Blood Components of the Early Lactation Cow

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ABSTRACT

Early lactation Holsteins cows (15 primiparous and 18 multiparous) were offered rations with dietary cation-anion difference, calculated as $\text{mEq (Na + K - Cl - S)/100 g}$ of feed dry matter (DCAD:S), of 20, 35, or 50 mEq from d 0 (calving) to 42 d postpartum (August 20, 2000 to January 9, 2001) to determine the effects of increasing DCAD:S on dry matter intake (DMI), milk yield, and blood metabolites. For DCAD:S of 20, 35, and 50, DMI was 3.30, 3.38, 2.96 kg/100 kg of body weight (BW); milk yield was 25.5, 24.2, and 22.4 kg/d, respectively. No differences were observed for concentration or yield of milk fat or milk protein. Serum Ca, P, Mg, Na, K, Cl, cation-anion difference, insulin, and glucose did not differ with DCAD. Serum HCO_3^- was 26.07, 25.88, and 27.64 mEq/L for 20, 35, and 50 DCAD:S. Serum Ca, Mg, Na, and K concentrations were greater for primiparous cows (9.52 mg/dL, 2.35 mg/dL, 140.03 mEq/L, 4.66 mEq/L, respectively) than for multiparous cows (9.27 mg/dL, 2.12 mg/dL, 137.63 mEq/L, 4.46 mEq/L, respectively). A DCAD:S between 23 and 33 mEq/100 g of dry matter (DM) appears to be adequate during cool weather for the milk yield that occurred in the present study based on DMI (kg/100 kg of BW), whereas DCAD:S of 50 mEq/100 g of DM may be excessive and could be too alkaline or unpalatable, resulting in decreased DMI (kg/100 kg of BW).

(Key words: dietary cation-anion difference, lactation, dry matter intake, milk yield)

Abbreviation key: CAD = cation-anion difference, DCAD = dietary cation-anion difference, mEq (Na + K - Cl) per 100 g of DM, **DCAD:S** = dietary cation-anion difference, $\text{mEq (Na + K - Cl - S)}$ per 100 g of DM.

INTRODUCTION

Sodium, potassium, and chloride play important roles in maintaining osmotic pressure and acid-base homeo-

stasis as well as in enzyme and nerve function. Dietary cation-anion difference may be a superior measure of the effects of electrolytes than the concentration of individual electrolytes provided there are no deficiencies or toxicities present. Dietary cation-anion difference has been defined by several equations, including $\text{mEq (Na + K - Cl - S)}$ per 100 g of DM (**DCAD:S**) and mEq (Na + K - Cl) per 100 g of DM (**DCAD**). The DCAD for lactating cows was determined to be more important than individual ingredients (Delaquis and Block, 1995b), cation source (West et al., 1992), or individual electrolyte concentrations (Tucker and Hogue, 1990); and increasing DCAD improved milk yield (Tucker et al., 1988a; Block, 1994). Early lactation cows are often in negative energy balance (Bauman and Currie, 1980) and may be more susceptible to metabolic acidosis because of the high concentrate diets fed to meet nutrient needs for lactation. Heat stress, which is often accompanied by a respiratory alkalosis, may also cause a slight metabolic acidosis as a compensatory response (Schneider et al., 1988).

Higher dietary Na and K concentrations increase the alkalinity of body fluids. The effect of DCAD is mediated through a change in acid-base chemistry (Block, 1984) and increasing DCAD negates a physiologic acidosis by raising buffering capacity of the blood, reflected by increased blood pH and blood bicarbonate (HCO_3^-) concentration. Blood pH and HCO_3^- concentration increased linearly as DCAD:S increased from -10 to 20 $\text{mEq (Na + K - Cl - S)/100 g}$ of DM, whereas DMI and milk yield improved by 11 and 9%, respectively, for DCAD of 20 vs. -10 (Tucker et al., 1988a). Increasing DMI paralleled a linear increase in blood pH for cows fed rations with DCAD ranging from 12 to 46 mEq (Na + K - Cl)/100 g of DM (West et al., 1992). Blood HCO_3^- increased from 19 to 28 mEq/L with DCAD:S ranging from 18 to 52 mEq (Na + K - Cl - S)/100 g of DM, and DMI increased from 20.5 to 24.9 kg/d (E. Block, Arm and Hammer Animal Nutrition, personal communication).

Cows exposed to hot weather also benefit from greater DCAD (Escobosa et al., 1984; West et al., 1991, 1992). Blood HCO_3^- was maximized with DCAD of 38 mEq (Na + K - Cl)/100 g of DM, whereas DCAD ranging from 17 to 38 and 25 to 40 mEq/100 g of DM maximized

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DMI and milk yield, respectively (Sanchez et al., 1994). Intake improved quadratically and milk yield improved linearly as DCAD increased from -12 to 31 mEq (West et al., 1991). These 2 studies implied that a higher DCAD may be required to support milk yield than for DMI. Cows in different stages of lactation may benefit from differing DCAD (Delaquis and Block, 1995a). Blood pH and HCO_3^- concentration became more alkaline as the cow progressed from early to late lactation (Erdman et al., 1982).

The optimal DCAD for prepartum dairy cows is negative (in the range of -5 to -10 mEq/100 g of DM), is relatively well defined, reduces the incidence of milk fever; and its effectiveness can be monitored via urine pH (Horst et al., 1997). A positive DCAD improved ADG in poultry (Mongin, 1981; Hulan et al., 1987) and swine (Patience et al., 1987). When numerous lactation studies were summarized to determine the response to DCAD in terms of DMI and milk yield definite trends were discovered, but the range within which a desirable response was elicited was relatively broad (Hu and Murphy, 2004; Sanchez et al., 1994). Thus, the optimal DCAD for lactating dairy cows has not been well defined, responses may differ depending on climatic conditions (cool or hot weather), and there are few data examining the response of dairy cows to DCAD immediately postpartum. The objectives of this trial were to determine the effects of increasing DCAD:S on DMI, milk yield, milk composition, serum electrolytes, and acid-base chemistry, and thus, to elucidate the optimal DCAD:S for the early lactation cow during moderately cool weather.

MATERIALS AND METHODS

Cows and General Management

Thirty-three Holstein cows (15 primiparous and 18 multiparous) entered the study at parturition. The study began on August 20, 2000, and ended on January 9, 2001. Cows were in the study from calving through 42 d postpartum. Cows were blocked into groups of 3 according to their 305-d mature equivalent milk for the previous lactation (multiparous cows) or estimated transmitting ability (primiparous cows). Cows were housed and fed in a barn with individual free stalls, a high metal roof, and open ridge vent, with fans and a high pressure misting system for cow cooling. Cows were trained to use electronic gate feeders (American Calan, Northwood, NH) before calving. Cows within blocks were assigned randomly to 1 of 3 treatments: DCAD:S of 20, 35, or 50 mEq ($\text{Na} + \text{K} - \text{Cl} - \text{S}$)/100 g of DM.

The DCAD:S was formulated using sodium sesquicarbonate and potassium carbonate (K-Minus, Church and

Dwight Co., Inc., Princeton, NJ). Composition of experimental diets is given in Table 1. Cows were fed a TMR once daily (1300 h) to provide approximately 10%orts for ad libitum consumption. Orts were removed daily, weighed, and daily feed intake was calculated. Ingredient proportions were adjusted for DM content each week. Cows were milked daily at approximately 0400 and 1500 h. Milk yield was recorded at each milking from day of calving through 42 d postpartum. Immediately following calving, milk was manually weighed in a bucket and yield was recorded. After colostrum cleared, milk yield was weighed by electronic meter (Alpro, Alfa Laval Agri, St. Louis, MO). Health-related incidents were recorded and cows were treated according to symptoms.

Sampling

Dietary ingredients and TMR were sampled weekly, dried in a forced-air oven at 60°C for 72 h, and DM content of TMR was used to calculate DMI for the week. Dried samples were ground to pass through a 6-mm screen using a Wiley mill (Arthur Thomas, Philadelphia, PA), labeled, and stored in sealed plastic bags. To improve grinding, whole cottonseed samples were frozen before and after grinding. Samples were composited for 3-wk intervals, ground to pass through a 1-mm screen using a Wiley mill, and stored for later analysis.

Cows were weighed once each week for the duration of the study. Weighing was conducted immediately following the p.m. milking and before cows had access to feed and water. Milk samples were collected each week from 2 consecutive milkings. Blood was collected in evacuated tubes via jugular venipuncture at 1400 h during wk 2 and 6 postpartum and placed on ice immediately following collection. Serum was harvested from blood samples and frozen for later determination of insulin and glucose.

Analyses

The DM content of feed ingredients and TMR was determined according to AOAC (1984). Feed N and S were determined (Leco Corporation, St. Joseph, MI) and CP was calculated as percentage $\text{N} \times 6.25$. Determination of ADF was by AOAC (1990) and NDF was by the method of Van Soest et al. (1991). The concentrations of Na, K, Ca, and Mg were measured by atomic absorption spectrophotometry (model 3030, Perkin-Elmer, Norwalk, CT) following acid digestion (AOAC, 1984). Phosphorus was determined colorimetrically using a Beckman DU-60 spectrophotometer (Beckman Instruments Inc., Fullerton, CA; AOAC, 1984). Chloride was extracted with a combination of acetic acid and nitric

Table 1. Ingredients and composition of experimental diets.

Item	DCAD:S, mEq/100 g of DM		
	20	35	50
Ingredient (% of DM)			
Corn silage	37.3	36.3	35.1
Alfalfa hay	10.6	10.6	10.6
Whole cottonseed	9.4	9.4	9.4
Steam-flaked corn	22.9	22.8	22.9
Base mix ¹	15.8	15.9	15.9
Sodium sesquicarbonate ²	0.82	1.18	1.58
Potassium carbonate ²	0.31	0.99	1.68
Magnesium sulfate ²	0.57	0.52	0.52
Soybean meal (48% CP) ²	8.7	8.7	8.7
Nutrients (dry basis)			
CP, %	16.7 ± 0.3	16.8 ± 0.7	16.2 ± 0.3
Undegradable protein, ³ % of CP	38.6	38.7	38.7
NE _L , ³ Mcal/kg	1.8	1.7	1.7
Ether extract, %	5.0 ± 0.1	4.3 ± 0.6	4.0 ± 0.4
ADF, %	20.7 ± 0.7	20.9 ± 0.5	21.1 ± 0.7
NDF, %	33.9 ± 0.6	33.0 ± 0.6	34.5 ± 0.8
NFC, % ³	40.9	40.3	39.8
Ca, %	1.01 ± 0.01	1.00 ± 0.04	1.02 ± 0.03
P, %	0.36 ± 0.02	0.36 ± 0.01	0.35 ± 0.01
Mg, %	0.39 ± 0.06	0.37 ± 0.01	0.37 ± 0.02
K, %	1.29 ± 0.02	1.58 ± 0.06	2.14 ± 0.11
Na, %	0.45 ± 0.01	0.49 ± 0.01	0.63 ± 0.04
Cl, %	0.30 ± 0.02	0.30 ± 0.05	0.29 ± 0.08
S, %	0.33 ± 0.02	0.31 ± 0.01	0.29 ± 0.02
DCAD:S, ⁴ mEq/100 g	22.72 ± 0.62	33.00 ± 2.02	54.64 ± 3.90

¹Contained 46.1% CP, 42.0% undegradable CP, 0.8 Mcal/kg NE_L, 11.9% ADF, 16.9% NDF, 19.1% NFC, 3.75% Ca, 1.51% P, 1.22% K, 0.60% Na, 0.91% Cl, 0.49% S, 9696 IU/kg of vitamin A, 3878 IU/kg of vitamin D, and 48.48 IU/kg of vitamin E.

²These ingredients were fed as a blended premix.

³Calculated using NRC (2001) values.

⁴Dietary cation-anion difference (DCAD:S) in mEq (Na + K - Cl - S) per 100 g of DM.

acid and was measured using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) by the method of Cotlove et al. (1958). Ration and ingredient fat content was determined by ether extraction (Soxtec System HT6, Tecator, Hoganas, Sweden; AOAC, 1984). Milk samples were analyzed (Southeast DHIA Laboratory, McDonough, GA) for fat and protein concentration by infrared analysis (Multispec; Foss Electric, Hillerød, Denmark). Blood serum was analyzed at the University of Georgia Veterinary Diagnostic Laboratory in Tifton for Na, K, Cl, Ca, P, Mg, HCO₃⁻ (Roche-Hitachi 912, Hoffmann-LaRoche Inc., Indianapolis, IN), and cation-anion difference (**CAD**) was calculated using the equation mEq (Na + K - Cl)/mL. Serum insulin was determined by radioimmunoassay as described by Bunting et al. (1994). Serum glucose was determined spectrophotometrically using a commercial kit (Tech. Bull. 315, Sigma Chemical, St. Louis, MO).

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2001). Analysis of variance was

conducted for a replicated randomized complete block design with 3 treatments. The model was:

$$Y_{ijkl} = \mu + \text{trt}_i + \text{wk}_j + \text{par}_k + \text{blk}_l + (\text{trt}_i \times \text{wk}_j) + (\text{wk}_j \times \text{par}_k) + (\text{par}_k \times \text{blk}_l) + (\text{trt}_i \times \text{par}_k) + (\text{trt}_i \times \text{blk}_l) + (\text{wk}_j \times \text{blk}_l) + (\text{trt}_i \times \text{wk}_j \times \text{par}_k) + (\text{wk}_j \times \text{par}_k \times \text{blk}_l) + (\text{trt}_i \times \text{par}_k \times \text{blk}_l) + \varepsilon_{ijkl}$$

where μ = the mean intercept, trt_i = the effect due to treatment i , wk_j = the effect due to wk j , par_k = the effect due to parity k , blk_l = the effect due to block l , and ε = error associated with each Y_{ijkl} .

Cow within treatment was included as a random effect and week was included as a repeated variable. Significance was declared at $P < 0.05$ unless stated otherwise. Treatment effects were compared using the multiple comparisons approach of Tukey (SAS Institute, 2001). Regression analyses were conducted with the Proc REG procedure, whereas correlation coefficients were obtained from the Proc CORR procedure of SAS (2001).

Table 2. Least square means of production measures for early lactation cows offered DCAD:S of 20, 35, or 50 mEq (Na + K – Cl – S) per 100 g of DM.

Variable	DCAD:S, ¹ mEq/100 g DM			SEM	Parity ²		SEM	<i>P</i> ³			
	20	35	50		1	>1		Trt	Par	Trt × Par	Trt × Wk
DMI, kg/d	16.4	17.1	15.3	0.8	15.6	17.0	0.7	NS	NS	*	NS
DMI, kg/100 kg BW	3.30 ^a	3.38 ^a	2.96 ^b	0.13	3.48	2.94	0.13	*	**	*	NS
BW, kg	505	509	525	10	456	570	9	NS	***	NS	*
Milk, kg/d	25.5	24.2	22.4	1.6	21.9	26.1	1.3	NS	*	†	NS
3.5% FCM, kg/d	27.8	25.6	25.7	2.4	23.6	29.1	2.0	NS	*	NS	NS
Fat, %	4.14	3.97	4.31	0.28	4.06	4.22	0.25	NS	NS	NS	*
Protein, %	2.89	2.95	2.91	0.07	2.89	2.94	0.07	NS	NS	NS	NS
Fat, kg	1.05	1.05	1.05	0.17	0.92	1.18	0.10	NS	†	NS	NS
Protein, kg	0.74	0.78	0.69	0.05	0.65	0.82	0.04	NS	**	NS	NS

^{a,b}Means within a row with different superscripts are different ($P < 0.05$).

¹Represents dietary cation-anion difference (DCAD:S) (Na + K – Cl – S).

²1 represents primiparous cows and >1 represents multiparous cows.

³Trt = Treatment; Par = Parity.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

RESULTS AND DISCUSSION

Intake and BW

There were no differences in DMI (kg/d) related to DCAD:S but the DMI (kg/100 kg of BW) was greater ($P < 0.05$) for diets with DCAD:S of 20 and 35 compared with the 50 DCAD:S treatment (Table 2). The latter treatment (50 DCAD:S) is equivalent to 73 mEq/100 g of DM DCAD (S excluded from the equation) and may have caused palatability problems or excess alkalinity, reducing DMI. Reduced palatability with cationic salts resulted in depressed feed intake (Stokes et al., 1986). There was a treatment by parity interaction ($P < 0.05$) for DMI (kg/100 kg of BW). Intake for primiparous cows (3.83 kg/100 kg of BW) was greater than that for multiparous cows (2.78 kg/100 kg of BW) at 20 DCAD:S, which was greater than for primiparous and multiparous cows consuming 35 DCAD:S (3.43 and 3.32 kg/100 kg of BW, respectively) and 50 DCAD:S (3.19 and 2.73 kg/100 kg of BW, respectively) treatments. Intake per 100 kg of BW for primiparous cows was consistently greater than for multiparous cows. West et al. (1992) reported that DMI increased linearly when lactating cows received DCAD treatments ranging from 12 to 46 mEq/100 g of DM during hot weather. In the present study, 20 and 35 DCAD:S were equivalent to DCAD of 43 and 52 mEq (Na + K – Cl)/100 g of DM.

Our findings are consistent with work by Roche et al. (2003), which demonstrated that a DCAD:S above 52 mEq/100 g was detrimental to feed intake. Cows offered the 50 DCAD:S treatment in the present study were actually consuming approximately 55 mEq/100 g of DCAD:S (Table 1). The DCAD equation without S was typically used in earlier studies with lactating cows and the very high level of DCAD used in the present

study is not common in the literature. The DCAD concentrations used by Tucker et al. (1988b) are comparable with this study; however, only plasma minerals were measured and feed intake and milk yield were not reported in the Tucker study.

Sanchez et al. (1994) reported a DCAD range from 12 to 62 mEq/100 g of DM and observed optimal DMI for midlactation cows when DCAD was between 30 and 50 mEq/100 g of DM. Results for the present study are in agreement with this range, in which DMI was numerically higher for 20 and 35 DCAD:S (equivalent to 43 and 52 mEq/100 g of DM Na + K – Cl) than for 50 DCAD:S. Although the 35 DCAD:S diet is slightly beyond the optimal range suggested by Sanchez et al. (1994), the present study was conducted with early lactation cows rather than midlactation cows. Early lactation cows may require a higher DCAD than cows in later lactation (Delaquis and Block, 1995a).

Milk Yield and Milk Composition

The DCAD:S did not affect milk yield or milk composition (Table 2), consistent with reports by Roche et al. (2003). Milk yield is dependent on DMI (Kertz et al., 1991), and in the present study, regression of milk yield on DMI yielded the model $y = 4.19 + 1.31x$, where y is milk yield (kg/d) and x is DMI (kg/d) ($R^2 = 0.54$, $P < 0.0001$). Lower DMI (kg/100 kg of BW) for 50 DCAD:S was accompanied by numerically lower milk yield compared with 20 and 35 DCAD:S. When DCAD is calculated without S in the equation, the 50 DCAD:S treatment is equivalent to 73 mEq (Na + K – Cl)/100 g of DM. Sanchez and Beede (1996) reported that performance was compromised when DCAD was over 50 mEq/100 g of DM. In contrast, Block (E. Block, Arm and

Hammer Animal Nutrition, personal communication) reported improved milk yield up to DCAD:S of 52 mEq/100 g of DM in a cool-weather study. West et al. (1991) reported linear increases in milk yield when DCAD was increased from -12 to 31 mEq/100 g of DM. Both milk yield and 3.5% FCM yield exhibited a parity effect ($P < 0.05$), in which production of mature cows was greater than for primiparous cows (Table 2).

Milk yield and DMI were moderate for the present study (Table 2). Because of the level of DMI, subsequent ruminal fermentation and organic acid production would be expected to be similarly moderate. It is plausible that cows with a higher intake of fermentable carbohydrates would benefit from a higher DCAD concentration. A meta-analysis conducted by Hu and Murphy (2004) demonstrated a quadratic milk yield response to increasing DCAD. The optimum range was broad, ranging from approximately 25 to 40 mEq/100 g of DM but average milk yield peaked at about 24 kg/d. Similarly, DMI peaked near 20 kg/d (again moderate), while blood pH and HCO_3^- concentration followed patterns similar to DMI (Hu and Murphy, 2004). Sanchez et al. (1994) reported a similar range of DMI and milk yield response to DCAD, and milk yield peaked at about 22 kg/d. In a summary of dietary buffer studies, Erdman (1988) reported that cows were responsive to sodium bicarbonate supplementation at approximately 30 kg/d milk yield for corn silage-based diets, but were less responsive when alfalfa and grass hays and silages were fed. Diets supplemented with sodium bicarbonate would have a greater DCAD value, whereas the diets containing alfalfa and grass hay and silage would have higher initial DCAD values and would be expected to be less responsive to greater cation content in the diet. Roche et al. (2003) used a wide DCAD range that extended to very high DCAD values (+21, +52, +102, and +127 mEq/100 g of DM). Those authors reported no improvement in milk yield, a small but linear decline in DMI, and a reduced daily weight gain with the relatively high and increasing DCAD. Reported milk yield was in the range of 23.2 to 25.4 kg/d and DMI varied around 15 kg/d, and was perhaps inadequate to challenge the systemic buffering capacity of the cow. Further DCAD research with high-producing dairy cows will be necessary to define the range necessary to provide ruminal and systemic buffering for cows consuming large quantities of DM.

Milk fat and protein percentage and yield were not affected by DCAD (Table 2), agreeing with West et al. (1992) and Delaquis and Block (1995a). Milk fat percentage was relatively high, probably reflecting mobilized adipose tissue in early lactation. Milk yield and milk protein percentage and yield for early lactation cows were not changed by sodium sesquicarbonate sup-

plementation (Cassida et al., 1988). Multiparous cows yielded greater fat and protein than primiparous cows due primarily to greater milk yield. Milk protein yield was positively correlated with DMI ($R^2 = 0.56$, $P < 0.0001$).

Serum Metabolites

Serum HCO_3^- was numerically higher for the 50 DCAD:S treatment (Table 3) although statistically, the 20 DCAD:S diet was similar ($P < 0.10$). Tucker et al. (1988b) reported that serum HCO_3^- was greater for diets containing DCAD:S of 55 vs. 48 mEq/100 g of DM. The 50 DCAD:S diet in the present study may have been excessively alkaline as reflected by serum HCO_3^- (27.6 mEq/L), which was at the high end of the physiological range (21.5 to 27.7 mEq/L) described by Benjamin (1981). An elevation of serum HCO_3^- may diminish serum Cl concentration because these anions are balanced and exchanged to maintain electroneutrality (Guyton, 1981). The actual DCAD:S calculated from ration analysis for 50 DCAD:S was 54.6 mEq/100 g of DM, slightly higher than the DCAD:S 52 mEq/100 g of DM used by Block (E. Block, Arm and Hammer Animal Nutrition, personal communication). However, Block reported improved DMI and milk yield even when blood HCO_3^- was 28 mEq/L for the 52 mEq DCAD:S diet.

Similarity among serum HCO_3^- for 20 and 35 DCAD:S treatments paralleled DMI (kg/100 kg of BW; Table 2). The analyzed DCAD:S for the 20 and 35 DCAD:S treatments were 23 and 33 mEq/100 g of DM, and the range was narrower than anticipated. Diets fed to lactating dairy cows without and with buffer supplementation (DCAD concentrations of 27 and 33 mEq/100 g of DM, respectively) resulted in no change in feed intake or milk yield (McKinnon et al., 1990). Sanchez et al. (1997) reported no difference for DMI, milk yield, or milk fat composition when DCAD ranged from 25 to 40 mEq/100 g of DM. Reports suggest that DCAD effects are mediated through blood buffering (West et al., 1992; Delaquis and Block, 1995b). The present trial agrees with the hypothesis because DMI (Table 2) and serum HCO_3^- (Table 3) were the only variables that responded to DCAD:S. Serum HCO_3^- declined ($P = 0.03$) from wk 2 to 6 postpartum, possibly due to the increasing DMI over time. Greater consumption of concentrates increases systemic acidity and decreases serum bicarbonate.

A treatment by week interaction occurred for serum insulin (Table 3). The serum insulin concentration for the 35 DCAD:S treatment at wk 2 postpartum was lower ($P < 0.10$) than that for wk 6 postpartum. The reason for this difference is not apparent. However, insulin concentrations are positively correlated with

Table 3. The effects of DCAD:S on serum metabolites for early lactation cows offered DCAD:S of 20, 35 or 50 mEq (Na + K – Cl – S) per 100 g DM.

Variable	Week ¹	DCAD:S ² , mEq/100 g DM				SEM	Parity ³		SEM	P ⁴			
		20	35	50	Mean		1	>1		Trt	Par	Trt × Par	Trt × Wk
HCO ₃ [–] , mEq/L	2	26.6	26.2	28.7	27.2 ^c								
	6	25.6	25.5	26.6	25.9 ^d								
	Mean	26.1 ^{ab}	25.9 ^a	27.6 ^b		0.60	26.17	26.89	0.49	†	NS	NS	NS
Insulin, μ IU/mL	2	9.4	6.0	9.8	8.4 ^c								
	6	11.1	14.1	10.2	11.8 ^d								
	Mean	10.3	10.1	10.0		1.77	10.25	9.94	1.54	NS	NS	NS	†
Glucose, mmol/L	2	3.37	3.00	3.23	3.20 ^c								
	6	3.84	3.62	3.46	3.64 ^d								
	Mean	3.60	3.31	3.34		0.14	3.39	3.44	0.11	NS	NS	NS	NS
Serum CAD, ⁵ mEq/L	2	46.5	46.0	48.9	47.1								
	6	45.6	46.0	46.2	45.9								
	Mean	46.1	46.0	47.5		0.69	46.78	46.24	0.57	NS	NS	NS	NS

^{a,b}Means within a row with different superscripts are different ($P < 0.05$).

^{c,d}Means within a column for each variable with different superscripts are different ($P < 0.05$).

¹Week = Week relative to parturition (2) = 2 wk postpartum (6) = 6 wk postpartum.

²Represents dietary cation-anion difference (Na + K – Cl – S).

³1 represents primiparous cows and >1 represents multiparous cows.

⁴Trt = Treatment; Par = Parity.

⁵CAD = Cation-anion difference (Na + K – Cl) in mEq/L.

† $P < 0.10$.

energy balance (Arieli et al., 2001), and body reserves are mobilized during negative energy balance (Bauman and Currie, 1980). Rastani et al. (2001) reported that a nadir of tissue energy balance occurred at wk 2 postpartum compared with wk 4 prepartum through wk 17 postpartum. Because serum insulin in the current study was lower for wk 2 (8.4 μ IU/mL) relative to wk 6 (11.8 μ IU/mL) postpartum (Table 3), energy balance for cows at wk 2 postpartum was probably more negative than at wk 6 postpartum.

There was no effect of DCAD:S on serum CAD, calculated as mEq (Na + K – Cl)/mL. However, serum HCO₃[–] had a significant ($P < 0.0001$) positive relationship with serum CAD (Figure 1). Tucker and Hogue (1990) reported a positive linear relationship between blood HCO₃[–] and serum CAD. Plasma CAD increased significantly from 44 to 49 mEq/L for cows fed diets with DCAD:S of 9 vs. 22 mEq/100 g of DM (Tucker et al., 1991). Because DCAD affects blood acid-base chemistry as reflected by serum CAD and serum HCO₃[–], nutrient manipulation can be useful in modifying the acid-base chemistry of cows to improve productivity. Serum CAD should be further investigated to determine the optimal concentration for performance enhancement.

No DCAD:S treatment effects were observed for serum mineral concentrations (Table 4). These results agree with previous reports (Tucker et al., 1988a; West et al., 1992; Delaquis and Block, 1995a). Homeostatic mechanisms typically maintain blood Ca and P concen-

trations within the normal range (Guyton, 1981), and DCAD had no effect on plasma P, Mg, Na, and K in the work by Tucker et al. (1991). In the present study, serum Mg was greater ($P = 0.03$) for primiparous cows (2.35 mg/dL) than for mature cows (2.12 mg/dL) and at wk 6 compared with wk 2 postpartum (Table 4), perhaps because of greater DMI (kg/100 kg of BW) for primiparous cows (Table 2) and greater DMI for wk 6 compared with wk 2 postpartum. Serum Cl concentrations follow serum Na and K concentrations because of the need to maintain electroneutrality (Rose, 1984). Regression analyses for serum Na on Cl and serum K on Cl yielded R² values of 0.51 and 0.24, respectively ($P < 0.0001$). A one-unit (1.0 mEq/L) increase in serum Cl concentration was accompanied by 0.7 mEq/L increase in serum Na and 0.04 mEq/L increase in serum K. The relationship is stronger with Na because Cl is found in the extracellular compartment with Na (Guyton, 1981). Serum Cl had a reciprocal relationship with serum HCO₃[–] ($r = -0.55$, $P < 0.0001$), which has been observed by others (Escobosa et al., 1984; West et al., 1992; Sanchez et al., 1994).

CONCLUSIONS

The DMI (kg/100 kg of BW) was similar for 20 and 35 DCAD:S treatments, which were greater compared with the 50 DCAD:S treatment. Serum HCO₃[–] tended to be higher for the 50 DCAD:S diet and the concentra-

Figure 1. Simple linear regression of serum HCO_3^- on serum cation-anion difference ($\text{Na} + \text{K} - \text{Cl}$, mEq/L) for cows offered dietary cation-anion difference (DCAD:S) of 20, 35, and 50 mEq ($\text{Na} + \text{K} - \text{Cl} - \text{S}$)/100 g of DM.

tions of serum HCO_3^- and CAD were positively correlated. The 20 and 35 DCAD:S treatments appeared adequate for the early lactation cow during cool weather; 50 DCAD:S may have been too alkaline or was unpalatable. In addition, in the presence of the moderate milk

yield and DMI encountered in the present study, the cows' acid-base balance may not have been challenged sufficiently to benefit from the highest DCAD treatment. However, additional work is needed to evaluate the response of high-yielding, early lactation dairy cows

Table 4. The effects of DCAD:S on serum electrolytes for early lactation cows offered DCAD:S of 20, 35, or 50 mEq ($\text{Na} + \text{K} - \text{Cl} - \text{S}$) per 100 g of DM.

Variable	Week ¹	DCAD:S, ² mEq/100 g DM				SEM	Parity ³		SEM	P ⁴			
		20	35	50	Mean		1	>1		Trt	Par	Trt × Par	Trt × Wk
Ca, mg/dL	2	9.56	9.16	9.25	9.32	0.11	9.52	9.27	0.09	NS	†	*	NS
	6	9.48	9.48	9.43	9.46								
	Mean	9.52	9.32	9.34									
P, mg/dL	2	6.07	6.44	5.80	6.10	0.26	6.37	6.10	0.21	NS	NS	NS	NS
	6	6.13	6.60	6.38	6.37								
	Mean	6.10	6.52	6.09									
Mg, mg/dL	2	2.22	2.16	2.12	2.17 ^a	0.13	2.35	2.12	0.07	NS	*	NS	NS
	6	2.34	2.31	2.29	2.31 ^b								
	Mean	2.28	2.24	2.21									
Na, mEq/L	2	138.8	140.1	140.7	139.9 ^a	0.94	140.03	137.63	0.77	NS	*	NS	NS
	6	135.6	138.9	138.8	137.8 ^b								
	Mean	137.2	139.5	139.8									
K, mEq/L	2	4.33	4.63	4.39	4.45 ^a	0.09	4.66	4.46	0.07	NS	†	NS	NS
	6	4.67	4.63	4.72	4.67 ^b								
	Mean	4.50	4.63	4.55									
Cl, mEq/L	2	96.7	98.8	96.2	97.2	1.14	97.91	95.86	0.93	NS	NS	NS	NS
	6	94.7	97.6	97.3	96.6								
	Mean	95.7	98.2	96.8									

^{a,b}Means within column for each variable with different superscripts are different ($P < 0.05$).

¹Week = Week relative to parturition (2) = 2 wk postpartum (6) = 6 wk postpartum.

²Represents dietary cation-anion difference ($\text{Na} + \text{K} - \text{Cl} - \text{S}$).

³1 represents primiparous cows and >1 represents multiparous cows.

⁴Trt = Treatment; Par = Parity.

† $P < 0.10$; * $P < 0.05$.

to DCAD to better define both the DCAD range and the benefits to cows consuming very high levels of feed DM, and for high-producing cows exposed to hot weather. The DCAD:S may be a better measure of macromineral requirements rather than individual concentrations, and further studies using DCAD:S in early lactation cows should categorize serum CAD as it correlates with serum bicarbonate in cool and hot weather, because blood bicarbonate can be influenced by environment. There may be an optimal serum CAD or HCO_3^- concentration for the lactating dairy cow.

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