Effects of Dietary Cation-Anion Difference and Potassium to Sodium Ratio on Lactating Dairy Cows in Hot Weather

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ABSTRACT

Forty-two lactating Holstein cows 188 ± 59 d in milk were used in an 8-wk randomized complete block trial with a 2×3 factorial arrangement of treatments. The objective was to determine the effects of high dietary cation-anion difference (DCAD) and K:Na ratio on milk yield and composition and blood acid-base chemistry. Treatments included DCAD concentrations of 45 or 60 mEq (Na + K – Cl)/100 g of feed dry matter and K:Na ratios of 2:1, 3:1, or 4:1. Mean DCAD values were later determined to be 41 and 58. Dry matter intake was similar across treatments. Yield of milk and energy corrected milk were lower for the 3:1 K:Na ratio compared with 2:1 and 4:1 ratios. Blood urea N was lower for the highest DCAD, suggesting that DCAD possibly reduced protein degradation or altered protein metabolism and retention. Mean temperature-humidity index was 75.6 for the duration of the trial, exceeding the critical value of 72 for all weeks during the treatment period. Cows maintained relatively normal body temperature with mean a.m. and p.m. body temperature of 38.5 and 38.7°C, respectively. These body temperatures suggest that cows were not subject to extreme heat stress due to good environmental control. Results of this trial indicate that the greatest effect on milk yield occurs when either Na or K is primarily used to increase DCAD, with the lowest yield of energy-corrected milk at a 3:1 K:Na ratio (27.1 kg/d) compared with ratios of 2:1 (29.3 kg/d) and 4:1 (28.7 kg/d). Results also suggest that greater DCAD improves ruminal N metabolism or N utilization may be more efficient with a high DCAD. **Key words:** dietary cation-anion difference, sodium, potassium, blood urea nitrogen

INTRODUCTION

The effect of DCAD on animal physiology and production has been investigated in several species including dairy cows, swine, and beef steers (Block, 1994; Ross

et al., 1994; Patience and Chaplin, 1997). The DCAD, defined as $\text{mEq (Na + K – Cl)/100 g of feed DM}$, is a method by which the acid-base chemistry of the cow can be altered by manipulating the amount of Na, K, or Cl included in the diet. Although considerable DCAD research has focused on the periparturient dairy cow and the reduction of incidence of milk fever (Block, 1984; Oetzel et al., 1988), the impact of DCAD on the lactating cow has also been studied. West et al. (1991) reported a linear increase in milk yield (MY) and a quadratic increase in DMI when cows were fed diets ranging in DCAD from -7.9 to 32.4 mEq/100 g of DM. Tucker et al. (1988b) reported higher DMI and MY in cows fed diets with DCAD level of $+20$ mEq/100 g of DM compared with those fed a DCAD level of -10 mEq/100 g of DM. Meta-analyses (Hu and Murphy, 2004) of multiple macromineral studies indicated that DMI peaked at a DCAD of 40 mEq/100 g of DM, and MY was maximized at 34 mEq/100 g of DM. Sanchez and Beede (1996) reported that DMI and MY were maximized at 38 mEq/100 g of feed DM and that the optimal range for DCAD was between 25 and 50 mEq/100 g of DM based on a similar analysis.

Results of previous research differ concerning the role of ion source in the formulation of lactating dairy cow rations. No differences in DMI or MY were observed when either Na or K was used as the cation source (West et al., 1992). Tucker et al. (1988a) also concluded that DCAD is more important than the individual ions used to alter the DCAD in lactating cows. These studies determined the effect of cation source; there is little information regarding the effect of varying ratios of dietary K to Na in lactating cow rations. Sanchez et al. (1997) compared dietary proportions of NaHCO_3 , NaCl, and KCl and observed that DMI was influenced by an interaction between Na and K and between Na and Cl. The authors also observed increased 3.5% FCM with higher dietary Na and concluded that interrelationships exist among Na, K, and Cl. Sanchez et al. (1994) reported that the DMI and MY response to one cation (Na or K) tends to be the greatest when the dietary level of the other cation is low. The objective of the present study was to determine the effects of adjusting K:Na ratios within high DCAD concentrations on DMI, MY, and serum and urinary electrolyte concentrations.

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Table 1. Ingredient composition of diets containing a DCAD of 41 or 58 mEq Na + K – Cl / 100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Ingredient	DCAD and K:Na ratio					
	41			58		
	2:1	3:1	4:1	2:1	3:1	4:1
	% of DM					
Corn silage	51.58	51.58	51.58	51.58	51.58	51.58
Cottonseed hulls	7.22	7.22	7.22	7.22	7.22	7.22
Ground corn	17.37	17.37	17.37	17.37	17.37	17.37
Base concentrate ¹	16.92	16.92	16.92	16.92	16.92	16.92
Mineral premix ²	2.07	2.07	2.07	2.07	2.07	2.07
Ground corn ³	2.48	2.50	2.58	1.22	1.36	1.44
NaHCO ₃ ³	1.82	1.20	0.83	2.41	1.61	1.16
K ₂ CO ₃ ³	0.02	0.58	0.87	0.63	1.29	1.66
MgSO ₄ ³	0.52	0.56	0.56	0.58	0.58	0.58

¹Base concentrate contained 78.79% soybean meal, 14.55% ProLak (H.J. Baker & Bro., Inc., Westport, CT), 3.64% Megalac Plus (Church & Dwight Co., Inc., Princeton, NJ), 2.42% Megalac (Church & Dwight Co., Inc.), and 0.60% urea (DM basis).

²Mineral premix contained 64.58% limestone, 10.42% salt, 10.42% distillers grain, 8.33% magnesium oxide, and 6.25% vitamin-trace mineral premix.

³These ingredients were mixed in a treatment premix for inclusion in each respective diet.

MATERIALS AND METHODS

Experimental Design

Forty-two multiparous Holstein cows averaging 188 ± 59 DIM were used in a randomized complete block design with a 2×3 factorial arrangement of treatments. The trial was conducted from May 23 to July 31 and consisted of a 2-wk standardization period (**SP**) followed by an 8-wk treatment period (**TP**). During the SP cows were offered the standard herd diet, and baseline measures were taken for DMI, MY, BW, milk composition, and serum and urinary electrolyte concentrations. Data collected during the SP were used for covariate analysis of TP data. Cows were ranked by DMI per BW (kg/100 kg) during the SP, blocked into groups of 6 by rank, and then assigned randomly to treatment within block.

Experimental diets contained 51.6% corn silage and 48.4% concentrate (dry basis) formulated to meet minimum NRC (2001) requirements (Table 1). Dietary treatments were formulated for DCAD of 45 (**LD**) or 60 (**HD**) mEq (Na + K – Cl)/100 g of DM, and K:Na ratio of 2:1 (**KNa2**), 3:1 (**KNa3**), or 4:1 (**KNa4**) within each DCAD treatment. The DCAD and K:Na ratios were adjusted using NaHCO₃ as a Na source and K₂CO₃ as a K source and were added to experimental premixes using ground corn as a carrier. Actual DCAD values were lower than originally formulated (Table 2). Mean DCAD for the 3 LD treatment combinations was 41 mEq/100 g of DM compared with 58 mEq/100 g of DM for the 3 HD treatment combinations. Although DCAD was slightly lower than formulated, the difference between the 2 treatments was larger than originally formulated, preserv-

ing the DCAD treatment difference. Calculation of the 4-element DCAD equation, defined as mEq (Na + K) – (Cl + S)/100 g of DM, is also included in Table 2.

Before the start of the SP, cows were trained to operate electronic gate feeders (American Calan, Inc., Northwood, NH). Cows were fed experimental diets once daily (0800 h) as a TMR, with the amount of feed offered recorded and adjusted daily based on the previous day's consumption to allow 10% orts. The TMR was pushed up at least 4 times daily. Feed DM content for adjustment of ration components was determined weekly by drying at 60°C for 72 h in a forced air oven. Cows were housed in a freestall barn with fans activated by thermostat when the ambient temperature exceeded 24°C. Cows were milked twice daily at approximately 0400 and 1500 h.

Sampling

Forage, concentrate, treatment mixes, and TMR were sampled 3 times weekly, dried at 60°C for 72 h, and composited by week for analysis. Samples were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltex System (Foss Tecator AB, Höganäs, Sweden) and CP was calculated as the percentage N $\times$ 6.25 (AOAC, 1990). Acid detergent fiber and NDF were determined according to the method of Van Soest et al. (1991). Samples were extracted with nitric and acetic acids for determination of chloride concentration using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) as outlined by Cottle et al. (1958). Phosphorus concentration was deter-

Table 2. Chemical composition¹ of diets containing a DCAD of 41 or 58 mEq (Na + K – Cl)/100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Item	Formulated DCAD and K:Na ratio					
	41			58		
	2:1	3:1	4:1	2:1	3:1	4:1
DM	55.00 ± 3.59	55.13 ± 3.29	55.52 ± 3.31	54.78 ± 3.22	54.52 ± 3.25	54.98 ± 3.11
CP	17.42 ± 0.69	17.29 ± 1.43	17.64 ± 1.08	17.83 ± 1.00	16.90 ± 1.05	18.08 ± 1.87
NDF	31.05 ± 1.88	29.79 ± 1.99	29.38 ± 1.75	29.03 ± 2.14	30.49 ± 2.09	30.23 ± 1.91
ADF	17.66 ± 1.14	17.43 ± 1.48	16.58 ± 1.66	16.65 ± 1.44	17.61 ± 1.06	17.53 ± 1.64
RUP, ² % of CP	37.42	37.43	37.43	37.35	37.35	37.36
Soluble protein, ² % of CP	27.30	27.30	27.29	27.41	27.40	27.36
NE _L , ² Mcal/kg	0.74	0.74	0.74	0.73	0.73	0.73
Ca	0.87 ± 0.10	0.90 ± 0.22	0.94 ± 0.16	0.87 ± 0.15	0.89 ± 0.09	0.98 ± 0.18
P	0.36 ± 0.02	0.38 ± 0.05	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.38 ± 0.01
Mg	0.36 ± 0.02	0.40 ± 0.05	0.37 ± 0.02	0.36 ± 0.04	0.37 ± 0.02	0.37 ± 0.04
K	1.03 ± 0.09	1.42 ± 0.21	1.45 ± 0.04	1.38 ± 0.11	1.76 ± 0.08	1.95 ± 0.12
Na	0.50 ± 0.08	0.46 ± 0.06	0.35 ± 0.04	0.74 ± 0.10	0.57 ± 0.06	0.45 ± 0.05
Cl	0.41 ± 0.03	0.39 ± 0.04	0.40 ± 0.01	0.36 ± 0.05	0.38 ± 0.02	0.38 ± 0.02
S	0.28 ± 0.01	0.29 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01
DCAD ³	36.5	45.1	40.8	56.9	58.8	58.5
DCAD:S ⁴	18.7	26.9	22.7	38.8	40.6	39.7
Calculated K:Na ratio	2.1:1	3.1:1	4.1:1	1.9:1	3.1:1	4.3:1

¹Mean value ± standard deviation.

²Calculated values using CPM-Dairy (The Center for Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; The Department of Animal Science, Cornell University, Ithaca, NY; and The William H. Miner Agricultural Research Institute, Chazy, NY)

³Calculated as mEq Na + K – Cl /100 g of DM.

⁴Calculated as mEq (Na + K) – (Cl + S)/100 g of DM.

mined by colorimetry (Beckman DU Series 500 spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) following wet ashing (AOAC, 1990). Other minerals were measured by atomic absorption spectrophotometry (model no. 3030, Perkin-Elmer, Norwalk, CT) following wet ashing (AOAC, 1990).

Milk yield was measured using electronic meters (DeLaval, Kansas City, MO). Milk samples were collected weekly from consecutive p.m. and a.m. milkings throughout the trial. Samples were shipped to Southeast Milk Inc. (Bellevue, FL) for analyses of fat and protein using infrared analysis (B2000, Bentley Instruments Inc., Chaska, MN).

Blood was collected by jugular venipuncture into evacuated tubes at approximately 1300 h at the end of the SP and 3 times during the TP. Samples were centrifuged at 2,500 × *g* for 20 min for separation of serum. Serum was harvested and analyzed immediately for Ca, P, Mg, Na, K, Cl, bicarbonate, BUN, creatinine, and glucose using a Boehringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN) at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Urine samples were collected via manual stimulation at the same time as blood collection for analysis of the same components as for blood serum for determination

of fractional excretion. Fractional excretion was calculated as ([urinary mineral]/[plasma mineral]) × ([plasma creatinine]/[urinary creatinine]) × 100 (Vander, 1991). Respiratory rates were recorded at 1300 h once during the SP and 3 times during the TP. Respiration rate was determined for each cow for 1 min by 2 individuals. Counts exceeding 10% error were re-counted until a difference of less than 10% was obtained.

Milk temperature has been highly correlated with deep body temperature (Bitman et al., 1982) and rectal temperature (Igono et al., 1985, 1987) due to the large volume of blood entering the mammary gland. Milk temperature was measured at each milking using a thermocouple in one short milk tube of each milker (Temp-Sense, Udder Health Systems, Bellingham, WA). Ambient temperature and relative humidity (RH) were recorded hourly at 3 locations in the freestalls and feeding area using a HOBO Pro RH/Temp Data Logger (Onset Computer Corp., Bourne, MA). Mean values across locations were used for analysis. Temperature-humidity index (THI) was calculated as THI = db – (0.55 – 0.55 × RH) × (db – 58), where db was the dry bulb temperature in degrees Fahrenheit and RH was expressed in decimals (NOAA, 1976).

Table 3. Environmental conditions for the trial

Item	Week ¹										Mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Maximum temperature, °C	30.4	31.1	29.1	31.2	31.0	31.5	33.4	31.3	30.4	30.8	31.0 $\pm$ 1.0
Minimum temperature, °C	18.8	21.7	22.5	22.2	21.8	22.5	23.7	22.0	22.2	22.9	22.0 $\pm$ 1.2
Maximum RH, ² %	90.4	96.1	97.6	94.0	93.4	93.9	93.7	94.3	95.2	96.5	94.5 $\pm$ 1.9
Minimum RH, %	51.0	52.7	67.9	59.6	53.7	60.7	55.9	48.2	58.5	63.2	57.1 $\pm$ 5.7
Maximum THI ³	79.0	79.9	79.5	81.4	80.1	82.0	83.9	80.2	80.1	81.8	80.8 $\pm$ 1.4
Minimum THI	63.2	69.8	71.6	70.1	69.2	70.6	72.6	71.1	71.5	72.9	70.3 $\pm$ 2.6
Mean THI	71.3	74.7	75.6	75.7	74.9	76.3	78.2	75.9	76.2	76.8	75.6 $\pm$ 1.9

¹Wk 1 and 2 were the standardization period; wk 3 through 10 were the treatment period.

²RH = Relative humidity.

³THI = Temperature-humidity index = $^{\circ}\text{F} - (0.55 - 0.55 \times \text{RH}) \times (^{\circ}\text{F} - 58)$; NOAA, 1976.

Statistical Analysis

Data were analyzed as a randomized complete block design with a factorial arrangement of treatments using the PROC MIXED procedure of SAS (SAS Institute, 1999). Cow within treatment was included as the random variable, with week as the repeated measure. The model was as follows:

$$Y_{ijklm} = \mu + I + B_i + D_j + R_k + DR_{jk} + W_l + DW_{jl} \\ + RW_{kl} + DRW_{jkl} + C_{m(ijk)} + e_{ijklm}$$

where μ = the mean intercept, I = the covariate, B_i = the effect due to the i th block, D_j = the effect due to the j th DCAD, R_k = the effect due to the k th K:Na ratio, W_l = the effect due to the l th week, $C_{m(ijk)}$ = the effect due to cow m being in the i th block with the j th DCAD and k th K:Na ratio, and e_{ijklm} = residual error associated with each Y_{ijklm} .

Least squares means with a significant F -test ($P < 0.10$) were compared using PDIF of SAS. Linear and quadratic orthogonal contrasts were included in the model to determine the effect of K:Na ratio.

RESULTS AND DISCUSSION

Environmental Conditions

Environmental conditions during the study are presented in Table 3. Mean maximum and minimum daily temperature averaged 31.0 to 22.0°C and were similar throughout the trial. Holstein cows become less productive due to heat stress when daily mean THI exceeds 72 (Johnson, 1987). The mean daily THI during this trial was 75.6, which greatly exceeded this critical value. Except for wk 1 of the SP, the mean weekly THI exceeded 72 for the duration of the trial (Table 3).

DMI

The DMI was similar for all treatments (Table 4). Early DCAD work by Tucker et al. (1988a) reported an

11% increase in DMI when DCAD was increased from -10 to $+20$ mEq/100 g of DM, although these DCAD concentrations were considerably lower than those used in the present study and are lower than the DCAD resulting from current NRC (2001) recommendations (27 mEq/100 g of DM) for Na, K, and Cl (0.19, 1.02, and 0.25%, respectively). West et al. (1991) observed a quadratic response for DMI when DCAD increased from -7.94 to 32.4 mEq/100 g of DM during cool weather and -11.7 to 31.2 mEq/100 g of DM during hot weather. The authors reported that DMI plateaued at a DCAD of approximately 20 mEq/100 g of DM, suggesting a limit in DMI response to DCAD with mineral concentrations above NRC (2001) recommendations for Na, K, and Cl. In later work, however, West et al. (1992) reported a positive linear response for DMI as DCAD was increased from 12 to 46 mEq/100 g of DM. Sanchez and Beede (1996) reported that DMI was maximized when DCAD ranged from 25 to 50 mEq/100 g of DM. In a similar analysis of data from 17 published studies, Hu and Murphy (2004) reported that DMI was maximal at a DCAD of 40 mEq/100 g of DM. In both reports, a curvilinear relationship was observed; DMI declined when DCAD concentrations were outside the optimal range. The absence of an effect of DCAD on DMI in the present study suggests that at ranges as narrow as 15 mEq/100 g of DM, the homeostatic controls handle the excess cations in the system via urinary excretion without decreasing DMI, as is reflected by greater urinary K excretion for HD. These data also suggest that the lowest DCAD was adequate for conditions in the present study.

The lack of response to K:Na is consistent with the results of Tucker et al. (1988a, 1991) and West et al. (1992) who reported that DCAD was more important than the ion used to alter DCAD in lactating cows. The reduction in DMI reported by Schneider et al. (1984) when KHCO_3 was used instead of NaHCO_3 as a cation source may have been caused by poor palatability from a relatively dry ration. Corn silage used in the present

Table 4. Dry matter intake, milk and milk component yield, and milk composition for cows fed diets containing a DCAD of 41 or 58 mEq (Na + K – Cl)/100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Item	DCAD and K:Na ratio						SEM	<i>P</i>		
	41			58				DCAD	Ratio	DCAD × Ratio
	2:1	3:1	4:1	2:1	3:1	4:1				
DMI										
kg/d	22.4	22.3	23.8	23.0	23.5	22.2	0.94	NS ¹	NS	NS
kg/100 kg of BW	3.84	3.82	3.92	3.73	3.66	4.07	0.17	NS	NS	NS
Yield, kg/d										
Milk	27.5	26.1	28.8	27.9	26.4	27.4	0.87	NS	Q ²	NS
ECM ³	28.8	26.4	29.7	29.8	27.7	27.6	1.04	NS	Q	NS
Fat	1.07	0.98	1.11	1.12	1.07	1.03	0.06	NS	NS	NS
Protein	0.83	0.79	0.86	0.83	0.81	0.80	0.03	NS	NS	NS
Composition, %										
Fat	3.85	3.82	3.90	3.91	3.84	3.92	0.08	NS	NS	NS
Protein	3.03	3.16	3.01	3.02	3.08	2.95	0.06	NS	Q	NS

¹NS = Not significant ($P > 0.10$).²Q = Quadratic effect ($P < 0.05$).³ECM = $0.3246 \times (\text{kg milk}) + 12.86 \times (\text{kg fat}) + 7.04 \times (\text{kg protein})$; Tyrrell and Reid, 1965.

study may have masked flavors and minimized palatability problems. Although DMI was similar across K:Na ratio, there was a trend ($P = 0.12$) for increased DMI/100 kg of BW for KNa4. However, initial BW for these cows was numerically lower than for other treatments (Table 4) and may have contributed to greater DMI/100 kg of BW.

MY and Milk Composition

Milk yield and composition were similar for both DCAD (Table 4), which is consistent with results for DMI. Previous research reporting greater MY with higher DCAD (Tucker et al., 1988a; West et al., 1991) indicated the greatest MY response at DCAD levels lower than those used in the present study. Because the objective of this trial was to determine the effect of altering K:Na ratio at high DCAD concentrations, and a quadratic relationship ($P < 0.05$) exists for MY and DCAD (Sanchez and Beede, 1996; Hu and Murphy, 2004), potential exists for depressed MY and DMI when DCAD exceeds the previously reported optimal range (Sanchez and Beede, 1994, 1996; Hu and Murphy, 2004). The absence of a DCAD response for DMI and MY suggests that at high DCAD, a 15 mEq/100 g of DM difference in DCAD concentration is not sufficient to depress DMI or MY.

A quadratic K:Na effect for MY ($P = 0.03$) and ECM ($P = 0.04$) was observed (Table 4). Mean MY decreased from 27.7 kg/d for KNa2 to 26.2 kg/d for KNa3. An increase was then observed to 28.1 kg/d for KNa4. A similar relationship was observed for ECM with mean yields of 29.3, 27.1, and 28.7 kg/d for KNa2, KNa3, and KNa4, respectively. These results are consistent with previous research in which interactions were observed

for Na and K. Sanchez et al. (1994) reported the greatest response in milk yield to either high Na or K when the concentration of the other ion was low. Erdman et al. (1980) and O'Connor et al. (1988), however, reported no effect of additional Na when fed with low, adequate, or high K.

A quadratic K:Na ratio effect was observed for milk protein percentage ($P = 0.04$) across DCAD with mean values of 3.02, 3.12, and 2.98% for KNa2, KNa3, and KNa4, respectively. This response is inversely related to that noted for MY and is most likely a dilution effect because milk protein yield was similar among treatments. No differences were noted among K:Na ratios for milk fat percentage or milk fat yield. Although not statistically different, milk fat yield was numerically lower for KNa3 within LD treatments. Further research with a larger number of cows might allow for the detection of a statistical difference in fat yield based on K:Na ratio.

BW and Temperature

No differences in initial BW or BW gain were observed for DCAD or K:Na ratio treatments (Table 5). No significant DCAD × K:Na ratio interactions were observed for a.m. milk temperature, p.m. milk temperature, or temperature difference (Table 5). However, a.m. milk temperature declined linearly ($P < 0.05$) with greater K:Na ratios. The difference in a.m. to p.m. temperature increased linearly ($P < 0.10$) as K:Na ratio increased, with the largest change observed in KNa4 (Table 5). West et al. (1992) observed decreased a.m. and p.m. milk temperature for cation sources added to the diet that was attributed to disparity in BW among treatment groups. This is consistent with the trend for

Table 5. Mean BW, respiration rate, and milk temperature for cows fed diets containing a DCAD of 41 or 58 mEq (Na + K – Cl)/100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Item	DCAD and K:Na ratio						SEM	<i>P</i>		
	41			58				DCAD	Ratio	DCAD × Ratio
	2:1	3:1	4:1	2:1	3:1	4:1				
Initial BW, kg	587.5	597.9	609.5	623.6	662.3	548.5	29.9	NS ¹	NS	NS
BW change, kg	8.1	4.5	1.1	−1.2	6.0	1.4	3.6	NS	NS	NS
Respiration rate, breaths/min	81	83	77	83	73	82	4	NS	NS	NS
Milk temperature, °C										
a.m.	38.7	38.5	38.3	38.6	38.6	38.5	0.1	NS	L ^{3*}	NS
p.m.	38.8	38.8	38.6	38.8	38.7	38.7	0.1	NS	NS	NS
Change ²	0.1	0.2	0.3	0.2	0.1	0.3	0.1	NS	L [†]	NS

¹NS = Not significant ($P > 0.10$).²Calculated as (p.m. temperature – a.m. temperature).³L = Linear effect.* $P < 0.05$; $†P < 0.10$.

a linear K:Na effect for a.m. to p.m. temperature change observed in the present study. Within the HD treatment, the greatest a.m. to p.m. temperature change occurred for lighter BW cows. The ability of lower BW animals to dissipate heat would improve body heat loss. Although respiratory rates were elevated across treatments due to heat stress, no differences were observed between DCAD or among K:Na ratio for respiratory rate (Table 5). Cows maintained relatively normal body temperatures (38.5°C a.m. temperature and 38.7°C p.m. temperature) and were not subject to severe heat stress due to good environmental control with high-velocity fans.

Blood and Urinary Metabolites

No differences were noted for serum Ca, Na, Cl, or bicarbonate concentration (Table 6). Increased serum bicarbonate concentration was positively correlated

with the positive DCAD effect on DMI (Tucker et al., 1988a; West et al., 1991). The absence of a DCAD effect for DMI and serum bicarbonate, coupled with the increase in urinary excretion of bicarbonate (Table 7) for HD suggests that the cow's buffering needs were satisfied by the DCAD of 45 mEq/100 g of DM. Blood urea N concentrations were higher ($P < 0.001$) for LD (17.0 mg/dL) compared with HD (14.5 mg/dL). Cai and Zimmerman (1995) reported increased plasma urea N and decreased plasma ammonia as DCAD increased, although there was no effect of DCAD on total plasma N (urea + ammonia + allantoin). Phillip (1983) reported reduced ruminal ammonia concentrations in lambs fed supplemental NaHCO₃. The author proposed that the addition of NaHCO₃ either enhanced microbial utilization of ruminal ammonia or raised ruminal pH such that absorption of ruminal ammonia increased. The lower BUN observed for the HD suggests that greater absorption of ruminal ammonia may not be occurring.

Table 6. Serum mineral and metabolite concentrations for cows fed diets containing a DCAD of 41 or 58 mEq (Na + K – Cl)/100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Item	DCAD and K:Na ratio						SEM	<i>P</i>		
	41			58				DCAD	Ratio	DCAD × Ratio
	2:1	3:1	4:1	2:1	3:1	4:1				
Ca, mg/dL	10.19	10.03	9.93	9.92	9.87	10.07	0.12	NS ¹	NS	NS
Mg, mg/dL	2.51	2.42	2.59	2.37	2.38	2.70	0.08	NS	L ^{2*}	NS
Na, mmol/L	144.8	144.6	144.6	143.9	144.4	144.7	0.4	NS	NS	NS
K, mmol/L	4.67	4.83	4.78	4.58	4.64	4.82	0.09	NS	L [†]	NS
Cl, mmol/L	102.5	102.4	101.9	101.2	102.1	101.7	0.6	NS	NS	NS
BUN, mg/dL	16.9	15.8	18.2	14.4	13.9	15.2	0.8	***	Q ^{3†}	NS
Bicarbonate, mmol/L	22.1	21.9	22.8	22.9	22.5	22.8	0.5	NS	NS	NS

¹NS = Not significant ($P > 0.10$).²L = Linear effect.³Q = Quadratic effect.† $P < 0.10$; * $P < 0.05$; *** $P < 0.001$.

Table 7. Urinary excretion of mineral and metabolites for cows fed diets containing a DCAD of 41 or 58 mEq (Na + K – Cl)/100 g of DM and K:Na ratio of 2:1, 3:1, or 4:1

Item	DCAD and K:Na ratio						SEM	<i>P</i>		
	41			58				DCAD	Ratio	DCAD × Ratio
	2:1	3:1	4:1	2:1	3:1	4:1				
Fractional excretion, ¹ %										
Ca	0.26	0.23	0.35	0.26	0.20	0.28	0.08	NS ²	NS	NS
Mg	12.6	10.1	16.4	10.9	7.4	14.7	2.2	NS	Q ^{3*}	NS
Na	1.80	1.45	1.12	2.16	1.38	1.32	0.19	NS	L ^{4***}	NS
K	42.9	60.3	70.8	52.6	68.7	95.6	3.9	***	L ^{4***}	†
Cl	1.17	1.23	1.27	1.29	1.16	1.50	0.11	NS	NS	NS
Urine metabolite/urine creatinine ⁵										
Urea N	10.6	10.3	10.7	10.1	10.2	10.6	0.4	NS	NS	NS
Bicarbonate	2.38	2.72	2.54	3.52	3.06	3.70	0.34	**	NS	NS

¹Fractional excretion = ([urinary mineral] / [plasma mineral]) × ([plasma creatinine] / [urinary creatinine]) × 100; Vander, 1991.

²NS = Not significant (*P* > 0.10).

³Q = Quadratic effect.

⁴L = Linear effect.

⁵Expressed as (milligrams of urine metabolite/100 mL of urine)/(milligrams of urine creatinine/100 mL of urine).

†*P* < 0.10; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Reduced BUN coupled with decreased rumen ammonia noted in previous research (Phillip, 1983) suggests the possibility for enhanced microbial utilization of ruminal ammonia for protein synthesis.

As the K:Na ratio increased, serum Mg increased linearly (*P* < 0.05). This is in contrast with previous research (Ram et al., 1998; Jittakhot et al., 2004) in which greater dietary K concentration reduced Mg absorption. Leonhard-Marek and Martens (1996) reported that in isolated ruminal epithelium, the relationship between ruminal K concentration and the mucosal to serosal Mg flux reach a plateau at high ruminal K concentrations, resulting in decreased suppression of Mg absorption. Jittakhot et al. (2004) reported that the plateau of mucosal to serosal Mg flux did not decrease suppression of Mg absorption at high K levels in non-pregnant, dry cows. Dietary K concentration in that study was considerably lower than that in the present study. Ruminal K concentration for the KNa4 ratio may have been sufficiently high to reach the plateau and the suppression in Mg absorption was alleviated. Serum Mg increased to 2.65 mg/dL for KNa4 from 2.44 and 2.40 mg/dL for KNa2 and KNa3, respectively.

A significant quadratic K:Na ratio effect was noted for BUN (*P* < 0.10; Table 6). The BUN concentration for KNa3 (14.9 mg/dL) was lower than that observed for KNa2 (15.7 mg/dL) and KNa4 (16.7 mg/dL). Greater BUN for KNa4 may be explained by the relationship between dietary K and intracellular AA concentration. Greater free muscle AA concentrations in rats (Arnauld and Lachance, 1980) and chicks (Austic and Calvert, 1981) were reported when dietary K was elevated. Greater dietary K in KNa4 may have led to increased

availability of free AA. If available in excess, these AA may be catabolized as an energy source, resulting in greater BUN. A linear increase (*P* < 0.05) for serum K concentration with increasing K:Na ratio was also observed, probably a result of increasing dietary K concentration (Table 2).

There was a trend for an interaction of DCAD × K:Na ratio (*P* < 0.10) for fractional excretion of K (Table 7). The increase in K excretion between KNa2 and KNa3 was similar between DCAD concentrations (17.4 and 16.1% increase for LD and HD, respectively), but the increase in K excretion between KNa3 and KNa4 was considerably larger for HD (26.9%) compared with LD (10.5%). This is consistent with the increase in dietary K for these treatments (Table 2). Fractional excretion of Na declined linearly (*P* < 0.001) as K:Na ratio increased because dietary Na concentration decreased with increasing K:Na ratio (Table 2), resulting in decreased Na fractional excretion.

CONCLUSIONS

The quadratic response for MY and ECM by K:Na ratio suggests that increasing DCAD primarily with Na or K affected milk yield, with the lowest ECM produced at a 3:1 K:Na ratio (27.1 kg/d) compared with ratios of 2:1 (29.3 kg/d) and 4:1 (28.7 kg/d). The absence of a DCAD effect for DMI, MY, ECM, and blood bicarbonate along with increased fractional excretion of bicarbonate with greater DCAD concentration suggests that blood buffering was adequate at either DCAD used in the present study. The absence of a DCAD × K:Na ratio interaction for DMI, MY, and ECM also suggests that

production is not improved when DCAD is elevated outside of the optimum range of 25 to 50 mEq (Na + K - Cl)/ 100 g of DM even with altered K:Na ratio. Lower BUN in cows fed a high DCAD concentration suggests the possibility of enhanced microbial ammonia utilization resulting in greater microbial CP. The mechanism behind these changes in protein metabolism is unclear and warrants further investigation. By elucidating these effects, the efficiency with which protein is fed can be improved.

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