Effect of dietary ratio of Na:K on feed intake, milk production, and mineral metabolism in mid-lactation dairy cows

W. Hu and L. Kung Jr.¹

Department of Animal and Food Sciences, University of Delaware, Newark 19716

ABSTRACT

The objective of this study was to determine the effect of altering the dietary ratio of Na:K while keeping the dietary cation-anion difference (DCAD) constant, on dry matter (DM) intake, milk production, and mineral metabolism in lactating dairy cows. Fifteen mid-lactation Holstein cows averaging 160 d in milk were used in a replicated 3×3 Latin square design with treatments varying in the molar ratio of Na:K (0.21, 0.53, and 1.06). Diets contained A) 0.25% Na and 2.00% K, B) 0.50% Na and 1.60% K, or C) 0.75% Na and 1.20% K (on a DM basis), and all contained the same DCAD of 33 mEq (Na + K – Cl – S)/100 g of DM. There was a quadratic effect of the ratio of Na:K on DM intake (28.4, 27.5, and 28.3 kg/d for diets A, B, and C, respectively). The ratio of Na:K did not affect milk yield (average 39.2 kg/d), milk composition (average 3.60% fat; 3.01% protein; and 8.62% solids-not-fat), or coccygeal venous plasma concentrations of HCO_3^- (average 29.3 mEq/L), Na^+ (average 136.7 mEq/L), K^+ (average 4.53 mEq/L), Cl^- (average 97.5 mEq/L), Ca (average 10.06 mg/dL), and Mg (average 2.49 mg/dL), and urinary pH (average 8.38) and ratio of Cl^- :creatinine (average 4.35). The ratios of urinary Na^+ :creatinine (1.80, 4.21, and 7.42), Ca:creatinine (0.035, 0.041, and 0.064), and Mg:creatinine (0.53, 0.60, and 0.77) increased linearly with increasing ratios of Na:K, whereas the ratio of urinary K^+ :creatinine decreased linearly as the ratio of Na:K increased (22.4, 15.9, and 10.3). Milk production and composition of mid-lactation cows was similar among dietary ratios of Na:K with the same DCAD of 33 mEq/100 g of DM.

Key words: sodium, potassium, dietary cation-anion difference, lactation

INTRODUCTION

Altering the levels of DCAD can affect the metabolism and performance of lactating dairy cows. It appears that DCAD improves acid-base balance and is positively related to productive performance in lactating dairy cows (Sanchez and Beede, 1996; Hu and Murphy, 2004). A higher DCAD can be achieved by the addition of NaHCO_3 and K_2CO_3 (NaCO_3 and KHCO_3 are not considered economically feasible). These can be fed alone or in combination in a diet for dairy cows.

Sodium and potassium cations contribute significantly to the pumping mechanisms of cells, osmotic balance, acid-base equilibrium, and kidney function in the animal body system. For instance, Na^+ - K^+ pumping of cells is often taken to illustrate the potential role of these strong ions in the metabolic process. This mechanism requires ATP and actively maintains high levels of K^+ and low levels of Na^+ intracellularly; excesses of one cation in relation to the other would obviously cause the pump to operate inappropriately beyond an optimal level (Block, 1994). Manipulating dietary levels of Na and K might therefore benefit physiological processes and subsequently improve production in lactating dairy cows. However, there is limited information on the effects of altering the ratio of Na:K on metabolism and production of lactating dairy cows. The responses of DMI and milk production to either high Na or K tend to be the greatest when the dietary concentration of the other cation is low (Sanchez et al., 1994). Wildman et al. (2007) reported a quadratic effect from altering the ratio of K:Na on milk production, whereas DMI was not affected by treatment. Further efforts need to be made to examine the effects of altering dietary ratios of Na:K at a fixed DCAD on DM intake, milk production, and mineral metabolism in mid-lactation dairy cows.

MATERIALS AND METHODS

This experiment was conducted at the University of Delaware dairy research farm, Newark, between June 19 and August 20, 2006. All experimental protocols used in the trial were approved by the University of Delaware,

Received April 3, 2008.

Accepted January 24, 2009.

¹Corresponding author: lksilage@udel.edu

Table 1. Composition of concentrate mixed into the experimental diets

Ingredient	%, DM basis
Corn, coarse	29.48
Soy hulls	22.93
Soybean meal, 48%	14.59
Wheat middlings	13.10
Corn gluten	9.83
Distillers dried grains	3.64
Stabilized fat	2.01
Limestone, fine	1.41
NaCl	0.88
Megalac ¹	0.84
Yeast culture	0.44
Magnesium oxide	0.34
KCl	0.16
Amino plus	0.08
Selenium premix, 0.06%	0.08
Organic selenium ²	0.08
A, D, and E vitamin premix ³	0.06
Ruminant trace minerals ⁴	0.06
Niacin, 99%	0.03

¹Arm and Hammer Animal Nutrition (Princeton, NJ).

²Contained 0.6 g of selenium/kg.

³Contained 23,935 kIU of vitamin A/kg, 5,984 kIU/kg of vitamin D, and 120 kIU of vitamin E/kg.

⁴Contained 10% Ca, 6% Mg, 77.3 g of Mn/kg, 2.6 g of I/kg, 41.2 g of Fe/kg, 124 g of Zn/kg, 13.9 g of Cu/kg, and 1.0 g of Co/kg.

College of Agriculture and Natural Resources, Agricultural and Animal Care and Use Committee.

Experimental Design and Animal Care

Fifteen multiparous Holstein cows averaging 160 ± 21 DIM were assigned randomly to replicated 3×3 Latin squares with the following dietary treatments: molar ratios of Na:K of 0.21 [0.25% Na (10.87 mEq/100 g) and 2.00% K (51.15 mEq/100 g) in the diet on DM basis], 0.53 [0.50% Na (21.75 mEq/100 g) and 1.60% K (40.92 mEq/100 g)], and 1.06 [0.75% Na (32.62 mEq/100 g) and 1.20% K (30.69 mEq/100 g)] at a fixed DCAD of 33 mEq (Na + K – Cl – S)/100 g of DM. The fixed DCAD was within the optimal range established for lactating dairy cows (Hu and Murphy, 2004). The concentrations of Na and K were designed to meet or exceed the nutrient requirements, but to be far less than the maximum tolerable concentrations in the diets for dairy cows (NRC, 2001). Concentrations of Cl and S in the diets were kept constant in all treatments. There were 3 experimental periods (periods 1 to 3 as lactation period proceeded), with each consisting of a 2-wk adjustment period followed by a 1-wk collection period. Cows were housed in a barn with Calan gates (American Calan, Northwood, NH) and sand-bedded freestalls. Feed offered was adjusted daily, and 110% of the previous day's consumption (as-fed basis) was

provided once daily at 0700 h. Water was available for ad libitum consumption. Cows were milked twice daily at approximately 0600 and 1600 h.

The TMR was composed of 48.3% concentrate mix (Table 1), 40.0% corn silage, and 10.0% alfalfa hay on a DM basis that met nutrient requirements (NRC, 2001). The ratio of Na:K was varied by using NaHCO_3 and K_2CO_3 ; these minerals were supplemented and mixed in the TMR (Table 2).

Sample Collection and Analysis

Feeds and Orts. Feed intake and refusals for each cow were recorded daily during each experimental period. Weekly samples of corn silage, alfalfa hay, concentrate mix, and the TMR were collected. Dry matter contents of these feeds were determined by drying in a forced-air oven at 60°C until constant weights were obtained. Diets were adjusted weekly based on DM content of feeds. Weekly samples of corn silage, alfalfa hay, concentrate mix, and the TMR were stored at –15°C until the end of each experimental period and then composited and pooled for later analysis. Nutrient contents of corn silage, alfalfa hay, concentrate mix, and the TMR were analyzed by wet chemistry for CP, ADF, NDF, and minerals (Cumberland Valley Analytical Services, Maugansville, MD). Briefly, CP was calculated as $\text{N} \times 6.25$ after analyses of N (AOAC, 2000) using a Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI). The ADF (AOAC, 2000) and NDF (Van Soest et al., 1991) contents of feeds were determined with the modification that Whatman 934-AH glass micro-fiber filters with 1.5- μm particle retention were used in place of fritted glass crucibles. In the analysis of NDF content of feeds, heat-stable amylase and sodium sulfite were added in the heating process before filtration (Van Soest et al., 1991). Mineral content of feeds (AOAC, 2000) was also determined using Perkin Elmer ICP (Perkin Elmer, Shelton, CT). In addition, energy concentration was calculated (Cumberland Valley Analytical Services). The nutrient composition of CP, NE_L , ADF, NDF and minerals is presented in Table 2 and was calculated on the basis of nutrient content analysis of corn silage, alfalfa hay, and concentrate mix supplemented with the minerals.

Urine. On d 19 of each experimental period, cows were manually stimulated to urinate at 0900 h, and midstream urine was collected in a 50-mL plastic container. Urine pH was measured immediately. Urine samples (30 mL for each cow) were stored at –15°C until further analyzed. Urine concentrations of Na^+ , K^+ , and Cl^- were determined using an ion selective electrode; urine concentrations of Ca, Mg, and creatinine were measured spectrophotometrically. All these assays were

Table 2. Ingredients and nutrient composition in experimental diets with 3 ratios of Na:K

Item	Ratio of Na:K		
	0.21	0.53	1.06
Ingredient, % of DM			
Corn silage ¹	40.0	40.0	40.0
Alfalfa hay ²	10.0	10.0	10.0
Concentrate	48.3	48.3	48.3
NaHCO ₃		0.79	1.60
K ₂ CO ₃	1.71	0.88	0.04
NaCl		0.02	0.02
KCl	0.02		
Composition ³			
CP, % of DM	16.9 ± 0.46	16.3 ± 0.36	16.7 ± 0.53
NE _L , Mcal/kg	1.70 ± 0.00	1.70 ± 0.00	1.71 ± 0.01
ADF, % of DM	20.8 ± 0.79	21.0 ± 1.23	20.4 ± 0.69
NDF, % of DM	32.3 ± 0.75	32.3 ± 0.96	31.8 ± 0.98
Ca, % of DM	0.70 ± 0.04	0.64 ± 0.06	0.69 ± 0.05
P, % of DM	0.35 ± 0.01	0.35 ± 0.02	0.35 ± 0.02
Mg, % of DM	0.32 ± 0.01	0.32 ± 0.02	0.32 ± 0.01
Na, % of DM	0.24 ± 0.03	0.42 ± 0.03	0.70 ± 0.04
K, % of DM	2.22 ± 0.10	1.73 ± 0.08	1.27 ± 0.12
Cl, % of DM	0.60 ± 0.01	0.60 ± 0.02	0.60 ± 0.01
S, % of DM	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01
DCAD, ⁴ mEq/100 g of DM	36.8 ± 3.1	32.0 ± 2.5	33.4 ± 3.4

¹Contained 38.4% DM, 37.1% NDF, 23.5% ADF, and 9.4% CP on a DM basis.

²Contained 86.7% DM, 47.7% NDF, 36.8% ADF, and 15.9% CP on a DM basis.

³Mean value ± standard deviation.

⁴DCAD in milliequivalents of (Na + K – Cl – S)/100 g of DM.

performed on the Hitachi 917 analyzer (Roche, Indianapolis, IN) using Roche diagnostic reagents.

Urinary mineral excretions (Na⁺, K⁺, Cl[–], Ca, and Mg) were expressed as ratios of minerals to creatinine concentration to overcome variations in urine volume among animals and as fractional clearance (FC) to indicate the renal clearance of electrolytes. The FC of electrolytes was calculated using the equation (Fleming et al., 1992)

$$FC_x = (U_x/P_x) \times (P_{\text{Creatinine}}/U_{\text{Creatinine}}) \times 100,$$

where FC_x = fractional clearance of X (%); U_x = urine concentration of X (mg/dL or mEq/L); P_x = plasma concentration of X (mg/dL or mEq/L); P_{Creatinine} = plasma concentration of creatinine (mg/dL); and U_{Creatinine} = urine concentration of creatinine (mg/dL).

Blood. On d 19 of each experimental period, coccygeal venous blood (there might be a slight chance for artery blood to be included in the blood sample) was collected at 0900 h using a Vacutainer (Becton Dickinson, Franklin Lakes, NJ) containing lithium heparin, placed on crushed ice, and immediately centrifuged at 1,500 × g for 15 min. Plasma was transferred to 5-mL plastic tubes and frozen at –15°C until analyzed.

Coccygeal venous plasma concentration of HCO₃[–] was measured spectrophotometrically. Coccygeal venous plasma concentrations of Na⁺, K⁺, Cl[–], Ca,

Mg, and creatinine were determined using the same analytical methods as for the urine. All of these assays were performed on the Hitachi 917 analyzer (Roche, Indianapolis, IN).

Milk Production and BW. Milk production was recorded twice daily at 0600 and 1600 h. On d 17 and 18 of each experimental period, milk samples were collected consecutively, and analyzed for milk fat, true protein, lactose, SNF, and SCC using a MilkoScan System 4000 (Foss North American, Eden Prairie, MN) by an infrared method (Dairy One Milk Laboratory, Ithaca, NY). Although BW was determined at the start of the trial and weekly thereafter, only BW measured during the last week of each experimental period was included in the statistical analyses.

Statistical Analysis

Dry matter intakes from d 15 to 20 and milk yields from d 15 to 21 in each experimental period were averaged for each cow. Mean milk composition from d 17 to 18 in each experimental period was calculated after being weighed for the proportion of milk produced at each milking. Urine pH was converted to free H⁺ concentration ([H⁺], assuming an activity coefficient of 1) and subjected to statistical analysis (Murphy, 1982). The resulting mean [H⁺] could still be transformed for convenience and reported as pH. Therefore, each

Table 3. Environmental conditions during the lactation trial¹

Item	Experimental period		
	1	2	3
Maximum temperature, °C	29.0	32.4	31.3
Minimum temperature, °C	19.0	20.6	18.5
Maximum RH, ² %	91.3	92.4	89.4
Minimum RH, %	52.3	49.1	42.3
Maximum THI ³	76.7	80.9	77.9
Minimum THI	66.1	68.6	65.1
Mean daily THI	71.5	75.2	72.4

¹Means of d 15 to 21 of each experimental period.

²Relative humidity.

³THI = temperature-humidity index = dry bulb temperature (°F) – (0.55 – 0.55 × RH) × (dry bulb temperature – 58).

mean was presented as both $[H^+]$ 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).

Data were analyzed with the GLM procedure in SAS (SAS Institute, 2004) according to the model for a replicated Latin square design:

$$Y_{ijkm} = \mu + C_i(S)_m + P_j + T_k + S_m + e_{ijkm},$$

where μ = overall mean; C_i = effect of cow i within square m ($i = 1, 2, 3$); P_j = effect of experimental period j ($j = 1, 2, 3$); T_k = effect of treatment k ($k = 1, 2, 3$); S_m = effect of square m ($m = 1, 2, 3, 4, 5$); and e_{ijkm} = error term.

Significance was defined as $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a trend. Two single degree-of-freedom orthogonal contrasts were constructed to infer the linear and quadratic effects of dietary ratio of Na:K.

RESULTS AND DISCUSSION

The environmental conditions during the lactation trial are presented in Table 3. Mean maximum and minimum daily temperature averaged 30.9 and 19.4°C across the 3 experimental periods. The minimum, mean, and maximum temperature-humidity index were close to or above the critical values of 64, 72, and 76 for Holstein cows, respectively (Igono et al., 1992), indicating that heat stress could have been induced in the cows throughout the trial.

BW, DMI, and Milk Yield and Composition

Body weight was not affected by treatments. A quadratic effect of the dietary ratio of Na:K was detected for DMI ($P = 0.03$; Table 4). The DMI was the lowest in the diet with a ratio of Na:K of 0.53 compared with

ratios of 0.21 and 1.06. In experimental period 2, the daily mean temperature-humidity index was 75.2, which greatly exceeded the critical value of 72 (Igono et al., 1992). Severe heat stress could dramatically decrease DMI and milk production. However, such decreases in DMI and milk production did not occur in experimental period 2. Decreasing trends of DMI and of milk production as lactation period proceeded were observed (data not shown).

Milk yield and 4% FCM were similar for mid-lactation cows fed diets with different ratios of Na:K (Table 4). The same was true for milk fat, true protein, lactose, SNF percentages and yields, and SCC.

Previous research has focused on the effect of Na or K cation source, rather than on the effect of the ratio of Na:K in diets of lactating cows. O'Connor et al. (1988) did not detect any difference in DM intake or milk production of mid-lactation Holstein cows fed 2 dietary concentrations of Na (0.24 vs. 0.62%) or K (1.14 or 1.59%). West et al. (1992) also reported that there was no effect on DMI and milk production when either Na or K was used as the cation source. It should be noted that, when Na or K ion source was compared in these studies, the concentration of the other ion or DCAD was not equalized (O'Connor et al., 1988; West et al., 1992), because there are potential interaction effects between the strong ions or between each strong ion with DCAD on lactating cows.

Sanchez et al. (1994) reported that, based on the empirical model analysis from a large data set, the interaction between Na and K influenced DMI and 4% FCM yield, with the greatest DMI and 4% FCM yield occurring in either high Na or K when the concentration of the other ion was relatively low. Wildman et al. (2007) examined the effect of dietary ratios of K:Na of 2:1, 3:1, and 4:1 (on DM percentage basis) on milk production in cows averaging 188 ± 59 DIM and showed that milk yield and ECM yield were lower (quadratic, $P < 0.05$) for a ratio of 3:1 K:Na (26.2 and 27.1 kg/d) compared with ratios of 2:1 and 4:1 (27.7 and 28.1; 29.3 and 28.7 kg/d, respectively), whereas DMI was similar across treatments. Interestingly, the lowest values of DMI in the present experiment and milk yield and ECM yield of Wildman et al. (2007), and the lowest values of both DMI and 4% FCM of Sanchez et al. (1994) occurred at the moderate, rather than the high or low, dietary ratio of Na:K. The underlying mechanism responsible for these observations remains unclear.

Coccygeal Venous Blood Minerals and Acid-Base Measures

Coccygeal venous plasma concentrations of Na^+ and K^+ were not altered by the ratios of Na:K even though

Table 4. Least squares means of DMI, BW, milk yield, 4% FCM, and milk composition for lactating cows fed experimental diets with 3 ratios of Na:K

Variable	Ratio of Na:K			SE	Orthogonal contrast	
	0.21	0.53	1.06		Linear	Quadratic
DMI, kg/d	28.4	27.5	28.3	1.0	NS	0.03
BW, kg	774	777	778	14	NS	NS
Milk						
Yield, kg/d	39.2	39.0	39.4	2.6	NS	NS
4% FCM, kg/d	37.2	36.3	36.8	3.5	NS	NS
Fat, %	3.68	3.53	3.59	0.28	NS	NS
Protein, %	3.00	3.00	3.02	0.08	NS	NS
Lactose, %	4.70	4.71	4.70	0.11	NS	NS
SNF, %	8.60	8.62	8.63	0.15	NS	NS
Urea, mg of N/dL	16.73	17.05	16.07	1.36	NS	NS
SCC, $\times 1,000/\text{mL}$	250	234	211	80	NS	NS

NS = $P > 0.10$.

dietary contents of Na or K were much higher for cows receiving diets with ratios of Na:K of 1.06 or 0.21, compared with that for cows with a ratio of 0.53. This is in agreement with Tucker et al. (1988), who found that concentrations of Na and K in the serum were not influenced by concentrations of these minerals in the diet. However, Roche et al. (2005) observed a quadratic increase in concentration of blood serum Na and a linear decrease in the concentration of blood serum K in early lactating cows with increased concentration of Na and same concentration of K in the diets, respectively, as DCAD increased. In addition, concentrations of other coccygeal venous plasma minerals (Cl^- , Ca, and Mg) were not affected in the present experiment (Table 5).

No difference in the concentration of coccygeal venous plasma HCO_3^- was found among the dietary ratios of Na:K. This is similar to that of Wildman et al. (2007). It is known that concentration of blood HCO_3^- increases quadratically with increasing DCAD (Hu and Murphy, 2004). The lack of an effect on the concentration of coccygeal venous plasma HCO_3^- is probably because

the DCAD was held the same for these dietary treatments, supporting the allegation that the DCAD, and not the individual element of Na or K, has the most influence on the status of systemic acid-base balance (Tucker and Hogue, 1990). Similarly, the coccygeal venous plasma cation-anion difference [expressed as mEq ($\text{Na} + \text{K} - \text{Cl}$)/L] and anion gap were not affected by dietary ratios of Na:K.

Urine pH and Minerals

Urinary excretion of creatinine is relatively constant (De Groot and Aafjes, 1960; Albin and Clanton, 1966; Asai et al., 2005); thus, changes in urinary concentrations of creatinine indicated changes in urine concentration. Total urine output was not measured for cows in the present experiment. Therefore, the concentration of creatinine in the urine was used as an index to estimate excretion of minerals. Urine concentration of creatinine tended to increase linearly with increasing ratios of Na:K ($P = 0.06$); higher concentrations of creatinine in

Table 5. Least squares means of coccygeal venous plasma acid-base measures, and mineral concentrations for lactating cows fed experimental diets with 3 ratios of Na:K

Variable	Ratio of Na:K			SE	Orthogonal contrast	
	0.21	0.53	1.06		Linear	Quadratic
HCO_3^- , mEq/L	30.0	28.2	29.6	5.0	NS	NS
Creatinine, mg/dL	0.73	0.80	0.79	0.05	0.03	0.07
Na^+ , mEq/L	137.2	136.1	136.7	1.3	NS	NS
K^+ , mEq/L	4.61	4.52	4.46	0.27	NS	NS
Cl^- , mEq/L	98.2	97.2	97.1	1.9	NS	NS
Ca, mg/dL	9.99	10.11	10.08	0.31	NS	NS
Mg, mg/dL	2.55	2.42	2.50	0.15	NS	NS
PCAD, ¹ mEq/L	34.4	34.4	35.1	1.6	NS	NS
Anion gap, ² mEq/L	13.6	15.2	14.4	5.2	NS	NS

¹PCAD = coccygeal venous plasma cation-anion difference ($\text{Na} + \text{K} - \text{Cl}$).²Anion gap = coccygeal venous plasma $\text{Na}^+ + \text{K}^+ - \text{Cl}^- - \text{HCO}_3^-$.NS = $P > 0.10$.

Table 6. Least squares means of urine pH and concentrations of urine components for lactating cows fed experimental diets with 3 ratios of Na:K

Variable	Ratio of Na:K			SE	Orthogonal contrast	
	0.21	0.53	1.06		Linear	Quadratic
H ⁺ concentration, nM	4.44	3.98	3.97	0.87	NS	NS
pH	8.35	8.40	8.40	0.10		
Creatinine, mg/dL	48	59	62	14	0.06	NS
Ratio, mg:mg						
Na ⁺ :creatinine	1.80	4.21	7.42	2.23	<0.01	NS
K ⁺ :creatinine	22.4	15.9	10.3	2.3	<0.01	NS
Cl ⁻ :creatinine	4.97	4.20	3.88	1.52	NS	NS
Ca:creatinine	0.035	0.041	0.064	0.018	<0.01	NS
Mg:creatinine	0.53	0.60	0.77	0.11	<0.01	NS
Fractional clearance, %						
Na ⁺	0.35	1.09	1.83	0.54	<0.01	NS
K ⁺	92.7	72.0	46.5	10.0	<0.01	NS
Cl ⁻	1.05	0.99	0.87	0.36	NS	NS
Ca	0.25	0.32	0.50	0.13	<0.01	NS
Mg	11.9	15.2	18.2	3.0	<0.01	NS

NS = $P > 0.10$.

cows with a higher ratio of Na:K (Table 6) suggested that urine was more concentrated and, consequently, total daily urine volume was less than that for cows with the lower ratio of Na:K. The mineral load, particularly Na and K that needs to be excreted, largely determines the urine volume (Bannink et al., 1999). In the kidney, the ratio of reabsorption to filtration of K⁺ was lower than that of Na⁺, and the reduction in the ratio of reabsorption to filtration in response to increased intake was much higher for K⁺ (Maltz and Silanikove, 1996). The different volumes of urine (indicated by urinary concentration of creatinine in the present experiment) resulting from different dietary ratios of Na:K suggested that urine volume might be more closely related to the need for urinary excretion of K.

The fractional clearance of minerals is a measure of the percentage of minerals in the urine versus the minerals reabsorbed by the kidney. It is measured in terms of plasma and urine minerals, rather than by urinary mineral concentration alone, as urinary mineral concentration can vary with water resorption. Therefore, fractional clearance compares the plasma and urinary mineral concentrations to obtain a more accurate picture of renal clearance. The measured fractional clearance of minerals (Table 6) is consistent with urinary mineral concentration expressed as the ratio of mineral:creatinine, suggesting that both of them are good indicators of the renal excretion of minerals.

There was no difference in urine pH across the different ratios of Na:K (Table 6). The pH of urine proved to be a very sensitive and practical means of assessing the degree of acidification following supplementation with acidogenic salts in dairy cows (Vagnoni and Oetzel, 1998; Charbonneau et al., 2006; Hu et al., 2007). In

contrast, a relatively small increase in urine pH (from 8.01 to 8.23) occurred as DCAD increased from 22 to 47 mEq/100 g of DM for dairy cows in early lactation (Hu et al., 2007). In the present experiment, the DCAD was 33 mEq/100 g of DM across dietary treatments, and was probably the reason that urine pH was similar among treatments (Table 6), regardless of different ratios of Na:K. The unchanged pH of urine together with similar concentrations of coccygeal venous plasma HCO₃⁻, as discussed above, indicated that the acid-base status of the cows was unaffected by the different ratios of Na:K in the diets.

The ratios of Na:K were manipulated by the addition of NaHCO₃ and K₂CO₃. Adding NaHCO₃ and K₂CO₃ increased the excretion of urinary Na and K. In the present experiment (Table 6), excretion of Na⁺, as the ratio of Na⁺:creatinine ($P < 0.01$) or FC of Na⁺ ($P < 0.01$), increased linearly, whereas excretion of K⁺, as the ratio of K⁺:creatinine ($P < 0.01$) or FC of K⁺ ($P < 0.01$), decreased linearly with increasing dietary ratios of Na:K. These changes reflected their dietary contents of Na and K (Table 2).

In contrast with previous research (Wildman et al., 2007), there was an effect of the dietary ratio of Na:K on the concentration of Ca in urine in the present experiment (Table 6). Excretion of Ca, as indicated by the ratio of Ca:creatinine ($P < 0.01$) or FC of Ca ($P < 0.01$), increased linearly with increasing dietary ratios of Na:K, although no difference was observed in concentrations of coccygeal venous plasma Ca among dietary treatments. Sodium, in the form of NaCl, increases urinary excretion of Ca; however, Na consumed as a salt with a metabolizable anion such as bicarbonate appears to exert no effect on excretion of Ca (Massey

and Whiting, 1996; Heaney, 2006). Decreased excretion of Ca with a lower ratio of Na:K might be attributed to the hypocalciuric effect of supplemental K in the diet. O'Connor et al. (1988) reported that urinary excretion of Ca decreased as dietary K increased from 1.14 to 1.58% in lactating dairy cows. Moreover, the significant role of dietary K to increase renal reclamation of Ca^{2+} , and consequently reduced excretion of Ca^{2+} in urine, is well documented in humans (Morris et al., 2006). Although the total amount of Ca recovered by reducing urinary excretion of Ca is considered relatively small (Goff, 2006), reducing excretion of Ca in urine might have a biological significance for cows when the Ca demand is very high.

All diets contained the same amount of Mg, but differences in the excretion of Mg by dairy cows fed the 3 diets were observed. Excretion of Mg, as indicated by the ratio of Mg:creatinine ($P < 0.01$) or FC of Mg ($P < 0.01$), increased linearly with increasing dietary ratios of Na:K (Table 6). This is in contrast with previous research (Wildman et al., 2007) in which a quadratic response of excretion of Mg to ratio of K:Na was observed. Urinary excretion of Mg reflects changes in the absorption of Mg and is considered a more reliable index of absorption of Mg than the difference between intake of Mg and fecal excretion of Mg (Jittakhot et al., 2004). In the present experiment, urinary excretion of Mg increased with increasing dietary ratios of Na:K; thus, more Mg was probably absorbed into the body as the ratio of Na:K increased. Active transport of Mg across the rumen wall is a Na-linked transport process. Addition of Na to the diet can improve the transport of Mg across the rumen when the dietary concentration of Na is low (Goff, 2006). Khorasani and Armstrong (1990) reported that the concentration of Na in the diet per se was not an important factor affecting Mg metabolism, but the ratio of dietary Na:K correlated positively with the net absorption of Mg before the small intestine in sheep. It was shown that the addition of K to the diet of ruminants inhibits the absorption of Mg (Ram et al., 1998; Schonewille et al., 1999), and consequently, reduces the urinary excretion of Mg (Khorasani and Armstrong, 1990; Jittakhot et al., 2004). In addition, the normal range of Mg in cow plasma is 1.8 to 2.4 mg/dL (Goff, 2006). The concentration of Mg in the plasma in the present experiment (Table 5) indicated that the homeostasis of Mg was maintained in the diets of dairy cows fed these ratios of Na:K.

CONCLUSIONS

The DMI of mid-lactation cows responded quadratically to different dietary ratios of Na:K, with the lowest DMI occurring at a ratio of Na:K of 0.53 compared

with the ratios of 0.21 and 1.06. Milk yield and composition and coccygeal venous plasma concentrations of HCO_3^- , Na^+ , K^+ , Cl^- , Ca, and Mg were similar among the treatments. Changes in urinary excretion of Na^+ and K^+ were consistent with changes in their dietary contents. The excretion of Ca and Mg in the urine increased linearly with increasing dietary ratios of Na:K. The dietary ratio of Na:K did not affect milk production in mid-lactation dairy cows fed with the same DCAD of 33 mEq/100 g of DM.

ACKNOWLEDGMENTS

The authors thank Richard Morris of the University of Delaware Dairy Research Farm for care and feeding the cows. We also thank the following graduate students in our laboratory: Candice Klingerman, Renato Schmidt, and Erin McDonell for assistance in blood and urine sample collection.

REFERENCES

- Albin, R. C., and D. C. Clanton. 1966. Factors contributing to the variation in urinary creatinine and creatinine-nitrogen ratios in beef cattle. *J. Anim. Sci.* 25:107–112.
- AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Arlington, VA.
- Asai, H., N. Hayashi, N. Takai, Y. Yoshimura, Y. Nakamura, H. Yokota, and K. Kita. 2005. Estimation of daily urinary potassium excretion using urinary creatinine as an index substance in prepartum dairy cows. *Anim. Sci. J.* 76:51–54.
- Bannink, A., H. Valk, and A. M. Van Vuuren. 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. *J. Dairy Sci.* 82:1008–1018.
- Block, E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. *J. Dairy Sci.* 77:1437–1450.
- Charbonneau, E., D. Pellerin, and G. R. Oetzel. 2006. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. *J. Dairy Sci.* 89:537–548.
- De Groot, Th., and J. H. Aafjes. 1960. On the constancy of creatinine excretion in the urine of the dairy cow. *Br. Vet. J.* 116:409–418.
- Fleming, S. A., E. L. Hunt, C. Brownie, A. Rakes, and B. McDaniel. 1992. Fractional excretion of electrolytes in lactating dairy cows. *Am. J. Vet. Res.* 53:222–224.
- Goff, J. P. 2006. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. *Anim. Feed Sci. Technol.* 126:237–257.
- Heaney, R. P. 2006. Role of dietary sodium in osteoporosis. *J. Am. Coll. Nutr.* 25:271S–276S.
- Hu, W., and M. R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. *J. Dairy Sci.* 87:2222–2229.
- Hu, W., M. R. Murphy, P. D. Constable, and E. Block. 2007. Dietary cation-anion difference and dietary protein effects on performance and acid-base status of dairy cows in early lactation. *J. Dairy Sci.* 90:3355–3366.
- Igono, M. O., G. Bjotvedt, and H. T. Sanford-Crane. 1992. Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate. *Int. J. Biometeorol.* 36:77–87.
- Jittakhot, S., J. T. Schonewille, H. Wouterse, C. Yuangklang, and A. C. Beynen. 2004. Apparent magnesium absorption in dry cows fed

- at 3 levels of potassium and 2 levels of magnesium intake. *J. Dairy Sci.* 87:379–385.
- Khorasani, G. R., and D. G. Armstrong. 1990. Effect of sodium and potassium level on the absorption of magnesium and other macrominerals in sheep. *Livest. Prod. Sci.* 24:223–235.
- Maltz, E., and N. Silanikove. 1996. Kidney function and nitrogen balance of high yielding dairy cows at the onset of lactation. *J. Dairy Sci.* 79:1621–1626.
- Massey, L. K., and S. J. Whiting. 1996. Dietary salt, urinary calcium, and bone loss. *J. Bone Miner. Res.* 11:731–736.
- Morris, R. C. Jr., O. Schmidlin, L. A. Frassetto, and A. Sebastian. 2006. Relationship and interaction between sodium and potassium. *J. Am. Coll. Nutr.* 25:262S–270S.
- Murphy, M. R. 1982. Analyzing and presenting pH data. *J. Dairy Sci.* 65:161–163.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- O'Connor, A. M., D. K. Beede, and C. J. Wilcox. 1988. Lactational responses to dietary magnesium, potassium, and sodium during winter in Florida. *J. Dairy Sci.* 71:971–981.
- Ram, L., J. T. Schonewille, H. Martens, A. T. Van't Klooster, and A. C. Beynen. 1998. Magnesium absorption by wethers fed potassium bicarbonate in combination with different dietary magnesium concentrations. *J. Dairy Sci.* 81:2485–2492.
- Roche, J. R., S. Petch, and J. K. Kay. 2005. Manipulating the dietary cation-anion difference via drenching to early-lactation dairy cows grazing pasture. *J. Dairy Sci.* 88:264–276.
- Sanchez, W. K., and D. K. Beede. 1996. Is there an optimal cation-anion difference for lactation diets? *Anim. Feed Sci. Technol.* 59:3–12.
- Sanchez, W. K., D. K. Beede, and M. A. Delorenzo. 1994. Macromineral element interrelationships and lactational performance: Empirical models from a large data set. *J. Dairy Sci.* 77:3096–3110.
- SAS Institute. 2004. *SAS system software: Release 9.1 (TS1M3)*. SAS Institute Inc., Cary, NC.
- Schonewille, J. T., A. T. Van't Klooster, H. Wouterse, and A. C. Beynen. 1999. Effects of intrinsic potassium in artificially dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. *J. Dairy Sci.* 82:1824–1830.
- Tucker, W. B., G. A. Harrison, and R. W. Hemken. 1988. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. *J. Dairy Sci.* 71:346–354.
- Tucker, W. B., and J. F. Hogue. 1990. Influence of sodium chloride or potassium chloride on systemic acid-base status, milk yield, and mineral metabolism in lactating dairy cows. *J. Dairy Sci.* 73:3485–3493.
- Vagnoni, D. B., and G. R. Oetzel. 1998. Effects of dietary cation-anion difference on the acid-base status of dry cows. *J. Dairy Sci.* 81:1643–1652.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- West, J. W., K. D. Haydon, B. G. Mullinix, and T. G. Sandifer. 1992. Dietary cation-anion balance and cation source effects on production and acid-base status of heat-stressed cows. *J. Dairy Sci.* 75:2776–2786.
- Wildman, C. D., J. W. West, and J. K. Bernard. 2007. Effects of dietary cation-anion difference and potassium to sodium ratio on lactating dairy cows in hot weather. *J. Dairy Sci.* 90:970–977.