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The effect of cation source and dietary cation-anion difference on rumen ion concentrations in lactating dairy cows

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ABSTRACT

Many studies have focused on the influence of dietary cation-anion difference (DCAD) on animal performance but few have examined the effect of DCAD on the rumen ionic environment. The objective of this study was to examine the effects of DCAD, cation source (Na vs. K), and anion source (Cl vs. bicarbonate or carbonate) on rumen environment and fermentation. The study used 5 rumen-fistulated dairy cows and 5 dietary treatments that were applied using a 5 × 5 Latin square design with 2-wk experimental periods. Treatments consisted of (1) the basal total mixed ration (TMR); (2) the basal TMR plus 340 mEq/kg of Na (dry matter basis) using NaCl; (3) the basal TMR plus 340 mEq/kg of K using KCl; (4) the basal TMR plus 340 mEq/kg of Na using $NaHCO_3$; and (5) the basal TMR plus 340 mEq/kg of K using K_2CO_3. On the last day of each experimental period, rumen samples were collected and pooled from 5 different locations at 0, 1.5, 3, 4.5, 6, 9, and 12 h postfeeding for measurement of rumen pH and concentrations of strong ions and volatile fatty acids (VFA). Dietary supplementation of individual strong ions increased the corresponding rumen ion concentration. Rumen Na was decreased by 24 mEq/L when K was substituted for Na in the diet, but added dietary Na had no effect on rumen K. Rumen Cl was increased by 10 mEq/L in diets supplemented with Cl. Cation source had no effect on rumen pH or total VFA concentration. Increased DCAD increased rumen pH by 0.10 pH units and increased rumen acetate by 4 mEq/L but did not increase total VFA. This study demonstrated that rumen ion concentrations can be manipulated by dietary ion concentrations. If production and feed efficiency responses to DCAD and ionophores in the diet are affected by rumen Na and K concentrations, then manipulating dietary Na and K could be used either to enhance or diminish those responses.

Key words: dietary cation-anion difference, rumen ions, dairy cattle

INTRODUCTION

Dietary cation-anion difference is used as a tool to evaluate the strong ion (Na, K, and Cl) balance in formulation of dairy cattle diets; DCAD (mEq/kg of DM) can be calculated as Na + K – Cl (Mongin, 1981; Tucker et al., 1988) or as Na + K – Cl – S (Ender et al., 1962). Although extensive research has shown that manipulating DCAD can increase performance by improving feed intake, milk production, and acid-base status of the animal (Mongin, 1981; Tucker et al., 1988; Hu and Murphy, 2004), little research has been done to examine the effects of the individual ions that contribute to DCAD or the effect of DCAD on the rumen environment. Although the effect of diet on the rumen ion environment has been studied previously (Bailey, 1961; Bennink et al., 1978), to our knowledge, only one study has investigated the effect of DCAD on rumen ion concentrations (Tucker et al., 1988).

Increasing DCAD enhances animal performance by increasing DMI, milk yield, and feed efficiency (Tucker et al., 1988; Sanchez et al., 1994; Hu and Murphy, 2004). In addition to total DCAD, there is some evidence that cation source may affect animal performance. Iwaniuk et al. (2015) found that substitution of Na for K as the supplemental cation source in DCAD constant diets increased milk fat percentage and fat yield. In contrast, Wildman et al. (2007) and Hu and Kung (2009) found no effect of cation source on these parameters when DCAD remained constant. Unfortunately, none of these studies measured rumen ion concentrations or considered the rumen cation-anion difference (**RCAD**). Tucker et al. (1988) did measure rumen parameters when using different combinations of ions to achieve specific DCAD values but only reported the results by DCAD concentration and not by the different ion concentrations used to achieve each DCAD.

Although Na is the predominant cation in rumen fluid, Bennink et al. (1978) demonstrated that dietary Na and K could influence the respective rumen concentrations of Na and K. Shifts in rumen Na and K concentrations could potentially mediate rumen fermentation responses to ionophores. Ionophores form complexes with specific cations, and these complexes

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bind to cell walls of bacteria, resulting in extracellular Na replacing intracellular K, limiting the bacteria's ability to function and divide (McGuffey et al., 2001; Ipharraguerre and Clark, 2003). Monensin binds Na ions with 10 times the affinity that it binds K ions, whereas lasalocid binds K ions with 3 to 10 times the affinity that it binds Na ions (McGuffey et al., 2001). Newbold et al. (2013) recently demonstrated increased monensin sensitivity in rumen bacteria by increasing media Na, but reduced monensin sensitivity in media with increased K. These results support previous in vivo evidence (Rumpler et al., 1986) that showed a much greater reduction in methane production in steers fed monensin or lasalocid when supplemented with added dietary Na but not with added dietary K. Further, rumen propionate responses to lasalocid were attenuated in lambs supplemented with K (Funk et al., 1986).

The previous in vivo (Funk et al., 1986; Rumpler et al., 1986; Iwaniuk et al., 2015) and in vitro studies (Newbold et al., 2013) suggested that, in addition to DCAD concentration, cation source and changes in rumen ion concentrations might also influence rumen fermentation and production responses. Our hypothesis was that dietary strong ion concentrations and DCAD affect rumen ion concentrations. The objective of this study was to examine the effects of DCAD, cation source, and anion source on the rumen ionic environment and further examine their effects on rumen fermentation.

MATERIALS AND METHODS

Research Facilities and Animals

All procedures involving animals were carried out as approved by the Institutional Animal Care and Use Committee at the University of Maryland. Animals were housed at the Central Maryland Research and Education Center in Ellicott City, Maryland. Five rumen-fistulated, multiparous Holstein cows in late lactation (245 ± 4.5 DIM at the start of the experiment) were used in a 10-wk study that was conducted between June and August 2014. Three cows were in early pregnancy and 2 cows were not pregnant at the start of the experiment. The cows were housed in tie stalls that were fitted with water mattresses and bedded with sawdust. Water was available ad libitum through automatic waterers located in between each stall. The cows were taken out of the barn for milking twice daily, at approximately 0615 and 1530 h. Stalls were cleaned and bedded while the cows were out of the barn for milking. Fans were used to help increase air circulation and reduce temperatures in the barn. The barn photoperiod during the study was 16 h of light and 8 h of dark. Cows were individually fed a TMR once daily, at 0700 h, and had continuous access to feed except when they were turned out for milking.

Experimental Diets

Cows were fed a TMR formulated to meet or exceed the NRC (2001) requirements for cows producing 40 kg of milk per day. The TMR basal diet consisted of 57.4% corn silage and 8.4% alfalfa hay as the forages (DM basis), with the remainder of the diet consisting of ground corn, soybean meal (48% CP), and a vitamin-mineral premix using corn gluten meal as the carrier. Ingredient composition of the basal and treatment diets is shown in Table 1. The basal TMR was prepared in a Calan Data Ranger feed mixer (American Calan, Northwood, NH). Feed was dispensed into individual feed bins, with each cow receiving enough feed to generate an as-fed feed refusal rate of 2 to 4 kg/d.

Experimental treatments consisted of (1) the basal TMR (Basal); (2) the basal TMR plus 340 mEq/kg added Na (DM basis) using supplemental sodium chloride (NaCl); (3) the basal TMR plus 340 mEq/kg added K using supplemental potassium chloride (KCl); (4) the basal TMR plus 340 mEq/kg added Na using supplemental sodium bicarbonate ($NaHCO_3$); and (5) the basal TMR plus 340 mEq/kg added K using supplemental potassium carbonate (K_2CO_3). The level of supplementation, 340 mEq per kg of diet DM equivalent to 0.78% and 1.33% added Na and K, respectively, was selected to ensure a treatment response while keeping within the expected potential range of DCAD and K concentrations observed when feeding ingredients with high K and Cl concentrations such as in diets containing small grain silages and alfalfa. Treatments were applied in a 5 × 5 Latin square design with 2-wk experimental periods.

During each experimental period, there was only one cow per dietary treatment. Thus, to prevent the loss of mineral supplements during the feed mixing process, treatment mineral supplements were added to the basal TMR for each cow in their individual feeding tubs and then mixed with a pitchfork. The mineral treatments supplied an additional 342 ± 0.4 (average ± SEM) mEq/kg of diet DM of either Na or K. Diets that were supplemented with chloride received an additional 347 ± 4.1 (average ± SEM) mEq/kg of diet DM of Cl. Table 2 shows the chemical composition of the experimental diets.

Measurements

Cows were weighed after the morning milking before feeding on the last day of wk 1 and 2 of each

Table 1. The ingredient composition of the experimental diets on a DM basis

	Treatment[1]				
Item	Basal	NaCl	KCl	$NaHCO_3$	K_2CO_3
Corn silage	57.41	56.26	55.87	55.76	56.03
Alfalfa hay	8.37	8.20	8.14	8.13	8.16
Ground corn	10.87	10.66	10.58	10.56	10.61
Soybean meal	15.30	15.00	14.89	14.87	14.94
Soyplus[2]	3.41	3.35	3.33	3.32	3.34
Corn gluten meal	0.60	0.59	0.59	0.59	0.59
Dynamate[3]	0.13	0.13	0.13	0.13	0.13
Biophos[4]	0.43	0.42	0.42	0.42	0.42
Limestone[5]	0.61	0.59	0.59	0.59	0.59
Magnesium oxide	0.34	0.33	0.33	0.33	0.33
ADE mix[6]	0.03	0.03	0.03	0.03	0.03
Vitamin E[7]	0.01	0.01	0.01	0.01	0.01
Megalac[8]	1.46	1.43	1.42	1.42	1.43
Selenium[9]	0.06	0.06	0.06	0.06	0.06
Omigen-AF[10]	0.20	0.19	0.19	0.19	0.19
TM-433[11]	0.03	0.03	0.03	0.03	0.03
4-Plex C[12]	0.01	0.01	0.01	0.01	0.01
Diamond V XP[13]	0.24	0.23	0.23	0.23	0.23
NaCl[14]	0.49	2.48	0.47	0.47	0.48
KCl	0.00	0.00	2.68	0.00	0.00
$NaHCO_3$	0.00	0.00	0.00	2.87	0.00
K_2CO_3	0.00	0.00	0.00	0.00	2.39

[1]The basal diet was supplemented with the indicated salt for the treatment diets. The NaCl diet contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet contained an additional 341 mEq/kg of Na. The K_2CO_3 diet contained an additional 343 mEq/kg of K.

[2]West Central Cooperative (Ralston, IA).

[3]Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN).

[4]Contained 17% Ca and 21% P.

[5]Contained 36% Ca and 0.02% P.

[6]Contained 5,454,545 IU/kg of vitamin A, 1,818,182 IU/kg of vitamin D, 9,091 IU/kg of vitamin E.

[7]Contained 56,818 IU/kg of vitamin E.

[8]Contained 9% Ca; 84.5% fat (Church & Dwight Co. Inc., Piscataway, NJ).

[9]Contained 600 mg Se/kg, 28% Ca.

[10]Contained 0.41 mg/kg of biotin, 15 mg/kg of choline, 31 mg/kg of D-pantothenic acid, 1.4 mg/kg of folic acid, 3.2 mg/kg of menadione, 102 mg/kg of niacin, 30 mg/kg of riboflavin, 4.5×10^{10} cfu/kg *Saccharomyces cerevisiae*, 15 mg/kg of thiamine, 8.2 mg/kg of vitamin B_6, and 41 μg/kg of vitamin B_{12} (Prince Agri Products, Inc., Quincy, IL).

[11]Contained 0.16% Co, 4.0% Cu, 3.0% Fe, 0.35% I, 15% Mn, and 16% Zn (Southern States Cooperative Inc., Richmond, VA).

[12]Contained 0.36% Co, 1.80% Cu, 2.86% Mn, and 5.15% Zn (Zinpro Corporation, Eden Prairie, MN).

[13]Contained *Saccharomyces cerevisiae* yeast (Diamond V, Cedar Rapids, IA).

[14]Additional salt was given to cows when receiving the NaCl treatment.

experimental period. Individual milk production was recorded electronically at each milking. Milk samples were collected during the last 6 milkings of each experimental period and were analyzed for milk fat, protein, SCC, other solids (lactose and minerals), and MUN at the Lancaster DHIA (Lancaster, PA). The amounts of feed offered and refused (weighed just before the next feeding) was measured once daily for each cow.

Samples of individual feed ingredients including corn silage, alfalfa hay, ground corn, soybean meal, and the vitamin mineral premix were collected weekly, pooled together by ingredient for the entire study, and sent to Cumberland Valley Analytical Service (Hagerstown, MD) for analysis of DM, CP, ADF, NDF, lignin and ether extract. Feed Ca, P, Mg, Na, and K were determined by inductively coupled plasma emission spectroscopy, Cl by potentiometric titration with silver nitrate, and S using a Leco S632 Sulfur Combustion Analyzer as reported in the feed laboratory procedures (Cumberland Valley Analytical Services, 2016). Corn silage DM was measured weekly using a Koster Tester (Koster Moisture Tester Inc., Brunswick, OH) to adjust the as-fed composition of the TMR to maintain a constant ingredient composition on a DM basis.

Rumen samples were collected on the last day of each period. Samples were collected at 0, 1.5, 3, 4.5, 6, 9, and

Table 2. The chemical composition of the experimental diets on a DM basis

	Treatment[1]					
Item	Basal	NaCl	KCl	$NaHCO_3$	K_2CO_3	SEM
DM, %	42.8	43.3	43.5	43.5	43.3	0.13
NE_L, Mcal/kg	1.63	1.59	1.58	1.58	1.59	0.01
CP, %	16.3	16.0	15.9	15.8	15.9	0.08
NDF, %	28.1	27.5	27.3	27.3	27.4	0.15
ADF, %	20.5	20.0	19.9	19.9	20.0	0.11
Lignin, %	3.51	3.44	3.42	3.41	3.43	0.02
Ash, %	7.53	9.38	10.01	10.2	9.74	0.479
Fat,[2] %	2.62	2.56	2.55	2.54	2.55	0.014
Na, %	0.25	1.03	0.24	1.03	0.24	0.192
K, %	1.56	1.53	2.85	1.51	2.86	0.325
Cl, %	0.57	1.77	1.80	0.55	0.56	0.300
S, %	0.23	0.22	0.22	0.22	0.22	0.001
Ca, %	0.91	0.89	0.89	0.88	0.89	0.005
P, %	0.53	0.52	0.51	0.51	0.51	0.003
Mg, %	0.39	0.38	0.38	0.38	0.38	0.002
DCAD,[3] mEq/kg	346	339	329	677	681	83.6
DCAD-S,[4] mEq/kg	204	199	190	538	541	83.7

[1]The basal diet was supplemented with the indicated salt for the treatment diets. The NaCl diet contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet contained an additional 341 mEq/kg of Na. The K_2CO_3 diet contained an additional 343 mEq/kg of K.

[2]Crude fat, which does not account for the 1.23% fatty acids provided by Megalac (Arm and Hammer Animal Nutrition, Piscataway, NJ) in the basal diet (84.5% of 1.46% diet DM) and the 1.20% and 1.21% fatty acids provided in the treatment diets (84.5% of 1.42% and 1.43% diet DM).

[3]DCAD = Na + K – Cl.

[4]DCAD-S = Na + K – Cl – S.

12 h postfeeding, with time 0 being just before feeding. Rumen fluid was collected in a 60-mL syringe using a 50-cm stainless steel tube with a mesh strainer at the end (RT Rumen Fluid Sampler Tube, Bar Diamond Inc., Parma, ID). Ten milliliters of fluid was collected and pooled from 5 different locations: the atrial, dorsal, ventral, caudodorsal, and caudoventral areas of the rumen, which gave a total of 50 mL of fluid collected at each sampling time.

The rumen fluid pH was measured immediately after sampling with a pH probe. A 10-mL aliquot was added to a 15-mL polypropylene Falcon tube containing 0.2 mL of 50% sulfuric acid and frozen at −20°C for later analysis of VFA. The remaining 40 mL of rumen fluid was frozen for later analysis of ion concentrations. Rumen fluid VFA concentrations were determined using gas-liquid chromatography. The frozen samples were thawed in a water bath at 34°C and then centrifuged at 2,100 × *g* for 30 min to clarify the rumen fluid. Samples were prepared by adding 0.7 mL of the supernatant to 0.3 mL of a phosphate buffer that contained 20 mEq/L 2-ethylbutyrate as an internal standard. A standard solution containing 80 mEq/L acetate, 35 mEq/L propionate, 5 mEq/L isobutyrate, 10 mEq/L butyrate, 5 mEq/L isovalerate, and 5 mEq/L valerate was used to determine the response factors of the various VFA. The standard was prepared for analysis by adding 0.7 mL to 0.3 mL of the phosphate buffer with 2-ethylbutyrate. Prepared samples were analyzed by a gas chromatograph (Agilent 6890, Agilent Technologies, Santa Clara, CA) with helium as the carrier gas, a flow rate of 49.1 mL/min, and a column temperature of 130°C. The ratio of the 2-ethylbutyrate concentration within each sample was compared with an internal standard to correct for variation in injection volume.

Rumen ion concentrations were determined using flame atomic absorption spectrometry (5100 PC, PerkinElmer, Norwalk, CT). Frozen rumen fluid samples were thawed in a water bath at 34°C and then centrifuged at 2,100 × *g* for 30 min with the supernatant used for mineral analyses. The protocols for measuring ion concentrations were adapted from procedures described by PerkinElmer (1996) and Agilent Technologies (2015). Sodium and potassium were directly measured from rumen fluid that had been diluted with deionized water 3,333 times to be within the linear range of absorption for the ion lamp used. Sodium concentrations were measured using a wavelength of 589.0 nm and a slit width of 0.4 nm. Potassium concentrations were measured using a wavelength of 766.5 nm and a slit width of 0.4 nm. Rumen chloride concentrations were measured indirectly using silver chloride precipitation. In this procedure, 2.5 mL of rumen fluid, 1 mL of HNO_3, 5 mL of 0.046 *M* $AgNO_3$, and 41.5 mL of deionized water

were combined, mixed, and then centrifuged at 1,303 × *g* for 10 min. The supernatant containing unprecipitated Ag was analyzed for Ag by atomic absorption using a wavelength of 338.3 nm and a slit width of 0.7 nm. The chloride concentration was then calculated by the following equation (PerkinElmer, 1996):

$$\text{Cl, mg/L} = [500 - (100 \times \text{Ag, mg/L})] \times 6.58 \times \text{dilution factor.} \quad [1]$$

All mineral concentrations in rumen fluid were converted to milliequivalents per liter by dividing by their respective atomic mass. Finally, the RCAD in rumen fluid was calculated using the following equation:

$$\text{RCAD (mEq/L)} = \text{Na} + \text{K} - \text{Cl}. \quad [2]$$

Data Summarization and Statistical Analysis

Measurements collected during the last 3 d of each experimental period were used to calculate DMI, milk production, milk composition and component yields, and feed efficiency for each cow. Milk fat, protein, and other solids yields were calculated as the individual component concentration multiplied by its respective milk yield at each milking to calculate a mean daily yield for each cow. The 3.5% FCM was determined as described by Erdman (2011) using the following equation:

$$\text{3.5\% FCM, kg/d} = [0.4318 \times \text{milk (kg/d)}] + [16.23 \times \text{milk fat (kg/d)}]. \quad [3]$$

Feed efficiency was calculated as 3.5% FCM divided by DMI.

Dry matter intake, milk production, milk composition, and feed efficiency data were summarized by cow within each experimental period and analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC) using a statistical model that included fixed effects of cow, period, and treatment, with cow within treatment serving as the experimental unit. A repeated-measures design with compound symmetry structure was used for the analysis of rumen pH, ion, and VFA concentrations with a model that included the effects of cow, treatment, period, cow within treatment, time postfeeding, and the interaction of time postfeeding and treatment. Cow within treatment and period were considered as random effects and used to test the effects of treatment, whereas time postfeeding and the time postfeeding by treatment interaction were tested using residual error. Four a priori comparisons were used to compare the effects of (1) cation source (NaCl and $NaHCO_3$ vs. KCl and K_2CO_3); (2) anion source (NaCl and KCl vs. $NaHCO_3$ and K_2CO_3); (3) DCAD (Basal, NaCl, and KCl vs. $NaHCO_3$ and K_2CO_3); and (4) Basal vs. NaCl and KCl using the contrast statement in the MIXED procedure.

A probability of $P < 0.05$ was considered statistically significant, and a probability of $0.05 < P < 0.10$ was considered to be a trend. Data from one cow receiving the $NaHCO_3$ treatment during the first period was omitted for that period because she developed a severe case of mastitis. However, the cow subsequently recovered and her data were used for the remainder of the experiment. Two of the samples used for VFA analysis, one for the NaCl treatment and one for the $NaHCO_3$ treatment, were excluded from the data analysis because they had outlier values for several of the VFA. To account for these missing data points, least squares means are provided, and the highest SEM is reported.

RESULTS

The chemical composition of the diets is presented in Table 2. The diets had similar nutrient composition but differed in Na, K, and Cl, which were manipulated by treatment. The experimental diets supplied an additional 342 ± 0.4 (mean ± SEM) mEq of Na or K/kg of diet DM compared with the basal diet. The NaCl and KCl diets were supplied with an additional 347 ± 4.1 (mean ± SEM) mEq of Cl/kg of diet DM compared with the other diets. The DCAD, calculated as Na + K – Cl, was 346, 339, 329, 677, and 681 mEq/kg of diet DM for the basal, NaCl, KCl, $NaHCO_3$, and K_2CO_3 diets, respectively. The corresponding DCAD-S, calculated as Na + K – Cl – S, were ~140 mEq/kg lower, as dietary S was 0.22%.

Due to the small number of cows and the relatively short (2-wk) experimental periods, the study was not designed to measure production responses. Therefore, detailed information on animal performance is only available in Supplemental Table S1 (http://dx.doi.org/10.3168/jds.2016-10853). We detected no treatment effects on DMI, milk production, FCM, fat yield, protein percentage and yield, other solids (lactose and minerals) percentage and yield, SCC (linear score), MUN, and feed efficiency, which averaged 23.8 kg/d, 31.2 kg/d, 31.0 kg/d, 1,125 g/d, 3.07%, 945 g/d, 5.52%, 1,726 g/d, 3.42, 14.3 mg/dL, and 1.30, respectively. Milk fat percentage was increased by 0.15 percentage units with increased DCAD ($P = 0.044$) in cows fed the $NaHCO_3$ and K_2CO_3 treatments.

The mean BW was 695 kg (SEM = 4.2). Anion source ($P = 0.001$) and DCAD ($P = 0.002$) had significant ef-

fects on mean BW. Cows fed the chloride diets weighed, on average, 17 kg more than when they were fed the carbonate diets, whereas Na increased BW by 5 kg. Because of the short experimental periods, we interpret these changes as changes in rumen volume and perhaps overall gut fill rather than true body tissue gains and losses.

Table 3 shows the effect of treatment on rumen pH, ion concentrations, and VFA concentrations. Rumen pH decreased with time postfeeding ($P = 0.001$; Figure 1). Mean rumen pH was 0.11 units higher in cows receiving the high DCAD diets ($P = 0.026$). Cation source (Na vs. K) affected rumen Na and K concentrations ($P = 0.001$; Table 3). Rumen Na concentrations were 11 mEq/L greater when cows were fed diets containing added Na compared with the basal diet ($P \leq 0.002$) and 24 mEq/L higher than when fed diets containing added K ($P = 0.001$). Rumen Na declined during the first 3 h postfeeding ($P = 0.001$; Figure 2A), and there was a trend for a greater decline in the cows fed added K (treatment by time postfeeding; $P = 0.091$).

Rumen K concentrations were, on average, 19.0 mEq/L higher in cows fed added K (cation; $P = 0.001$).

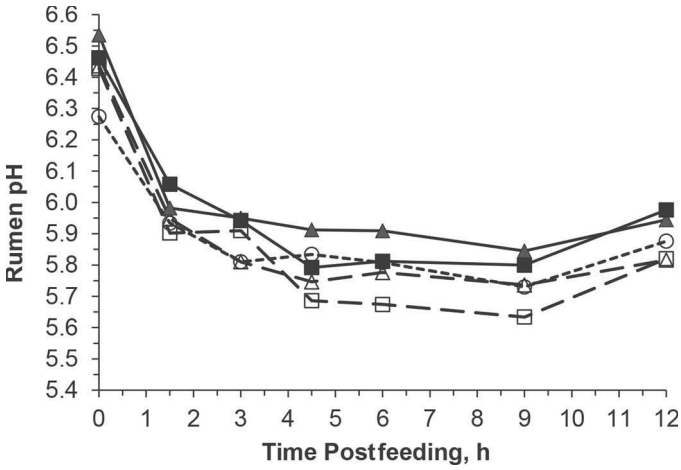

Figure 1. Time postfeeding ($P = 0.001$) and treatment by time postfeeding ($P = 0.940$) effects on rumen pH (SEM = 0.088). The basal diet (---○---) was supplemented with the indicated salt for the treatment diets. The NaCl diet (– – Δ – –) contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet (– –□– –) contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet (—▲—) contained an additional 341 mEq/kg of Na. The K_2CO_3 diet (—■—) contained an additional 343 mEq/kg of K.

Table 3. Treatment effects on rumen pH, strong ion, and VFA concentrations

	Treatment[1]						*P*-value			
Item	Basal	NaCl	KCl	$NaHCO_3$	K_2CO_3	SEM	Cation[2]	Anion[3]	DCAD[4]	Basal vs. Cl[5]
No. of cows	5	5	5	4	5					
No. of samples	35	35[6]	35	28[7]	35					
Rumen pH	5.90	5.90	5.86	6.01	5.98	0.052	0.499	0.030	0.026	0.785
Rumen ions (mEq/L)										
Na	83.8	93.1	71.8	96.3	69.5	1.98	0.001	0.797	0.999	0.510
K	27.0	25.5	47.9	26.0	42.5	1.46	0.001	0.091	0.509	0.001
Cl	8.16	18.2	23.0	11.2	10.6	1.43	0.126	0.001	0.001	0.001
RCAD[8]	103	100	97	111	101	2.4	0.010	0.004	0.009	0.125
Na:K ratio	3.36	3.97	1.63	3.95	1.79	0.154	0.001	0.608	0.376	0.005
Rumen VFA (mEq/L)										
Acetate (A)	71.5	70.5	73.3	75.0	76.9	1.91	0.187	0.035	0.020	0.833
Propionate (P)	24.3	22.4	23.4	21.7	23.4	1.12	0.192	0.731	0.381	0.234
Isobutyrate	1.04	0.99	1.00	1.02	1.07	0.033	0.381	0.138	0.239	0.262
Butyrate	10.8	10.5	10.8	10.8	11.2	0.42	0.378	0.358	0.396	0.712
Isovalerate	1.94	1.89	1.82	2.21	1.89	0.172	0.232	0.217	0.248	0.639
Valerate	1.54	1.49	1.45	1.46	1.60	0.063	0.362	0.265	0.457	0.252
Total VFA	111	108	112	112	116	3.1	0.178	0.141	0.151	0.674
A:P	3.06	3.25	3.28	3.49	3.32	0.120	0.518	0.222	0.058	0.119

[1]The basal diet was supplemented with the indicated salt for the treatment diets. The NaCl diet contained (dry matter basis) an additional 343 mEq/kg of Na and Cl. The KCl diet contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet contained an additional 341 mEq/kg of Na. The K_2CO_3 diet contained an additional 343 mEq/kg of K.

[2]Comparison of added dietary Na (NaCl and $NaHCO_3$) vs. added dietary K (KCl and K_2CO_3).

[3]Comparison of added dietary Cl (NaCl and KCl) with added dietary carbonate and bicarbonate ($NaHCO_3$ and K_2CO_3).

[4]Comparison of dietary DCAD effects (Basal, NaCl, and KCl vs. $NaHCO_3$ and K_2CO_3).

[5]Comparison of Basal vs. NaCl and KCl.

[6]Thirty-five samples were used for the analysis of rumen ion concentrations. Thirty-four samples were used for the analysis of VFA concentrations.

[7]Twenty-eight samples were used for the analysis of rumen ion concentrations. Twenty-seven samples were used for the analysis of VFA concentrations.

[8]Rumen cation-anion difference: Na + K – Cl (mEq/L).

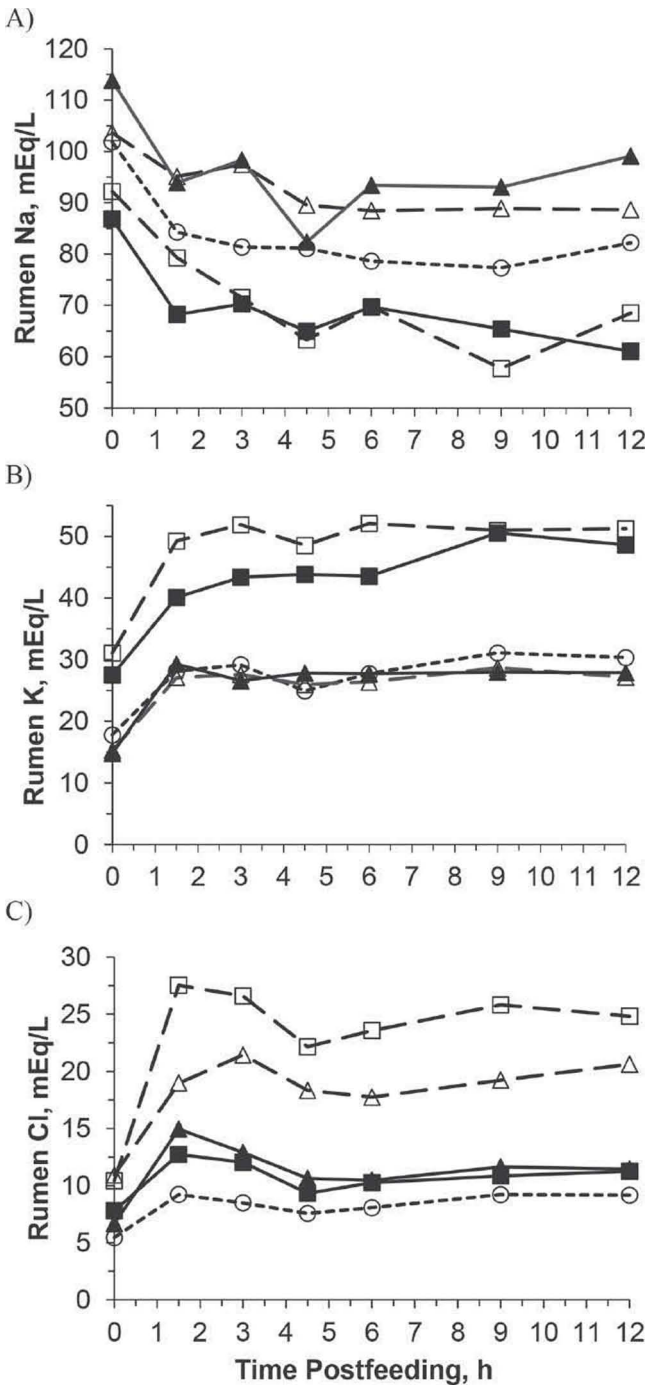

Figure 2. Time postfeeding effects on rumen strong ion concentrations. The basal diet (---○---) was supplemented with the indicated salt for the treatment diets. The NaCl diet (– – Δ – –) contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet (– –□– –) contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet (—▲—) contained an additional 341 mEq/kg of Na. The K_2CO_3 diet (—■—) contained an additional 343 mEq/kg of K. (A) Rumen Na: time postfeeding ($P = 0.001$); treatment by time postfeeding interaction ($P = 0.091$); SEM = 3.88. (B) Rumen K: time postfeeding ($P = 0.001$); treatment by time postfeeding interaction ($P = 0.340$); SEM = 2.60. (C) Rumen Cl: time postfeeding ($P = 0.001$); treatment by time postfeeding interaction ($P = 0.260$); SEM = 2.36.

In contrast to the decline in rumen Na with time postfeeding, rumen K increased with time postfeeding ($P = 0.001$) and there was a trend for the effects of anion source ($P = 0.091$), where added dietary Cl reduced rumen K (Figure 2B). Rumen Cl concentrations increased ($P = 0.001$) nearly 2-fold immediately after feeding and then remained nearly constant thereafter during the 12-h sampling period (Figure 2C). The absolute magnitude of the increase was greatest in the cows fed diets with added Cl (anion source; $P = 0.001$), where cows fed added Cl had an 11 mEq/L increase in rumen Cl (Table 3).

The combination of reduced rumen Na and increased rumen K with time postfeeding resulted in a decline in rumen Na:K ratio with time postfeeding ($P = 0.001$; Figure 3A). The Na:K ratio was on average 2.3-fold higher in cows fed diets supplemented with Na compared with those supplemented with K ($P = 0.001$). A plot of the raw data for rumen K versus Na (Figure 3B) showed an inverse relationship between rumen Na and K, where a 1 mEq/L increase in rumen Na corresponded to a 0.53 mEq/L decrease in rumen K.

Changes in RCAD followed changes in dietary DCAD, where increased DCAD increased RCAD ($P = 0.009$; Table 3). As expected, added dietary Cl reduced RCAD ($P = 0.004$); RCAD decreased with time postfeeding ($P = 0.001$; data not presented).

Acetate was the only rumen VFA affected by dietary treatment (Table 3). Acetate averaged 4.2 mEq/L higher ($P = 0.020$) in cows fed the high DCAD diets ($NaHCO_3$ and K_2CO_3). Rumen acetate concentrations increased rapidly after feeding ($P = 0.001$) and remained elevated thereafter. There was no effect of treatment on the remaining rumen VFA. We detected time effects for propionate ($P = 0.002$), isobutyrate ($P = 0.001$), butyrate ($P = 0.001$), and valerate ($P = 0.001$), where concentrations increased immediately after feeding. There was a trend for a DCAD effect in rumen acetate-to-propionate ratio ($P = 0.058$) but no time postfeeding effect. Total VFA concentrations (Figure 4) were affected by time ($P = 0.001$) but not by treatment ($P = 0.332$; Table 3).

DISCUSSION

The results of this study support the hypothesis that dietary ion concentrations affect rumen ion concentrations. Rumen Na concentrations increased with increased dietary Na irrespective of the source of added Na (NaCl vs. $NaHCO_3$). Previously, Bailey (1961) stated that salivary Na had a greater effect on rumen Na than dietary Na, but that conclusion was based on the composition of Na in saliva versus feed and not actual flows of Na from saliva versus feed entry. Bailey and Balch

(1961) found that types of feeds in the diet did not have a significant influence on salivary Na concentrations. We did not measure saliva Na in this experiment, but the present study clearly demonstrated that dietary Na has an effect on rumen Na concentration. Compared with the basal diet, Na addition increased rumen Na by 11 mEq/L. We also showed that substituting dietary K for Na resulted in a 24 mEq/L or a 25% decrease in rumen Na concentration. The decrease in rumen Na concentrations postfeeding might be explained by increased Na absorption. Stacy and Warner (1966) found that Na absorption out of the rumen increased after feeding, and they concluded that the increased absorption was the response to increased osmotic pressure in the rumen. In addition, Warner and Stacy (1972) showed that increasing Na or K concentrations within the rumen increased the rate of sodium absorption.

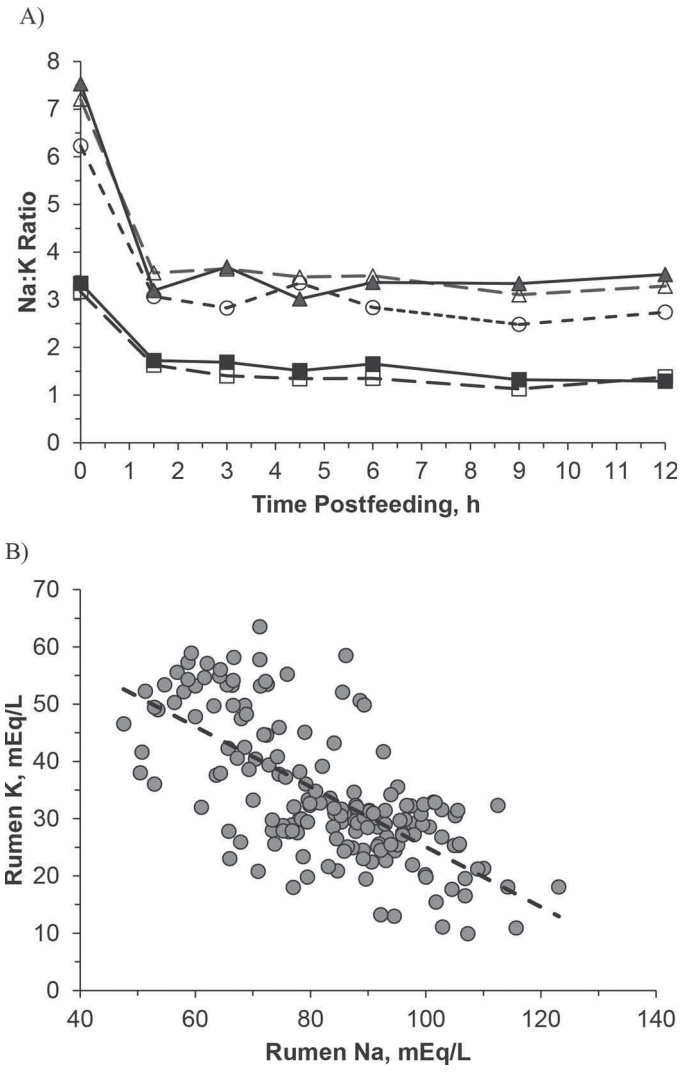

Figure 3. Time postfeeding effects on rumen Na:K ratio (mEq/L) and the relationship between rumen Na and K. A) Time postfeeding effects ($P = 0.001$) and treatment by time postfeeding interaction ($P = 0.011$; SEM = 0.360). The basal diet (---○---) was supplemented with the indicated salt for the treatment diets. The NaCl diet (– – Δ – –) contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet (– –□– –) contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet (—▲—) contained an additional 341 mEq/kg of Na. The K_2CO_3 diet (—■—) contained an additional 343 mEq/kg of K. (B) Negative correlation between rumen Na and K concentrations across sampling times, where rumen K (mEq/L) = 0.525 × rumen Na (mEq/L) + 77.6; intercept ($P = 0.001$); intercept SE = 3.63; slope ($P = 0.001$); slope SE = 0.043; $R^2 = 0.470$; root mean square error = 8.76.

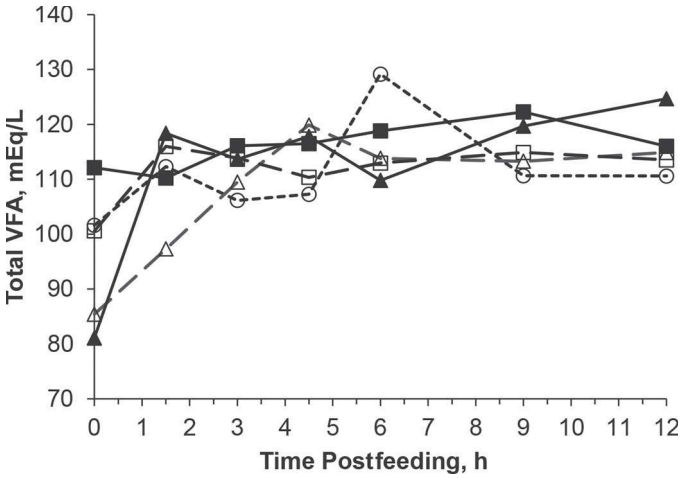

Figure 4. Time postfeeding ($P = 0.001$) and treatment by time postfeeding ($P = 0.144$) effects on rumen total VFA concentration (mEq/L). Time effects were significant ($P = 0.001$), but there were no time by treatment interactions ($P = 0.144$; SEM = 8.01). The basal diet (---○---) was supplemented with the indicated salt for the treatment diets. The NaCl diet (– – Δ – –) contained (DM basis) an additional 343 mEq/kg of Na and Cl. The KCl diet (– –□– –) contained an additional 343 mEq/kg of K and 351 mEq/kg of Cl. The $NaHCO_3$ diet (—▲—) contained an additional 341 mEq/kg of Na. The K_2CO_3 diet (—■—) contained an additional 343 mEq/kg of K.

Adding dietary K increased rumen K by 19 mEq/L demonstrating that diet K concentration alters rumen K. Several studies have shown that dietary K influences rumen K concentration (Bailey, 1961; Bennink et al., 1978). Bailey (1961) found that salivary K was always lower than rumen K concentration but there was a correlation between salivary K and rumen K. In contrast to dietary K addition, which reduced rumen Na compared with the basal diet, addition of dietary Na had no effect on rumen K. Perhaps K absorption across the rumen wall is much slower and therefore Na absorption across the rumen wall is increased to maintain osmotic pressure and electrochemical neutrality.

In the present study, rumen K increased within the first 1.5 h after feeding with a greater time postfeeding effect in cows fed added K. Bennink et al. (1978) did not show clear time postfeeding effects on rumen K concentrations. However, in their experiments, dietary K was altered by selection of feed ingredients that var-

ied in K content rather than by direct supplementation of K, where K from supplements might be solubilized more rapidly than inherent K within feed ingredients. Tucker et al. (1988) found that DCAD did not affect rumen Na or K concentrations, but they did note an inverse relationship between the 2 ions in the rumen. Other studies have also discussed the inverse relationship between rumen Na and K concentrations (Poutiainen, 1968; Warner and Stacy, 1972). Although the basal, KCl, and K_2CO_3 diets contained similar amounts of Na, the basal diet had 13.2 mEq/L higher rumen Na concentrations than the K diets.

In our study, rumen Cl increased with increased dietary Cl. Bennink et al. (1978) found that dietary Cl did not directly influence rumen Cl concentrations, but again, this was by selecting feeds with high Cl rather than using direct supplementation of NaCl and KCl that we used. The alfalfa pellet diet used in the Bennink et al. (1978) study had the highest dietary Cl concentration but resulted in the lowest rumen Cl concentration. Bennink et al. (1978) suggested that this could have been due to differences in the elution rate of ions from the different types of feeds. Alternatively, alfalfa also has high K content so there could have been confounding effects of dietary Cl and K. In contrast, Bailey (1961) reported that dietary Cl concentrations did affect rumen Cl concentrations, with dietary concentrations having more influence than saliva on rumen Cl. Cows had similar rumen Cl concentrations when they were fed the same diets, and diets with higher Cl concentrations tended to have higher rumen Cl concentrations (Bailey, 1961).

Although the data from this study suggest a significant DCAD effect on rumen Cl concentrations, it is likely that the effect was the result of the increased Cl in the lower DCAD diets (NaCl and KCl) and not the actual DCAD itself. The basal diet produced rumen Cl concentrations similar to that of the higher DCAD diets. Tucker et al. (1988) found that increased DCAD tended to decrease rumen Cl concentrations; however, the present study contradicts those results. The discrepancy between the 2 studies could be the result of the different ways DCAD was manipulated. Tucker et al. (1988) altered the DCAD using different combinations of Na, K, and Cl for each specific DCAD that they studied. Unfortunately, they pooled results for the rumen ion concentrations by DCAD instead of reporting them for the 3 different dietary combinations that they used to achieve each DCAD, so direct dietary comparison with their rumen Cl concentrations is not possible (Tucker et al., 1988).

The increase in rumen pH with increased DCAD was consistent with the predicted response from a meta-analysis of DCAD effects (Iwaniuk and Erdman, 2015). In that analysis, an increase in DCAD of 1 mEq/kg of diet DM was predicted to result in a 0.0003-unit increase in rumen pH. In the current study, addition of K_2CO_3 and $NaHCO_3$ increased DCAD by 345 mEq/kg and would have been predicted to increase rumen pH by 0.1035 units, which is nearly identical to the measured change of 0.108 pH units.

In this study, cation source did not affect rumen pH. A literature review by Erdman (1988) discussed the benefits of using buffers such as $NaHCO_3$ and K_2CO_3 to reduce the decrease in rumen pH associated with time postfeeding. When Vagnoni and Oetzel (1998) used anionic salts to decrease DCAD, they found that rumen pH also decreased. Similarly, Tucker et al. (1988) reported that rumen pH was lower when using a negative DCAD than when using a neutral or positive DCAD.

In this study, rumen VFA concentration was not affected by cation source or DCAD. Similarly, Apper-Bossard et al. (2010) reported no significant changes in VFA concentration with altered DCAD. Although rumen acetate was 4.2 mEq/L higher with the higher DCAD diets, there were no differences for the other VFA or total VFA concentration. Tucker et al. (1988) only noted a tendency for decreased total VFA concentrations and increased isovalerate concentrations with increased DCAD.

Changes in dietary Na and K were clearly shown to affect their respective rumen ion concentrations, especially when dietary K was increased, which resulted in a 19.0 mEq/L increase in rumen K and a 24 mEq/L decrease in rumen Na. Increasing dietary K by 340 mEq/kg of DM reduced the rumen Na:K ratio from 3.96:1 to 1.71:1 such that Na was reduced from 80 to 63% of the total strong ion concentration in the rumen. Hypothetically, such a shift in rumen Na and K concentrations might be sufficient to mediate the effects of ionophores on rumen fermentation. Monensin preferentially binds Na ions over K ions (McGuffey et al., 2001). Although lasalocid preferentially binds K ions, it can also bind Na ions (McGuffey et al., 2001).

Using pure cultures of *Eubacterium ruminantium* 2388, *Streptococcus bovis* C277, *Lactobacillus casei* LB17, and *Prevotella albensis* M384, Newbold et al. (2013) demonstrated increased monensin sensitivity in rumen bacteria by increasing media Na from 132 to 177 mEq/L. Conversely, increasing media K concentrations from 19 to 35 mEq/L decreased monensin sensitivity in these same species. Although even the lowest Na concentrations used by Newbold et al. (2013) were greater than the rumen Na concentrations observed in the present study, the K concentrations were not dissimilar from the range (26 to 48 mEq/L) measured herein. However, measurement of differences in monensin sensitivity in pure culture to varying Na and K media concentrations

is quite different from determination of their potential in vivo responses. Feeding experiments with varying dietary Na and K would be required to determine if there are potential mediating effects of strong ion sources on animal performance in responses to monensin feeding. This experiment demonstrated only that Na, K, and Cl, the major strong ions in the rumen, could be modulated by varying the dietary concentrations of those ions.

Finally, it is likely that added mineral supplements increased water intake, but we did not measure water intake. Regression equations developed by Murphy et al. (1983) suggested a 0.05 kg/d increase in water intake per gram increase in sodium intake. Spek et al. (2012) reported a 44 kg/d increase in water intake as Na intake increased from 67 to 417 g/d. The mean Na intakes were 59, 247, 58, 247, and 56 g/d and K intakes were 366 367, 687, 362, and 672 g/d in the Basal, NaCl, KCl, $NaHCO_3$, and K_2CO_3 treatments, respectively. Predicted urine output based on Na and K intake (Bannink et al., 1999) would be 28, 51, 47, 50, and 46 kg/d for the respective treatments. However, it is uncertain how changes in water intake would affect rumen ion concentrations because concentrations would depend on a variety of factors, including rumen water balance, rumen ion absorption and influx across the rumen wall, rates of liquid passage from the rumen, and salivary flow in addition to feed ion concentrations.

CONCLUSIONS

This study demonstrated that manipulation of dietary strong ion concentrations can alter rumen ion concentrations. Increasing dietary concentrations of Na, K, and Cl increased their respective rumen ion concentrations, and we detected an inverse relationship between rumen Na and K concentrations. Increasing DCAD by substitution of bicarbonate and carbonate salts for Cl salts of Na and K had a respective effect on RCAD and the balance between Na, K, and Cl ion concentrations in the rumen. Therefore, if production and feed efficiency responses to DCAD and potentially ionophores in the diet are affected by rumen Na and K concentrations, then manipulating dietary Na and K could be used to either enhance or diminish those responses.

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