

## ABSTRACT

Title of Document: THE IMPACT OF DIETARY IONS ON RUMEN ION CONCENTRATIONS

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Dietary cation-anion difference (DCAD) evaluates the strong ion balance in dairy cattle diets. We performed two experiments to study the impacts of strong dietary ions (Na, K, and Cl) on the rumen environment. Our overall hypothesis was that dietary strong ions would influence their corresponding rumen concentrations. Our objective was to determine the impact of the dietary strong ions on the rumen environment. In the first experiment, five fistulated dairy cows consumed the following treatments: (1) a basal diet composed of corn silage and alfalfa hay and then the basal diet supplemented with (2) NaCl, (3) KCl, (4) NaHCO<sub>3</sub>, and (5) K<sub>2</sub>CO<sub>3</sub>. Rumen samples were collected 0, 1.5, 3, 4.5, 6, 9, and 12 h post-feeding to measure ion concentrations, pH, and volatile fatty acid concentrations. In the second experiment, we conducted a meta-analysis of published literature that measured rumen strong ion concentrations.

THE IMPACT OF DIETARY IONS ON RUMEN ION CONCENTRATIONS

By

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## **Dedication**

To Spike and all of the other ruminants I have had the pleasure of knowing,  
especially the lovely cows that made this thesis possible

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## List of Abbreviations

<b>A:P</b>	acetate to propionate ratio
<b>Ag</b>	silver
<b>AgNO<sub>3</sub></b>	silver nitrate
<b>BW</b>	body weight
<b>CaCl<sub>2</sub></b>	calcium chloride
<b>Cl</b>	chloride
<b>cm</b>	centimeter
<b>DCAD</b>	dietary cation-anion difference
<b>DCAD-S</b>	dietary cation-anion difference when considering sulfur
<b>DM</b>	dry matter
<b>DMI</b>	dry matter intake
<b>DHIA</b>	Dairy Herd Improvement Association
<b>FCM</b>	fat corrected milk
<b>HNO<sub>3</sub></b>	nitric acid
<b>K</b>	potassium
<b>K<sub>2</sub>CO<sub>3</sub></b>	potassium carbonate
<b>KCl</b>	potassium chloride
<b>KHCO<sub>3</sub></b>	potassium bicarbonate
<b>L</b>	liter
<b>M</b>	molar
<b>mOsm</b>	milliosmole

<b>mV</b>	millivolt
<b>mEq</b>	milliequivalent
<b>MUN</b>	milk urea nitrogen
<b>n</b>	number of observations
<b>Na</b>	sodium
<b>NaCl</b>	sodium chloride
<b>NaHCO<sub>3</sub></b>	sodium bicarbonate
<b>NH<sub>4</sub>Cl</b>	ammonium chloride
<b>NRC</b>	National Research Council
<b>PCA</b>	principal components analysis
<b>RCAD</b>	rumen cation-anion difference
<b>RMSE</b>	root mean square error
<b>SD</b>	standard deviation
<b>SE</b>	standard error
<b>SEM</b>	standard error of the means
<b>SCC</b>	somatic cell count
<b>TMR</b>	total mixed ration
<b>TPF</b>	time post-feeding
<b>TVFA</b>	total volatile fatty acids
<b>VFA</b>	volatile fatty acid
<b>ZnCl<sub>2</sub></b>	zinc chloride

## Chapter 1: Introduction

In the 1980s, the dietary cation-anion difference (**DCAD**), began receiving attention for use in formulating dairy cattle diets. DCAD considers the strong ions of the diet, and it is calculated as follows:  $DCAD \text{ (mEq/kg DM)} = Na + K - Cl$  (Mongin, 1981; Tucker et al., 1988). Manipulation of DCAD has been shown to improve dairy cow performance by increasing dry matter intake, milk yield, and feed efficiency (Tucker et al., 1988; Hu and Murphy, 2004). Although considerable research has been done on the impact of DCAD on physiological state, volatile fatty acid (**VFA**) concentration, and animal performance (Tucker et al., 1988; Sanchez et al., 1994; Hu and Murphy, 2004; Roche et al., 2005), none of those studies focused on the impacts of the dietary strong ions on rumen strong ion concentrations.

DCAD can be manipulated by the selection of different feed ingredients or by mineral supplementation. For example, alfalfa hay has a higher potassium concentration than corn silage and timothy hay, and thus, it has a higher DCAD (NRC, 2001). Erdman et al. (2011) reported that the perceived benefit of supplementing corn silage diets with alfalfa hay to improve feed efficiency was due to the increased DCAD of the diets, which could also be achieved by mineral supplementation.  $NaHCO_3$ ,  $KHCO_3$ , and  $K_2CO_3$  are dietary minerals that can be used to increase DCAD, whereas  $NH_4Cl$ ,  $CaCl_2$ , and  $ZnCl_2$  are some of the minerals used to decrease DCAD.  $NaCl$  and  $KCl$  are DCAD neutral salts because they contain equivalent amounts of cation and anions. According to Iwaniuk et al. (2015), dietary potassium supplements generally cost 3 to 4 times more than sodium supplements on a weight basis, so understanding the impacts of the individual strong ions on animal performance and the rumen environment can have economic consequences for farmers.

Ionophores are another tool commonly used in the dairy industry to improve animal performance (Duffield et al., 2008). Ionophores are feed additives that facilitate the transportation of cations across the cell walls of susceptible bacteria (McGuffey et al., 2001; Ipharraguerre and Clark, 2003). This causes a shift in the rumen bacteria towards those that produce propionate and away from those that produce acetate and butyrate, improving rumen fermentation (Ipharraguerre and Clark, 2003). There is evidence that dietary concentrations of sodium and potassium can impact the efficacy of ionophores (Funk et al., 1983; Rumpler et al., 1986), so further understanding the relationship between dietary and rumen strong ions can lead to better use of ionophores.

Our overall hypothesis was that dietary strong ion concentrations impact their corresponding rumen ion concentrations. We tested this hypothesis using two experiments. In the first experiment, our objective was to examine the effects of DCAD, cation source, and anion source on the rumen environment and to further examine their effects on rumen fermentation. Five lactating fistulated dairy cows were fed the following experimental diets in a 5 x 5 Latin square design: 1) the basal total mixed ration (**TMR**); 2) the basal TMR plus 340 mEq/kg (dry matter basis) sodium and chloride using NaCl; 3) the basal TMR plus 340 mEq/kg potassium and chloride using KCl; 4) the basal TMR plus 340 mEq/kg sodium using NaHCO<sub>3</sub>; and 5) the basal TMR plus 340 mEq/kg potassium using K<sub>2</sub>CO<sub>3</sub>. These diets allowed us to compare sodium to potassium as a cation source and to compare chloride to bicarbonate and carbonate as an anion source. In addition, we compared the basal diet and those supplemented with chloride to the bicarbonate and carbonate diets to make DCAD comparisons.

In the second experiment, our objective was to explore the relationships between dietary strong ions, rumen ion concentrations, pH, and VFA. We conducted a meta-analysis using previously published data that measured rumen strong ion concentrations in fistulated cows at multiple times post-feeding and our data from Experiment 1. The final dataset consisted of three studies, which highlights the need for more research to be conducted about these relationships. Potential implications of these relationships include better use of DCAD to improve animal performance and better manipulation of rumen ion concentrations to increase the efficacy of ionophores.

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## Chapter 2: Literature Review

### INTRODUCTION

During changes in the dietary supply of ions, the ion concentrations within the rumen remain closely regulated, with other systems of the body compensating for these changes (Warner and Stacy, 1972a). Rumen ion concentrations can be influenced by several factors, including: the content and state of the feed, salivary and blood concentrations, and rate of absorption (Bennink et al., 1978). Table 2.1 shows the physiological concentrations of sodium, potassium, and chloride in parotid saliva, serum, and rumen fluid. Parotid saliva is the main component of mixed saliva, which gets secreted continuously throughout the day (McDougall, 1948).

In addition, differences in measured rumen ion concentrations can be caused by the method and location of sampling (Duffield et al., 2004; Shen et al., 2012). Sampling methods include use of rumen fistulated cows, rumenocentesis, and oral stomach tubes or probes (Duffield et al., 2004; Shen et al., 2012). Over the past three decades, the major rumen ions (sodium, potassium, and chloride) have received increased attention in diet formulation because of their effect on dietary cation-anion difference (**DCAD**) (Mongin, 1981; Tucker et al., 1988). DCAD has been shown to improve the performance of lactating dairy cows (Tucker et al., 1988; Sanchez et al., 1994a; Hu and Murphy, 2004; Roche et al., 2005). Ionophores, which increase the movement of cations into susceptible bacteria, have also been shown to enhance animal performance by altering rumen fermentation (Russell and Strobel, 1989; Duffield et al., 2008).

## SALIVATION

As shown in Table 2.1, parotid saliva contains 157 to 168 mEq/L Na, 6 to 14 mEq/L K, and 7.4 to 34 mEq/L Cl (Kay, 1960; Bailey and Balch, 1961a). Bailey and Balch (1961a, 1961b) showed that the composition of mixed saliva closely matched the composition of parotid saliva. The amount of saliva secreted into the rumen per day, and thus, the amount of ions introduced into the rumen from saliva, depend on the type of diet fed to an animal (Bailey, 1961a). Although saliva is secreted continuously, the rate of secretion depends on whether the cow is eating, ruminating, or at rest (Bailey and Balch, 1961a), and the total amount of saliva secreted per day depends on the amount of time spent on each activity (Bailey, 1961a).

Bailey and Balch (1961a) showed that saliva was secreted at a rate two times faster while eating and 2.5 times faster while ruminating compared to when the animal was at rest. In addition, cows fed hay diets spent more time eating and ruminating than cows fed high concentrate diets (Bailey, 1961a). Cows fed a forage diet spent 5 hours eating per day (Johnstone-Wallace and Kennedy, 1944), while cows fed concentrate diets spent 2 to 3 hours eating per day (Balch et al., 1955). Cows fed high forage diets spent 7 to 8 hours ruminating per day, while cows fed concentrate diets spent 2 to 5 hours ruminating (Johnstone-Wallace and Kennedy, 1944; Balch et al., 1955; Bailey, 1961a).

As shown in Table 2.2, cows produce between 108 and 308 liters of saliva/day, with an average of 180 liters of saliva/day, and cows fed hay diets produce more saliva/kg dry matter intake (**DMI**) than those fed concentrate diets (Bailey, 1961a; Cassida and Stokes, 1986). Balch (1958) also found that the amount of saliva added to the rumen while eating was higher with hay (5 to 7 L/kg DMI) than with concentrate diets (1 to 2 L/kg DMI). The

rate of saliva secretion was 1.4 to 2 times higher when fed concentrate diets, but hay diets took longer to consume, causing more saliva secretion while eating hay diets (Balch, 1958). Compared to concentrate diets, hay diets resulted in higher saliva secretion during eating and ruminating per kg DMI, but an 87.5% concentrate diet increased resting saliva secretion by 2 L/kg DMI compared to the hay diet (Bailey, 1961a; Cassida and Stokes, 1986). Scott (1966) showed that saliva is the primary source of sodium in the rumen, so the varying amounts of saliva secreted into the rumen with different diets impacts rumen sodium concentrations (Bennink et al., 1978).

## **SODIUM**

Sodium is the most prevalent cation found in rumen fluid (Bailey, 1961b; McCullough and Smart, 1968; Bennink et al., 1978; Durand and Kawashima, 1980; Duffield et al., 2004). As shown in Table 2.1, rumen sodium concentration ranges from 74.5 to 169.5 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012). Scott (1966) showed that preventing parotid saliva from entering the rumen of sheep decreased rumen sodium concentrations from between 80 and 131 mEq/L to between 30 and 81 mEq/L, indicating that saliva was the main source of rumen sodium. Although cows fed alfalfa hay or alfalfa pellets consumed similar quantities of sodium, 5 g/24 h and 4 g/24 h respectively, alfalfa hay resulted in higher rumen sodium concentrations (97.5 mEq/L) compared to the pellets (74.5 mEq/L) (Bennink et al., 1978). This was attributed to higher saliva secretion when cattle were fed the hay diet (Bennink et al., 1978).

Bennink et al. (1978) also found that rumen sodium concentrations tended to have more hourly fluctuations than other ions due to differences in salivation and absorption. As shown in Figure 2.1, a diet of alfalfa hay resulted in more dramatic hourly fluctuations than

a diet of alfalfa pellets (Bennink et al., 1978). The average rumen sodium concentration for the alfalfa hay was 97.5 mEq/L, while the average rumen sodium concentration for the alfalfa pellet diet was 74.5 mEq/L (Bennink et al., 1978). Hourly rumen samples of cows fed exclusively rye grass hay indicated that sodium concentrations had a mean of 103 mEq/L, started at 260 mEq/L pre-feeding, decreased to their minimum concentration of 100 mEq/L around 5 hours post feeding, and then began to increase (Fenner et al., 1969). Similar to Bennink et al. (1978), Fenner et al. (1969) noted that hourly concentration changes in rumen Na were the result of rumination throughout the day and absorption across the rumen wall.

Although salivation increased while eating and ruminating (Bailey, 1961a; Cassida and Stokes, 1986), increases in rumen sodium concentrations were prevented by dilution from water intake and by absorption across the rumen wall (Bailey, 1961b). Bailey (1961b) observed that rumen sodium concentrations were proportional to the salivary concentrations. However, rumen Na was 17% lower in the rumen than in the saliva. As shown in Table 2.1, the serum concentration for sodium was between 136 mEq/L and 161 mEq/L (Tucker et al., 1988; Leroy et al., 2004; Cozzi et al., 2011), while the rumen fluid showed a broader range of 74.5 mEq/L to 169.5 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012). Parthasarathy and Phillipson (1953) found that sodium is absorbed from the rumen when its concentration is higher than that of the blood, and it is added into the rumen when its concentration is lower than the blood concentration. Sheep were used to show that the venous-arterial difference for sodium ranged from -0.4 mEq/L to -1.4 mEq/L for rumen concentrations of 0 to 58 mEq/L, but increased to 1.7 mEq/L when the concentration in the rumen was 254 mEq/L, indicating

that sodium was not absorbed from the rumen into the carotid artery until the concentration exceeded the concentration of the blood (Parthasarathy and Phillipson, 1953). The negative venous-arterial differences were attributed to the movement of sodium into the rumen from the carotid artery and the absorption of water from the rumen (Parthasarathy and Phillipson, 1953).

Dobson (1959), however, found that absorption of sodium out of the rumen did occur when the rumen sodium concentration was lower than the concentration in the blood, suggesting that sodium could be transported against its concentration and electrochemical gradients. When sheep had higher rumen sodium concentrations than blood concentrations, between 21.0 and 28.7 mEq of sodium were absorbed out of the rumen (Dobson, 1959). When sheep had lower rumen sodium concentrations than blood concentrations, between 1.8 and 24.7 mEq of sodium were absorbed out of the rumen (Dobson, 1959). Dobson (1959) suggested that the methods used by Parthasarathy and Phillipson (1953) were less accurate and not reliable enough to judge the direction the ions moved.

Bailey (1961b) noted that in sodium deficient cows, the sodium concentration of the rumen did not fall below 60 mEq/L even though the saliva sodium concentration was only 28 mEq/L. This suggested changes in the balance between the addition and removal of both water and sodium from the rumen to maintain appropriate rumen sodium concentrations (Bailey, 1961b). Parthasarathy and Phillipson (1953) found that changes in tonicity of the rumen fluid caused differences in the rumen volume. When hypotonic solutions ranging from 0.08 M to 0.165 M were placed in the rumen, the rumen volume decreased by between 70 and 304 mL, while hypertonic solutions ranging from 0.22 M and

0.33 M caused the addition of 30 to 340 mL to the rumen (Parthasarathy and Phillipson, 1953). Warner and Stacy (1965) showed that sheep that had been fasted for 18 hours had hypotonic rumen fluid compared to the blood, with the degree of hypotonicity ranging from 10 to 52 mOsm/kg, and they suggested that the rumen would normally be hypotonic for some part of the day.

Stacy and Warner (1966) showed that sodium absorption out of the rumen increased when osmotic pressure of the rumen increased, indicating that lowered sodium concentrations in hypertonic solutions are not necessarily caused by water entering the rumen. They found that the flow of water into the rumen occurred at 0.29 L/hour prior to feeding but occurred at 0.85 L/hour during the first hour post-feeding (Stacy and Warner, 1966). Stacy and Warner (1966) attributed this to a flow of 1.1 L/hour of saliva into the rumen, 0.03 L/hour of water being absorbed out of the rumen and into the blood, and 0.6 L/hour of water entering the abomasum from the rumen. Prior to feeding, sodium was absorbed from the rumen of sheep at the rate of 8.25 mEq/hour, but for 2 hours post-feeding, sodium was absorbed at an average rate of 47.25 mEq/hour when sheep were fed a diet containing around 85 mEq of sodium, showing that the decrease in sodium was the result of sodium absorption and not the introduction of water into the rumen (Stacy and Warner, 1966). Stacy and Warner (1966) also showed that tonicity of the rumen and sodium absorption can also be impacted by potassium concentrations.

Increased dietary potassium concentrations increased sodium absorption from the rumen (Stacy and Warner, 1966; Warner and Stacy, 1972a, 1972b). Warner and Stacy (1972a) found that rumen sodium concentrations decreased from 118 mEq/L to 100 mEq/L within 4 days and remained stable at 100 mEq/L for the next 24 days when sheep began

consuming a high potassium diet containing 680 mEq potassium/day, despite both diets having an intake of 30 mEq sodium/day. In the same period, urinary sodium excretion increased from 12 mEq/day to 39 mEq/day within 4 days before stabilizing to 20 mEq/day by 28 days (Warner and Stacy, 1972a). There was a total sodium balance of -15 mEq/day before stabilizing to a balance of 6 mEq/day, compared to the balance of 10 mEq/day when fed the basal diet (Warner and Stacy, 1972a). When the diet was switched back to the basal diet, the change in rumen sodium concentration was more gradual, and the rumen sodium concentrations increased to 103 mEq/L at 14 days before returning to 118 mEq/L at 28 days (Warner and Stacy, 1972a). Urinary sodium excretion decreased to 1 mEq/day at 14 days, indicating the kidneys were retaining sodium, and it was 12 mEq/day at 28 days, while the sodium balance increased to 28 mEq/day at 14 days and was 11 mEq/day at 28 days (Warner and Stacy, 1972a).

Warner and Stacy (1972a) attributed these physiological responses to the rumen's ability to maintain its own electrolyte balance at a total concentration of sodium and potassium between 129 and 137 mEq/L throughout the whole study, despite increases in rumen potassium concentrations of up to 14 mEq/L, at the expense of the electrolyte balance of the animal as a whole. When dietary potassium levels were manipulated, the sum of the rumen sodium and potassium concentrations remained constant, with potassium concentrations changing inversely to that of sodium (Warner and Stacy, 1972a).

## **POTASSIUM**

As shown in Table 2.1, rumen potassium concentrations range from 28.2 to 68.9 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012) while parotid saliva concentrations are 6 to 14 mEq/L (Kay, 1960; Bailey and Balch, 1961a), and



serum concentrations are 4 to 7 mEq/L (Tucker et al., 1988; Leroy et al., 2004; Cozzi et al., 2011). Rumen potassium concentrations are influenced by the diet more than by salivary concentrations (Bailey, 1961b; Poutiainen, 1968). Poutiainen (1968) found that rumen fluid had potassium concentrations 60 to 65% higher than that of the saliva, and that 79% of the potassium entering the rumen came from the diet and drinking water.

Bennink et al. (1978) found that higher rumen potassium concentrations tended to be associated with higher potassium intakes, but this was not consistent for all feeds. For example, cows fed alfalfa hay or corn silage ingested 96 grams of potassium per 24 hours, but the alfalfa hay diet resulted in mean rumen potassium concentrations of 67.9 mEq/L compared to 43.7 mEq/L for the corn silage diet (Bennink et al., 1978). Cows fed a diet of dairy concentrate, corn silage, and alfalfa hay consumed 142 grams of potassium per 24 hours, but the mean rumen potassium concentration was only 32.5 mEq/L, which was 5.3 mEq/L higher than rumen concentrations for a milo diet that had the lowest potassium consumption of 22 grams per 24 hours (Bennink et al., 1978).

In addition to the amount of potassium consumed, the elution rate of potassium from the feed also has an impact on rumen potassium concentrations (Bennink et al., 1978). Tyler et al. (1967) compared alfalfa pellets and alfalfa hay to show that potassium ions eluted more quickly from alfalfa pellets than from the hay in rumen fluid and in water. Potassium ions from the alfalfa pellets eluted 1.6 times faster than from alfalfa hay during the first 15 minutes in a water solution (Tyler et al., 1967). Bennink et al. (1978) found that in cows fed alfalfa hay, the rumen potassium concentration increased by 22 mEq/L within the first 40 minutes post-feeding, but in cows fed alfalfa pellets, the rumen potassium concentration increased by 29 mEq/L. A study of six different forages showed

that 98 to 100% of the potassium within the forage is immediately released within the rumen without fermentation (Emanuele and Staples, 1990). Similarly, Playne et al. (1978) used different hays to show that the elution rate of potassium from the feed was faster than that of sodium, with between 85% and 95% of potassium being removed from the dry matter within 24 hours of digestion compared to 40% to 85% removal of sodium.

Potassium is absorbed from the rumen when its concentration is higher than that of blood (Parthasarathy and Phillipson, 1953). Because serum potassium concentrations range from 4 to 7 mEq/L (Tucker et al., 1988; Leroy et al., 2004; Cozzi et al., 2011), and rumen potassium concentrations range from 28.2 to 68.9 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012), potassium is constantly absorbed from the rumen into the blood (Parthasarathy and Phillipson, 1953). Parthasarathy and Phillipson (1953) showed that 0.2 to 0.4 grams of potassium per hour were absorbed from isotonic solutions when rumen potassium concentration ranged from 34 to 57 mEq/L, but absorption increased to between 0.6 and 0.8 grams of potassium per hour when the solutions were hypertonic and slightly basic. Slightly acidic, hypertonic solutions did not result in the same increased absorption (Parthasarathy and Phillipson, 1953). Warner and Stacy (1972a, 1972b) showed that increased rumen potassium concentrations increased potassium absorption from the rumen. When potassium intake increased from 190 mEq/day to 700 mEq/day, absorption from the rumen increased from 50 mEq/day to 235 mEq/day, while plasma potassium concentrations only increased from 4.7 mEq/L to 5.3 mEq/L (Warner and Stacy, 1972a). Urinary excretion increased from 145 mEq/day to 600 mEq/day (Warner and Stacy, 1972a).

In addition to the previously described relationship with sodium, potassium also has a relationship with magnesium. Hypomagnesic tetany, commonly referred to as grass tetany, results from low serum magnesium concentrations, and is also associated with feeding forage diets that are high in potassium (Fontenot, 1979). Several studies have shown that increased potassium intake decreased magnesium absorption (Newton et al., 1972; Greene, et al. 1983; Jittakhot et al., 2004). When potassium intake increased from 2.1% to 7.6% of the diet, apparent magnesium absorption decreased from 18.6% of magnesium intake to 6.9% (Jittakhot et al., 2004). Jittakhot et al. (2004) showed that supplementing the diet of dry cows with potassium and magnesium increased the concentrations of the respective ions in the rumen, but it did not impact the concentration of the other ions (Jittakhot et al., 2004). Similarly, Holtenius et al. (2008) showed that supplementing the diet of lactating cows with magnesium increased rumen magnesium concentrations, but supplementing the diet with potassium did not impact rumen magnesium concentrations.

## **CHLORIDE**

Similar to rumen potassium concentrations, work by Bailey (1961b) indicates that rumen chloride concentrations are impacted more by the diet than by saliva. As shown in Table 2.1, rumen chloride concentrations range from 6.7 to 25.6 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012), while parotid saliva concentrations are 7.4 to 34 mEq/L (Kay, 1960; Bailey and Balch, 1961a). Although only four out of the five diets in Bailey's (1961b) study supplied higher dietary concentrations of chloride than that found in the saliva, all five diets resulted in higher concentrations in rumen fluid than in the saliva. For example, cows fed a hay diet consumed 33 grams of chloride per day and had between 3.0 and 8.9 mEq/L chloride in their saliva, while their

rumen chloride concentrations were between 7 and 18 mEq/L (Bailey, 1961b). However, when Bailey (1961b) fed a diet of hay, flaked maize, and groundnut cake, the cows consumed only 7 grams of chloride/day, while saliva concentrations were between 6.3 and 9.7 mEq/L, and the rumen concentrations were higher at 9 to 15 mEq/L.

Furthermore, different cows receiving the same diets had similar rumen chloride concentrations, although rumen chloride concentrations varied between different diets (Bailey, 1961b). Chloride concentrations in the rumen increased within 1 hour post-feeding (except for the hay, flaked maize, and groundnut cake diet) and returned to concentrations similar to that of the saliva by 14 hours post feeding (Bailey, 1961b). Although Bailey (1961b) indicated that there was a dietary effect on rumen chloride concentrations, no statements were made about the nature of this effect. Bennink, et al. (1978) suggested that other factors besides diet chloride concentration affect rumen chloride concentrations. While there was a diet effect, differences in rumen chloride concentrations were not directly correlated to differences in chloride consumption (Bennink et al., 1978). For example, cows fed alfalfa pellets had higher rumen chloride concentrations (25.6 mEq/L) than cows being fed other diets, including alfalfa hay, but chloride consumption (2 g/24 hours) was the lowest for this diet (Bennink et al., 1978). Conversely, cows fed corn silage had the highest chloride intake (10 g/24 hours), but had the lowest rumen chloride concentrations (6.7 mEq/L) (Bennink et al., 1978). However, this does not suggest that there was an inverse relationship, as the diet with the second highest chloride consumption (6 g/24 hours), a mixture of dairy concentrate, corn silage, and alfalfa hay, also had the second highest rumen chloride concentration (13.8 mEq/L) (Bennink et al., 1978).

Rumen chloride concentration has been shown to vary with time post-feeding, but the post-feeding effect varies with the diet being fed (Bailey, 1961b). Bailey (1961b) found that rumen chloride concentrations increased to between 15 and 32 mEq/L within one hour post-feeding for lucerne silage, rye grass and clover hay, hay and dairy cubes, and cocksfoot and rye grass diets. By 14 hours post-feeding, the chloride concentrations within the rumen had decreased to between 9 and 16 mEq/L, which was near the concentrations found in saliva. No post-feeding increase in chloride concentration was observed in cattle fed a diet containing hay, flaked maize, and groundnut cake, which had an intake of 26 to 117 less grams chloride/day (Bailey, 1961b). Bennink et al. (1978) found that in only two of the six diets they studied, there was increased rumen chloride concentration post-feeding. Alfalfa pellets and a mixed diet containing dairy concentrate, corn silage, and alfalfa hay resulted in increased rumen chloride concentrations within one and two hours post-feeding (Bennink et al., 1978).

It has been shown that chloride can be absorbed into the blood from the rumen against a concentration gradient (Parthasarathy and Phillipson, 1953; Dobson and Phillipson, 1958) and down an electrical gradient (Dobson and Phillipson, 1958; Dobson, 1959). As shown in Table 2.1, rumen chloride concentrations range from 6.7 to 25.6 mEq/L (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012), and serum concentrations range from 99 to 107 mEq/L (Tucker et al., 1988; Leroy et al., 2004; Cozzi et al., 2011). Similarly, Bailey (1961b) found that the highest concentration of rumen chloride in his study of different diets, 29 mEq/L, was less than a third of the chloride concentration in plasma, and he suggested that the electrochemical gradient must have been

exceeded in order for the movement of chloride out of the rumen and into the blood to occur.

Parthasarathy and Phillipson (1953) showed that chloride could move from the rumen of anesthetized sheep into the blood against its concentration gradient after the concentration in the rumen exceeds a certain amount. When rumen chloride concentrations were below 28.2 mEq/L, chloride was absorbed into the rumen, when rumen chloride concentrations were between 28.2 and 38.0 mEq/L, chloride was absorbed into and out of the rumen at equal rates, and when rumen chloride concentrations exceeded 38.0 mEq/L, chloride was absorbed out of the rumen (Parthasarathy and Phillipson, 1953). These rumen chloride concentrations exceed those from Table 2.1 that are normally found in cows, but Parthasarathy and Phillipson (1953) noted that the concentration levels for the movement of chloride may be lower in unanaesthetized sheep than in the animals used in their study. Dobson and Phillipson (1958) found that chloride moved against its concentration gradient from the rumen into the blood because there was a -30 mV difference between the electrochemical potential of the rumen fluid and the blood, and chloride was moving down its electrical potential gradient.

Dobson (1959) observed that small amounts of chloride could move against the electrochemical gradient in the presence of high concentrations of potassium and low concentrations of sodium. When rumen potassium concentrations were around 100 mEq/L and rumen sodium concentrations were around 80 mEq/L, chloride entered the rumen from the blood (Dobson, 1959). Dobson (1959) maintained high rumen potassium concentrations and manipulated the chloride concentrations. The study found that at low (12.1 mEq/L) and high (49.0 mEq/L) chloride concentrations, 1.2 mEq and 4.6 mEq

chloride moved along the electrochemical gradient, respectively, but at a moderate chloride concentration (31.3 mEq/L), 1.4 mEq chloride moved against the electrochemical gradient within a half hour (Dobson, 1959).

Other minerals have also been shown to affect rumen chloride concentration. For example, Yano and Kawashima (1979) conducted a study in sheep that suggested phosphorus concentrations in the rumen have an impact on chloride concentration. Feeding trials indicated a negative correlation between rumen phosphorus and chloride concentrations because increased phosphorus in the diet decreased rumen chloride concentrations (Yano and Kawashima, 1979). Sheep fed diets containing 0.36% phosphorus had rumen concentrations of 12.7 mEq chloride/L 3 hours post-feeding, while diets containing 0.72% phosphorus resulted in 11.8 mEq chloride/L, and diets containing 1.08% phosphorus resulted in 10.2 mEq chloride/L (Yano and Kawashima, 1979). These diets caused rumen phosphorus concentrations of 41.3, 53.2, and 66.2 mEq/L, respectively, at 3 hours post-feeding (Yano and Kawashima, 1979). However, when Yano and Kawashima (1979) increased dietary magnesium from 0.21% to 0.89%, the rumen phosphorus concentration at 3 hours post-feeding decreased from 39.1 mEq/L to 34.0 mEq/L, and the rumen chloride concentration increased from 8.4 mEq/L to 10.5 mEq/L, which indicated that magnesium may have indirectly increased chloride concentrations by decreasing phosphorus concentrations.

## **SAMPLING METHODS**

### ***Sampling Technique***

The technique used to collect rumen samples can cause variations in measured rumen ion concentrations (Duffield et al., 2004; Shen et al., 2012). Sampling methods include

use of rumen cannulated cows, rumenocentesis, and oral stomach tubes or probes (Duffield et al., 2004; Shen et al., 2012). Rumen cannulation allows for long-term access to rumen contents with minimal disturbance to the cow after the initial surgical procedure and post-surgical care period (Laflin and Gnad, 2008). According to Phillipson and Innes (1939), details of the first successful rumen fistulation procedure in oxen were published by Colin in 1886, but no attempts to create permanent fistulas that could be closed were reported until the 1920s. Although some of the earlier techniques involved the use of wood blocks or metal cannulas (Phillipson and Innes, 1939), modern cannulas with removable plugs are often made out of silicone (Horigane, 1989) or plastic (Harmon and Richards, 1997). The cannulation surgery, which can be performed in either a one-step or two-step process, involves the placement of a cannula into an opening made in the abdominal and rumen walls (Martineau et al., 2015). Martineau et al. (2015) reported on a two-step procedure that could be performed in 30 minutes under local anesthesia. Rumen cannulation allows for easy collection of rumen fluid (Garrett et al., 1999), and it provides more representative rumen samples (Nocek, 1997) because it provides more access to the rumen.

Rumenocentesis has received attention as a tool for collecting rumen samples for the diagnosis of subacute rumen acidosis (Garrett et al., 1999; Duffield et al., 2004; Kleen et al., 2004). When samples are collected by rumenocentesis, rumen fluid is aspirated from a needle that has been inserted through the abdominal wall and into the rumen (Norlund and Garrett, 1997). While samples collected from cannulated cows can be collected from any location in the rumen, samples collected from rumenocentesis are collected from only one location, about 15 to 20 cm from the last rib (Norlund and Garrett, 1997; Duffield et al., 2004). Although some studies (Garrett et al., 1999; Morgante et al., 2007) have



reported no problems with infection associated with rumenocentesis, others have (Norlund and Garrett, 1997; Kleen et al., 2004). Norlund and Garrett (1997) observed abscesses at the rumenocentesis site in 1 to 2% of several hundred samplings. Similarly, Kleen et al. (2004) reported abscesses and hematomas at the rumenocentesis site in 9 of 164 cows (5.5%). In addition to the risk of infection, rumenocentesis also increases the risk of restraint injuries (Norlund and Garrett, 1997).

Collecting rumen samples with oral stomach tubes requires less restraint of the animal in general but more head restraint compared to collecting samples by rumenocentesis (Norlund and Garrett, 1997). Saliva contamination is a major concern for samples collected using this technique (Nocek, 1997; Norlund and Garrett, 1997; Duffield et al., 2004). In addition, when using either weighted stomach tubes or guidable probes, it is difficult to know the exact location of the rumen sample (Norlund and Garrett, 1997). There are also concerns about the tubes and probes not being able to penetrate the fiber mat of the rumen (Norlund and Garrett, 1997; Duffield et al., 2004).

Duffield et al. (2004) compared samples obtained from oral stomach probes, rumenocentesis, and rumen cannulation and found minor, but significant differences. In this study, it was determined that the stomach probe used was unable to penetrate the fiber mat and obtained samples more dorsally than ventrally, although the preferred site for both oral stomach probes and rumenocentesis was the cranial ventral portion of the rumen (Duffield et al., 2004). The sodium concentration in the first 200 mL of rumen fluid collected via an oral stomach probe was 100.5 mEq/L, which was significantly different from the caudal ventral and central rumen, which had sodium concentrations of 93.4 and 91.7 mEq/L, respectively, but it was not significantly different from the second 200 mL of

fluid collected from the stomach probe, rumenocentesis, or other parts of the rumen (Duffield et al., 2004).

Potassium concentrations in samples obtained from rumenocentesis and the cranial ventral rumen were 33.9 and 33.6 mEq/L, respectively, and these concentrations were significantly different from samples obtained from the caudal ventral rumen, which had a potassium concentration of 31.0 mEq/L, but not significantly different from samples obtained from the oral stomach probe or other parts of the rumen (Duffield et al., 2004). The chloride concentration obtained within the first 200 mL of fluid collected with an oral stomach probe was 23.8 mEq/L, which was significantly different from the second 200 mL of fluid collected from the stomach probe, rumenocentesis, and the rumen cannula (Duffield et al., 2004). The second 200 mL of fluid collected from the oral stomach probe had a chloride concentration of 22.6 mEq/L, and was similar to samples obtained via rumenocentesis and the cranial ventral rumen (Duffield et al., 2004). Duffield et al. (2004) concluded that samples obtained from the oral stomach probe were likely contaminated by saliva, and that the first 200 mL of fluid obtained with this method should not be used for measuring rumen parameters.

Shen et al. (2012) examined the effects of inserting oral stomach tubes to different depths and collecting different batches of fluid to minimize saliva contamination. An oral stomach tube inserted to a depth of 180 cm would be in the cranial dorsal portion of the rumen, while a tube inserted to a depth of 200 cm would be in the central rumen, and these samples were compared to fluid obtained from the ventral rumen using a cannula (Shen et al., 2012). Compared to samples obtained from a rumen cannula, samples collected using an oral stomach tube at a depth of 180 cm had higher sodium concentrations, lower

potassium concentrations, and similar chloride concentrations (Shen et al., 2012). Samples from the oral stomach tube at 180 cm had sodium concentrations ranging from 100.0 to 103.7 mEq/L, while samples from the rumen cannula had a sodium concentration of 90.4 mEq/L (Shen et al., 2012).

Potassium concentrations of samples collected with the oral stomach tube at 180 cm ranged from 32.3 to 33.0 mEq/L, while samples from the rumen cannula had a potassium concentration of 41.1 mEq/L (Shen et al., 2012). At a depth of 200 cm, there were no differences in the measured ion concentrations obtained from the oral stomach tube or rumen cannula (Shen et al., 2012). In addition, when batches of rumen fluid were collected via the stomach tubes in 150 mL increments, no differences in rumen ion concentrations were found between different batches (Shen et al., 2012). Based on these results, Shen et al. (2012) concluded that oral stomach tubes that were inserted into the central rumen resulted in more accurate ion measurements by limiting saliva contamination.

### ***Sampling Location***

The location of rumen sampling can also cause variation in measured rumen parameters (Bryant, 1964; Duffield et al., 2004; Li et al., 2009; Shen et al., 2012). According to Bryant (1964), the most representative sample of rumen fluid would be obtained from the central region, or a composite of the central, ventral, and dorsal regions. Variations in pH of rumen fluid samples from different locations result from rumination and addition of saliva into the rumen (Bryant, 1964). Li et al. (2009) suggested that cranial regions of the rumen had a higher pH than more caudal regions due to the proximity to the esophagus, where saliva would be entering the rumen. In addition to carrying buffers associated with a higher rumen pH (Li et al., 2009), saliva is the primary source of sodium in the rumen (Scott,

1966). Shen et al. (2012) showed that the cranial dorsal rumen had the highest sodium concentration at 111.9 mEq/L, which was not significantly different from the sodium concentrations in the cranial ventral or central rumen, but it was significantly different from the ventral, caudal dorsal, and caudal ventral rumen, which ranged from 99.4 to 102.2 mEq/L (Shen et al., 2012). Similarly, Duffield et al. (2004) found that the cranial dorsal and cranial ventral rumen sites had similar sodium concentrations, which were statistically different from the caudal ventral and central rumen sites.

Shen et al. (2012) found no significant differences in potassium or chloride concentrations among these different sampling sites. Duffield et al. (2004), however, found that the potassium concentration in samples from the cranial ventral rumen was 33.6 mEq/L, which was significantly different from samples obtained from the caudal ventral rumen, which had a concentration of 31.0 mEq/L. Duffield et al. (2004) also found a location difference for chloride. The cranial ventral rumen had the highest chloride concentration at 21.6 mEq/L, which was significantly different from the caudal ventral, central, and cranial dorsal rumen, which had concentrations of 18.9, 19.2, and 19.8 mEq/L, respectively, but no comments were made regarding possible reasons for the higher concentration (Duffield et al., 2004).

## **DIETARY CATION-ANION DIFFERENCE**

### ***Background***

Dietary cation-anion difference is a measure of the balance between the strong cations and the strong anions in the diet (Mongin, 1981). The strong ions include sodium, potassium, and chloride which are the major contributors to osmotic pressure in body fluids (Sanchez et al., 1994a). In addition, these strong ions typically have dietary absorption

rates of 90% or greater (Delaquis and Block, 1995), and the major route of their excretion is in the urine (Warner and Stacy, 1972a; Delaquis and Block, 1995; Apper-Bossard et al., 2010). Sodium and potassium are the main sources of cations, whereas chloride is the main source of anions (Mongin, 1981). Dietary cation-anion difference is calculated as follows:  
DCAD (mEq/kg) = Na + K – Cl (Mongin, 1981; Tucker et al., 1988).

Other sources of dietary cations include calcium and magnesium, while sulfur and phosphate are other sources of dietary anions (Mongin, 1981). Some researchers prefer to use DCAD-S, which is calculated as DCAD-S (mEq/kg) = Na + K – Cl – S, instead of DCAD (Tucker et al., 1991; Roche et al., 2005; Apper-Bossard et al., 2006). However, DCAD is used more often, and Mongin (1981) reported that additional ions were not as important as the strong ions for these calculations. Regardless of which equation is used, the DCAD has implications on determining the acid-base balance of the animal and animal productivity (Mongin, 1981).

### ***Acid-Base Status***

Animal performance is impacted by the acid-base status of the animal because bodily fluids must remain neutrally charged (Sanchez et al., 1994a). The strong ions contribute charges to bodily fluids since they are absorbed without being metabolized (Sanchez et al., 1994a). In the gastrointestinal tract, the absorption of sodium and potassium ions is coupled with the secretion of H<sup>+</sup> ions out of the blood, and the absorption of chloride is coupled with the secretion of bicarbonate (HCO<sub>3</sub><sup>-</sup>) out of the blood (Chien and Stevens, 1972; Martens and Blume, 1987; Tucker et al., 1988). These couplings allow for fluids to maintain their neutral charges (Chien and Stevens, 1972). Tucker et al. (1988) found that increased DCAD increased the cation-anion difference of the serum, resulting in increased

production of bicarbonate and secretion into the blood, and they proposed that this altered bicarbonate concentration in the blood was how DCAD impacted blood pH.

In mammals, blood pH is normally ~7.4, with a pH below 7.35 indicating acidosis, and a pH above 7.45 indicating alkalosis (Sherwood et al., 2005). The carbon dioxide-bicarbonate buffer system is critical for maintaining an appropriate blood pH (Sherwood et al., 2005). In this system, which is illustrated by the following equation:  $H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$ ,  $H_2CO_3$  is formed by the spontaneous reaction of  $H_2O$  and  $CO_2$ , which are the products of metabolic processes, and most of that  $H_2CO_3$  then dissociates into  $H^+$  and  $HCO_3^-$  (Sherwood et al., 2005). Under normal conditions, there are approximately 600,000 times more  $HCO_3^-$  ions than  $H^+$  ions, so there are plenty of  $HCO_3^-$  ions to react with excess  $H^+$  ions, and there are enough  $H_2O$  and  $CO_2$  ions to allow for the production of more  $H^+$  ions during times of proton deficiency (Sherwood et al., 2005). According to the Henderson-Hasselbalch equation, the ratio of  $HCO_3^-$  to dissolved  $CO_2$  in the blood must be kept constant in order for blood pH to remain constant (Sanchez et al., 1994a; Sherwood et al., 2005). Animals can maintain their acid-base balance by controlling both input and output of contributors to acidity (Mongin, 1981).

The respiratory system maintains carbon dioxide concentrations, and the kidneys maintain bicarbonate concentrations (Sherwood et al., 2005). Changing the respiration rate alters the rate that  $CO_2$  ions, which lead to the generation of  $H^+$  ions, are removed from the body (Sherwood et al., 2005). The respiratory system is only able to compensate for pH deviations by 75%, while the kidneys are able to compensate up to 100% (Sherwood et al., 2005). The kidneys maintain the proper pH of bodily fluids by controlling the excretion of  $H^+$  and  $HCO_3^-$  and the secretion of  $NH_3$  (Sherwood et al., 2005). The excretion of  $H^+$  is

related to CO<sub>2</sub> concentrations, with higher amounts of H<sup>+</sup> being excreted under higher CO<sub>2</sub> concentrations, but there are no mechanisms for H<sup>+</sup> reabsorption in the kidneys, so they can only reduce the amount of H<sup>+</sup> excretion by secreting less of the ion (Sherwood et al., 2005).

There are two methods the kidneys use to regulate the amount of HCO<sub>3</sub><sup>-</sup> removed for excretion based upon the concentrations of H<sup>+</sup> in the plasma (Sherwood et al., 2005). The first method is by altering the reabsorption of filtered HCO<sub>3</sub><sup>-</sup> from the urine back to the plasma, and the second method is by altering the addition of new HCO<sub>3</sub><sup>-</sup> (Sherwood et al., 2005). H<sup>+</sup> ions are secreted into the urine against a concentration gradient, but once the urine becomes 800 times more acidic than the plasma, the secretory processes can no longer overcome the concentration gradient (Sherwood et al., 2005). HCO<sub>3</sub><sup>-</sup> is not secreted into the urine at the same time as H<sup>+</sup>, so it is unable to serve as a buffer in the urine (Sherwood et al., 2005). Instead, NH<sub>3</sub> secreted into the urine reacts with H<sup>+</sup> ions to form NH<sub>4</sub><sup>+</sup>, decreasing the acidity, and allowing more H<sup>+</sup> to be added to the urine (Sherwood et al., 2005).

Uncompensated disturbances to the acid-base status of an animal can lead to disruptions in homeostasis. Increased CO<sub>2</sub> concentrations can lead to respiratory acidosis, while decreased concentrations can lead to respiratory alkalosis (Sherwood et al., 2005). Heat stressed cows use panting and sweating for evaporative cooling (Kibler and Brody, 1950; Bianca, 1955; West, 2003), and the increased respiration rate from panting decreases blood CO<sub>2</sub> concentrations and leads to respiratory alkalosis (Bianca, 1955; West, 2003). Metabolic acidosis is associated with decreased plasma HCO<sub>3</sub><sup>-</sup> concentrations, while metabolic alkalosis is associated with increased HCO<sub>3</sub><sup>-</sup> concentrations (Sherwood et al.,

2005). Although these conditions can have negative long term consequences, producers have found that inducing minor metabolic acidosis in periparturient dairy cows can be beneficial in preventing milk fever (Goff et al., 2004; Charbonneau et al., 2006).

### ***Impacts of a Negative DCAD***

The original interest in DCAD in the dairy industry was the use of negative DCAD to prevent or minimize the incidence of hypocalcemia at calving called milk fever (Hu and Murphy, 2004). While diets high in anions have been shown to reduce the incidence of milk fever (Ender et al., 1971; Block, 1984; Charbonneau et al., 2006), diets high in cations have been shown to increase its incidence (Ender et al., 1971; Block, 1984; Goff and Horst, 1997). Milk fever occurs when cows are unable to meet the demand for increased calcium at parturition and the beginning of lactation, and many studies have shown that feeding cows diets high in calcium prior to calving increases their risk for the disease (Boda and Cole, 1954; Goings et al., 1974; Jorgensen, 1974; Green et al., 1981).

In some situations, however, feeding diets with a low calcium to phosphorus ratio is impossible due to the availability of feeds in a geographic area (Block, 1984). Ender et al. (1971) found that feeding acidic diets instead of alkaline diets increased intestinal calcium absorption and influenced the amount of calcium available from the bones. Block et al. (1984) studied the impacts of positive, 33.05 mEq/kg DM, and negative, -12.85 mEq/kg DM, DCAD on diets that contained high ratios of calcium to phosphorus, 2.63 and 2.89. Milk fever occurred in 47.4% of the cows while they were on the positive DCAD diet, but no cows got milk fever while they were on the negative DCAD diet (Block et al., 1984). Despite the diet having a high calcium to phosphorus ratio, Block et al. (1984) suggested



that the negative DCAD allowed for improved mobilization of calcium from bones during the calcium stress associated with calving and lactation.

Charbonneau et al. (2006) used a meta-analysis to confirm the findings of Block et al. (1984) and other studies, and the analysis was able to find significance that was not attained in the individual studies. Lowering DCAD by 300 mEq/kg induced minor metabolic acidosis, which was illustrated by a reduction in blood pH by 0.018 units, an 8.6% decrease in blood  $\text{HCO}_3^-$ , and a 4.5% reduction of blood  $\text{CO}_2$  concentrations (Charbonneau et al., 2006). Charbonneau et al. (2006) suggested that the reduced blood  $\text{CO}_2$  indicated a respiratory response to help compensate for the induced acidosis. Total blood Ca was increased by 10.7%, and ionized blood Ca was increased by 12.2% (Charbonneau et al., 2006).

Charbonneau et al. (2006) also found that dry matter intake was decreased by 11.3%, which might be caused by the metabolic acidosis or by decreased palatability of the diet caused by the addition of anionic salts (Vagnoni and Oetzel, 1998; Hu and Murphy, 2004). In order to compensate for decreased intake of prepartum anionic diets, Moore et al. (2000) recommended increasing the energy content of the feed. In contrast to the decreased dry matter intake observed in low DCAD diets (Charbonneau et al., 2006), high DCAD diets have been associated with increased dry matter intake and have been used to enhance the performance of lactating cows (Tucker et al., 1988; Hu and Murphy, 2004).

### ***Impacts of a Positive DCAD***

Feeding DCAD positive diets to lactating cows improves their performance by increasing dry matter intake, increasing milk yield, and improving acid-base status (Tucker et al., 1988; Hu and Murphy, 2004). Apper-Bossard et al. (2010) indicated that positive

DCAD maintained improved acid-base status, allowing for increased dry matter intake. Dry matter intake increased linearly with increased DCAD, and higher increases between 1.2 and 4.0 kg/DM per day were observed when cows were fed high concentrate diets compared to low concentrate diets (Apper-Bossard et al., 2006, 2010). Tucker et al. (1988) found a trend for increased feed intake with increased DCAD, with the -100 mEq/kg DM balance diets resulting in an average feed intake of 16.8 kg DMI and the 200 mEq/kg DM balance diets resulting in a feed intake of 18.6 kg DMI, but they suggested that the  $\text{CaCl}_2$  used to decrease the DCAD may have decreased palatability and intake in the rations with lower DCAD. As mentioned previously, metabolic acidosis induced by the low DCAD diet could have also decreased dry matter intake (Hu and Murphy, 2004). Hu and Murphy (2004) identified a quadratic relationship between DCAD and dry matter intake, and they determined that maximum dry matter intake occurred when the DCAD was 400 mEq/kg DM. Sanchez et al. (1994a) identified a cubic relationship between DCAD and dry matter intake.

Increased dry matter intake is associated with increased milk yield (NRC, 2001). Tucker et al. (1988) found a trend for increased milk yield with increased DCAD. The actual milk yield averaged 18.5 kg for the -100 mEq/kg DM balance diets, while it averaged 20.1 kg for the 200 mEq/kg DM balance diets (Tucker et al., 1988). In a meta-analysis, Hu and Murphy (2004) found that milk yield was increased quadratically by DCAD, and milk yield reached its maximum at 340 mEq/kg DM. The study indicated that the 4% fat corrected milk yield was also quadratically increased by DCAD, and it was maximum at a DCAD of 490 mEq/kg DM (Hu and Murphy, 2004). Similarly, Sanchez et al. (1994a) found a quadratic relationship between DCAD and 3.5% fat corrected milk. There was a

decrease in milk yield associated with negative DCAD (Tucker et al., 1988; Sanchez et al., 1994a).

Hu and Murphy (2004) suggested that milk fat percentage and milk protein percentage were not directly influenced by DCAD because increased milk yield had a diluting effect, even though milk fat yield and milk protein yield both increased quadratically with DCAD. Similarly, Delaquis and Block (1995) attributed decreased milk fat percentage from cows in mid-lactation to increased milk production resulting from increased DCAD. Cows in late lactation maintained milk fat percentage because increased DCAD did not impact milk production (Delaquis and Block, 1995). While Hu and Murphy (2004) observed that milk fat yield increased with increased DCAD over a range of -200 to 600 mEq/kg DM, Delaquis and Block (1995) concluded that milk fat yield was not impacted when positive DCAD were increased by 200 mEq/kg. However, Iwaniuk et al. (2015) reported results similar to those of Hu and Murphy (2004). In one experiment, milk fat yield increased linearly as DCAD increased from 277 to 406 mEq/kg DM (Iwaniuk et al., 2015). In another experiment, milk fat yield increased linearly and quadratically as DCAD increased from 257 to 603 mEq/kg DM (Iwaniuk et al., 2015). In addition, a meta-analysis examining a range of DCAD from 100 to 655 mEq/kg DM indicated that each 100 mEq/kg DM increase in DCAD increased milk fat yield linearly by 39.9 g/day (Iwaniuk, 2013).

Sanchez et al. (1994a) used regression to determine that maximum lactational performance occurred between 300 and 500 mEq/kg DM. Hu and Murphy (2004) concluded that a slightly more narrow DCAD range between 340 and 490 mEq/kg DM optimized dry matter intake, milk yield, and 4% fat corrected milk yield. Sanchez et al. (1994b) used a large data set to model the effects of DCAD, and they concluded that a

DCAD of 380 mEq/kg DM allowed for maximum performance. Sanchez and Beede (1996) recommended monitoring diets for optimum DCAD but suggested that most commercial diets would be within the range of maximum performance.

Other studies found no significant differences with varied DCAD, but it has been suggested that there is a range where altering DCAD has no effect (Sanchez et al., 1994a, 1994b, 1997). Sanchez et al. (1994b) and Sanchez and Beede (1996) found only small differences within a DCAD range of 250 and 500 mEq/kg DM. Sanchez et al. (1997) used a DCAD between 250 and 400 mEq/kg DM, and they suggested that normal acid-base status and milk yield were maintained by homeostatic mechanisms at these differences. The only significant effect of increased DCAD in that study was decreased milk magnesium concentrations (Sanchez et al., 1997). Similarly, Coppock et al. (1982) found no significant differences in milk yield with DCAD between 209 and 417 mEq/kg DM. They suggested that although their diets resulted in differences in sodium and chloride concentrations, the mammary gland was able to meet its ion needs for milk production by altering the homeostasis of the animal in response to changes in dietary ion intake (Coppock et al., 1982).

Although all blood pH values were found to be within normal limits, there was a trend for increased blood pH with increased DCAD, with -100 and 0 mEq/kg DM balance diets being significantly different from 100 and 200 mEq/kg DM balance diets (Tucker et al., 1988). Hu and Murphy (2004) showed that blood pH increased quadratically with increased DCAD, with a maximum at 350 mEq/kg DM. Tucker et al. (1988) found that DCAD increased the cation-anion difference of the serum, which resulted in increased blood  $\text{HCO}_3^-$  concentrations, and they suggested that DCAD impacted blood pH by these

altered  $\text{HCO}_3^-$  concentrations. Quadratic relationships between DCAD and blood  $\text{HCO}_3^-$  concentrations have been identified (Sanchez et al., 1994a; Hu and Murphy, 2004). Hu and Murphy (2004) concluded that maximum  $\text{HCO}_3^-$  concentrations were attained when DCAD was 470 mEq/kg DM. Cubic (Sanchez et al., 1994a) and linear (Hu and Murphy, 2004) relationships between DCAD and blood  $\text{CO}_2$  concentrations have been observed. Increased  $\text{CO}_2$  in response to increased DCAD reflected respiratory compensation for the increased  $\text{HCO}_3^-$  (Hu and Murphy, 2004).

Compared to the other ions, blood chloride concentrations were most impacted by DCAD (Tucker et al., 1988; Hu and Murphy, 2004). Tucker et al. (1988) found that serum chloride concentrations decreased with increasing DCAD. Sanchez et al. (1994a) described a negative linear relationship between DCAD and blood chloride concentrations, while Hu and Murphy (2004) described a quadratic relationship. This could be explained by an inverse relationship between blood chloride and  $\text{HCO}_3^-$  concentrations (Hu and Murphy, 2004). Tucker et al. (1988) suggested that the decrease could be from increased chloride being excreted into the urine, which would counter the charges of increased sodium and potassium excretion. Hu and Murphy (2004) found that blood chloride concentrations increased with increased dietary chloride, which would lower the DCAD, but sodium and potassium concentrations were unaffected by their dietary concentrations due to increased excretion by the kidneys. In one study, serum sodium concentrations were not impacted by DCAD (Tucker et al., 1988), but a negative linear relationship was observed in another (Hu and Murphy, 2004). In both studies, serum potassium concentrations were not impacted by DCAD (Tucker et al., 1988; Hu and Murphy, 2004).

Tucker et al. (1988) found that urine pH increased with increased DCAD, and they suggested that this was due to increased  $\text{HCO}_3^-$  excretion by the kidneys. The increase was only significant when the DCAD increased from 100 to 200 mEq/kg DM, where the urine pH increased from 6.4 to 7.6 (Tucker et al., 1988). At DCAD of -100 and 0 mEq/kg DM, urine pH was 6.1 (Tucker et al., 1988). Hu and Murphy (2004) showed that urine pH increased quadratically with DCAD, and it reached its maximum pH, around 8.5, with a DCAD of 620 mEq/kg DM. They suggested that increased DCAD decreased the acid load in lactating cows (Hu and Murphy, 2004). Urine ion concentrations were related to the ion concentrations of the diets (Tucker et al., 1988; Tucker and Hogue, 1990).

Tucker and Hogue (1990) examined the impact of manipulating sodium, potassium, and chloride while maintaining a constant DCAD around 320 mEq/kg DM. Although there was a trend for plasma chloride concentrations to increase with increased dietary chloride, the differences were not statistically significant, and manipulating different ions did not impact the cation-anion difference of the plasma (Tucker and Hogue, 1990). Blood pH ranged from 7.38 to 7.40 for the different diets, and blood  $\text{HCO}_3^-$  and  $\text{CO}_2$  concentrations also only had small, insignificant variations between the different diets (Tucker and Hogue, 1990). Diet did not significantly impact milk yield or milk composition, but milk fat and milk protein tended to be higher in the diet that did not contain salts (Tucker and Hogue, 1990). Tucker and Hogue (1990) attributed this to decreased feed intake in the diets containing the salts. Sanchez et al. (1997) found that cows fed KCl and NaCl had similar dry matter intake, milk yield, 3.5% fat corrected milk yield, milk fat percentage, and milk protein percentage, but the DCAD in this study ranged from 250 to 400 mEq/kg DM, and they were not as similar as the DCAD used by Tucker and Hogue (1990).

## ***Impacts on Rumen Environment***

Roche et al. (2005) suggested that increased rumen pH caused by increased DCAD could enhance rumen fermentation. Tucker et al. (1988) showed that rumen pH tended to increase with increased DCAD. Diets that had DCAD of -100 and 0 mEq/kg DM had an average rumen pH of 6.45 and 6.63, respectively (Tucker et al., 1988). The rumen pH with the negative DCAD diet was significantly different from diets that had DCAD of 100 and 200 mEq/kg DM, which had an average rumen pH of 6.73 and 6.76, respectively (Tucker et al., 1988). While Tucker et al. (1988) manipulated DCAD with bicarbonate salts, a study that added anionic salts to the diet to achieve DCAD between -63 and -40 mEq/kg DM similarly found that negative DCAD diets reduced rumen pH by 0.12 units compared with cows fed a diet with a DCAD of 203 mEq/kg DM (Vagnoni and Oetzel, 1998). Apper-Bossard et al. (2010) observed that increasing DCAD had an impact on rumen pH within a DCAD range of 82 to 391 mEq/kg DM, and they indicated a trend for maximum rumen pH to occur in the middle DCAD range of 235 to 238 mEq/kg DM. When high concentrate diets were fed, increased DCAD decreased the rumen pH range that occurred throughout the day, but this was not observed with low concentrate diets (Apper-Bossard et al., 2010).

Tucker et al. (1988) found that increased DCAD tended to decrease the total VFA concentration, but isovalerate tended to increase. At a DCAD of -100 mEq/kg DM, the total VFA concentration was 104.9 mEq/L, which was significantly different from the total VFA concentration of 91.5 mEq/L that occurred at a DCAD of 100 mEq/kg DM, but there were no significant differences between the other DCAD (Tucker et al., 1988). Apper-Bossard et al. (2010) found that increasing DCAD did not impact total VFA or individual VFA concentrations, but their study only included diets with positive DCAD. Vagnoni

and Oetzel (1998) observed that total VFA concentrations were impacted by diet, but not necessarily by a change in DCAD. They found no differences in individual VFA concentrations or the acetate to propionate ratio, and they suggested that rumen fermentation was minimally impacted by anionic salts (Vagnoni and Oetzel, 1998). Tucker et al. (1988) observed that rumen fluid mineral concentrations were not impacted by DCAD, but chloride tended to decrease as DCAD increased. At a DCAD of -100 mEq/kg DM, the rumen chloride concentration was 12.6 mEq/L, while the chloride concentration was 8.4 mEq/L at a DCAD of 200 mEq/kg DM (Tucker et al., 1988).

## **IONOPHORES**

Ionophores are used as feed additives to improve feed efficiency in lactating dairy cattle (Duffield et al., 2008). A meta-analysis by Duffield et al. (2008) showed that monensin, an ionophore now commonly used by the dairy industry, decreased dry matter intake and increased milk production. The mechanism of ionophore action is to form complexes with specific cations, and these complexes bind to cell walls of bacteria, limiting the bacteria's ability to function and divide (McGuffey et al., 2001; Ipharraguerre and Clark, 2003). There have been some studies that have examined the impact of dietary ion concentrations on the effects of ionophores (Funk et al., 1983; Rumpler et al., 1986) or the impact of ionophores on rumen ion concentrations (Starnes et al., 1984).

Monensin binds Na ions with 10X the affinity that it binds K ions, while lasalocid, another ionophore, binds K ions with between 3X and 10X the affinity that it binds Na ions (McGuffey et al., 2001). Because intracellular K concentrations tend to be higher than the extracellular concentrations, the transport of cations into the cell through these ionophore complexes decreases intracellular K concentrations, with increased H and Na



concentrations, forcing the cell to use active transport mechanisms to restore optimal concentrations (Russell and Strobel, 1989; McGuffey et al., 2001). Gram negative bacteria, which produce propionate, tend to be resistant to ionophores, while gram positive bacteria, which produce acetate, butyrate, and other methane precursors, tend to be susceptible (Ipharraguerre and Clark, 2003). This explains the decreased acetate-to-propionate ratio, reduced methane production, and improved rumen fermentation with ionophore feeding (Ipharraguerre and Clark, 2003; McGuffey et al., 2004).

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 137 mEq/L to 172 mEq/L. They also demonstrated reduced monensin sensitivity in media where K was increased from 19 mEq/L to 35 mEq/L (Newbold et al., 2013). Although even the lowest Na concentration used by Newbold et al. (2013) was at the high end of the 74.5 mEq/L to 169.5 mEq/L range typically found in the rumen (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012), the K concentrations were more within the range of 28.2 to 68.9 mEq/L that would be measured in vivo (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012). Although the cation concentrations used by Newbold et al. (2013) may be outside the range of in vivo concentrations, there is also in vivo evidence that dietary ion concentrations can impact the efficacy of ionophores (Funk et al., 1983; Rumpler et al., 1986), which is likely a result of changes in rumen ion concentrations.

It could be hypothesized that through their effects on rumen ion concentrations, dietary ion concentrations might also influence the effects of ionophores on rumen fermentation.

Monensin preferentially binds Na ions over K ions (McGuffey et al., 2001), and increasing Na in the rumen fluid would increase the amount of Na ions available to be imported into the bacteria. Although lasalocid preferentially binds K ions, it can also bind Na ions (McGuffey et al., 2001). Starnes et al. (1984) measured rumen ion concentrations when growing beef steers were fed ionophores and found that monensin and lasalocid both decreased rumen K (Starnes et al., 1984). They attributed the decreased rumen K to increased association with feed particles in the rumen fluid or increased K absorption from the rumen. Funk et al. (1983) showed an interaction between lasalocid and dietary K in growing lambs. At 0.9% dietary K, the addition of lasalocid increased rumen propionate and decreased acetate (Funk et al., 1983). However, rumen propionate and acetate were not affected when lasalocid was added to diets that contained 2.5% K, suggesting that higher dietary K decreased the effectiveness of lasalocid in selecting for favorable rumen bacteria (Funk et al., 1983).

Rumpler et al. (1986) studied the impact of cation concentrations and ionophores on methane production in steers fed diets that were supplemented with NaCl and KCl to increase dietary Na and K to 2.5% of diet DM. In animals fed control diets, added dietary Na had no effect while added dietary K increased methane production by 17 L/day. In monensin fed steers, high dietary Na and K decreased methane production by 23 and 13 L/day, respectively, suggesting a potential monensin by cation interaction. In lasalocid fed steers, added Na reduced methane production by 13 L/day while added K increased methane production by 10 L/day, again suggesting a dietary cation by ionophore interaction (Rumpler et al., 1986).

## CONCLUSIONS

Rumen ion concentrations can be impacted by the diet, time post-feeding, salivary and blood concentrations, and their rate of absorption (Bennink et al., 1978). They can also be impacted by the concentrations of other ions in the rumen (Warner and Stacy, 1972a). Although these factors can all influence rumen ion concentrations, other systems in the body compensate for them to prevent drastic changes in rumen ion concentrations, indicating close regulation of ion concentrations in the rumen (Warner and Stacy, 1972a). DCAD (Mongin, 1981; Tucker et al., 1988) and ionophores (Duffield et al., 2008) are both tools used by the dairy industry to improve animal performance and feed efficiency. A better understanding of the effects of dietary strong ion concentrations on rumen ion environment might lead to a better understanding of the use of these tools.

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**Table 2.1** The physiological concentrations of sodium, potassium, and chloride found in parotid saliva, serum, and rumen fluid of cows. All concentrations are on a mEq/L basis.

Fluid	Ion Concentrations		
	Na	K	Cl
Parotid saliva <sup>1</sup>	157 – 168	6 – 14	7.4 – 34
Serum <sup>2</sup>	136 – 161	4 – 7	99 – 107
Rumen fluid <sup>3</sup>	74.5 – 169.5	28.2 – 68.9	6.7 – 25.6

<sup>1</sup>Compiled from (Kay, 1960; Bailey and Balch, 1961a)

<sup>2</sup>Compiled from (Tucker et al., 1988; Leroy et al., 2004; Cozzi et al., 2011)

<sup>3</sup>Compiled from (Bennink et al., 1978; Tucker et al., 1988; Duffield et al., 2004; Shen, et al., 2012)

**Table 2.2** The amount of saliva produced during eating, ruminating, and resting per day when cows are fed different diets. Saliva production is given on a liters per day (L/day) and liters per kilogram dry matter (L/kg DM) basis.

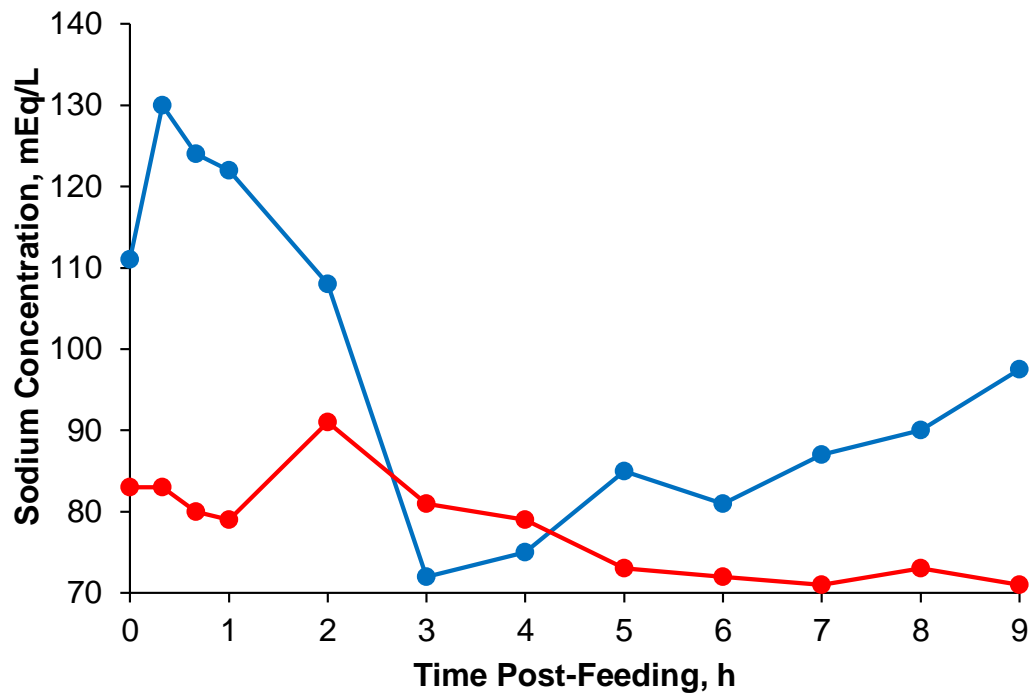
Diet	DMI <sup>3</sup>	Eating		Ruminating		Resting		Total	
	kg/day	L/day	L/kg DM	L/day	L/kg DM	L/day	L/kg DM	L/day	L/kg DM
Medium quality hay <sup>1</sup>	5.5	26	4.7	70	12.7	56	10.2	149	27.1
Alfalfa silage <sup>1</sup>	7.7	21	2.7	44	5.7	45	5.8	110	14.2
40% hay, 20% concentrate <sup>1</sup>	7.7	17	2.2	42	5.5	64	8.3	123	16.0
12.5% hay, 87.5% concentrate <sup>1</sup>	6.4	12	1.9	18	2.8	78	12.2	108	16.9
Fresh grass <sup>1</sup>	5.5	38	6.9	57	10.4	83	15.1	178	32.4
30% hay crop silage, 70% grain <sup>2</sup>	20	67	3.4	172	8.6	69	3.5	308	15.4
40% corn silage, 60% grain <sup>2</sup>	20	62	3.1	135	6.8	87	4.4	284	14.2
Average	10.4	34.7	3.6	76.9	7.5	68.9	8.5	180.0	19.5

<sup>1</sup>Adapted from (Bailey, 1961a)

<sup>2</sup>Adapted from (Cassida and Stokes, 1986)

<sup>3</sup>Dry matter intake

**Figure 2.1** The hourly variations in rumen sodium concentrations when cows were fed diets of either alfalfa hay or alfalfa pellets. Cows fed the alfalfa hay diet consumed 5 grams of sodium daily, while cows fed the alfalfa pellet diet consumed 4 grams of sodium (Bennink et al. 1978). Legend: Alfalfa hay (blue); alfalfa pellets (red).



## **Chapter 3: Experiment 1**

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

## INTERPRETIVE SUMMARY

**The impact of cation source and dietary cation-anion difference on rumen ion concentrations in lactating dairy cows.** *Catterton et al., page 000.* Dietary cation-anion difference (DCAD) is used to evaluate the strong ion balance in dairy cattle diets, but few studies have examined the impact of DCAD on rumen ion concentrations. In this study, cows were fed diets supplemented with NaCl, KCl, NaHCO<sub>3</sub>, and K<sub>2</sub>CO<sub>3</sub> to examine the effects of DCAD and cation source on the rumen ion environment. Rumen Na, K, and Cl concentrations increased when dietary supplements containing those elements were fed, and rumen Na decreased with increased dietary K. Further research about the relationship between dietary and rumen ion concentrations can lead to better utilization of DCAD and ionophores to improve dairy cattle performance.

### RUNNING HEAD: IMPACT OF CATION SOURCE AND DCAD ON RUMEN IONS

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

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## ABSTRACT

Although many studies have focused on the influence of dietary cation-anion difference (DCAD) on animal performance, few of them 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 the rumen environment and fermentation. The study used five rumen fistulated dairy cows, and treatments were applied using a 5 x 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 Na (DM basis) using NaCl; 3) the basal TMR plus 340 mEq/kg K using KCl; 4) the basal TMR plus 340 mEq/kg Na using NaHCO<sub>3</sub>; and 5) the basal TMR plus 340 mEq/kg K using K<sub>2</sub>CO<sub>3</sub>. On the last day of each experimental period, rumen samples were collected and pooled from five different locations at 0, 1.5, 3, 4.5, 6, 9, and 12 h post-feeding for measurement of rumen pH, strong ion, and VFA concentrations. 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 total rumen VFA and rumen pH. Increased DCAD increased rumen pH by 0.10 pH units and increased rumen acetate concentration, but did not increase total VFA. This study demonstrated that rumen ion concentrations can be manipulated by dietary ion concentration. Potential implications of changes in rumen ion concentrations include responses to dietary ionophores that influence rumen fermentation by transporting cations into affected bacterial cells and disrupting cellular homeostasis.



**Key words:** dietary cation-anion difference, rumen ion, dairy cow

## INTRODUCTION

Dietary cation-anion difference (**DCAD**) is used as a tool to evaluate the strong ion balance in formulation of dairy cattle diets. DCAD is calculated as follows: DCAD (mEq/kg DM) = Na + K – Cl (Mongin, 1981; Tucker et al., 1988). 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 on the effects of the individual ions that contribute to DCAD or the impact of DCAD on the rumen environment. While the impact of diet on the rumen ion environment has been studied previously (Bailey, 1961; Bennink et al., 1978), to our knowledge, only one study has studied the effect of DCAD on rumen ion concentrations (Tucker et al., 1988).

Increasing DCAD enhances animal performance by increasing dry matter intake, 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 impact animal performance (Iwaniuk et al., 2015). Iwaniuk et al. (2015) found that substitution of sodium for potassium as the supplemental cation source increased milk fat percentage and fat yield. In contrast, Wildman et al. (2007) and Hu and Kung (2009) found no impact 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.

In addition, ionophores are another tool used to improve feed efficiency. Several studies have examined the impact of dietary ion concentrations on the effects of ionophores (Funk et al., 1983; Rumpler et al., 1986) or the impact of ionophores on rumen ion concentrations (Starnes et al., 1984). Ionophores form complexes with specific cations, and these complexes bind to cell walls of bacteria, limiting the bacteria's ability to function and divide (McGuffey et al., 2001; Ipharraguerre and Clark, 2003).

Monensin binds Na ions with 10X the affinity that it binds K ions, while lasalocid binds K ions with between 3X and 10X the affinity that it binds Na ions (McGuffey et al., 2001). Because intracellular K concentrations tend to be higher than the extracellular concentrations, the transport of cations into the cell through these ionophore complexes decreases intracellular K concentrations, with increased H and Na concentrations, forcing the cell to use active transport mechanisms to restore optimal concentrations (Russell and Strobel, 1989; McGuffey et al., 2001). Gram-negative bacteria, which produce propionate, tend to be resistant to ionophores, while gram positive bacteria, which produce acetate, butyrate, and other methane precursors, tend to be susceptible (Ipharraguerre and Clark, 2003). This allows for a decreased acetate-to-propionate ratio and reduced methane production, improving rumen fermentation (Ipharraguerre and Clark, 2003; McGuffey et al., 2004). Newbold et al. (2013) demonstrated increased monensin sensitivity in rumen bacteria by increasing media Na and demonstrated reduced monensin sensitivity in media with increased K. There is further in vivo evidence that dietary ion concentrations can

impact the efficacy of ionophores (Funk et al., 1983; Rumpler et al., 1986), which is likely a result of changes in rumen ion concentrations.

The objective of this study was to examine the effects of DCAD, cation source, and anion source on the rumen 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 ( $245 \pm 4.5$  day-in milk at the start of the experiment) were used in a 10 week study that was conducted between June and August of 2014. Three cows were in early pregnancy, and 2 cows were non-pregnant at the start of the experiment. The cows were housed in tie stalls that were fitted with water mattresses and bedded with saw dust. 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 hours. Stalls were cleaned and rebedded 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 dark. Cows were individually fed a total mixed ration (**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 2001 National Research Council requirements for cows producing 40 kg of milk per day. The TMR basal diet consisted of 57.41% corn silage and 8.37% alfalfa hay as the forages (DM basis), with the remainder of diet consisting of ground corn, soy bean meal (48% crude protein), Soyplus<sup>®</sup> (West Central Cooperative, Ralston, IA), and a vitamin-mineral premix using corn gluten meal as the carrier. Ingredient composition of the basal and treatment diets are shown in Table 3.1. The total mixed ration 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 to generate a 2 to 4 kg/d feed refusal rate.

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<sub>3</sub>**); and 5) the basal TMR plus 340 mEq/kg added K using supplemental potassium carbonate (**K<sub>2</sub>CO<sub>3</sub>**). The corresponding mineral supplements included NaCl (Champion's Choice<sup>®</sup> Mix-N-Fine<sup>®</sup>, Cargill, Minneapolis, MN), KCl (Dyna-K<sup>®</sup>, The Mosaic Company, Plymouth, MN), NaHCO<sub>3</sub> (The Normalizer<sup>®</sup>, Church & Dwight Co., Inc, Piscataway, NJ), and K<sub>2</sub>CO<sub>3</sub> (DCAD Plus<sup>®</sup>, Church & Dwight Co., Inc, Piscataway, NJ). The level of supplementation (340 mEq per kg diet DM) was selected to ensure a response to the experimental treatments while keeping within the expected potential range of DCAD and K concentrations observed when feeding ingredients with high K and Cl concentrations

such as small grain silages and alfalfa. Treatments were applied in a 5 x 5 Latin square design with 2-wk experimental periods.

At any given time during the experiment, each treatment diet was being fed to only one cow. Thus, in order 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 by hand in the feeding tubs with a pitchfork. The mineral treatments supplied an additional  $342 \pm 0.4$  (average  $\pm$  SEM) mEq/kg diet DM of either Na or K. Diets that were supplemented with chloride received an additional  $347 \pm 4.1$  (average  $\pm$  SEM) mEq/kg diet DM of Cl. Table 3.2 shows the chemical composition of the experimental diets.

### ***Measurements***

Cows were weighed weekly, beginning on the first day of the study. 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, somatic cell count (**SCC**), other solids (lactose and minerals), and milk urea nitrogen (**MUN**) at the Lancaster DHIA (Lancaster, PA). The amounts of feed offered and refused (weighed just prior to the next feeding) were measured daily for each cow.

Samples of individual feed ingredients including corn silage, alfalfa hay, ground corn, soybean meal, Soyplus<sup>®</sup>, and the vitamin mineral premix were collected weekly, pooled together by component for the entire study, and sent to Cumberland Valley Analytical Service (Hagerstown, MD) for analysis of DM, crude protein, acid detergent fiber, neutral detergent fiber, lignin, ether extract, Ca, P, Mg, Na, K, Cl and S. Feed DM was measured weekly to adjust the as fed composition of the total mixed ration.

Rumen samples were collected on the last day of each period. Samples were collected 0, 1.5, 3, 4.5, 6, 9, and 12 h post-feeding, with time 0 being just before the cows were fed. Rumen fluid was collected in a 60 mL syringe using a 10-centimeter stainless steel tube with a mesh strainer at the end (RT Rumen Fluid Sampler Tube, Bar Diamond, Inc., Parma, ID). Ten mL of fluid was collected and pooled from five 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. Next, 10 mL of the fluid was placed into a tube containing 0.2 mL of 50% sulfuric acid and frozen at -20° C for later analysis of volatile fatty acids (VFA). The remaining 40 mL of rumen fluid was frozen for later analysis for rumen ion concentrations. Rumen VFA were analyzed using gas-liquid chromatography. The frozen samples were thawed in a water bath at 34° C and then centrifuged at 2100 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 using an Agilent 6890 Gas Chromatograph (Agilent Technologies, Santa Clara, CA) with helium as the carrier gas, a flow rate of 49.1 mL/minute, and a column temperature of 130° C. The ratio of the 2-ethylbutyrate concentration within each sample was compared to an internal standard to correct for variation in injection volume.

Rumen ion concentrations were determined using flame atomic absorption spectrometry (PerkinElmer 5100 PC, Norwalk, CT). Frozen rumen fluid samples were thawed in a water bath at 34° C and then centrifuged at 2100 g for 30 minutes 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 HNO<sub>3</sub>, 5 mL 0.046 M AgNO<sub>3</sub>, and 41.5 mL deionized water were combined, mixed, and then centrifuged at 1303 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):

$$\text{Cl, mg/L} = [500 - (100 \times \text{Ag, mg/L})] \times 3.29 \times \text{dilution factor} \quad (1)$$

All mineral concentrations in rumen fluid were converted to mEq/L by dividing by their respective atomic mass. Finally, the rumen cation-anion difference (**RCAD**) in rumen fluid was then 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 three days of each experimental were used to calculate dry matter intake (**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% fat corrected milk (**FCM**) was determined as described by Erdman (2011) using the following equation:

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

Feed efficiency was also calculated as described by Erdman (2011) by using the following equation:

$$\text{Feed efficiency} = \frac{3.5\% \text{ FCM (kg/day)}}{\text{DMI (kg/day)}} \quad (4)$$

DMI, milk production, milk composition, and feed efficiency data, summarized by cow within each experimental period were analyzed using the MIXED procedure in SAS (SAS Institute Inc.) using a statistical model that included the 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 post-feeding and time-post-feeding by treatment interactions. Cow within treatment and period were considered as random effects and used to test the effects of treatment whereas time post-feeding and time post-feeding by treatment interaction were tested using residual error. Four *a priori* comparisons were used to compare treatment effects using the contrast option within the MIXED procedure of SAS (SAS Institute Inc.)



to compare the effects of: 1) cation source (NaCl and NaHCO<sub>3</sub> vs. KCl and K<sub>2</sub>CO<sub>3</sub>); 2) anion source (NaCl and KCl vs. NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>); 3) DCAD (Basal, NaCl, and KCl vs. NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>); 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<sub>3</sub> 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<sub>3</sub> treatment, were excluded from the data analysis due to having outlier values for several of the VFAs. To account for these missing data points, the least square means are provided, and the highest SEM is reported.

## RESULTS

The chemical composition of the diets on a dry matter basis is presented in Table 3.2. The diets had similar amounts of all components, except for Na, K, and Cl, which were manipulated by treatment. The experimental diets supplied an additional  $342 \pm 0.4$  (average  $\pm$  SEM) mEq of Na or K/kg diet DM compared to the basal diet. The NaCl and KCl diets were supplied with an additional  $347 \pm 4.1$  (average  $\pm$  SEM) mEq Cl/kg diet DM compared to the other diets. The DCAD, calculated as  $\text{Na} + \text{K} - \text{Cl}$  (Mongin, 1981; Tucker et al., 1988) of the different diets was 346 mEq/kg diet DM for the basal diet, 339 mEq/kg diet DM for the NaCl diet, 329 mEq/kg diet DM for the KCl diet, 677 mEq/kg diet DM for the NaHCO<sub>3</sub> diet, and 681 mEq/kg diet DM for the K<sub>2</sub>CO<sub>3</sub> diet.

Due to the small number of cows and the relatively short (2 wk) experimental periods, the study was not designed to measure production responses. Treatment effects on body weight (**BW**), DMI, milk production, milk composition, and feed efficiency are shown in Table 3.3. Anion source ( $P = 0.001$ ) and DCAD ( $P = 0.002$ ) had significant effects on mean BW. Cows fed the chloride diets weighed on average 17 kg more than when they were fed the carbonate diets, while 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 changes in body tissue gains and losses. Dry matter intake, milk production, FCM, milk, fat yield, protein percentage, other solids (lactose and minerals) percentage and yield, somatic cell count, MUN, and feed efficiency were not affected by treatment.

Milk fat percentage was increased by 0.15 percentage units by increased DCAD ( $P = 0.044$ ). There was no difference ( $P = 0.853$ ) between basal diet and chloride diets (NaCl and KCl) for milk fat percentage. Protein yield was reduced by 61 g/d by increasing DCAD ( $P = 0.043$ ), and similarly, there was a trend for an anion source effect ( $P = 0.053$ ), but there was no difference between the basal and chloride diets ( $P = 0.135$ ). Milk from cows receiving the chloride diets contained on average 66 more grams of protein per day than milk from cows receiving the carbonate diets. Cows receiving the low DCAD diets produced on average 51 more grams milk protein per day than cows receiving the high DCAD diets. While the differences in milk fat and protein were significant, the degree of inference is limited due to the small number of cows and short experimental periods used in the experiment. Therefore, intake and production responses will not be further discussed

Table 3.4 shows the effect of treatment on rumen pH, ion concentrations, and volatile fatty acid concentrations. Rumen pH decreased with time post-feeding ( $P = 0.001$ ; Figure 3.1). 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 3.4). Rumen sodium concentrations were 11 mEq/L greater when cows were fed diets containing added sodium as 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 post-feeding ( $P = 0.001$ ; Figure 3.2), and there was a trend for a greater decline in the cows fed added K (treatment by time post-feeding;  $P = 0.091$ ).

Rumen K concentrations were on average 19.0 mEq/L higher in cows fed added K (Cation;  $P = 0.001$ ). In contrast with the decline in rumen Na with time post-feeding, rumen K increased with time post-feeding ( $P = 0.001$ ) and there was a trend for the effects of anion source ( $P = 0.091$ ) and a cation by anion interaction ( $P = 0.089$ ) where added dietary Cl reduced rumen K (Figure 3.3).

Rumen Cl concentrations increased ( $P = 0.001$ ) nearly two-fold immediately after feeding and then remained nearly constant thereafter during the 12 h sampling period. 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 resulted in an 11 mEq/L increase in rumen Cl (Table 3.4).

The combination of reduced rumen Na and increased rumen K with time post-feeding resulted in a decline in rumen Na:K ratio with time post-feeding ( $P = 0.001$ ; Figure 3.5). The Na:K ratio was on average 2.3-fold higher in cows fed diets supplemented with Na as

compared to those supplemented with K ( $P = 0.001$ ). A plot of the raw data for rumen K vs. Na (Figure 3.6) showed an inverse relationship between rumen Na and K.

Changes in rumen RCAD followed changes in dietary DCAD where increased DCAD increased RCAD ( $P = 0.009$ ; Table 3.4). Cation source in the high DCAD diets had an effect on RCAD where cows fed added  $\text{NaHCO}_3$  had a greater increase in RCAD than those fed  $\text{K}_2\text{CO}_3$  (Table 3.4;  $P = 0.010$ ). As expected, added dietary Cl reduced RCAD ( $P = 0.004$ ). RCAD decreased with time post-feeding ( $P = 0.001$ ).

Acetate was the only rumen VFA that was affected by dietary treatment (Table 3.4). Acetate was increased ( $P = 0.020$ ) in cows fed the high DCAD diets ( $\text{NaHCO}_3$  and  $\text{K}_2\text{CO}_3$ ) where acetate concentrations were on average 4.2 mEq/L higher. Rumen acetate concentrations increased rapidly after feeding ( $P = 0.001$ ) and remained elevated thereafter (Figure 3.7). There was no effect of treatment on the remaining rumen VFA. There were 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 (Figures 3.9 and 3.11 to 3.14). Figure 3.10 shows the changes in rumen acetate-to-propionate (**A:P**) ratio by treatment and time post-feeding. There was a trend for a DCAD effect on A:P ( $P = 0.058$ ) but no time post-feeding effect on rumen A:P. Total VFA concentrations (Figure 3.14) were affected by time ( $P = 0.001$ ), but they were not significantly impacted by treatment ( $P = 0.332$ ; Table 3.4).

## 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 ( $\text{NaCl}$  vs.  $\text{NaHCO}_3$ ). 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 vs. 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 25 percent decrease in rumen Na concentration. The decrease in rumen Na concentrations post-feeding 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.

Adding dietary K increased rumen K by 19 mEq/L and showed that diet K also has an effect on 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 with dietary K addition, which reduced rumen Na compared to the basal diet, addition of dietary Na had no effect on rumen K.

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

direct supplementation of K supplements. Tucker et al. (1988) found that DCAD did not impact rumen Na or K concentrations, but they did note an inverse relationship between the two 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<sub>2</sub>CO<sub>3</sub> 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 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 of dietary Cl and K. In contrast, Bailey (1961) reported that dietary Cl concentrations did impact 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 suggests a significant DCAD effect on rumen Cl concentrations, it is likely that the effect is 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 two 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 three different dietary combinations that they used to achieve each DCAD, so direct dietary comparison to their rumen Cl concentrations is not possible (Tucker et al., 1988).

Rumen pH was 0.108 units higher when cows were fed the higher DCAD diets, which were achieved using supplementation with  $\text{NaHCO}_3$  and  $\text{K}_2\text{CO}_3$ . However, cation source did not impact rumen pH. A literature review by Erdman (1988) discussed the benefits of using buffers such as  $\text{NaHCO}_3$  and  $\text{K}_2\text{CO}_3$  to reduce the fall in rumen pH associated with time post-feeding. 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. 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 concentrations. Tucker et al. (1988) only noted a tendency for decreased total VFA concentrations and increased isovalerate concentrations with increased DCAD. Similarly, Apper-Bossard et al. (2010) reported no significant changes in VFA concentration with altered DCAD.

It could be hypothesized that through their effects on rumen ion concentrations, dietary ion concentrations might also influence 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). Starnes et al. (1984) measured rumen ion concentrations when growing beef steers were fed ionophores and found that monensin and lasalocid both decreased rumen K (Starnes et al., 1984). They attributed the decreased rumen K to increased association with feed particles in the rumen fluid or increased K absorption from the rumen.

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. While 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) that we measured in our study.

There are in vivo results that support differential responses to ionophores by altering dietary K and Na. Funk et al. (1983) showed an interaction between lasalocid and dietary K in growing lambs. At 0.9% dietary K, the addition of lasalocid increased rumen propionate and decreased acetate (Funk et al., 1983). However, rumen propionate and acetate were not affected when lasalocid was added to diets that contained 2.5% K, suggesting that higher dietary K decreased the effectiveness of lasalocid in selecting for favorable rumen bacteria (Funk et al., 1983).

Rumpler et al. (1986) studied the impact of cation concentrations and ionophores on methane production in steers fed diets that were supplemented with NaCl and KCl to



increase dietary Na and K to 2.5% of diet DM. In animals fed control diets, added dietary Na had no effect while added dietary K increased methane production by 17 L/d. In monensin fed steers, high dietary Na and K decreased methane production by 23 and 13 L/d respectively suggesting a potential monensin by cation interaction. In lasalocid fed steers, added Na reduced methane production by 13 L/d while added K increased methane production by 10 L/d, again suggesting a dietary cation by ionophore interaction (Rumpler et al., 1986).

Similar to the *in vivo* results obtained by Funk et al. (1983) and Rumpler et al. (1986), we suggest that increasing rumen K decreases the effects of ionophores such as monensin and lasalocid on rumen fermentation. Although those experiments did not measure rumen ion concentrations, it can be assumed that the increased dietary K would have increased rumen K concentrations and reduced rumen Na concentrations as we demonstrated in our study. The reduced responses to monensin and lasalocid in high K diets are consistent with the reduced monensin sensitivity in rumen bacteria where K was increased in the culture media (Newbold et al., 2013). While our study demonstrated that rumen ion concentrations could be manipulated by dietary ion content, more experiments are needed to demonstrate both the direct effects of rumen Na, K and Cl on rumen fermentation and their potential modulation of ionophore responses.

## **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 there was 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 has a respective effect on RCAD or the balance between Na, K, and Cl ion concentrations in the rumen. Therefore, if production and feed efficiency responses to ionophores in the diet are affected by rumen Na and K concentrations, then theoretically manipulating dietary Na and K could either mute or enhance the responses to ionophores in the diet.

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**Table 3.1** The ingredient composition of the experimental diets (% of diet DM)

Item	Treatment <sup>1</sup>				
	Basal	NaCl	KCl	NaHCO <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>
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 <sup>2</sup>	3.41	3.35	3.33	3.32	3.34
Corn gluten meal	0.60	0.59	0.59	0.59	0.59
Dynamate <sup>3</sup>	0.13	0.13	0.13	0.13	0.13
Biophos <sup>4</sup>	0.43	0.42	0.42	0.42	0.42
Limestone <sup>5</sup>	0.61	0.59	0.59	0.59	0.59
Magnesium oxide	0.34	0.33	0.33	0.33	0.33
ADE mix <sup>6</sup>	0.03	0.03	0.03	0.03	0.03
Vitamin E <sup>7</sup>	0.01	0.01	0.01	0.01	0.01
Megalac <sup>8</sup>	1.46	1.43	1.42	1.42	1.43
Selenium <sup>9</sup>	0.06	0.06	0.06	0.06	0.06
Omigen-AF <sup>10</sup>	0.20	0.19	0.19	0.19	0.19
TM-433 <sup>11</sup>	0.03	0.03	0.03	0.03	0.03
4-Plex C <sup>12</sup>	0.01	0.01	0.01	0.01	0.01
Diamond V XP <sup>13</sup>	0.24	0.23	0.23	0.23	0.23
NaCl <sup>14</sup>	0.49	2.48	0.47	0.47	0.48
KCl	0.00	0.00	2.68	0.00	0.00
NaHCO <sub>3</sub>	0.00	0.00	0.00	2.87	0.00
K <sub>2</sub> CO <sub>3</sub>	0.00	0.00	0.00	0.00	2.39

<sup>1</sup>The basal diet was supplemented with the indicated salt for the treatment diets.

<sup>2</sup>(West Central Cooperative, Ralston, IA).

<sup>3</sup>Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN).

<sup>4</sup>Contained 17% Ca and 21% P.

<sup>5</sup>Contained 36% Ca and 0.02% P.

<sup>6</sup>Contained 5,454,545 IU/kg Vitamin A, 1,818,182 IU/kg Vitamin D, 9,091 IU/kg Vitamin E.

<sup>7</sup>Contained 56,818 IU/kg Vitamin E.

<sup>8</sup>Contained 9% Ca; 84.5% Fat (Church & Dwight Co., Inc., Piscataway, NJ).

<sup>9</sup>Contained 0.3 IU/g of selenium, 28% Ca.

<sup>10</sup>Contained 0.41 mg/kg Biotin, 15 mg/kg Choline, 31 mg/kg d-Pantothenic Acid, 1.4 mg/kg Folic Acid, 3.2 mg/kg Menadione, 102 mg/kg Niacin, 30 mg/kg Riboflavin, 4.5 x 10<sup>10</sup> CFU/kg *Saccharomyces cerevisiae*, 15 mg/kg Thiamine, 8.2 mg/kg Vitamin B-6, and 41 mcg/kg Vitamin B-12 (Prince Agri Products, Inc., Quincy, IL).

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

<sup>12</sup>Contained 0.36% Co, 1.80% Cu, 2.86% Mn, and 5.15% Zn (Zinpro Corporation, Eden Prairie, MN).

<sup>13</sup>Contained *Saccharomyces cerevisiae* yeast (Diamond V, Cedar Rapids, IA).

<sup>14</sup>Additional salt was given to cows when receiving the NaCl treatment.

**Table 3.2** The chemical composition of the experimental diets on a DM basis

Item	Treatment <sup>1</sup>					SEM
	Basal	NaCl	KCl	NaHCO <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>	
DM, %	42.8	43.3	43.5	43.5	43.3	0.127
NE <sub>L</sub> , Mcal/kg	1.63	1.59	1.58	1.58	1.59	0.008
CP, %	16.3	16.0	15.9	15.8	15.9	0.084
NDF, %	28.1	27.5	27.3	27.3	27.4	0.146
ADF, %	20.5	20.0	19.9	19.9	20.0	0.106
Lignin, %	3.51	3.44	3.42	3.41	3.43	0.018
Ash, %	7.53	9.38	10.01	10.2	9.74	0.479
Fat <sup>2</sup> , %	2.62	2.56	2.55	2.54	2.55	0.014
Na, %	0.250	1.03	0.243	1.03	0.244	0.192
K, %	1.56	1.53	2.85	1.51	2.86	0.325
Cl, %	0.569	1.77	1.80	0.552	0.555	0.300
S, %	0.229	0.224	0.223	0.222	0.223	0.001
Ca, %	0.910	0.892	0.885	0.884	0.888	0.005
P, %	0.527	0.516	0.513	0.512	0.514	0.003
Mg, %	0.392	0.384	0.382	0.381	0.383	0.002
DCAD <sup>3</sup> mEq/kg	346	339	329	677	681	83.57
DCAD-S <sup>4</sup> mEq/kg DM	275	269	260	608	611	83.74

<sup>1</sup>The basal diet was supplemented with the indicated salt for the treatment diets.

<sup>2</sup>Crude fat, which does not account for the 1.23% fatty acids provided by Megalac 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). (Arm and Hammer Animal Nutrition, Piscataway, NJ).

<sup>3</sup>DCAD = Na + K - Cl.

<sup>4</sup>DCAD-S = Na + K - Cl - S.

**Table 3.3** Treatment effects on DMI, milk production, milk composition, and feed efficiency

Item	Treatment					SEM	P- value			
	Basal	NaCl	KCl	NaHCO <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>		Cation <sup>1</sup>	Anion <sup>2</sup>	DCAD <sup>3</sup>	Basal vs Cl <sup>4</sup>
Number of cows	5	5	5	4	5					
BW, kg	696	706	701	689	684	4.2	0.191	0.001	0.002	0.107
DMI, kg/d	23.5	24.0	24.1	24.0	23.5	0.69	0.788	0.639	0.854	0.444
Milk, kg/d	30.9	32.9	31.4	30.2	30.4	2.47	0.759	0.425	0.504	0.633
3.5% FCM, kg/d	30.4	32.7	30.9	30.7	30.2	0.95	0.200	0.145	0.299	0.175
Fat, %	3.64	3.63	3.61	3.80	3.76	0.085	0.731	0.053	0.044	0.853
Fat yield, g/d	1,100	1,179	1,114	1128	1,105	38.5	0.222	0.391	0.651	0.258
Protein, %	3.07	3.07	3.08	3.03	3.08	0.027	0.272	0.481	0.475	0.905
Protein yield, g/d	936	1005	956	909	921	27.0	0.456	0.019	0.043	0.135
OS <sup>5</sup> , %	5.49	5.57	5.50	5.54	5.52	0.029	0.113	0.911	0.665	0.204
OS <sup>5</sup> yield, g/d	1,698	1,833	1,728	1690	1,683	59.4	0.314	0.101	0.197	0.206
SCC, linear score	3.57	3.19	3.38	3.37	3.61	0.299	0.433	0.455	0.661	0.375
MUN, mg/dL	15.1	14.5	13.8	14.0	14.2	0.45	0.581	0.860	0.314	0.067
Feed Efficiency <sup>6</sup>	1.30	1.37	1.28	1.28	1.29	0.035	0.231	0.186	0.245	0.490

<sup>1</sup>Comparison of added dietary Na (NaCl and NaHCO<sub>3</sub>) vs. added dietary K (KCl and K<sub>2</sub>CO<sub>3</sub>).

<sup>2</sup>Comparison of added dietary Cl (NaCl and KCl) vs. added dietary carbonate and bicarbonate (NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>).

<sup>3</sup>Comparison of dietary DCAD effects (Basal, NaCl, and KCl vs. NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>).

<sup>4</sup>Comparison of Basal vs. NaCl and KCl.

<sup>5</sup>Other solids (lactose and minerals).

<sup>6</sup>3.5% FCM/DMI.

**Table 3.4** Treatment effects on rumen pH, strong ion, and VFA concentrations

Item	Treatment					SEM	P- value			Basal vs CI <sup>7</sup>
	Basal	NaCl	KCl	NaHCO <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>		Cation <sup>4</sup>	Anion <sup>5</sup>	DCAD <sup>6</sup>	
Number of cows	5	5	5	4	5					
Number of samples	35	35 <sup>1</sup>	35	28 <sup>2</sup>	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 <sup>3</sup>	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 volatile fatty acids (mEq/L)										
Acetate	71.5	70.5	73.3	75.0	76.9	1.91	0.187	0.035	0.020	0.833
Propionate	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 <sup>8</sup>	3.06	3.25	3.28	3.49	3.32	0.120	0.518	0.222	0.058	0.119

<sup>1</sup>35 samples were used for the analysis of rumen ion concentrations. 34 samples were used for the analysis of VFA concentrations.

<sup>2</sup>28 samples were used for the analysis of rumen ion concentrations. 27 samples were used for the analysis of VFA concentrations.

<sup>3</sup>Rumen cation-anion difference: Na + K – Cl (mEq/L).

<sup>4</sup>Comparison of added dietary Na (NaCl and NaHCO<sub>3</sub>) vs. added dietary K (KCl and K<sub>2</sub>CO<sub>3</sub>).

<sup>5</sup>Comparison of added dietary Cl (NaCl and KCl) vs. added dietary carbonate and bicarbonate (NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>).

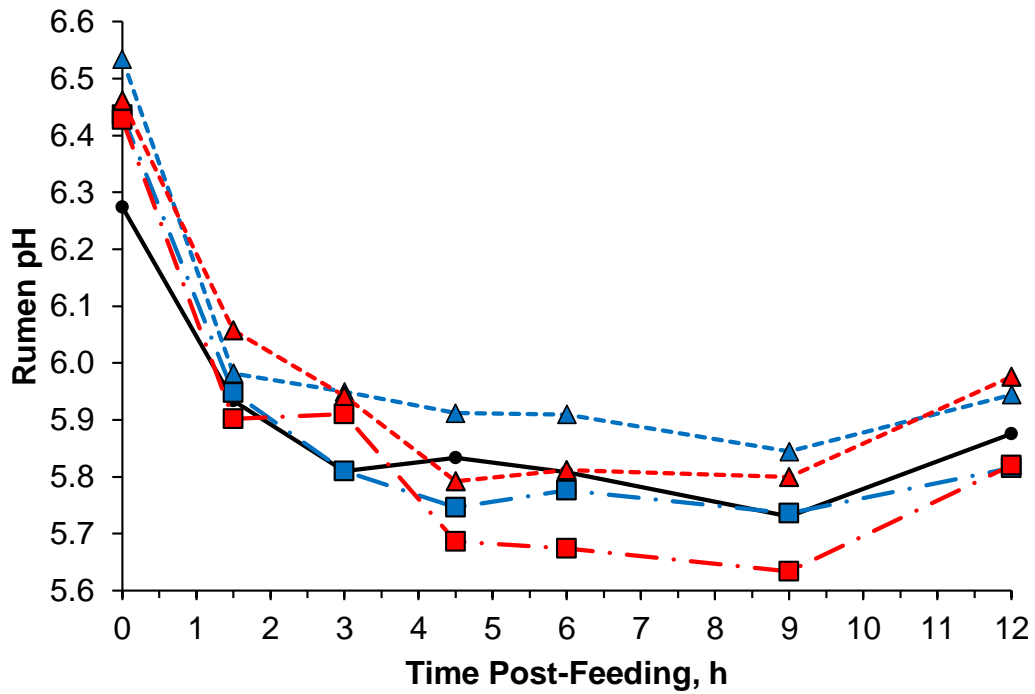
<sup>6</sup>Comparison of dietary DCAD effects (Basal, NaCl, and KCl vs. NaHCO<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>).

<sup>7</sup>Comparison of Basal vs. NaCl and KCl.

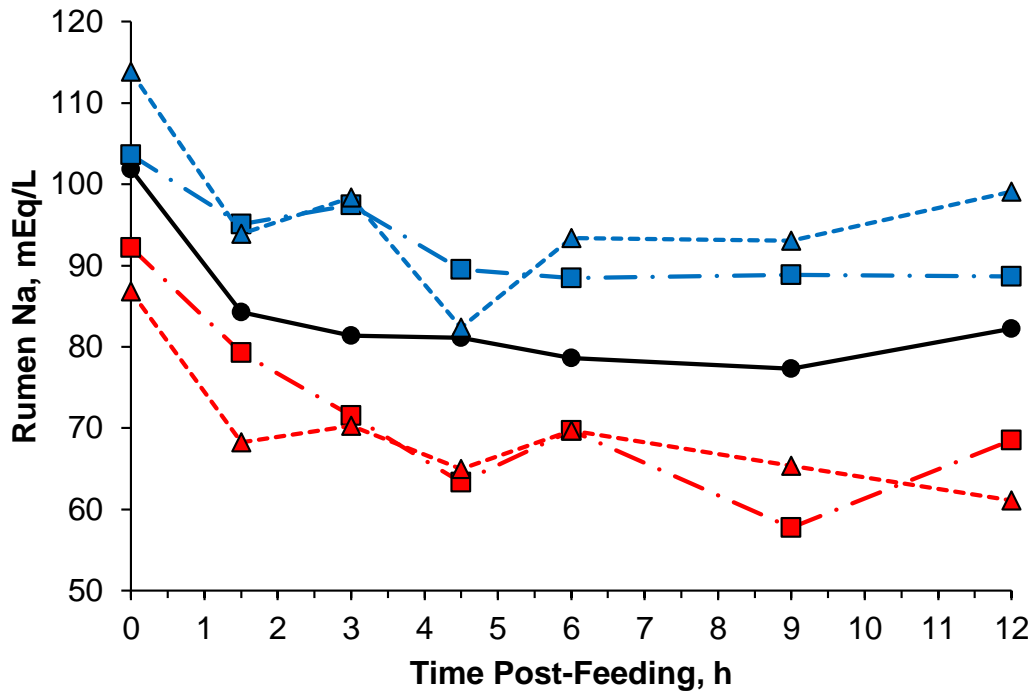
<sup>8</sup>Acetate:propionate ratio.



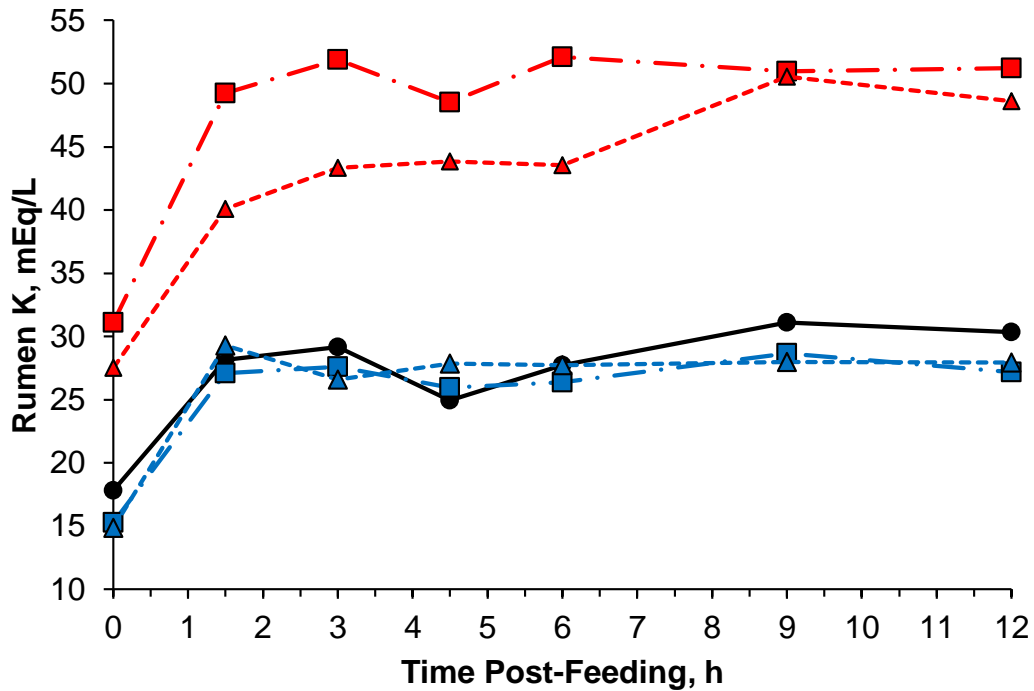
**Figure 3.1** Time post-feeding and treatment by time post-feeding effects on rumen pH. The effect of time post-feeding on rumen pH was significant ( $P = 0.001$ ) but there were no treatment by time interactions ( $P = 0.940$ ). (SEM = 0.088). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



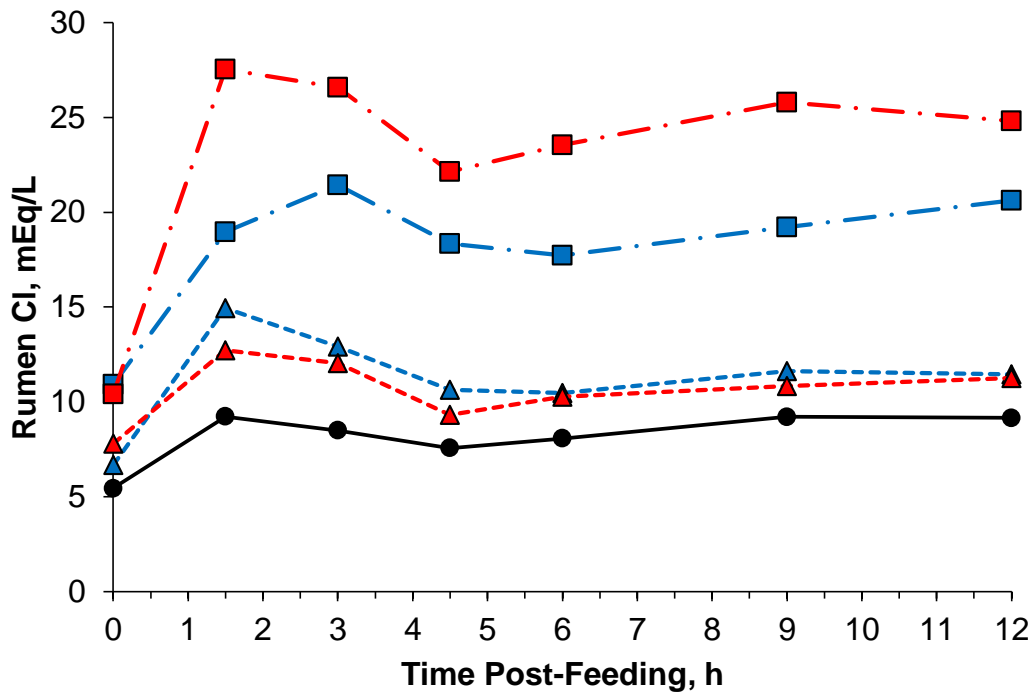
**Figure 3.2** Time post-feeding and treatment by time post-feeding effects on rumen Na concentration. Treatment ( $P = 0.001$ ) and time ( $P = 0.001$ ) effects were significant, and there was a trend for a treatment by time interaction ( $P = 0.091$ ). Cation source (added dietary Na) increased rumen Na ( $P = 0.001$ ), but there were no effects of anion source ( $P = 0.797$ ) or DCAD ( $P = 0.999$ ). (SEM = 3.88). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



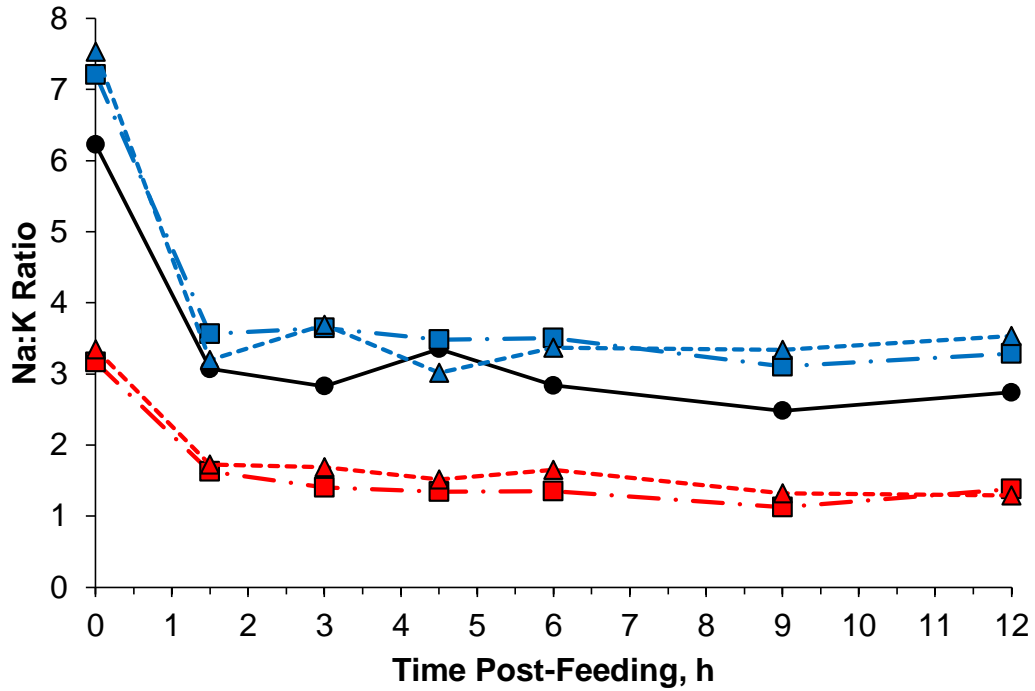
**Figure 3.3** Time post-feeding and treatment by time post-feeding effects on rumen K concentration. Time had a significant ( $P = 0.001$ ) effect on rumen K but there were no treatment by time interactions ( $P = 0.340$ ). The cation source ( $P = 0.001$ ) effect was significant where added dietary K increased rumen K concentration, and there was a trend for anion source ( $P = 0.091$ ) where added dietary Cl reduced rumen K. (SEM = 2.60). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



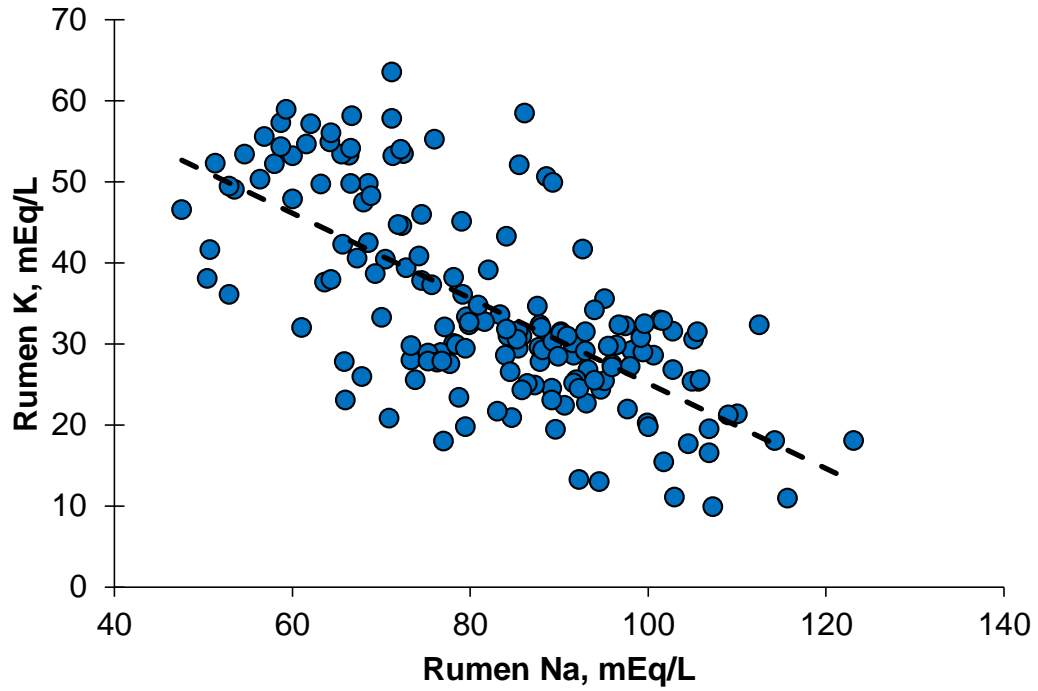
**Figure 3.4** Time post-feeding and treatment by time post-feeding effects on rumen Cl concentrations. There was a significant effect of time ( $P = 0.001$ ) where rumen Cl increased immediately after feeding but there was no treatment by time interaction ( $P = 0.260$ ). Cows fed the basal diet had significantly lower rumen Cl than cows fed diets that received added Cl ( $P = 0.001$ ). (SEM = 2.36). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



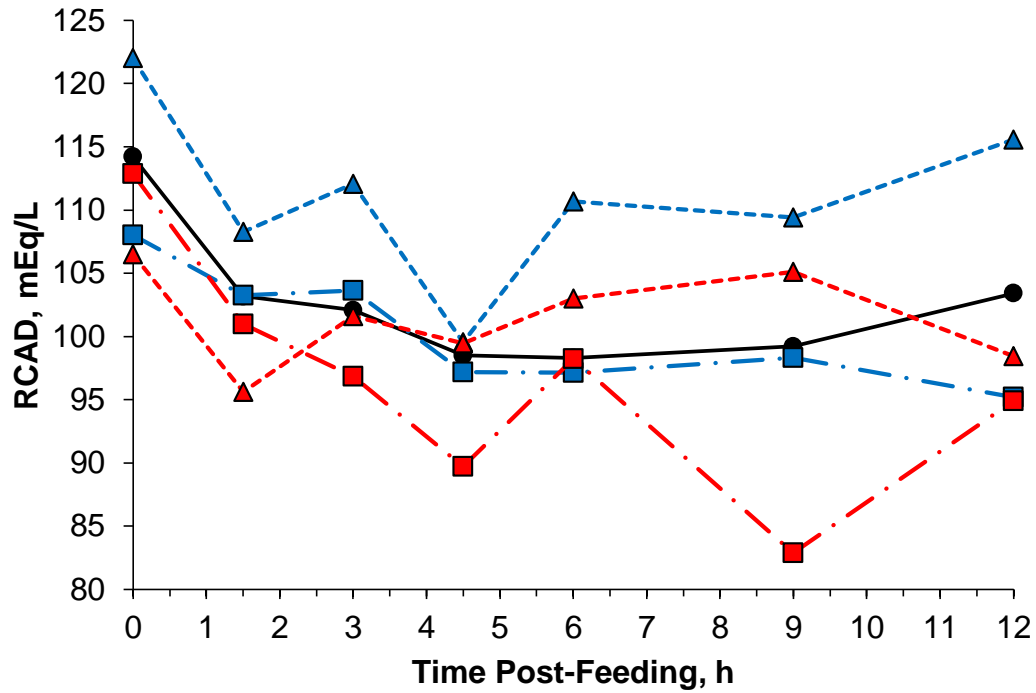
**Figure 3.5** Time post-feeding and treatment by time post-feeding effects on rumen Na:K ratios. Time ( $P = 0.001$ ) and the treatment by time interactions ( $P = 0.011$ ) were significant where feeding added K increased rumen K in relation to rumen Na. Cation source was significant ( $P = 0.001$ ) where cows fed added dietary K had reduced rumen Na:K. (SEM = 0.363). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



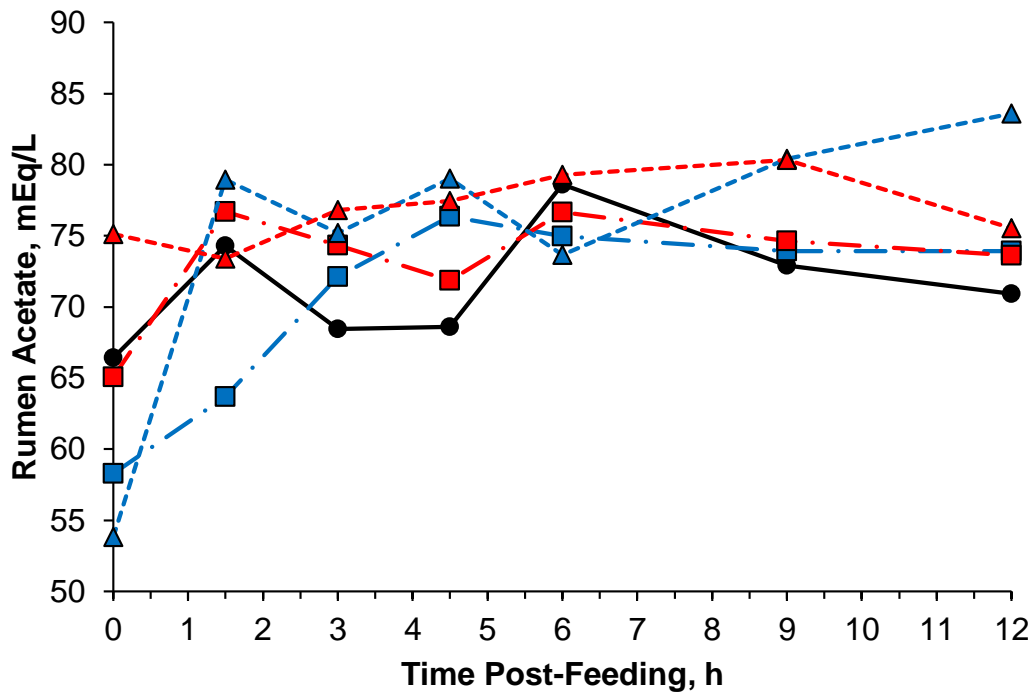
**Figure 3.6** Negative correlation between rumen Na and K concentrations across sampling times where Rumen K (mEq/L) = 0.525 x Rumen Na (mEq/L) + 77.6; Intercept  $P = 0.001$ ; Intercept SE = 3.63; Slope  $P = 0.001$ ; Slope SE = 0.043;  $R^2 = 0.470$ ; RMSE = 8.76).



**Figure 3.7** Time post-feeding and treatment by time post-feeding effects on rumen cation-anion difference (RCAD). Time effects were significant ( $P = 0.001$ ), but there was no treatment by time interaction ( $P = 0.222$ ). Increased DCAD ( $P = 0.009$ ) and sodium as a cation source ( $P = 0.010$ ) increased RCAD while chloride as anion source decreased ( $P = 0.004$ ) RCAD. (SEM = 4.66). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.

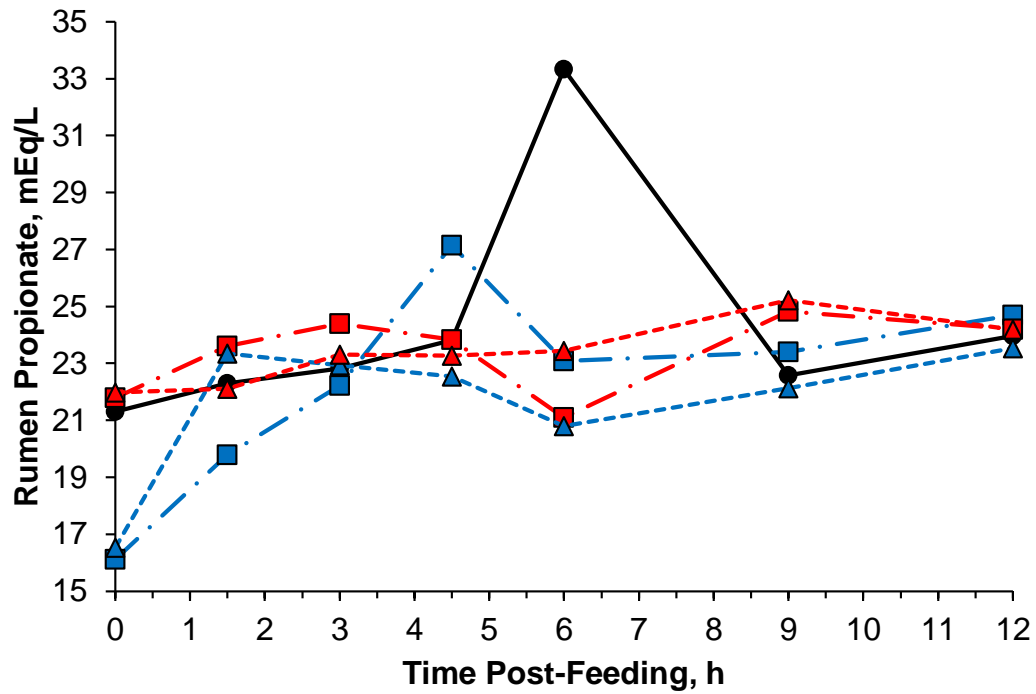


**Figure 3.8** Time post-feeding and treatment by time post-feeding effects on rumen acetate concentrations. Time post-feeding was significant ( $P = 0.001$ ), and there was a trend for a treatment by time interaction ( $P = 0.095$ ). Cation source was not significant ( $P = 0.187$ ). Increased DCAD ( $P = 0.020$ ) increased rumen acetate. (SEM = 4.80). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.

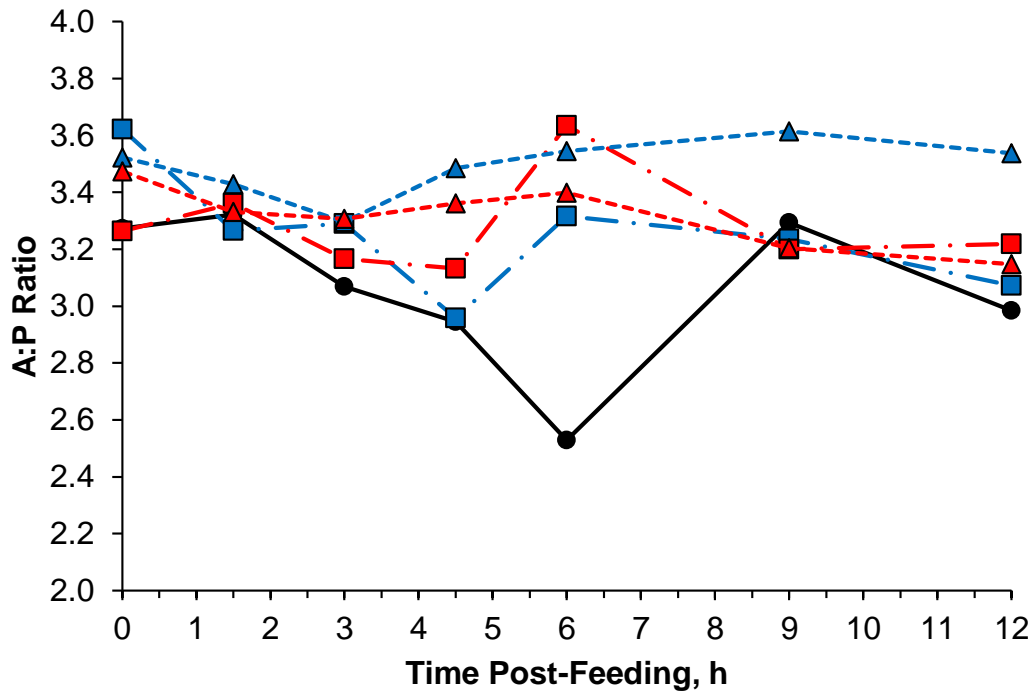




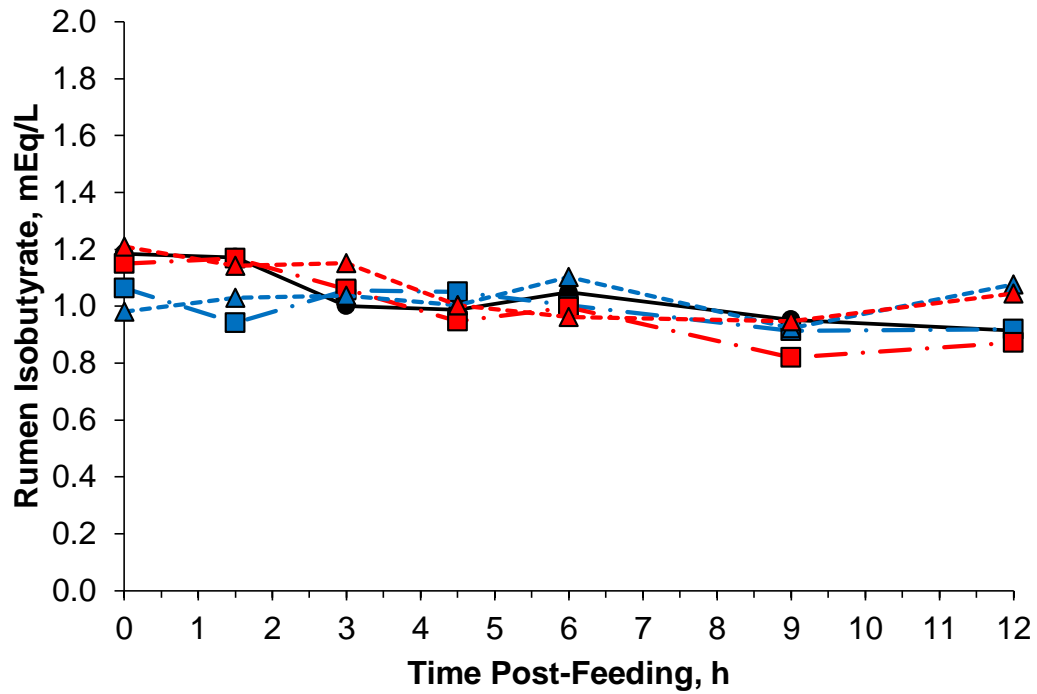
**Figure 3.9** Time post-feeding and treatment by time post-feeding effects on rumen propionate concentrations. Time effects were significant ( $P = 0.002$ ), and there was a trend for a treatment by time interaction ( $P = 0.055$ ). (SEM = 2.61). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



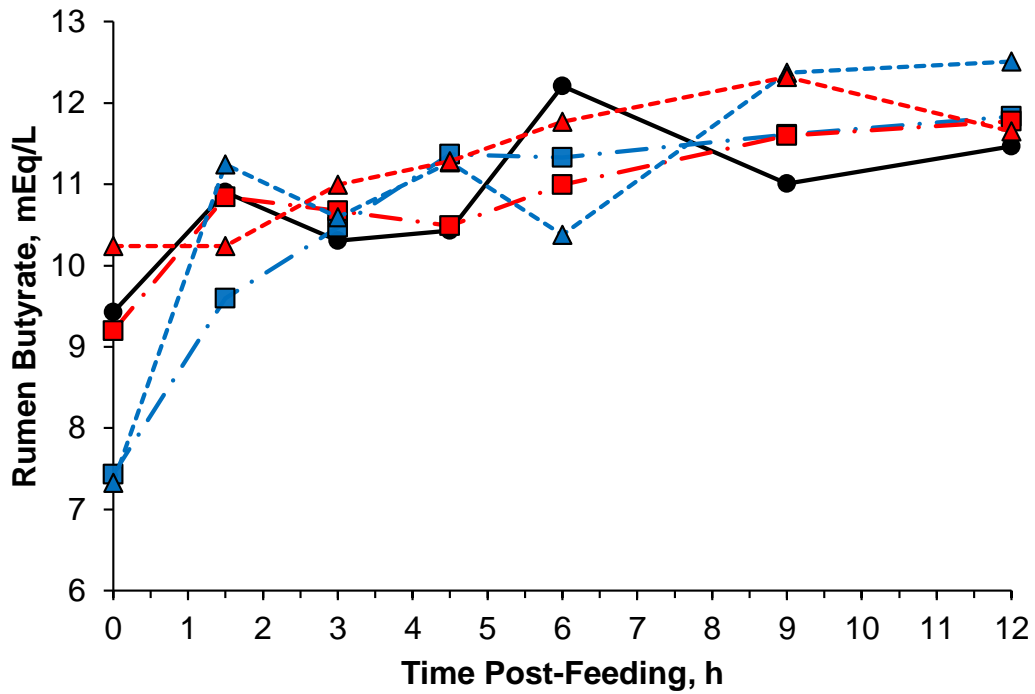
**Figure 3.10** Time post-feeding and treatment by time post-feeding effects on rumen acetate-to-propionate (A:P) ratio. There were no time effects ( $P = 0.120$ ), but there was a trend for a treatment by time interaction ( $P = 0.095$ ). (SEM = 0.220). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



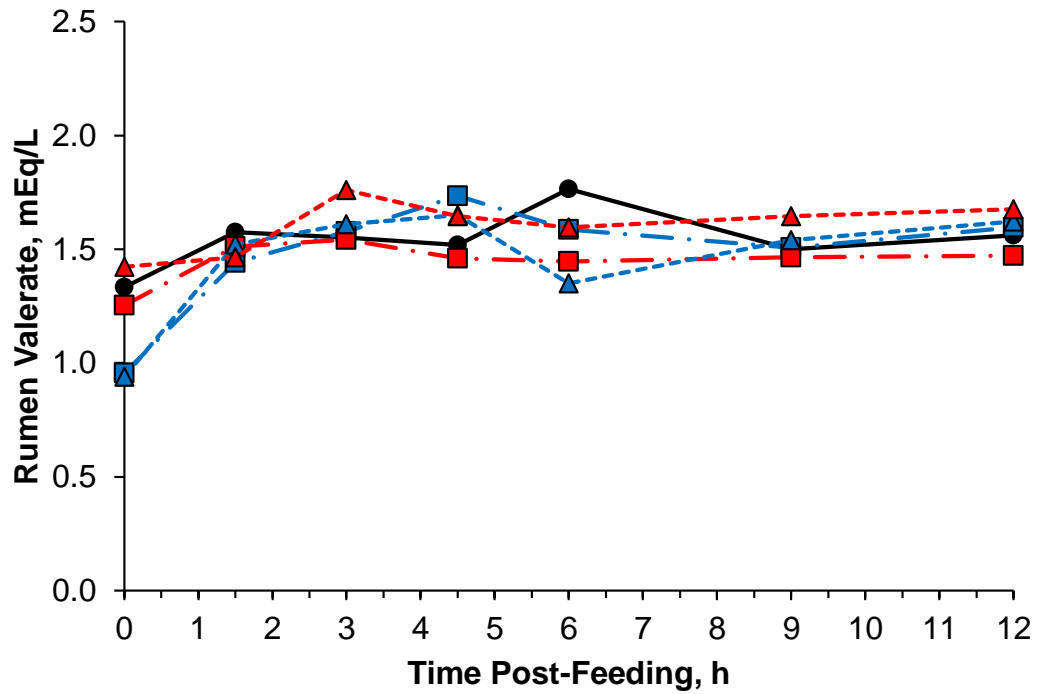
**Figure 3.11** Time post-feeding and treatment by time post-feeding effects on rumen isobutyrate concentrations. Time effects were significant ( $P = 0.001$ ), but there was no treatment by time interaction ( $P = 0.357$ ). (SEM = 0.088). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



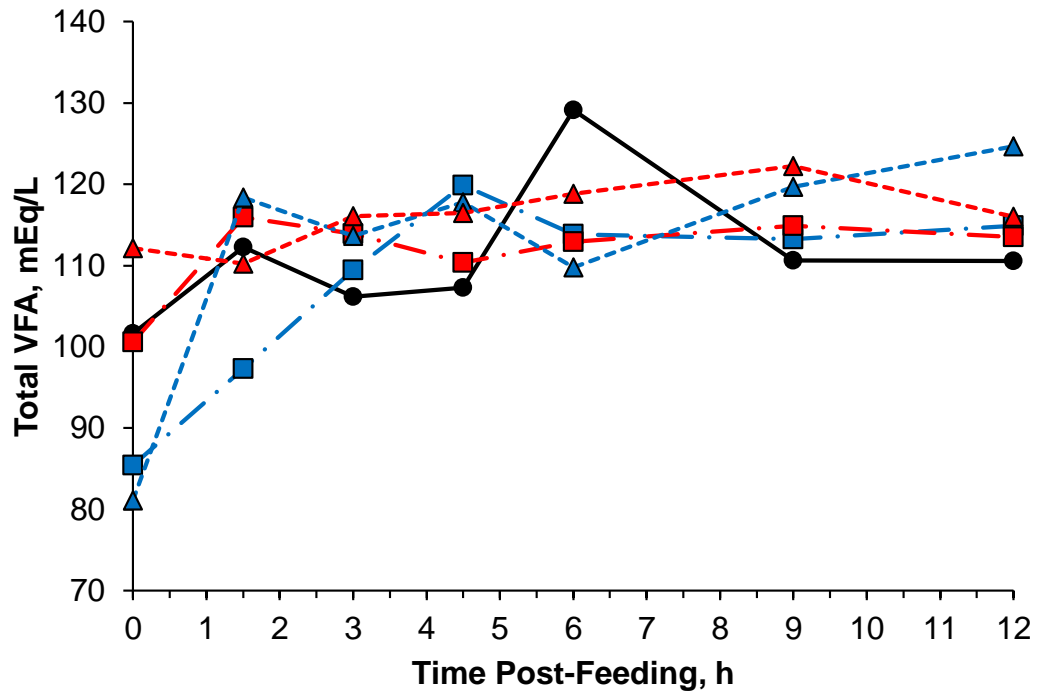
**Figure 3.12** Time post-feeding and treatment by time post-feeding effects on rumen butyrate concentrations. Time effects were significant ( $P = 0.001$ ), but there was no treatment by time interaction ( $P = 0.627$ ). (SEM = 0.987). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



**Figure 3.13** Time post-feeding and treatment by time post-feeding effects on rumen valerate concentrations. The time effect was significant ( $P = 0.001$ ), but there was no treatment by time interaction ( $P = 0.792$ ). (SEM = 0.170). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



**Figure 3.14** Time post-feeding and treatment by time post-feeding effects on rumen total volatile fatty acid (VFA) concentration. Time effects were significant ( $P = 0.001$ ), but there were no time by treatment interactions ( $P = 0.144$ ). (SEM = 8.01). Legend: ● Basal; ■ NaCl; ■ KCl; ▲ NaHCO<sub>3</sub>; ▲ K<sub>2</sub>CO<sub>3</sub>.



## **Chapter 4: Experiment 2**

**A meta-analysis of the influence of dietary strong ions on rumen ion concentrations and their effects on pH and volatile fatty acids**

## INTERPRETIVE SUMMARY

**A meta-analysis of the influence of dietary strong ions on rumen ion concentrations, pH, and volatile fatty acids.** *Catterton et al., page 000.* Dietary cation-anion difference (DCAD) evaluates the strong ion balance in dairy cattle diets. We conducted a meta-analysis based on 3 published studies where rumen sodium, potassium, and chloride concentrations were measured at various times post-feeding. Rumen ion concentrations could be predicted from dietary ion concentrations, the amounts of other strong ions in the rumen, and time post-feeding. Rumen ions increased with their respective dietary concentrations and impacted the concentrations of other rumen ions. Rumen pH and total volatile fatty acid concentrations could be predicted from dietary and rumen ion concentrations. The results of this analysis can be used to better predict rumen pH and fermentation responses to changes in DCAD and dietary strong ion concentrations.

### **RUNNING HEAD: META-ANALYSIS OF DIET AND RUMEN STRONG IONS**

**A meta-analysis of the influence of dietary strong ions on rumen ion concentrations and their effects on rumen pH and volatile fatty acids**

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## ABSTRACT

Dietary cation-anion difference (**DCAD**) is being used as a basis for diet formulation in the dairy industry. While extensive research has been conducted on the effects of DCAD on animal performance and rumen characteristics, the effects of the individual strong ions (Na, K, and Cl) that contribute to DCAD on the rumen environment have yet to be determined. Our objective is to determine the relationship between dietary strong ions and rumen ion concentrations, pH, and VFA. An initial dataset of 32 published papers was assembled for a meta-analysis with the selection criteria that a study had to have measured the concentration of at least one ion in the rumen. The initial list of papers was narrowed down to 3 published studies by using the following selection criteria: studies that collected samples from rumen fistulated cows at multiple times post-feeding and provided dietary and rumen concentrations for all 3 of the strong ions. The three studies used a total of 12 animals, 16 dietary treatments, and a maximum of 127 treatment means (including time post-feeding means) for rumen Na, K, and Cl. Data were evaluated using correlation, principal components analysis, and multiple regression analysis. Rumen ion concentrations were impacted by both their dietary ion concentrations and interactions with other strong ions in the rumen. Rumen Na had negative relationships with rumen K and rumen Cl while rumen K and Cl were positively correlated. Dietary and rumen ion concentrations were significant predictors for rumen pH and volatile fatty acid (**VFA**) concentrations. This analysis served as an initial survey of the available data, and additional research about the relationship between dietary ion concentrations, rumen ion concentrations, pH, and VFA can lead to better use of DCAD to improve dairy cattle performance.

**Key words:** dietary cation-anion difference, rumen pH, sodium, potassium, and chloride

## INTRODUCTION

Although several studies have measured rumen ion concentrations (Bailey, 1961b; Bennink et. al, 1978; Tucker et. al, 1988), to our knowledge, there has been no systematic analysis of the impact of dietary ion concentrations on rumen strong ion concentrations. The strong ions include sodium, potassium, and chloride, which are major contributors to acid-base status and osmotic pressure in bodily fluids (NRC, 2001). Most studies have used a limited number of animals and have measured rumen ion concentrations at inconsistent times with respect to feeding. Although work by Bailey (1961b) and Bennink et al. (1978) showed that rumen ion concentrations fluctuate after feeding, several studies only measured rumen concentrations at one time point or only reported the means of multiple time points, making it difficult to compare results across different studies.

To date, the most comprehensive study of rumen ion concentrations (Bennink et al., 1978) used only three rumen fistulated cattle (two Holstein steers and one Guernsey heifer) weighing 400 to 500 kg that were fed six different diets. Bennink et al. (1978) collected samples every 20 minutes for the first hour post-feeding and then hourly until 9 h post-feeding. That study demonstrated that rumen Na, K, and Cl concentrations varied with diet and time after feeding. However, it is difficult to make broad inferences about the diet and time post-feeding effects on rumen ion concentrations based on one study with just 3 cows.

A majority of our knowledge of rumen strong ion concentrations has been derived from studies conducted by Parthasarathy and Phillipson (1953), Bailey (1961b), Warner and Stacy (1965, 1972a, 1972b), Stacy and Warner (1966), and Poutiainen (1968). However, in the 1980s, the dietary strong ions began receiving increased attention as a tool for diet

formulation (Mongin, 1981; Tucker et al. 1988). The dietary cation-anion difference (**DCAD**) considers the strong ions of the diet, and is calculated as follows:  $DCAD \text{ (mEq/kg DM)} = Na + K - Cl$  (Mongin, 1981; Tucker et al., 1988). Manipulation of DCAD can enhance animal performance by increasing feed intake and milk production by improving the acid-base status of the animal (Mongin, 1981; Tucker et al., 1988; Hu and Murphy, 2004). To our knowledge, Tucker et al. (1988) and Catterton and Erdman (2016) conducted the only published studies that measured the impact of DCAD on rumen ion concentrations. Although most of the studies that measured rumen ion concentrations were published prior to the concept of DCAD, we can calculate it post-hoc for those that provided dietary information for Na, K, and Cl content. Although Tucker et al. (1988) used different dietary concentrations of the strong ions to achieve certain DCAD, they reported rumen ion concentrations based on each DCAD instead of the different dietary combinations used to achieve the DCAD. By using data from studies that did not initially consider DCAD, we can gain a better understanding of the influence of both individual dietary ion concentrations and DCAD on rumen ion concentrations.

While some studies provided dietary concentrations for all three strong ions, many were missing information for at least one ion, with chloride being excluded most often. Adams (1975) and Coppock (1986) reported on the need for more detailed analyses of different types of feed ingredients. Coppock (1986) discussed the limited information available about chloride concentration in different feed ingredients and the requirements of chloride in dairy cattle. Compared to the United States–Canadian Tables of Feed Composition (NRC, 1982) referenced by Coppock (1986), the current dairy NRC (2001) contains more complete information about the chloride content of different feed ingredients.

To identify the relationships between the ions involved in DCAD and the rumen environment, we conducted a meta-analysis of studies that provided information about dietary and rumen strong ion concentrations. Meta-analysis of published data in the literature allows for a systematic approach to summarization of the data across experiments and provides more definitive information about that factors that influence rumen ion concentrations. Our hypothesis was that individual rumen ion concentrations increase with respective increases in corresponding dietary ion concentrations, and that rumen pH and rumen volatile fatty acid (VFA) concentrations relate to diet and rumen strong ion concentration. Our objective was to determine the relationship between dietary strong ions and rumen ion concentrations, pH, and VFA.

## **MATERIALS AND METHODS**

### ***Data collection***

A dataset of rumen ion concentrations was assembled from published reports in the literature after a search using rumen ion and rumen minerals as key words. We identified 32 published papers that measured at least one rumen ion in either cows or sheep. Many of these papers were selected by looking at the references used for a literature review conducted by Durand and Kawashima (1981). Although their review contained both dietary and rumen ion concentrations for sodium, potassium, phosphorus, magnesium, and calcium, it did not provide data about rumen chloride concentrations. The review also failed to include other information such as sampling time with respect to feeding, sampling method, and VFA concentrations. Other studies were then located by conducting searches in the *Journal of Dairy Science*, the *British Journal of Nutrition*, and the *Journal of Animal Science*.

Although the initial dataset consisted of data from both cow and sheep studies, we eliminated all of the data from the 13 sheep studies because information about dietary chloride was not provided for any of them, so we could not calculate DCAD. Of the remaining 19 published papers with cattle, rumen sampling methods included the use of rumen cannulated cows, rumenocentesis, and stomach tubes (Duffield et al., 2004; Shen et al., 2012). Rumen cannulation allows for the collection of more representative samples of the rumen contents and multiple samples over time post-feeding. Samples collected by oral stomach tube may be contaminated by saliva (Nocek, 1997). Because saliva contains high concentrations of sodium and buffers (Bailey and Balch, 1961), this can alter rumen ion concentrations and pH measurements. Duffield et al. (2004) compared samples obtained by stomach tube, rumenocentesis, and rumen cannulation. They found minor but significant differences in ion and pH measurements between the different sampling sites (Duffield et al., 2004). In addition, Shen et al. (2012) found that sampling method influenced rumen ion, pH, and VFA measurements. Based on the much greater number of treatment means from studies that collected samples from fistulated cows compared to those that collected samples using stomach tubes or rumenocentesis, we decided we would not be able to make any meaningful comparisons about differences in sampling technique with this dataset. This decreased our dataset to 14 published studies.

Previous research indicated that rumen ion concentrations vary with time post-feeding (**TPF**) (Bailey, 1961b; Bennink, 1978). Of the 14 remaining studies, ten only measured rumen ion concentrations at a single time post-feeding or alternatively only reported the means across several different time points, and these studies were removed. Finally, we removed one more paper because it did not provide rumen chloride measurements. Our

final dataset contained information from 3 published studies that included 16 dietary treatments where all three dietary and rumen strong ion concentrations were measured at several times pre and post-feeding.

### ***Incomplete Data***

Dietary information was calculated or estimated when enough information was provided to do so. For example, the dry matter intake (**DMI**) for cattle fed the diets used by Bailey (1961b) had been reported in a previous study (Bailey, 1961a). Bailey (1961b) reported the daily dietary intake of each mineral on an as-fed basis, so we calculated the diet ion concentrations based on the dry matter intakes provided in the previous paper (Bailey, 1961a). The study published by Bennink et al. (1978) did not clearly state whether the average daily feed consumption and mineral intakes were on an as-fed or a dry matter basis, so we assumed that as-fed values were provided. Dry matter intake for each diet was estimated using average dry matter percentages for each feed ingredient from the 2001 NRC. The mineral composition of each diet on a dry matter basis was then estimated using the average daily intake of each ion and the estimated dry matter of the diet.

### ***Data Summarization and Statistical Model***

To examine the general relationships between dietary and rumen strong ions, rumen pH, TPF, total volatile fatty acid (**TVFA**), and individual VFA, we examined simple Pearson correlations used the CORR procedure in SAS (SAS Institute Inc.). TVFA was calculated as the sum of acetate, propionate, and butyrate concentrations. These initial correlations were based on data from Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016).

Next, principal components analysis (**PCA**) was used to visually identify interrelationships between dietary ions, rumen ions, DCAD, rumen cation-anion difference (**RCAD**), TPF, and DMI using the PRINCOMP procedure in SAS (SAS Institute Inc.) with data from Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016). Subsequently, the GLIMMIX procedure (SAS Institute Inc.) was used to verify rumen ion interrelationships and to determine the potential proportion of variance in rumen ion concentrations that can be accounted for by dietary ion concentration, TPF, and the concentrations of other ions in the rumen fluid. The model consisted of the dietary and rumen strong ion concentrations, DMI, TPF, and TPF quadratic as the fixed effects. Study was considered a random effect in the model. DCAD and RCAD were not included in the model because they would confounded the effects of the individual ions. We were unable to weight observations by their standard errors for this model and the following model because Bailey (1961b) and Bennink et al. (1978) did not provide this information. Study adjusted rumen ion concentrations were generated for comparison to the model predicted concentrations for rumen Na and K. The estimate for the study effect for rumen Cl was 0, so we used the observed values. We generated simple linear regression statistics for the study adjusted (or observed) vs predicted values from GLIMMIX using the REG procedure (SAS Institute Inc.). At that point, data outliers for each model were identified and removed based on Cook's distance  $> 4/n$ . After removing the outliers, the procedures in GLIMMIX were repeated, and the new study adjusted (or observed) and predicted values were used for regressions.

We then looked at the relationship between rumen ion concentrations and rumen pH and TVFA concentration using the GLIMMIX procedure (SAS Institute Inc.) with data

from Bennink et al. (1978) and Catterton and Erdman (2016). Data from Bailey (1961b) was excluded from the model because rumen pH and TVFA information was not provided. The fixed effects were the diet ion concentrations, rumen ion concentrations, rumen pH, TVFA, DMI, TPF, and TPF quadratic. Study was a random effect. The study adjusted rumen pH values were generated for comparison to the model predicted values. The estimate for the study effect for TVFA was 0, so we used the observed values for comparison to the model predicted values. We then followed the same procedures as in the previous model to detect and eliminate outliers before generating new models.

Finally, we also wanted to look at the impact of rumen ion concentrations on individual VFA concentrations. However, this would have narrowed the dataset down to only the study by Catterton and Erdman (2016), and we felt that we could not make appropriate conclusions based on the data from only one experiment.

## **RESULTS**

Table 4.1 summarizes the final dataset used for our meta-analysis. All of the data for rumen strong ion concentrations collected at multiple TPF were from a total of 12 rumen fistulated cows across 3 studies. Although two studies (Bennink et al., 1978; Catterton and Erdman, 2016) provided data for TVFA concentrations, only one of them (Catterton and Erdman, 2016) provided individual concentrations for acetate, propionate, and butyrate.

Table 4.2 shows the Pearson correlations between dietary strong ions, rumen strong ions, rumen pH, TPF, TVFA, acetate, propionate, and butyrate. Rumen Na was negatively correlated with rumen K and rumen Cl. Rumen K and rumen Cl were positively correlated. TVFA was positively correlated with rumen Na and rumen K. The concentrations of



acetate, propionate, and butyrate on a mEq/L basis negatively correlated with rumen Na but positively correlated with rumen K.

Table 4.3 provides a summary mean, range, and standard error for DMI, dietary Na, K, and Cl while Figure 4.1 illustrates the range of Na, K, Cl, and DCAD concentrations among the different experimental diets fed by Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016). The average  $\pm$  standard error (**SE**) for DMI was  $11.8 \pm 2.11$  kg/day. The average Na concentration was  $120 \pm 33.6$  mEq/kg diet DM. The average K concentration was  $481 \pm 64.4$  mEq/kg diet DM. The average Cl concentration was  $183 \pm 50.2$  mEq/kg diet DM. The average DCAD concentration was  $418 \pm 45.9$  mEq/kg diet DM. The dietary means presented in Table 4.1 differ from those in Table 4.3 because the means in Table 4.1 are based on the number of sampling times, while the means in Table 4.3 weight each diet equally.

Principal components analysis (Figure 4.2) was used to illustrate the relationships between dependent variables such as rumen Na, K, and Cl, and rumen pH and independent (predictor) variables such as DMI, time post-feeding, dietary Na, K, Cl and DCAD, rumen Na, K, Cl, and RCAD. A fundamental advantage of PCA is that it minimizes problems associated with co-linearity among independent variables that are common in multiple regression analysis. In this analysis, principal components 1 and 2 accounted for 58.6% of the variance. As expected, rumen Na clustered with RCAD since Na is the primary strong in rumen fluid, but surprisingly, it was opposite diet Na. Rumen Na was opposite rumen K and Cl. Since dietary K is the primary cation in most feed ingredients, DCAD was closely associated with diet K. Rumen K and Cl clustered together.

Table 4.4 shows the regression statistics when considering the impacts of dietary ions, other rumen ions, DMI, and TPF on the rumen ion concentrations. Figures 4.3, 4.4, and 4.5 show the predicted (or observed) vs study adjusted rumen ion concentrations for Na, K, and Cl, respectively. The mean rumen Na concentration was 122 mEq/L, and the statistical model accounted for 92.3% of the variance between study adjusted rumen Na and predicted rumen Na (Figure 4.3). Rumen Na was predicted to increase with increased diet Na ( $P = 0.033$ ), diet Cl ( $P = 0.003$ ), and DMI ( $P = 0.001$ ), but it was predicted to decrease with increasing diet K ( $P = 0.021$ ), rumen K ( $P = 0.001$ ), rumen Cl ( $P = 0.001$ ), and TPF ( $P = 0.019$ ).

Mean rumen K concentration was 39.1 mEq/L, and the statistical model accounted for 85.1% of the variance between study adjusted rumen K and predicted rumen K (Figure 4.4). Rumen K was predicted to increase with increasing diet K ( $P = 0.001$ ) and rumen Cl ( $P = 0.001$ ), and was predicted to decrease with increasing diet Cl ( $P = 0.019$ ), and rumen Na ( $P = 0.001$ ). Finally, mean rumen Cl concentration was 13.0 mEq/L across studies and treatments. The statistical model accounted for 71.6% of the variance in rumen Cl concentration. In the regression model, rumen Cl was predicted to increase with increased diet Na ( $P = 0.004$ ), diet K ( $P = 0.004$ ), diet Cl ( $P = 0.001$ ), and rumen K ( $P = 0.005$ ), but decrease with increasing DMI ( $P = 0.005$ ).

Table 4.5 shows the regression statistics when considering the influence of diet ions, rumen ions, DMI, and TPF on rumen pH and TVFA concentration using data from Bennink et al. (1978) and Catterton and Erdman (2016). Figure 4.6 shows the predicted vs study adjusted rumen pH, and Figure 4.7 shows the predicted vs observed TVFA concentration. The average rumen pH was 6.16, and the model accounted for 92.4% of the variance in

rumen pH due to diet, rumen ions, DMI, time post-feeding, and study. The model predicted that rumen pH would increase with increasing diet Na ( $P = 0.001$ ), diet K ( $P = 0.001$ ), rumen K ( $P = 0.001$ ) and TPF quadratic ( $P = 0.003$ ), but it would decrease with increasing rumen Na ( $P = 0.006$ ), rumen Cl ( $P = 0.001$ ), TVFA ( $P = 0.001$ ), and TPF ( $P = 0.001$ ). The average TVFA concentration was 104 mEq/L, and the model accounted for 85.0% of the variance. The TVFA concentration was predicted to increase with increasing diet Na ( $P = 0.001$ ), diet K ( $P = 0.001$ ), and rumen K ( $P = 0.002$ ), but it would decrease with increasing diet Cl ( $P = 0.023$ ), rumen pH ( $P = 0.001$ ), and DMI ( $P = 0.001$ ).

## DISCUSSION

As shown in Table 4.4, DMI increased rumen Na but decreased rumen Cl and numerically decreased rumen K. Putnam et al. (1966) showed that increasing the DMI resulted in increased salivation rate, and Cassida and Stokes (1986) suggested that the cows used in their study had high saliva production due to high DMI. Because saliva is the primary source of Na in the rumen (Bailey, 1961b; Poutiainen, 1968), increased DMI would be expected to be associated with increased rumen Na since Na is the primary cation in ruminant saliva. A negative relationship between rumen Na and rumen K has been previously reported (Poutiainen, 1968; Warner and Stacy, 1972), and this could explain why increased DMI decreased rumen K. Also, since the rumen osmotic pressure remains relative constant throughout the day (Bennink et al., 1978) and Na and K are the principal cations in the rumen, it makes sense that as Na or K increases, that the opposite cation would decrease to maintain a neutral charge in the rumen. PCA and regression analysis (Table 4.4) also showed that rumen Na was decreased in the presence of elevated rumen Cl concentrations. Elevated Cl may result in increased Na absorption across the rumen

wall into blood. Previous studies have proposed coupled active transport mechanisms in the rumen for Na and Cl, although the exact mechanism is unknown (Chien and Stevens, 1972; Martens and Blume, 1987). Martens and Blume (1987) showed that rumen Na and Cl absorption increased when rumen Na was increased, and they found that replacing Na with Li inhibited Cl absorption. Chien and Stevens (1972) observed that Na absorption decreased when Cl was removed from the solution in an isolated rumen. They also found that the active transport of Na and Cl was inhibited when the bicarbonate buffering system was replaced with tricine, and they suggested a dual exchange mechanism of Na for H and Cl for  $\text{HCO}_3$  (Chien and Stevens, 1972). Our PCA (Figure 4.2) and regression analysis (Tables 4.3 and 4.4) also showed a positive relationship between rumen K and Cl. Because rumen K increases with decreased rumen Na, increased rumen Cl may indirectly increase rumen K by decreasing rumen Na.

Rumen pH and TVFA were negatively related since increased TVFA reflects increased acid and hydrogen ion concentrations. Regression equations in Table 4.5 illustrated the positive correlation between rumen pH and rumen Cl. Because chloride is an anion, it would be expected to decrease rumen pH (Stewart, 1983). Na and K would be expected to increase rumen pH (Stewart, 1983). A meta-analysis by Singh and Kohn (2004) showed that dietary  $\text{NaHCO}_3$  increased the strong ion difference in the rumen and increased rumen pH. Tables 4.2 and 4.5 show a positive relationship between rumen K and rumen pH. The correlations shown in Table 4.2 indicates a numerical positive relationship between rumen Na and rumen pH; however, the regression in Table 4.5 shows that rumen Na significantly decreased rumen pH. This contradiction highlights one of the downfalls of having such a small dataset to work with.

## **CONCLUSIONS**

To our knowledge, this is the first systematic analysis of the literature to examine relationships between dietary strong ion and rumen ion concentrations. The analysis demonstrated the rumen ion concentrations were impacted by both their dietary ion concentrations and interactions with other ions in the rumen. Rumen ion concentrations are also associated with changes in rumen pH and TVFA, suggesting that manipulation of diet strong ions and DCAD alter rumen fermentation. Due to limitations in reporting of dietary and rumen ion concentration, our dataset was small, which limits broad inferences from the results. Still, these analyses serve as a starting point for future research about the relationships between the strong ions and the rumen environment. Having a better understanding of these relationships could allow for better use of DCAD to improve dairy cattle performance.

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**Table 4.1** A summary of the data provided by the final dataset that included Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016).

Item	Total	Diet <sup>1</sup>			Rumen							
		Na	K	Cl	Na <sup>2</sup>	K <sup>2</sup>	Cl <sup>2</sup>	pH	Acetate <sup>2</sup>	Propionate <sup>2</sup>	Butyrate <sup>2</sup>	TVFA <sup>3</sup>
Mean		110	452	135	111	43.3	14.5	6.44	73.4	23.0	10.8	134
SD		127	228	179	30.4	17.9	6.56	0.548	6.14	2.77	1.18	28.7
Studies, N	3	3	3	3	3	3	3	2	1	1	1	2
Treatments, N	16	16	16	16	16	16	16	11	5	5	5	11
TPF, N <sup>4</sup>	23	23	23	23	23	23	23	19	7	7	7	19
Total means, N <sup>5</sup>	127	127	127	127	127	127	127	107	35	35	35	107
Total animals, N	12	12	12	12	12	12	12	8	5	5	5	8

<sup>1</sup>Mean and standard deviation (SD) are expressed on a mEq/kg diet dry matter basis.

<sup>2</sup>Mean and SD are expressed on a mEq/L basis.

<sup>3</sup>Total volatile fatty acids; mean and SD are expressed on a mEq/L basis.

<sup>4</sup>Times post-feeding.

<sup>5</sup>Treatment x TPF means.



**Table 4.2** The Pearson correlations between variables using data from Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016). For each item, row 1 is the estimate, row 2 is the *P*-value, and row 3 is the number of observations.

Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Diet Na, mEq/kg DM		-0.054	0.428	-0.131	-0.441	0.009	-0.511	0.120	-0.349	-0.094	-0.299	-0.117	0.307	-0.322	0.004
		0.549	0.001	0.143	0.001	0.918	0.001	0.180	0.000	0.591	0.081	0.503	0.073	0.060	0.983
		127	127	127	127	127	107	127	107	35	35	35	35	35	35
2. Diet K, mEq/kg DM			0.512	-0.543	0.399	0.585	0.028	0.025	-0.164	0.221	0.115	0.122	0.047	-0.028	-0.073
			0.001	0.001	0.001	0.001	0.777	0.784	0.092	0.202	0.511	0.485	0.789	0.874	0.678
			127	127	127	127	107	127	107	35	35	35	35	35	35
3. Diet Cl, mEq/kg DM				-0.329	-0.243	0.311	-0.543	0.077	-0.548	-0.206	-0.040	-0.122	-0.130	0.131	0.015
				0.001	0.006	0.001	0.001	0.390	0.001	0.234	0.821	0.486	0.457	0.453	0.931
				127	127	127	107	127	107	35	35	35	35	35	35
4. Rumen Na, mEq/L					-0.364	-0.377	0.124	-0.213	0.485	-0.451	-0.468	-0.496	0.276	-0.195	-0.320
					0.001	0.001	0.204	0.016	0.001	0.007	0.005	0.002	0.109	0.261	0.061
					127	127	107	127	107	35	35	35	35	35	35
5. Rumen K, mEq/L						0.491	0.537	-0.024	0.210	0.468	0.323	0.453	-0.078	0.003	0.274
						0.001	0.001	0.789	0.030	0.005	0.058	0.006	0.655	0.988	0.111
						127	107	127	107	35	35	35	35	35	35
6. Rumen Cl, mEq/L							0.00 <sup>1</sup>	-0.055	0.124	0.173	0.141	0.210	-0.080	0.025	0.202
							1.00 <sup>2</sup>	0.536	0.204	0.321	0.420	0.227	0.649	0.887	0.245
							107	127	107	35	35	35	35	35	35
7. Rumen pH								-0.274	0.089	-0.615	-0.553	-0.747	0.316	-0.140	-0.660
								0.004	0.361	0.001	0.001	0.001	0.064	0.424	0.001
								107	107	35	35	35	35	35	35
8. Time post-feeding, hour									0.026	0.469	0.412	0.730	-0.307	0.072	0.858
									0.793	0.005	0.014	0.001	0.073	0.680	0.001
									107	35	35	35	35	35	35
9. Total volatile fatty acid, mEq/L										0.965	0.809	0.945	-0.290	0.160	0.490
										0.001	0.001	0.001	0.092	0.357	0.003
										35	35	35	35	35	35
10. Acetate, mEq/L											0.630	0.904	-0.029	-0.099	0.446
											0.001	0.001	0.870	0.572	0.007
											35	35	35	35	35
11. Propionate, mEq/L												0.738	-0.772	0.708	0.337
												0.001	0.001	0.001	0.048
												35	35	35	35
12. Butyrate, mEq/L													-0.318	0.117	0.746
													0.062	0.504	0.001
													35	35	35
13. Acetate, mol/100 mol														-0.961	-0.286
														0.001	0.096
														35	35
14. Propionate, mol/100 mol															0.010
															0.956
															35
15. Butyrate, mol/100 mol															

<sup>1</sup>The actual estimate is -0.00003.

<sup>2</sup>The actual *P* = 0.9997.

**Table 4.3** A summary of the strong ion content of diets used by Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016). All values are expressed as mEq/kg diet dry matter.

Item	DMI <sup>1</sup>	Dietary Ion			DCAD <sup>2</sup>
		Na	K	Cl	
Mean	11.8	120	481	183	418
Standard Error	2.11	33.6	64.4	50.2	45.9
Range	3.99 - 24.1	6.33 - 448	141 - 1134	11.0 - 636	169 - 793

<sup>1</sup>Dry matter intake.

<sup>2</sup>Dietary cation-anion difference; DCAD = Na + K – Cl.

**Table 4.4** Regression statistics between dietary and rumen ion concentrations using data from Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016).

Regression statistics	Rumen Ion <sup>1</sup>								
	Na			K			Cl		
Observations	122			117			121		
Mean <sup>1</sup>	102			39.1			13.0		
Root MSE <sup>1</sup>	13.1			5.54			4.21		
Adjusted R <sup>2</sup>	0.923			0.851			0.716		
Regression coefficients	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>
Bailey	49.0	43.17	0.259	8.50	5.902	0.153	0.000	0.000	1.000
Bennink et al.	35.3	43.14	0.414	-0.516	5.8260	0.930	0.000	0.000	1.000
Catterton and Erdman	-84.3	44.16	0.059	-7.99	7.027	0.258	0.000	0.000	1.000
Intercept <sup>1</sup>	109	44.5	0.135	63.4	7.67	0.014	7.65	4.844	0.117
Diet Na <sup>2</sup>	0.032	0.0149	0.033	-0.005	0.0063	0.468	0.013	0.0046	0.004
Diet K <sup>2</sup>	-0.020	0.0087	0.021	0.025	0.0038	0.001	0.009	0.0031	0.004
Diet Cl <sup>2</sup>	0.039	0.0126	0.003	-0.013	0.0054	0.019	0.014	0.0036	0.001
Rumen Na <sup>1</sup>	--	--	--	-0.308	0.0308	0.001	-0.018	0.0230	0.437
Rumen K <sup>1</sup>	-0.744	0.1188	0.001	--	--	--	0.121	0.0421	0.005
Rumen Cl <sup>1</sup>	-0.949	0.2766	0.001	0.592	0.1172	0.001	--	--	--
DMI <sup>3</sup>	4.01	0.963	0.001	-0.529	0.3691	0.155	-0.308	0.1078	0.005
TPF <sup>4</sup>	-2.74	1.154	0.019	-0.512	0.5096	0.317	-0.192	0.3719	0.606
TPF, quad. <sup>5</sup>	0.192	0.1105	0.086	0.060	0.0480	0.211	0.009	0.0354	0.802

<sup>1</sup>Means, root mean square errors (MSE) estimates, and standard errors (SE), and intercept are expressed on a mEq/L basis.

<sup>2</sup>Estimates and SE are expressed on a mEq/kg diet dry matter basis.

<sup>3</sup>Dry matter intake; estimates and SE are expressed on a kg/day basis.

<sup>4</sup>Time post-feeding; estimates and SE are expressed on an hourly basis.

<sup>5</sup>Time post-feeding, quadratic; estimates and SE are expressed on a quadratic basis.

**Table 4.5** Regression statistics for the influence of dietary and rumen strong ions on pH and total volatile fatty acids (TVFA) using data from Bennink et al. (1978) and Catterton and Erdman (2016).

Regression statistics	Rumen Measurement					
	pH			TVFA		
Observations	106			104		
Mean	6.16			133 <sup>1</sup>		
Root MSE	0.139			10.8 <sup>1</sup>		
Adj. R <sup>2</sup>	0.924			0.850		
Regression coefficients	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>
Bennink et al.	0.747	0.7660	0.332	0.000	0.000	1.000
Catterton and Erdman	-0.747	0.7660	0.332	0.000	0.000	1.000
Intercept	7.37	0.786	0.068	429 <sup>1</sup>	34.9 <sup>1</sup>	0.001
Diet Na <sup>2</sup>	0.001	0.0002	0.001	0.046	0.0136	0.001
Diet K <sup>2</sup>	0.001	0.0001	0.001	0.062	0.0102	0.001
Diet Cl <sup>2</sup>	0.000	0.0002	0.885	-0.029	0.0126	0.023
Rumen Na <sup>1</sup>	-0.003	0.0011	0.006	0.231	0.0651	0.001
Rumen K <sup>1</sup>	0.008	0.0015	0.001	0.188	0.1382	0.177
Rumen Cl <sup>1</sup>	-0.022	0.0039	0.001	-0.258	0.3075	0.404
Rumen pH	--	--	--	-48.1	4.52	0.001
TVFA <sup>1</sup>	-0.009	0.0009	0.001	--	--	--
DMI <sup>3</sup>	0.006	0.0128	0.619	-4.06	0.428	0.001
TPF <sup>4</sup>	-0.061	0.0158	0.001	2.05	1.172	0.084
TPF, quad. <sup>5</sup>	0.004	0.0014	0.003	-0.169	0.1043	0.109

<sup>1</sup>Mean, root mean square error (MSE), estimates, standard errors (SE), and intercept are expressed on a mEq/L basis.

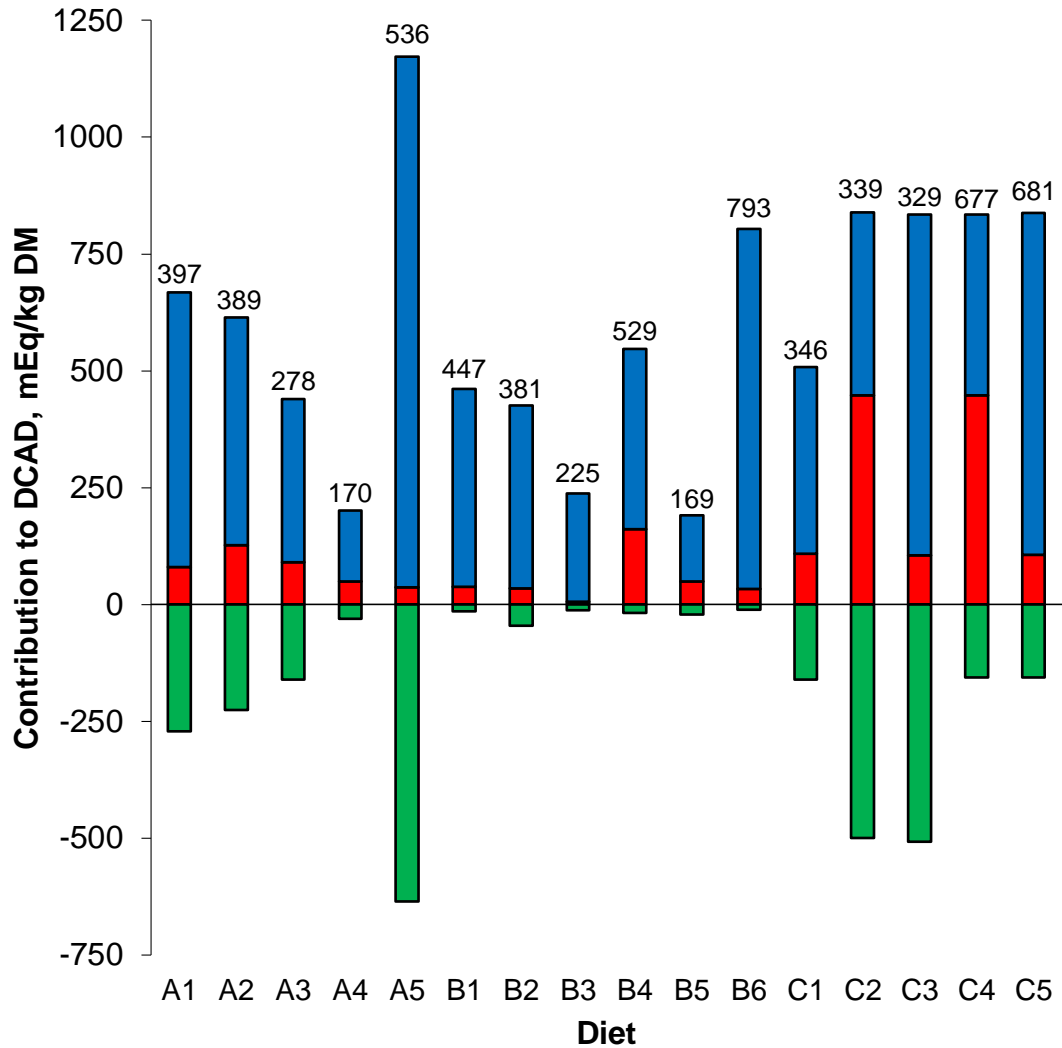
<sup>2</sup>Estimates and SE are expressed on a mEq/kg diet dry matter basis.

<sup>3</sup>Dry matter intake; estimates and SE are expressed on a kg/day basis.

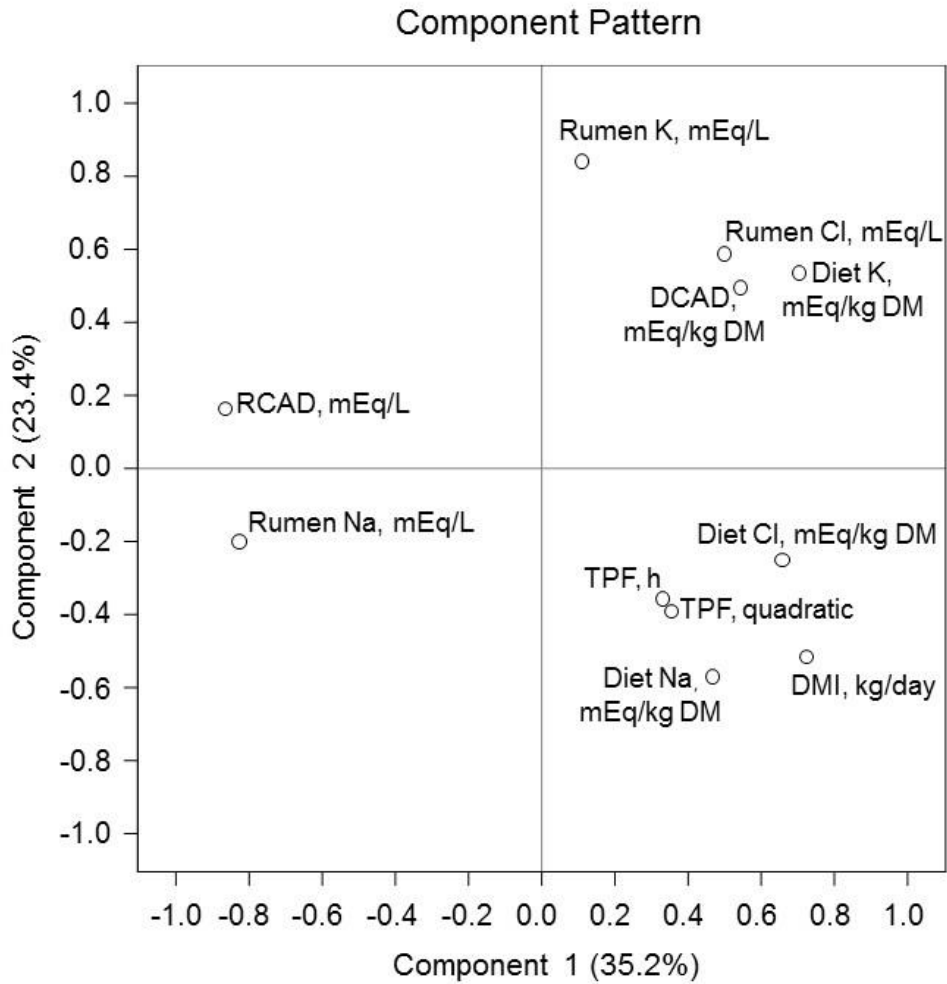
<sup>4</sup>Time post-feeding; estimates and SE are expressed on an hourly basis.

<sup>5</sup>Time post-feeding, quadratic; estimates and SE are expressed on a quadratic basis.

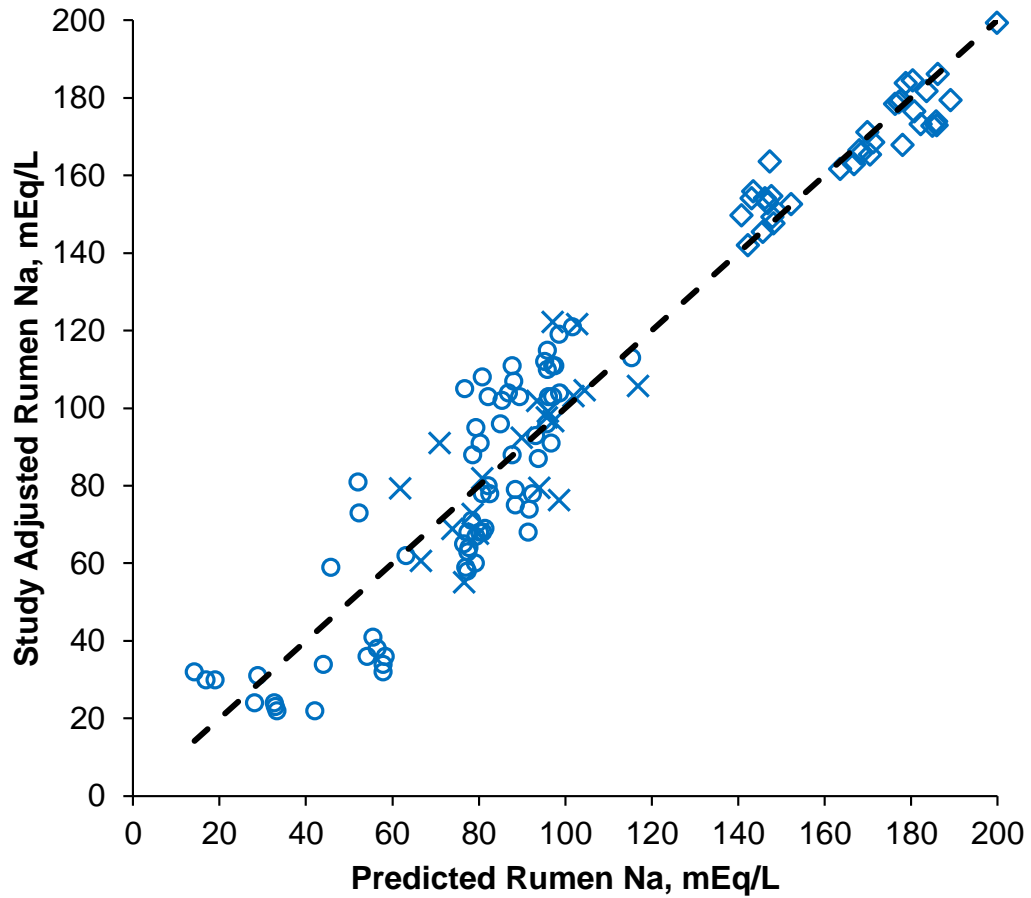
**Figure 4.1** The strong ion concentrations of the diets used by Bailey (1961b), Bennink et al. (1978), and Catterton and Erdman (2016). Legend: (Blue) sodium; (red) potassium; (green) chloride. The number above each bar is the calculated DCAD for each diet. (A1) alfalfa silage; (A2) hay; (A3) hay and dairy cubes; (A4) hay, flaked maize, and ground nut cake; (A5) fresh grass; (B1) alfalfa hay; (B2) corn silage and cottonseed meal; (B3) flaked corn, corn silage, and cottonseed meal; (B4) concentrate, corn silage, and alfalfa hay; (B5) rolled milo; (B6) alfalfa pellets; (C1) corn silage, alfalfa hay, and concentrate; (C2) corn silage, alfalfa hay, concentrate, and NaCl; (C3) corn silage, alfalfa hay, concentrate, and KCl; (C4) corn silage, alfalfa hay, concentrate, and NaHCO<sub>3</sub>; (C5) corn silage, alfalfa hay, concentrate, and K<sub>2</sub>CO<sub>3</sub>. Diets with A are from Bailey (1961b), diets with B are from Bennink et al. (1978), and diets with C are from Catterton and Erdman (2016).



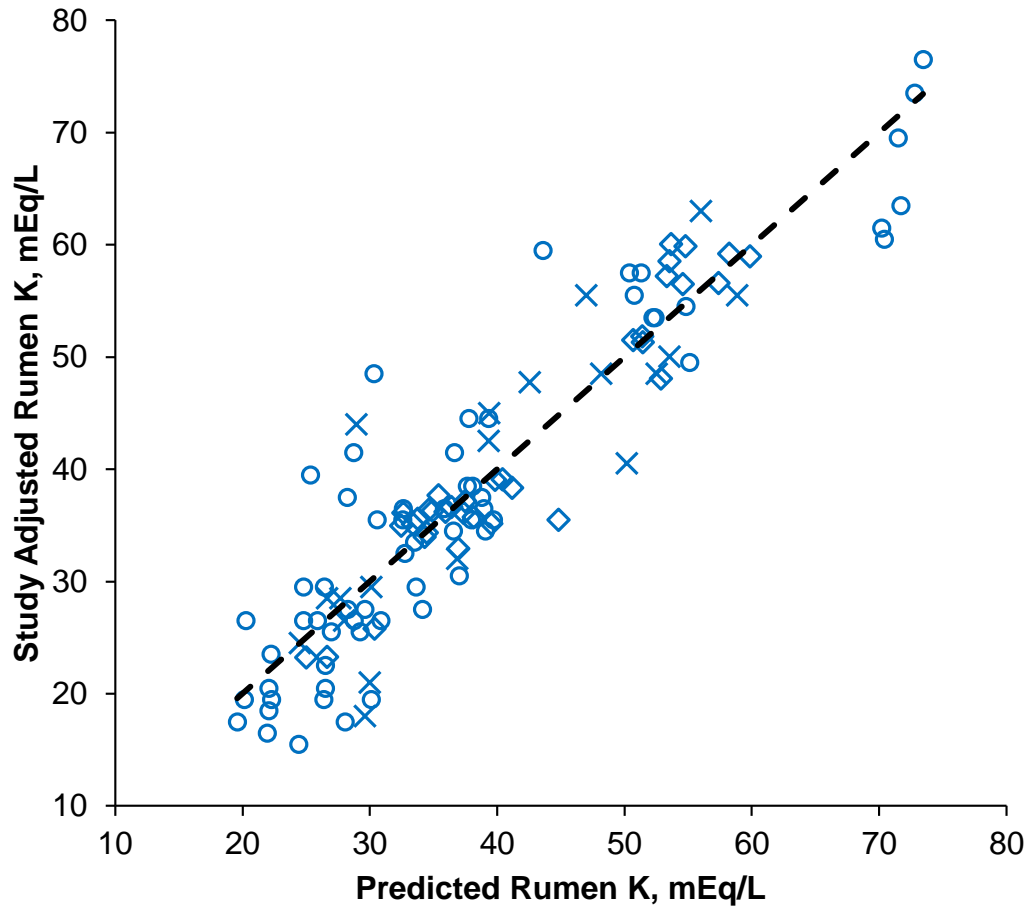
**Figure 4.2** A principal components analysis of dietary and rumen strong ions, dietary cation-anion difference (DCAD), rumen cation-anion difference (RCAD), time post-feeding (TPF), and dry matter intake (DMI). Principal components 1 and 2 accounted for 58.6% of the variance.



**Figure 4.3** The predicted vs study adjusted rumen Na concentrations. The model included diet Na, diet K, diet Cl, rumen K, rumen Cl, dry matter intake, time post-feeding, and time post-feeding quadratic. (intercept =  $2.22 \times 10^{-8}$ ;  $P = 1.00$ ; intercept SE = 2.93; slope = 1.00; slope  $P = 0.001$ ; slope SE = 0.026; adjusted  $R^2 = 0.923$ ; RMSE = 13.1;  $n = 122$ ). Legend: (---)  $Y = X$ ; (x) Bailey, 1961b; (o) Bennink et al., 1978; (◇) Catterton and Erdman, 2016.

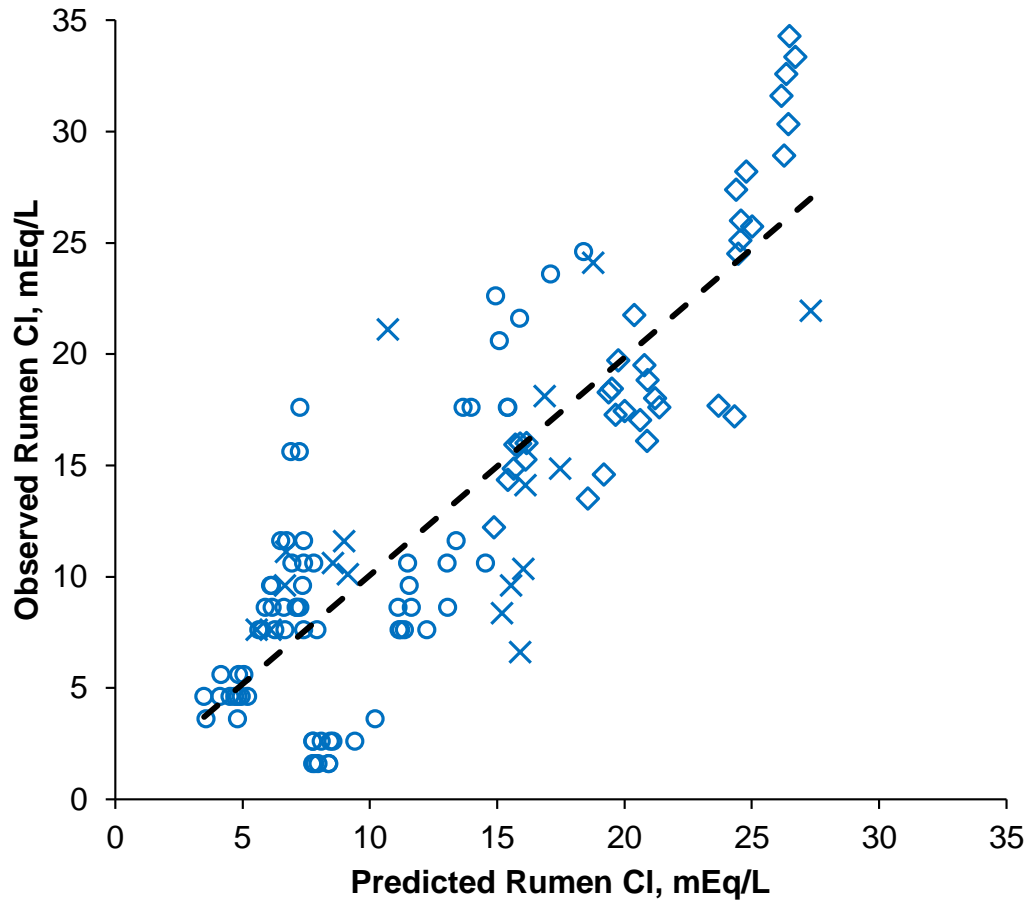


**Figure 4.4** The predicted vs study adjusted rumen K concentrations. The model included diet Na, diet K, diet Cl, rumen Na, rumen Cl, dry matter intake, time post-feeding, and time post-feeding quadratic. (intercept =  $4.32 \times 10^{-8}$ ; intercept  $P = 1.00$ ; intercept SE = 1.60; slope = 1.00; slope  $P = 0.001$ ; slope SE = 0.039; adjusted  $R^2 = 0.851$ ; RMSE = 5.54;  $n = 117$ ). Legend: (---)  $Y = X$ ; (x) Bailey, 1961b; (o) Bennink et al., 1978; (◇) Catterton and Erdman, 2016.

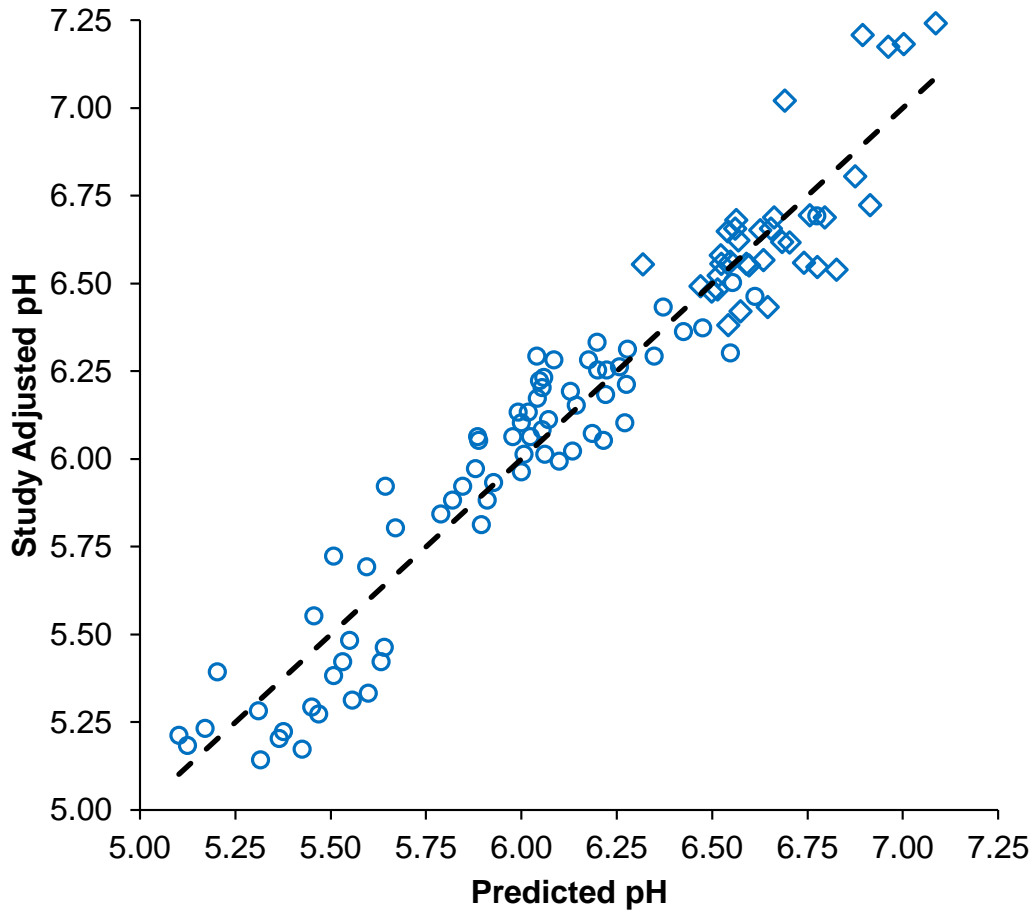




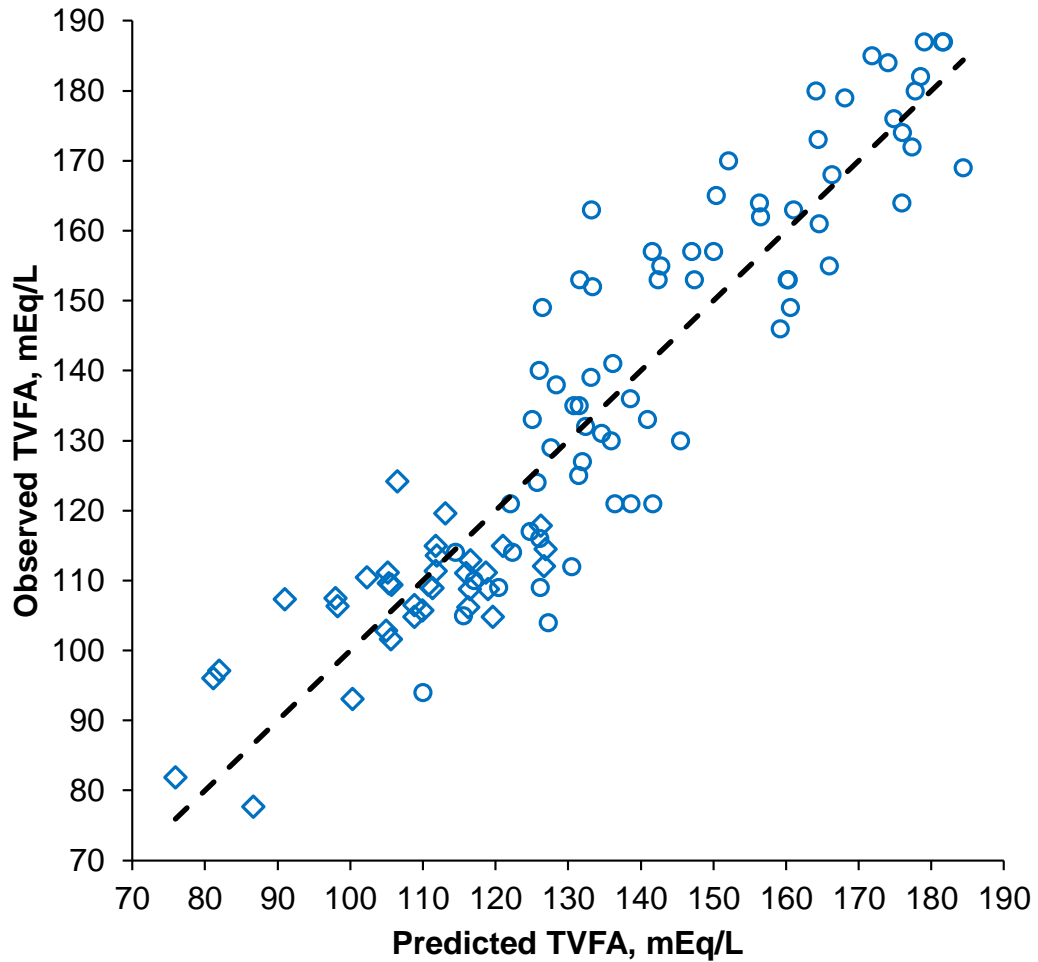
**Figure 4.5** The predicted vs observed rumen Cl concentrations. The model included diet Na, diet K, diet Cl, rumen Na, rumen K, dry matter intake, time post-feeding, and time post-feeding quadratic. (intercept = 0.289; intercept  $P$  = 0.727; intercept SE = 0.824; slope = 0.987; slope  $P$  = 0.001; slope SE = 0.056; adjusted  $R^2$  = 0.716; RMSE = 4.21;  $n$  = 121). Legend: (---)  $Y = 0.987x + 0.289$ ; (x) Bailey, 1961b; (o) Bennink et al., 1978; ( $\diamond$ ) Catterton and Erdman, 2016.



**Figure 4.6** The predicted vs study adjusted rumen pH. The model included diet Na, diet K, diet Cl, rumen Na, rumen K, rumen Cl, total volatile fatty acids, dry matter intake, time post-feeding, and time post-feeding quadratic. (intercept =  $3.94 \times 10^{-8}$ ; intercept  $P = 1.00$ ; intercept SE = 0.173; slope = 1.00; slope  $P = 0.001$ ; slope SE = 0.028; adjusted  $R^2 = 0.924$ ; RMSE = 0.139;  $n = 106$ ). Legend: (---)  $Y = X$ ; (○) Bennink et al., 1978; (◇) Catterton and Erdman, 2016.



**Figure 4.7** The predicted vs observed total volatile fatty acid (TVFA) concentrations. The model included diet Na, diet K, diet Cl, rumen Na, rumen K, rumen Cl, rumen pH, dry matter intake, time post-feeding, and time post-feeding quadratic. (intercept =  $1.09 \times 10^{-11}$ ; intercept  $P = 1.00$ ; intercept SE = 5.61; slope = 1.00; slope  $P = 0.001$ ; slope SE = 0.041; adjusted  $R^2 = 0.850$ ; RMSE = 10.8; n = 104). Legend: (---)  $Y = X$ ; (○) Bennink et al., 1978; (◇) Catterton and Erdman, 2016.



## Chapter 5: Summary and Conclusions

The results of these experiments support our hypothesis that changes in dietary ion content can be used to manipulate rumen ion concentrations. In Experiment 1, we showed that increasing dietary strong ion concentrations increased their respective concentrations in the rumen. We also showed that increasing dietary K decreased rumen Na, but increasing dietary Na did not have the same impact on rumen K. While rumen ion concentrations were impacted by dietary ion concentrations, volatile fatty acid concentrations were not significantly impacted. Although we are unable to draw conclusions about production responses to cation source and DCAD, there is evidence that these factors do impact animal performance (Sanchez et al., 1994; Iwaniuk et al., 2015).

In Experiment 2, we combined our data from Experiment 1 with the results from two previously published studies to further examine the relationship between dietary strong ions and rumen ion concentrations. The dataset used in Experiment 2 consists of rumen ion measurements from only 12 rumen fistulated cows that were fed a total of 16 different diets across 3 studies, which indicates the lack of research that has focused on the rumen strong ions. We generated models that showed that dietary ion concentrations influence rumen ion concentrations, rumen pH, and total volatile fatty acid concentrations.

Gaining a better understanding of these relationships has several implications for the dairy industry. First, it can allow farmers to optimize the use DCAD to improve feed efficiency. Because feed is one of the greatest costs of milk production (Buza et al., 2014), farmers can increase their profits by improving feed efficiency and selecting feed ingredients that have lower costs. According to the equation  $DCAD \text{ (mEq/kg DM)} = Na + K - Cl$  (Mongin, 1981; Tucker et al., 1988), increasing either dietary Na or K has the

same effect on DCAD. Although some studies suggest that the actual DCAD is more important than the cation source used to increase the DCAD (Wildman et al., 2007; Hu and Kung, 2009), other studies have shown that cation source influences the production response to DCAD (Sanchez et al., 1994; Iwaniuk et al., 2015). Iwaniuk et al. (2015) found that substitution of Na for K as the supplemental cation source increased milk fat percentage and fat yield. Because dietary K supplements generally cost 3 to 4 times more than Na supplements on a kilogram basis, understanding the impacts of the individual strong ions on animal performance and the rumen environment can help farmers determine which cation source would be most cost effective for improving milk production (Iwaniuk et al., 2015).

In addition, understanding how dietary ion concentrations influence rumen ion concentrations can allow for optimized use of ionophores. Although we did not use ionophores in our experiments, it could be hypothesized that through their effects on rumen ion concentrations, dietary ion concentrations might also influence the effects of ionophores on rumen fermentation. *In vivo* studies have shown that monensin and lasalocid are less effective when used with high dietary K concentrations (Funk et al., 1983; Rumpler et al., 1986), so using these ionophores in conjunction with a high K diet would not be an efficient feeding strategy. Similarly, an *in vitro* study showed that increasing potassium in the culture media protected bacteria from the effects of monensin (Newbold et al., 2013). In contrast, increasing the Na concentration of the diet (Rumpler et al., 1986) and the culture media (Newbold et al., 2013) increased the effects of monensin, which suggests that using monensin in conjunction with a high Na diet may be a more efficient feeding strategy.

More research about the influence of dietary strong ions on rumen ion concentrations is needed to draw broader conclusions about how this also impacts animal performance. Because it is difficult to justify doing cannulation surgery on many animals, most studies with fistulated cows use small numbers of animals. Although meta-analysis is a useful tool for working with data from multiple studies, our final dataset only contained data from 12 cows, which is significantly less than the number of animals used in most production studies. Additional research using greater numbers of cows are needed to establish the relationships between cation source and DCAD on rumen ion concentrations, animal performance, and responses to ionophores.

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