THE RELATIONSHIP BETWEEN DIETARY CATION-ANION DIFFERENCE AND NITROGEN METABOLISM IN LACTATING DAIRY COWS

by

CHRISTOPHER DWAYNE WILDMAN

Under the Direction of Joe W. West

ABSTRACT

Positive dietary cation-anion difference (**DCAD**) enhances blood bicarbonate concentrations in lactating cows, improving blood buffering which may improve animal health and performance. The objectives of this research were to determine the effects of DCAD on nitrogen metabolism in lactating dairy cows, including changes in amino acid (**AA**) metabolism that may be influenced by DCAD. Potassium:sodium ratios were compared to elucidate interactions between 2:1, 3.5:1, and 5:1 dietary K:Na ratios within DCAD treatments of 45 and 60 meq / 100 g dry matter (**DM**). No differences were observed for DM intake (**DMI**) or milk yield (**MY**) for DCAD treatments. Milk yield was lower for the 3.5:1 K:Na ratio. Blood bicarbonate was similar among treatments, but blood urea N declined as DCAD increased from 45 to 60 meq Na + K – Cl / 100 g suggesting reduced systemic protein catabolism because of reduced need for protein in systemic buffering. No differences were observed for blood urea N concentration among potassium:sodium ratios. In late lactation cows, serum total AA and total essential AA concentrations were greater for DCAD of 50 meq / 100 g DM compared with 25 meq / 100 g DM. Milk yield declined as DCAD increased although no difference in DMI was noted. Blood bicarbonate concentration was greater for high DCAD diets, indicating improved blood buffering

with high DCAD. In early lactation cows, improved DMI, MY, and urinary uric acid, which is indicative of microbial crude protein yield, was observed for cows fed diets low in dietary crude protein when DCAD was increased from 25 to 50 meq / 100 g DM. Blood bicarbonate concentrations were also higher. Improved DMI, MY, total blood AA, and total essential AA concentration was observed in diets low in dietary crude protein and rumen degradable protein when DCAD increased from 25 to 50 meq / 100 g DM. Results of this research indicate that increasing DCAD enhances amino acid status in lactating dairy cows. The mechanism is not known, but could be a result of decreasing systemic buffering role of amino acids.

INDEX WORDS: dietary cation-anion difference, potassium, sodium, nitrogen metabolism, amino acid metabolism

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B.S., Louisiana State University, 1996

M.S., Louisiana State University, 2000

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2005

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December 2005

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank first, and foremost, my lord and savior, Jesus Christ, for his unconditional love and guidance as I have worked to further my education. I would also like to express my appreciation to my family for their continuous support. To my mother, Janice Wildman, my sister, Kelli Wildman, and my father, Dwayne Wildman, thanks for believing in me, even when I doubted myself. You have been willing to share both my joy and pain, and have been supportive through both. I am eternally grateful. To my uncle, Edwin Wildman, you have always been a tremendous cheerleader for me in education and life. You will never know how much that has meant and will continue to mean to me.

My deepest gratitude goes to Dr. Joe W. West for his guidance, encouragement, and most importantly, friendship. Not only have you provided me with the opportunity to glean from your vast knowledge, but you have also given me the opportunity to grow both as a person and as a scientist. Thank you! I would also like to thank Dr. John K. Bernard for his advice, expertise, and friendship. Many thanks go to Dr. Scott Brown, Dr. Robert Dove, and Dr. Bill Dozier for the time and effort they have put forth in serving on my graduate committee.

Sincerest thanks also go to Lani Pacetti and Kelly Cooke, who put up with me as long-term office mates in Tifton. Thank you for your moral support and for making me laugh when I needed it. I wish you the greatest success. Thanks to Heath Cross, Sue Trammell, Melissa Tawzer, and Elaine Henry for all of your assistance at the dairy and in the lab. I could not have done it without you.

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CHAPTER 1

INTRODUCTION

Dietary cation-anion difference (**DCAD**) is the proportion of specific fixed ions or the balance between positively charged and negatively charged fixed ions and considers the physiologic effects on acid-base chemistry of the electrical charge among these ions. Dietary cation-anion difference affects acid-base chemistry and can alter blood buffering through changes in blood bicarbonate concentration. Through altered blood buffering, the metabolism of nutrients such as minerals, energy, and nitrogen can be affected by changing DCAD.

The DCAD is calculated as either meq Na + K – Cl / 100 g DM or meq (Na + K) – (Cl + S) / 100 g DM. The presence of more cation equivalents in the diet constitutes a positive DCAD while a greater number of anion equivalents comprise a negative DCAD diet. In addition to the concentrations of each respective ion present in feedstuffs, dietary concentrations of these ions are modified through the addition of cationic or anionic salts.

Much of the DCAD research for lactating dairy cows has focused on the feeding of negative DCAD (anionic) diets for reduction of hypocalcemia and parturient paresis. This work has focused on the use of DCAD to enhance Ca mobilization and absorption, especially in the peripartum cow. Greater blood acidity increases blood ionized Ca and improves blood Ca resorption at parturition (Block, 1984; Goff et al., 1991).

Lactating dairy cows are fed diets high in soluble carbohydrates that provide increasing challenges to the animal's homeostatic buffering system. Feeding high DCAD diets improves blood buffering by increasing blood bicarbonate concentration which raises blood pH, which can

result in increased dry matter intake (**DMI**), milk yield (**MY**), and yield of milk components. More rapid respiration rate due to heat stress increases loss of carbon dioxide, causing alkalosis. In response to this respiratory alkalosis, excess bicarbonate is excreted in the urine to maintain physiological pH. The loss of bicarbonate compromises systemic buffering, increasing the opportunity for acidosis. Increasing blood concentrations of Na and K by feeding positive DCAD diets results in greater retention of bicarbonate, reducing the effects of heat stress.

The interaction of DCAD and N metabolism has received extensive attention in several species. In ruminants, results have been inconclusive. Delaquis and Block (1995b; 1995c) reported no DCAD effect on N metabolism in lactating or dry dairy cows. Phillip (1983), however, reported greater N retention and digestibility in sheep when $NaHCO_3$ was included in a low protein diet. Mixed results have also been reported in swine. Patience et al. (1986) reported no difference in total N digestibility or balance when 1.3 or 2.6% $NaHCO_3$ was fed. However, Haydon and West (1990) observed a linear increase in N digestibility at the terminal ileum when DCAD increased.

An interaction between DCAD and amino acid (**AA**) metabolism has been well established in rats (Arnauld and Lachance, 1980; Iacobellis et al., 1956), chicks (Austic and Calvert, 1981; Robbins et al., 1982), and swine (Austic et al., 1982; Patience, 1990). Changes in AA metabolism with high DCAD diets resulted in increased availability of essential AA for protein synthesis. Changes brought on by high DCAD include decreasing the role of AA in intracellular buffering, alleviation of the lysine-arginine antagonism, and enhanced buffering when sulfur-containing AA are fed in excess of the animals needs and are catabolized as an energy source. Research into the interaction between DCAD and AA metabolism in lactating dairy cows is very limited.

The purpose of this review is to investigate various aspects of DCAD in dairy cows including concentrations that have been fed, effects on metabolism of other nutrients, and proposed mechanisms by which DCAD affects the metabolism of other minerals. In addition, this review will explore the relationship between DCAD and N metabolism, especially in ruminants, to elucidate effects that DCAD may have on AA metabolism.

CHAPTER 2

LITERATURE REVIEW

Acid-base chemistry

Acid-base homeostasis is the tendency of an animal to maintain a constant intracellular and extracellular hydrogen ion ($\mathbf{H^+}$) concentration (Patience, 1990). The physiological pH range of blood is a slightly alkaline 7.36 to 7.44 when acid-base homeostasis is attained (Robinson, 1975). There are two main metabolic sources of H^+ in animals (Kronfeld, 1976); incomplete combustion of carbohydrates and fats yields lactic, citric, acetoacetic, or 3-hydroxybutyric acids, and sulfuric and phosphoric acid result from the incomplete combustion of sulfur and P containing proteins. The second source is the complete oxidation of carbohydrates and fats that yields H_2CO_3 and water. Carbonic acid is decomposed by carbonic anhydrase to CO_2 and water, and CO_2 is expelled via the lungs during respiration while water is excreted in urine. According to Kronfeld (1976), CO_2 elimination usually equals formation, so expired CO_2 is derived from complete oxidation of carbohydrate and fat without the production of excess H^+. The endogenous acids formed from incomplete combustion of carbohydrates and fats are formed inside cells, where they are immediately buffered by proteins (Kronfeld, 1976). These acids eventually leave the cell and are transported by blood for excretion in urine.

There are three mechanisms by which the kidney preserves normal body pH (Hilwig, 1976). Bicarbonate reabsorption, in conjunction with carbonic anhydrase results in the recovery of approximately 99% of the bicarbonate filtered. It is almost completely reabsorbed along the proximal tubule. Carbonic anhydrase type II facilitates the generation of H^+ and bicarbonate in

tubular cells. Hydrogen ions are secreted into the lumen via a Na^+/H^+ exchanger in the apical membrane and bicarbonate ions are transferred via a basolateral Na^+-HCO_3^- co-transporter (Unwin et al., 2002). Some primary active H^+ secretion via an H^+-ATPase also occurs. The secreted H^+ ions react with filtered bicarbonate to form H_2CO_3. In the luminal membrane of the proximal tubule, carbonic anhydrase type IV rapidly converts H_2CO_3 to CO_2 and H_2O, which diffuse into the cell (Laing et al., 2005). This process is neutral in terms of net urinary H^+ excretion, because the secreted H^+ are used to reclaim filtered bicarbonate. Reabsorption of bicarbonate is affected by pCO_2 in the arterial blood due to changes in intracellular H^+ concentration. An increase in pCO_2 within the tubular cells increases formation of H_2CO_3 via CA to improve the intracellular availability of H^+ for bicarbonate reabsorption. Direct bicarbonate reabsorption from the tubular lumen also occurs without the CA reaction (Hilwig, 1976).

The second method by which the body preserves normal pH is through the exchange of H^+ and Na^+ in non-bicarbonate buffer systems. The phosphate buffer system is of greatest importance. In this system, H^+ that has been secreted into the tubular lumen converts dibasic phosphate to the monobasic form, increasing net acid excretion (Laing et al., 2005).

The third method by which the renal system preserves physiological pH is through ammonia excretion. Van Slyke et al. (1943) reported that acid-base status and method of N excretion were related in that the metabolism of glutamine, the primary amino acid (**AA**) involved in ammonia-genesis, was altered depending on whether the system was acidic or alkaline. The authors observed that administration of glutamine to a dog in acidosis markedly increased ammonia excretion. Hilwig (1976) and Laing et al. (2005) reported that under conditions of low urinary buffer excretion, the highly diffusible free base NH_3 is formed in the

renal cortex and then diffuses through the tubular epithelium into the acid tubular fluid where it is converted to ammonium ion ($\mathbf{NH_4^+}$). Ammonium ion is poorly diffusible. Hydrogen ion secretion must accompany NH_3 secretion in order to prevent back diffusion. Therefore, the activity of CA, which provides a source of H^+, plays a key role in excretion of ammonium ions. Ammonium ion will then pair with an anion such as Cl^- prior to excretion in the urine. The formation of NH_4^+ from glutamine by the proximal tubule also generates new bicarbonate that is added to plasma. Net acid excretion occurs via the α-intercalated cell of the distal nephron where it is secreted into the lumen of the distal tubule and collecting duct by an H^+/K^+-ATPase (Laing et al., 2005).

In addition to the acid load created at the cellular level from incomplete oxidation of carbohydrates and fats, ruminants must metabolize additional exogenous acids created via ruminal fermentation. These are primarily acetic, propionic, and butyric acids. Ruminants often exhibit an acid tide in the urine following a meal, especially one with a large proportion of fermentable carbohydrates (Kronfeld, 1976). Causes of this acid tide are secretion of bicarbonate in the saliva and the absorption of exogenous acids from the rumen. Ruminal fermentation contributes to a constant tendency towards metabolic acidosis. This is more prominent in ruminants such as lactating dairy cows, which are fed diets rich in soluble carbohydrates, compared with those whose diet consists primarily of fibrous carbohydrates such as grazing beef cattle. Digestion of fibrous diets creates less acid because diets are more slowly fermented and are more abundant in alkaline materials. Soluble carbohydrates are fermented more rapidly and contain less alkaline material, leading to a greater acid load.

Monitoring acid-base status

The acid-base status of an animal is not easily determined. Changes in acid-base chemistry of the animal are highly regulated and compensated by the renal and respiratory systems. For example, plasma pH is not necessarily related to buffering capacity. It is the sum of all reactions in the body and is highly regulated by both the renal and respiratory systems (Block, 1994). Houpt (1977) observed that as long as the ratio of bicarbonate to carbonic acid (H_2CO_3) is kept at 20 to 1, there is no change in plasma pH. This is illustrated by the Henderson-Hasselbach equation:

$$pH = pKa + \log ([HCO_3^-]/[H_2CO_3])$$

where physiological pH = 7.4, pKa at 38^oC = 6.10 and the ratio of bicarbonate to carbonic acid equals 20 to 1. Blood pH and partial pressure of carbon dioxide (pCO_2) are measured for calculation of bicarbonate concentration. Haskins (1977) reported that blood bicarbonate concentration is not reliable as a sole indicator of acid-base status. Calculated values such as base excess and anion gap are better indicators of acid-base chemistry because they take multiple metabolites into account. Anion gap, a good indicator of metabolic acidosis, is calculated as [Na – (Cl +HCO_3^-)] or [(Na + K) – (Cl + HCO_3^-) (Gossett et al., 1987). Normal range of anion gap for cattle is 13.9 to 20.3 meq/L (Garry et al., 1988). Base excess is defined as the amount of acid or base needed to restore 1L of blood to normal acid-base composition at pCO_2 of 40 mm Hg. Base excess reflects bicarbonate concentration. Normal blood bicarbonate concentration is 24 meq/L (Kronfeld, 1979), while the normal range for base excess is 0 $\pm$ 4 meq/L (Haskins, 1977). Therefore the normal range for bicarbonate concentration would be 20 to 28 meq/L.

Alkalosis and acidosis are states that result from net accumulation of alkali or acid in the system (Kronfeld, 1976). Metabolic acidosis occurs from the loss of bicarbonate or gain of a

strong acid. It can be caused by diarrhea or ketosis, as well as the consumption of large amounts of fermentable carbohydrates relative to roughage. Metabolic alkalosis is caused by a primary increase in bicarbonate. This can be brought on by a loss of strong acids in the body such as HCl from emesis or sequestration of HCl in a twisted or displaced abomasum (Kronfeld, 1976). It can also be caused by overconsumption of buffer salts or K deficiency. During metabolic alkalosis, the ratio of bicarbonate to carbonic acid is greater than 20 to 1. Respiratory alkalosis occurs when respiratory rate is increased such as during heat stress. Elevated respiration rate leads to increased expiration of CO_2. In order to compensate and maintain bicarbonate:carbonic acid at a 20 to 1 ratio, the renal system increases excretion of bicarbonate, which can reduce the total amount of bicarbonate systemically.

Definition of DCAD

Dietary cation-anion difference (**DCAD**) is defined as the proportion of specific fixed ions or the balance between positively charged and negatively charged fixed ions (Beede, 1992). The DCAD is calculated as either meq (Na + K – Cl) or (Na + K) – (Cl + S) per 100 g DM or per kg DM. The meq of an element per 100 g DM is calculated by dividing the concentration of the element (% of diet DM) by the meq weight of the element. Milliequivalent weights for Na, K, Cl, and S are 0.0233, 0.0397, 0.0355, and 0.0160 respectively. The ions involved in the calculation of DCAD, with the exception of S, are non-metabolized bioavailable ions, or fixed ions. Fixed ions play a major role in determining acid-base balance of biological fluids (Stewart, 1978). Sulfur, while not a fixed ion, can be included in the calculation because sulfates directly acidify biological fluids when fed at high dietary concentrations, thus altering acid-base balance (Cole and Zlotkin, 1983; Whiting and Draper, 1981). Sodium, K, Cl, and S have been chosen to calculate DCAD for ruminants because of their contribution to osmotic potential, acid-base

chemistry and pumping mechanisms of cell membranes (Block, 1994). Other terms have been used to describe concepts similar to DCAD. Stewart (1978) defined strong ion difference as the sum of all strong cations in solution minus all strong anions.

Dietary electrolyte balance is the term coined by Mongin (1981) for use in poultry. It is expressed as meq (Na + K – Cl) per 100 g DM, the same three-element DCAD equation mentioned previously. The three elements were included in this equation since Na, K, and Cl are fixed ions with the greatest absorption and thus the greatest effect on acid-base homeostasis from a dietary standpoint. Wheeler (1981) referred to dietary electrolyte balance as fixed ion balance. Other names for the equation include dietary cation-anion balance (Tucker et al., 1988a; West et al., 1992), anion-cation balance (Block, 1984; Oetzel et al., 1988), and fixed cation-anion balance (Fredeen et al., 1988b). Elements with poor absorption that still play a role in acid-base chemistry have also been included in the DCAD calculation. These elements were included with modifying coefficients to take into account absorption and led to the calculation described as meq ($Na^+ + K^+ + 0.15\ Ca^{2+} + 0.15\ Mg^{2+}$) – ($Cl^- + 0.20\ SO_4^{2-} + 0.30\ P^{3-}$) per 100 g DM (Goff and Horst, 1997).

DCAD and acid-base chemistry

The DCAD can be adjusted to alter or restore acid-base base balance in the dairy cow to improve health and performance (Block, 1994). Sodium sesquicarbonate, Na_2CO_3, $KHCO_3$, K_2CO_3, and $NaHCO_3$ are all buffering agents that have been added to the diets of lactating dairy cows to buffer hydrogen ions. The impact of these cationic salts may or may not be observed in blood pH. As mentioned previously, blood pH is highly regulated via respiration and kidney function. Vagnoni and Oetzel (1998) and Pehrson et al. (1999) reported no significant differences in blood pH for diets with decreasing DCAD. Wang and Beede (1992a) however,

observed lower blood pH when low DCAD diets were fed to prepartum cows. Tucker et al. (1988a) reported a linear increase in both blood and urine pH when DCAD increased from –10 to 20 meq (Na + K – Cl) per 100 g DM. West et al. (1992) reported a linear increase in blood pH when DCAD increased from 12 to 46 meq (Na + K – Cl) per 100 g DM. In finishing steers, there was a linear increase in blood pH for the first 28 days, followed by a quadratic response on day 84 for steers fed diets with DCAD varying from 0 to 45 meq (Na + K – Cl) per 100 g DM (Ross et al., 1994). Not all research has shown increased blood pH with positive DCAD. Tucker et al. (1988b) reported no increase in blood pH for diets with DCAD of 42, 55, or 63 meq per 100 g DM, suggesting that at higher DCAD concentrations, blood pH reaches a plateau and compensation occurs through urinary excretion of excess base. Patience (1988) suggested that resistance to increases in blood pH may be due to the comparative ease of excess base excretion compared with the excretion of excess acids.

Changes in blood and urine bicarbonate and blood pCO_2 are also indicative of acid-base status and are affected by DCAD. Tucker et al. (1992) reported an inverse relationship between blood bicarbonate and blood hydrogen ion concentration, while pCO_2 parallels blood bicarbonate. Higher blood and urine bicarbonate concentrations were reported with increased DCAD (Tucker et al., 1988a; West et al., 1992). When diets low in DCAD were fed to prepartum cows, a reduction in blood and urine bicarbonate content was observed (Moore et al., 2000; Vagnoni and Oetzel, 1998). During lactation, increased blood bicarbonate concentration improves blood buffering, resulting in improved DMI and milk yield in the dairy cow. However, the optimum range for systemic bicarbonate has yet to be determined.

Tucker and Hogue (1990) illustrated the close relationship between DCAD and acid-base chemistry when they reported a uniform response of acid-base measures with constant DCAD

(about 32 meq/100 g DM) even though the diet contained varying levels of sodium chloride and potassium chloride. Although Vagnoni and Oetzel (1998) and Pehrson et al. (1999) did not observe changes in blood pH for differing DCAD, they did observe improved Ca mobilization, which indicates a positive DCAD effect on acid-base chemistry in prepartum cows.

The diet of the dairy cow changes considerably after calving in order to support milk production. Postpartum diets are higher in concentrates relative to roughage, which can lead to metabolic acidosis. Positive DCAD diets benefit the lactating cow by improving blood and rumen buffering, however, the magnitude of this benefit is yet unknown.

DCAD and ruminal buffering

A portion of the DCAD effect on lactating cows may result from altered rumen fermentation. Ruminal pH is reduced by lactic acid production when high grain concentrations are fed (Kennelly et al., 1999; Russell, 1998), which decreases fiber digestibility (Rogers et al., 1982; Snyder et al., 1983). According to Terry et al. (1969) and Mertens (1979) ruminal pH of 6.4 to 6.8 is optimal for cellulose digestion. Early research with buffers focused on the benefits associated with the addition of buffers to lactating cow rations. Positive results from the addition of cationic salts were attributed to changes in ruminal fermentation as opposed to any systemic buffering effects. The effect of buffers on rumen pH has been well documented. Erdman et al. (1982) reported increased rumen pH for 6 to 8 h postfeeding and increased fiber digestibility with the inclusion of $NaHCO_3$. West et al. (1987) reported increased ruminal pH for 6 h postfeeding with the inclusion of either $NaHCO_3$ or K_2CO_3. These authors also noted that fiber digestibility was higher for diets that included buffers compared with the control diet. High concentrate diets increase production of propionate in the rumen (Murphy et al., 2000; Van Soest, 1963). Most buffers increase ruminal acetate production and decrease ruminal propionate

(Erdman et al., 1982; Snyder et al., 1983) by increasing fiber digestion. Acetate's role as a precursor for fat synthesis results in greater milk fat percentage. The bicarbonate and carbonate ions buffer the rumen primarily by buffering excess H^+ ions produced from fermentation of soluble carbohydrates. They also buffer by stimulating increased water intake and increased fluid dilution rate. Increased fluid dilution rate increases the amount of ruminally undegraded starch by decreasing the amount of time dietary starch resides in the rumen, resulting in decreased ruminal propionate production (Russell and Chow, 1993). Similar results, however, have not been observed for all buffer salts. Emery et al. (1964) noted reduced milk fat percentage and ruminal acetate concentration and increased ruminal propionate concentration with the inclusion of $CaCO_3$ compared with $NaHCO_3$. These differences may be caused by greater particle size for the $CaCO_3$ as well as reduced solubility in the rumen. Buffer salts with Na or K as the cation had the greatest effect on ruminal volatile fatty acid production and pH changes in addition to improvements in milk fat percentage (Belibasakis and Triantos, 1991; Emery et al., 1964; West et al., 1987). Because of the role of Na and K in base generation, the overall DCAD effect may be an additive systemic Na or K and ruminal effect.

Simultaneous comparisons of ruminal and systemic measures have been inconclusive. Tucker et al. (1988a) reported a linear increase in both ruminal pH and blood pH when DCAD was increased from –10 to 20 meq/100 g DM, while West et al. (1987) reported no difference in blood pH or bicarbonate concentration, yet noted an increase in ruminal pH when either $NaHCO_3$ or K_2CO_3 was included in the diet. Although DCAD was not calculated in this study, the increase in Na or K concentration in the diet should have increased the DCAD. Because the sources of the additional Na and K were cationic salts, however, differentiation of a DCAD or buffering salt effect is difficult.

Balancing DCAD in rations

The DCAD of a ration is calculated by subtracting milliequivalents of anions from cations. No recommendation for DCAD for lactating dairy cattle has been made by the National Research Council (NRC, 2001), however, using NRC (2001) recommendations for Na, K, and Cl (0.19, 1.02, and 0.25%, respectively) a DCAD of 27 meq/100 g DM is derived. The DCAD calculated from NRC recommendations does not take into account other factors that affect acid-base balance and consequently the appropriate DCAD. Factors such as stage of lactation and environment affect the optimum DCAD needed for particular cows. Several studies (Escobosa et al., 1984; Schneider et al., 1984; West et al., 1991) reported improved blood buffering of heat-stressed dairy cows from diets with more positive DCAD than that derived from NRC (2001) values for Na, K, and Cl. Block (1984) and Delaquis and Block (1995b) reported improved milk yield with higher DCAD in early and midlactation cows, however, no response in milk yield was observed in late lactation. Early and midlactation cows, therefore, may require higher or more positive DCAD than that required by late lactation cows. Erdman (1982) reported that the acid-base status of the lactating dairy cow tends toward alkalinity as stage of lactation increases with blood pH, HCO_3 and pCO_2 all increasing. Meta-analyses have been conducted to elucidate optimum DCAD for lactating dairy cows. Hu and Murphy (2004) analyzed data from 12 studies and reported dry matter intake (**DMI**) and milk yield were maximized at 40 and 34 meq Na + K – Cl/100 g DM, respectively. Sanchez and Beede (1996) analyzed data from ten studies and reported that DMI and milk yield were maximized at 38 meq Na + K – Cl/100 g DM with the optimal DCAD ranging from 25 to 50 meq/100 g DM.

Ingredients

Positive DCAD diets for lactating cows are achieved primarily through the use of cationic salts including $NaHCO_3$ (Gaynor et al., 1989; Sanchez et al., 1997), sodium sesquicarbonate (Cassida et al., 1988; Tucker et al., 1994), K_2CO_3 (West et al., 1991), and $KHCO_3$ (Schneider et al., 1984).

Anionic salts are used to acidify late gestation cow diets. Ammonium chloride (Gaynor et al., 1989; Oetzel et al., 1988), $(NH_4)_2SO_4$ (Oetzel et al., 1988; Takagi and Block, 1991), $MgCl_2$ (Gaynor et al., 1989; Goff et al., 1991), and $MgSO_4$ (Takagi and Block, 1991) are anionic salts which are used to acidify these diets. Based on reduction in urine pH, NH_4Cl is the most acidic and $MgSO_4$ is the least acidic of the anionic salts used (Oetzel, 1991). Reduced DMI has been associated with feeding anionic salts due to poor palatability and metabolic acidosis (Phillipo et al., 1994; Tucker et al., 1992; Tucker et al., 1991b). Feeding these salts in a total mixed ration, especially one based on silage improves palatability (Goff et al., 1991; Kilmer et al., 1980).

DCAD and nitrogen metabolism

Feeding livestock in confinement can lead to large concentrations of minerals such as N and P in the waste stream. The majority of these minerals are excreted in urine and manure that can subsequently be used for fertilizer. However, significant quantities of N are lost to the atmosphere (Bierman et al., 1999) as either dinitrogen gas or ammonia (Harper et al., 2000; Hutchinson et al., 1982). Therefore, it is important to investigate mechanisms to enhance N metabolism in order to improve utilization and reduce waste.

The relationship between acid-base chemistry and N metabolism is illustrated by the role of ammonia in hydrogen ion excretion. Ammonia serves as a major urinary/systemic buffer

during metabolic acidosis (Heitmann and Bergman, 1978). Acid load stimulates renal ammoniagenesis (Madias and Zelman, 1986) and inhibits hepatic ureagenesis (Kashiwagura et al., 1984). This change in ammonia- and ureagenesis comes about not only because of the role of ammonia in urinary and systemic buffering, but also as a means by which bicarbonate, which is needed for urea production, can be conserved. During alkalosis urea production is the primary means of N excretion (Guder, 1987; Waring et al., 1974). Ammonia- and ureagenesis are generally in synchrony such that total N excretion remains constant (Cai and Zimmerman, 1995). Blood ammonia concentrations also affect method of N excretion. The presence of excess ammonia leads to increased urea synthesis, even in cases of metabolic acidosis when ammoniagenesis would otherwise predominate. McKinnon et al. (1990) reported greater blood urea N (**BUN**) concentration in cows and heifers fed diets supplemented with NH_4Cl (6.65 mM) compared with those fed a control diet (5.96 mM). Elevated BUN may have been due to greater blood ammonia concentration from feeding NH_4Cl , masking any potential acid-base chemistry effect on ammonia- and ureagenesis.

The relationship between acid-base chemistry and N metabolism suggests that N metabolism can be modified using DCAD. Escobosa et al. (1984) observed greater BUN in lactating cows fed high DCAD diets (11.92 mg/dl) compared with those fed low DCAD (8.73 mg/dl). Cai and Zimmerman (1995) reported the same positive relationship between DCAD and plasma urea N in castrated male pigs. Increased ammonium excretion in pigs (Golz and Crenshaw, 1991) and cows (Wang and Beede, 1990) was observe when low DCAD was fed.

Results of prior research investigating the effect of DCAD on N metabolism have been inconclusive. Delaquis and Block (1995a; 1995b) observed no effect on N metabolism in lactating or dry dairy cows. However, Phillip (1983) reported a marked improvement in N

retention and digestibility in sheep when $NaHCO_3$ was included in a low protein diet. In swine, no difference in total N digestibility or balance was reported with the addition of 0, 1.3 or 2.6% $NaHCO_3$ (Patience et al., 1986). The authors also reported no significant effect on digestibility of N, lysine, or tryptophan measured at the terminal ileum in growing pigs. Haydon and West (1990), however, reported a linear increase in N digestibility measured at the terminal ileum when DCAD was increased from –5 to 40 meq (Na + K – Cl)/100 g DM. Urinary N excretion exhibited a quadratic response to DCAD, increasing by 11% when DCAD increased from –5 to 10 meq/100 g, then decreasing by 13 and 23% as DCAD increased further to 25 and 40 meq/100 g DM. The authors also reported a linear improvement in retention of consumed and absorbed N as DCAD increased. Patience and Chaplin (1997) reported no differences in N balance when dietary undetermined anion, calculated as (Na + K + Ca + Mg) – (Cl + P + $S_{inorganic}$), was changed. Nitrogen retention declined with the addition of $NaHCO_3$ to the diet of lambs (Mees et al., 1985; Rogers and Phillip, 1987). In each of these studies, the negative effect of $NaHCO_3$ seemed to be mediated through increased excretion of urinary N. It has been proposed that urea is synthesized in the liver as a mechanism for disposal of excess blood bicarbonate and thereby maintenance of acid-base balance (Atkinson and Camien, 1982). However, Halperin et al. (1986) refuted this and reported that the need to dispose of excess bicarbonate does not primarily determine the rate of ureagenesis, which is determined instead by the amount of ammonium to be removed. The mechanism explaining the relationship between acid-base balance and N balance and retention is unclear, especially in ruminants.

Research to determine the effects of DCAD on ruminal N metabolism has also been inconclusive. Phillip (1983) reported a trend for decreased ruminal ammonia concentrations with the inclusion of $NaHCO_3$ in the diet, with a larger decrease in ruminal ammonia content for

high crude protein diets compared with low protein diets. The author proposed that the reduction was caused either by increased microbial growth, which would increase the supply of AA to the duodenum (Harrison and McAllan, 1980), or increased rate of ammonia absorption at higher ruminal pH. However, the mechanism behind reduced ruminal ammonia has not been elucidated.

DCAD and amino acid metabolism

While the relationship between DCAD and N metabolism is still unclear, extensive results have been reported on the interaction between DCAD and AA metabolism. Ammonia plays a vital role in excretion of excess hydrogen ions during metabolic acidosis (Heitmann and Bergman, 1978). Van Slyke et al. (1943) established the role of glutamine in ammoniagenesis. Under conditions of acid-base homeostasis, sufficient glutamine is present in the diet or can be generated from other AA to meet the need for ammoniagenesis (Patience, 1990). However, when dietary glutamine or glutamine precursors are inadequate, skeletal tissue may be catabolized to release glutamine (Heitmann and Bergman, 1978), severely restricting productivity.

Amino acid metabolism also influences acid-base status through the contribution of sulfur-containing AA to total acid load. Oxidation of sulfur containing AA occurs only when methionine and cysteine are present in excess of the amount required for protein synthesis (Patience, 1990). Excess sulfur-containing AA contribute 13 to 26% of the net acid excreted by a growing pig consuming 2 kg/day of a typical corn-soybean meal diet (Patience, 1990). The amount of additional acid contributed by the oxidation of cysteine and methionine is dependent on the concentrations of other essential AA. The additional blood buffering offered by a high DCAD could buffer acidity generated by oxidation of sulfur-containing AA.

Dietary K concentration appears to affect the utilization of basic AA, especially lysine and arginine, for intracellular buffering. Potassium-depleted rat muscle exhibited a tremendous increase in free lysine and arginine concentration (Arnauld and Lachance, 1980; Eckel et al., 1958; Iacobellis et al., 1956). Arnauld and Lachance (1980) also reported that feeding excess lysine to rats increased free-muscle lysine, reduced muscle K, and decreased replacement ratio of K with Na. In poultry, the relationship between lysine metabolism and the content of dietary alkaline or acid salts is also well established (Austic and Calvert, 1981). O'Dell and Savage (1966) and Stutz et al. (1970) reported that the growth-depressing effects of the lysine-arginine antagonism were alleviated with the addition of dietary K. Scott and Austic (1978) reported increased activity of hepatic lysine-α-ketoglutarate reductase, an initial enzyme in the catabolism of lysine, in response to increased dietary K. These authors proposed that the alleviation of the lysine-arginine antagonism could be due to greater lysine catabolism when excess K is fed. Robbins et al. (1982) reported that as dietary K increased from 0.12 to 0.18%, muscle concentrations of free glutamine, lysine, and arginine increased. Concentrations of these AA in muscle tissue decreased when dietary K exceeded 0.18%.

In a review, Patience (1990) summarized the results of several studies on the effect of the inclusion of alkaline salts on lysine metabolism. The author reported that the greatest effect on growth was observed when larger quantities of alkaline salts were fed. Supplemental Na or K ranged from 26 meq/kg to over 400 meq/kg. It was postulated that the growth response was greater as lysine deficiency became more severe. The proposed explanation was that experimental diets were slightly above the K requirement for growing pigs (Combs et al., 1985). The basal diet was also severely deficient in lysine. Because of the low growth rates associated with the low lysine concentration in the diet, the requirement for other AA such as cysteine and

methionine was also reduced making these AA in excess. The AA were catabolized, producing approximately 50 mmoles of excess acid per day. The combination of excess acid and limited dietary K may have established ideal conditions for a response to alkaline salts such as $NaHCO_3$ or $KHCO_3$.

DCAD and heat stress

Heat stress poses a serious problem to lactating dairy cows because of the inability to dissipate the large quantity of metabolic heat generated. The amount of metabolic heat formed is greater for high producing cows (Purwanto et al., 1990) and for cows in hot climates compared with those in thermoneutral conditions due to greater physical activity such as panting needed to facilitate cooling in hot conditions. Robinson et al. (1986) reported greater heat production in beef cattle exposed to 35^{o}C ambient temperature compared with those exposed to 20^{o}C. Heat stress in dairy cows results in reduced DMI and milk yield (Her et al., 1988). West et al. (2003) reported a linear decline in DMI and milk yield as ambient temperature and temperature-humidity index increased. West et al. (1992) reported a linear increase occurred in DMI as DCAD increased from 12 to 46 meq (Na + K – Cl)/100 g DM during heat stress. Reduced DMI and milk yield in dairy cows under heat stress was reported by Sanchez et al. (1994b) when high concentrations of chloride and sulfate were included in drinking water. Chloride and sulfate decrease DCAD, implying that a more positive DCAD is better for lactating dairy cows under heat stress. Greater DMI and milk fat percentage was reported with 32 meq/100 g DM DCAD diet during hot weather when compared with –19 and 17 meq/100 g DM (Escobosa et al., 1984). The authors proposed that the higher milk fat percentage with greater DCAD was due to increased ruminal buffering as opposed to blood buffering. Milk fat percentage is depressed during heat stress due to decreased fiber intake (Emery et al., 1965). Shalit et al. (1991) reported

decreased DMI during heat stress when DCAD was increased from 43 to 95 meq/100 g DM. A DCAD of 95 is extremely alkaline, however, and may have caused the depression in DMI.

DCAD and mineral metabolism

Dietary cation-anion difference plays a role in regulating blood Ca concentrations by altering acid-base chemistry, which affects hormones involved in excretion and reabsorption. Gaynor et al. (1989) reported decreased plasma Vitamin D_3 concentration with high versus low DCAD diets. Reduced plasma calcitonin concentration was reported by Romo et al. (1991) when DCAD was reduced from 23 to –8 meq (Na + K) – (Cl + S)/100 g DM. Greater dietary alkalinity (Lomba et al., 1978) also depresses absorption efficiency of Ca in the gastrointestinal tract. West et al. (1991) also reported a quadratic decline in serum Ca concentrations as DCAD increased from -12 to 31 meq (Na + K – Cl)/100 g DM. The authors also noted that both serum Ca and urinary Ca excretion were highest for the lowest DCAD diet.

Calcium and P are found at a constant ratio of 2 to 1 in bone tissues (Fontenot and Church, 1979). Phosphorus also serves as a buffer in saliva of dairy cows. Much like plasma Ca, plasma P concentration drops considerably at parturition due to a sudden demand caused by the onset of lactation (Hove, 1986; Wohlt et al., 1984). Previous research has not elucidated the relationship between DCAD and P metabolism. Acidogenic diets increased serum P in dairy calves (Beighle et al., 1988), but resulted in no change in either lactating (Romo et al., 1991) or dry cows (Van Mosel et al., 1994). Borucki Castro et al. (2004) reported a tendency for a linear decrease in plasma P as DCAD increased from 14 to 45 meq (Na + K) – (Cl + S)/100 g DM were fed to lactating cows. LeClerc and Block (1989) reported that DCAD had no effect on apparent absorption of P in dry cows. Phosphorus absorption in lactating goats, however, was enhanced for diets with low DCAD (Fredeen et al., 1988a). Increased urinary P excretion was reported for

acidogenic diets in dairy heifers (Roby et al., 1987), but was not observed in calves (Jackson et al., 2001). Borucki Castro et al. (2004) reported no change in urinary or fecal P excretion when cows were fed diets ranging in DCAD from 14 to 45 meq (Na + K) – (Cl + S)/100 g DM.

High dietary K, such as high DCAD diets, decreased plasma Mg concentration in rats (Charlton and Armstrong, 1989), Mg absorption and urinary excretion in sheep (Khorasani and Armstrong, 1990; Ram et al., 1998), and Mg absorption in dairy cows (Jittakhot et al., 2004). High DCAD (alkaline) diets also tend to depress plasma Mg concentration in dairy cows (Gaynor et al., 1989; LeClerc and Block, 1989; West et al., 1991) and steers (Ross et al., 1994). Gaynor et al. (1989) and Wang and Beede (1990) observed elevated urinary Mg excretion when low DCAD diets were fed to dairy cows. However, Wang and Beede (1992b), reported a decrease in urinary Mg excretion when acidic diets were fed to dry cows. Waterman et al. (1991) reported no difference in either plasma or urinary Mg in lactating cows. Leonhard-Marek and Martens (1996) investigated the relationship between ruminal K concentrations and the mucosal to serosal K and Mg flux in isolated ruminal epithelium. They observed a plateau in the inhibition of Mg absorption at high ruminal K concentrations, which resulted in a reduction in the suppression of Mg absorption by elevated K. Consequently, the relationship between DCAD and Mg metabolism is unclear, especially in the dairy cow. Because of the antagonistic relationship between K and Mg absorption, DCAD would only seem to affect Mg absorption in cases where high DCAD diets were fed and a cationic potassium salt such as K_2CO_3 or $KHCO_3$ was used as the cation source.

Plasma chloride is closely associated with metabolic acidosis (Coppock, 1986), therefore the interaction between chloride balance and DCAD is very strong. Tucker et al. (1988a) proposed that chloride might be primarily responsible for altering acid-base status in dairy cows.

Tucker et al. (1992; 1988b; 1991b) reported increased urinary chloride excretion in dairy cows when DCAD was reduced by increasing dietary chloride concentration. Higher plasma chloride concentrations were observed in cows (Bigner et al., 1997) and pigs (Yen et al., 1981) experiencing metabolic acidosis. It is not surprising then that low DCAD or acidogenic diets also increased plasma chloride in steers (Ross et al., 1994) and dairy cows (Hu and Murphy, 2004; Phillipo et al., 1994; Tucker et al., 1991a). Hu and Murphy (2004) reported a linear increase in blood chloride with increased dietary chloride content, and a quadratic decrease in blood chloride as DCAD increased. This decrease is probably due to the inverse relationship between blood chloride and bicarbonate. Blood bicarbonate increases as DCAD increases (Hu and Murphy, 2004; West et al., 1992; West et al., 1991).

The effect of DCAD on Na and K metabolism is unclear. West et al. (1991) reported no DCAD effect for serum Na and a quadratic effect for serum K when DCAD ranged from –12 to 31 meq (Na + K – Cl)/100 g DM. Block (1984) and Waterman et al. (1991), however, reported increased plasma Na in cows when DCAD was increased. Block (1984) also reported reduced plasma K for cationic diets compared with anionic diets. In a meta-analysis of twelve prior DCAD studies, Hu and Murphy (2004) reported no changes in plasma or serum concentration of Na and K by dietary Na and K, respectively, even when diets contained high concentrations of these minerals. These authors hypothesized that excess Na and K were excreted via the kidney. The authors also reported a linear reduction in blood K as DCAD increased but blood Na was not affected.

DCAD and energy metabolism

Glucose absorption in the gastrointestinal tract and kidney occurs through a Na - K pump and is therefore closely related to the major electrolytes affecting DCAD. Little research, however, has been reported on the relationship between DCAD and energy metabolism. Increased plasma glucose concentrations were noted in cows fed diets with low DCAD or diets that caused acidosis (Bigner et al., 1996; Murphy et al., 2000; Owens et al., 1998). Belibasakis and Triantos (1991) reported no effect on plasma glucose concentration when cows were supplemented with Na_2CO_3. Tesseraud et al. (1992) also reported no effect on plasma glucose concentration when goats were infused with potassium chloride and sodium chloride. Changes to DCAD, however, in this study would be minimal due to the inclusion of chloride as the anion. Bigner et al. (1996) reported increased insulin sensitivity in Jersey cows when metabolic alkalosis was induced by feeding diets with 12 meq (Na + K) – (Cl + S)/100 g DM DCAD. The results of these studies suggest improved energy metabolism with increased DCAD, however, because of the limited number of studies reported the strength of the relationship is unclear.

Conclusions

Dietary cation-anion difference affects many aspects of nutrient metabolism through its alteration of acid-base chemistry. The specific mechanism by which metabolism of energy and N are altered is unknown. Feeding a cationic diet improves dry DMI, milk yield, and blood buffering in early and midlactation dairy cows. A cationic diet also improves DMI, milk yield, and blood buffering in heat stressed dairy cows. The relationship between acid-base chemistry and N metabolism implies that possibilities exist for improvements in N utilization by altering DCAD. Before improvements can be made, however, a better understanding of the relationship between DCAD and N metabolism must be obtained. The unique metabolic challenges faced by

the lactating dairy cow may also affect the DCAD:N interaction in ways that are different from other species. Further research should be conducted to better understand the relationship between DCAD and ruminal, as well as systemic, N metabolism in the dairy cow. Research should also be conducted on the relationship between DCAD and AA status in ruminants, more specifically the dairy cow. Although earlier research explored the DCAD:AA interaction in other species, a better understanding of the lactating dairy cow is needed. The objectives of this research are to gain a better understanding of the interaction between acid-base chemistry, DCAD, and N utilization in the lactating dairy cow and elucidate changes that occur in ruminal and systemic N and AA metabolism.

CHAPTER 3

DIETARY CATION-ANION DIFFERENCE AND POTASSIUM:SODIUM RATIO EFFECTS ON PERFORMANCE OF HEAT-STRESSED LACTATING DAIRY COWS[1]

[1] Wildman, C. D, J. W. West, and J. K. Bernard. 2005. To be submitted to Journal of Dairy Science.

ABSTRACT

Forty-two lactating Holstein cows 188 ± 59 days in milk were used in an 8 wk randomized complete block trial with a 2 x 3 factorial arrangement of treatments. The objective was to determine the effects of high dietary cation-anion difference (**DCAD**) and K:Na ratio on milk yield and composition, and acid-base chemistry. Treatments included two DCAD concentrations of 45 or 60 meq Na + K – Cl / 100 g feed DM and three K:Na ratios of 2:1, 3.5:1, or 5:1. Dry matter intake was similar across treatments. Yield of milk and energy corrected milk were lower for 3.5:1 K:Na ratio compared with 2:1 and 5:1 K:Na ratio. Blood urea N was lower with the highest DCAD, suggesting that DCAD possibly reduced protein degradation or altered protein metabolism and retention. Results of this trial indicate that the greatest effect on milk yield occurs when either Na or K is primarily used to increase DCAD. Results also suggest that greater DCAD improves ruminal N metabolism or N utilization may be more efficient with a high DCAD.

(**Key words:** dietary cation-anion difference, sodium, potassium, blood urea nitrogen)

Abbreviation key: **BUN** = blood urea N, **DCAD** = dietary cation-anion difference, **DCAD45** = 45 meq Na + K – Cl / 100 g DM treatment, **DCAD60** = 60 meq Na + K – Cl / 100 g DM treatment, **ECM** = energy-corrected milk yield, **KNa2** = 2:1 K:Na ratio treatment, **KNa3** = 3.5:1 K:Na ratio treatment, **KNa5** = 5:1 K:Na treatment, **MY** = milk yield, **RH** = relative humidity, **SP** = standardization period, **THI** = temperature-humidity index, **TP** = treatment period.

INTRODUCTION

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

Results of previous research differ concerning the role of ion source in the formulation of lactating dairy cow rations. No differences in DMI or MY were observed when either Na or K were used as cation sources (West et al., 1992) . Tucker et al. (1988a) also concluded that DCAD is more important than the individual ions used to alter the DCAD in lactating cows. While these studies determined the effect of cation source, there is little information regarding the effect of varying ratios of dietary K to Na in lactating cow rations. Sanchez et al. (1997) compared dietary proportions of $NaHCO_3$, NaCl and KCl and observed an interaction for DMI

between Na and K and between Na and Cl. The authors also observed increased 3.5% FCM with higher dietary Na, and concluded that interrelationships exist among Na, K, and Cl. Sanchez et al. (1994a) reported that the DMI and MY response to one cation (Na or K) tends to be the greatest when the dietary level of the other cation is low. The objectives of the present study were to determine the effects of adjusting K:Na ratios within high DCAD concentrations on DMI, MY, and serum and urinary electrolyte concentrations.

MATERIALS AND METHODS

Experimental Design

Forty-two multiparous Holstein cows averaging 188 $\pm$ 59 DIM were used in a randomized complete block design with a 2 x 3 factorial arrangement of treatments. The trial was conducted from May 23 to July 31, 2001 and consisted of a 2 wk standardization period (**SP**) followed by an 8 wk treatment period (**TP**). During the SP cows were offered the standard herd diet and baseline measures were taken for DMI, MY, BW, milk composition, and serum and urinary electrolyte concentration. Data collected during the SP were used for covariate analysis of TP data. Cows were ranked by DMI per BW (kg/100kg) during the SP, blocked into groups of six by rank, and then assigned randomly to treatment within block.

Experimental diets contained 51.6% corn silage and 48.4 % concentrate (dry basis) formulated to meet NRC (2001) requirements (Table 3.1). Dietary treatments consisted of two DCAD (45 (**DCAD45**) or 60 (**DCAD60**) meq Na + K – Cl / 100 g DM) and three K:Na ratios (2:1 (**KNa2**) , 3.5:1 (**KNa3**) , or 5:1 (**KNa5**)) within each DCAD treatment. The DCAD and K:Na ratios were adjusted using $NaHCO_3$ as a Na source and K_2CO_3 as a K source and were added to experimental premixes using ground corn as a carrier.

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

Sampling

Forage, concentrate, treatment mixes, and TMR's were sampled three times weekly, dried at 60^{o} C for 72 h, and composited by week for analysis. Samples were ground to pass through a 1-mm screen using a Wiley Mill (Arthur B. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltec System (Foss Tecator AB, Hoeganaes, Sweden) and CP was calculated as the percentage N x 6.25 (AOAC, 1990). Acid detergent fiber and NDF were determined according to the method of Van Soest et al. (1991). Samples were extracted with nitric and acetic acids for determination of chloride concentration using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) as outlined by Cotlove et al. (1958). Phosphorus concentration was determined by colorimetry (Beckman DU Series 500 Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) following wet ashing (AOAC, 1990). Other minerals were measured by atomic absorption spectrophotometry (Perkin-Elmer Model Number 3030, Norwalk, CT) following wet ashing (AOAC, 1990).

Milk yield was measured using electronic meters (DeLaval, Kansas City, MO). Milk samples were collected weekly from consecutive p.m. and a.m. milkings throughout the trial.

Samples were shipped to Southeast Milk Inc. (Belleview, FL) for analyses of fat and protein using infrared analysis (B2000; Bentley Instruments Inc., Chaska, MN).

Blood was collected by jugular venipuncture into evacuated tubes at the end of the SP and three times during the TP at 1300 h. Samples were centrifuged at 3300 rpm for 20 minutes for separation or serum. Serum was harvested and analyzed immediately for Ca, P, Mg, Na, K, Cl, bicarbonate, urea N (**BUN**) , creatinine, and glucose using a Bodhringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN) at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Urine samples were collected via manual stimulation at the same time as blood collection for analysis of the same components as for blood serum.

Respiratory rates were recorded at 1300 h once during the SP and three times during the TP. Respiration rate was determined for each cow for 1 minute by two individuals. Counts exceeding 10% error were recounted until a difference of less than 10% was obtained.

Milk temperature, an indicator of body temperature, was measured at each milking using a thermocouple in one short milk tube of each milker (Temp-Sense, Udder Health Systems, Bellingham, WA). Ambient temperature and relative humidity (**RH**) were recorded hourly at three locations in the free stalls and feeding area using a HOBO Pro RH/Temp Data Logger (Onset Computer Corp., Bourne, MA). Mean values across locations were used for analysis. Temperature-humidity index (**THI**) was calculated as THI = db – (0.55 – 0.55 RH) x (db – 58), where db was the dry bulb temperature in degrees Fahrenheit and RH was expressed in decimals (NOAA, 1976).

Statistical Analysis

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

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

where

μ = the mean intercept;

I = the covariate;

B_i = the effect due to the ith block;

D_j = the effect due to the jth DCAD;

R_k = the effect due to the kth K:Na ratio;

W_l = the effect due to the lth week;

$C_{m(ijk)}$ = the effect due to cow m being in the ith block with the jth DCAD and kth K:Na ratio (error a); and

e_{ijklm} = residual error associated with each $Y_{ijklm.}$

Treatment means were separated by least square means in the presence of a significant F-test ($P < 0.10$) using pdiff. Linear and quadratic orthogonal contrasts were included in the model to determine the effect of K:Na ratio.

RESULTS AND DISCUSSION

Chemical Composition of Diets

The DCAD across K:Na ratios was lower than expected (Table 3.2). Mean DCAD for the three DCAD 45 treatment combinations was 41 meq/100 g DM, compared with 58 meq/100 g DM for the three DCAD 60 treatment combinations. While DCAD was slightly lower than

formulated, the difference between the two treatments was larger than originally formulated, preserving the potential for a DCAD treatment difference.

The K:Na ratios were lower for the 3.5:1 and 5:1 treatments than formulated. Mean values for these treatments were 3.1:1 and 4.2:1 respectively. Mean K:Na ratio for the 2:1 treatment was as formulated. Although the 3.5:1 and 5:1 ratios were smaller than formulated, similar ratios were observed between DCAD treatments.

Environmental Conditions

Environmental conditions during the study are presented in Table 3.3. Mean maximum and minimum daily temperatures averaged 31.0 and 22.0^{o} C and were similar throughout the trial. Holstein cows become less productive due to heat stress when daily mean THI exceeds 72 (Johnson, 1987). The mean daily THI during this trial was 75.6, which greatly exceeded this critical value.

Body Weight and Temperature

No differences in initial BW or BW gain were observed for DCAD or K:Na ratio treatments (Table 3.4). No significant DCAD x K:Na ratio interactions were observed for AM milk temperature, PM milk temperature, or temperature difference (Table 3.4). However, AM milk temperature declined linearly (P<0.05) as K:Na ratio increased. The change in AM to PM temperature measures increased linearly (P<0.10) as K:Na ratio increased with the largest change observed in KNa5 (Table 3.4). West et al. (1992) observed decreased AM and PM milk temperature for cation sources added to the diet that was attributed to disparity in BW among treatment groups. This is consistent with the trend for a linear K:Na effect for AM to PM temperature change observed in the present study. Within the DCAD60 treatment, the greatest AM to PM temperature change occurred for lighter BW cows. The ability of lower BW animal

to dissipate heat would improve body heat loss. Although respiratory rates were elevated across treatments due to heat stress, no differences were observed between DCAD or among K:Na ratio for respiratory rate (Table 3.4).

Dry Matter Intake

The DMI was similar for all treatments (Table 3.5). Early DCAD work by Tucker et al. (1988a) reported an 11% increase in DMI when DCAD was increased from -10 to +20 meq / 100 g DM, although these DCAD concentrations were lower than those used in the present study, and are lower than the DCAD resulting from current NRC (2001) recommendations (27 meq / 100 g DM) for Na , K , and Cl (0.19, 1.02, and 0.25%, respectively). West et al. (1991) observed a quadratic response for DMI when DCAD increased from -7.94 to 32.4 meq / 100 g DM during cool weather and -11.7 to 31.2 meq / 100 g DM during hot weather. The authors reported that DMI plateaued at a DCAD of approximately 20 meq / 100 g DM, suggesting a limit in DMI response to DCAD with mineral concentrations above NRC (2001) recommendations for Na, K, and Cl. In later work, however, West et al. (1992) reported a positive linear response in DMI as DCAD was increased from 12 to 46 meq / 100 g DM. Sanchez and Beede (1996) reported that DMI was maximized when DCAD was 25 to 50 meq / 100 g DM. In a similar analysis of data from 17 published studies, Hu and Murphy (2004) reported that DMI was maximized at a DCAD of 40 meq / 100 g DM. In both reports, a curvilinear relationship was observed for DCAD in which DMI decreased when DCAD concentrations were outside the optimal range. The absence of a DCAD effect on DMI in the present study suggests that at ranges as narrow as 15 meq / 100 g DM, the homeostatic controls handle the excess cations in the system via urinary excretion without decreasing DMI (Table 3.7). These data also suggest that the lowest DCAD was adequate in this situation.

The lack of response to K:Na ratio is consistent with the results of Tucker et al. (1988a; 1991a) and West et al. (1992) who reported that DCAD was more important than the ion used to alter DCAD in lactating cows. The decrease in DMI reported by Schneider et al. (1984) when $KHCO_3$ was used as a cation source compared with $NaHCO_3$ may have been caused by poor palatability whereas the corn silage used in the present study may have masked any possible palatability problems. Although DMI was similar across K:Na ratio, there was a trend ($P = 0.12$) for increased DMI/100 kg BW for KNa5. Initial BW for these cows was numerically lower than for other treatments (Table 3.4) and may have contributed to greater DMI/100 kg BW.

Milk Yield and Composition

Milk yield and composition were similar for both DCAD, which is consistent with DMI. Previous research reporting increased MY with increased DCAD (Tucker et al., 1988a; West et al., 1991) indicated the greatest DCAD effect at levels lower than those used in the present study. Because the objective of this trial was to determine the effect of altering K:Na ratio at high DCAD concentrations, and a quadratic relationship ($P<0.05$) exists for MY (Hu and Murphy, 2004; Sanchez and Beede, 1996), potential exists for depressed MY due to reduced DMI when DCAD exceeds (60 meq Na + K – Cl / 100g DM) the previously reported optimal range (Hu and Murphy, 2004; Sanchez and Beede, 1994; Sanchez and Beede, 1996). The absence of a DCAD response for DMI and MY suggests that at high DCAD, a 15 meq / 100 g DM difference in DCAD concentration is not sufficient to depress DMI or milk yield.

A quadratic K:Na effect for MY ($P = 0.03$) and energy-corrected milk (**ECM**) ($P = 0.04$) was observed (Table 3.5). Mean MY decreased from 27.7 kg/d for KNa2 to 26.2 kg/d for KNa3. An increase was then observed to 28.1 kg/d for KNa5. A similar relationship was observed for ECM with mean yields of 29.3, 27.1, and 28.7 kg/d for KNa2, KNa3, and KNa5, respectively.

These results are consistent with previous research in which interactions were observed for Na and K. Sanchez et al. (1994a) reported the greatest response to either high Na or K when the concentration of the other ion was low. Erdman et al. (1980) and O'Connor et al. (1988) reported no effect for additional Na when fed with either low, adequate, or high K. The lack of effect in these studies may be explained by the fact that the diets were not isochloridic. Because dietary Cl affects DCAD (meq Na + K – Cl / 100 g DM), the addition of Na or K may be offset by the presence of different Cl concentrations. Mean MY and ECM were highest when the concentrations of either K or Na were highest.

A quadratic K:Na ratio effect was observed for milk protein percentage ($P = 0.04$) across DCAD with mean values of 3.02, 3.12, and 2.98 % for KNa2, KNa3, and KNa5, respectively. This response is inversely related to that noted for MY and is most likely a dilution effect since milk protein yield was similar among treatments. No differences were noted among K:Na ratios for milk fat percentage.

Blood and Urinary Metabolites

No differences were noted for serum Ca, Na, Cl, or bicarbonate concentration (Table 3.6). Increased serum bicarbonate concentration was positively correlated with the positive DCAD effect on DMI (Tucker et al., 1988a; West et al., 1991). The absence of a DCAD effect for DMI and serum bicarbonate, coupled with the increase in urinary excretion of bicarbonate (Table 3.7) for DCAD60 suggests that the cow's buffering needs were satisfied by the DCAD of 45 meq / 100 g DM. Blood urea N concentrations were higher ($P<0.001$) for DCAD 45 (17.0 mg/dl) compared with DCAD 60 (14.5 mg/dl). Cai and Zimmerman (1995) reported increased plasma urea N and decreased plasma ammonia as DCAD increased. These researchers did not observe any effect of DCAD on total plasma N (urea + ammonia + allantoin). Phillip (1983)

reported reduced ruminal ammonia concentrations in lambs fed supplemental $NaHCO_3$. The author proposed that the addition of $NaHCO_3$ either increased microbial utilization of ruminal ammonia or raised ruminal pH such that absorption of ruminal ammonia increased. The lower BUN observed for the DCAD60 suggests that greater absorption of ruminal ammonia may not be occurring. Reduced BUN coupled with decreased rumen ammonia noted in previous research (Phillip, 1983) may suggests the possibility for a protein sparing effect caused by a reduction of protein degradation in the rumen.

As the K:Na ratio increased, serum Mg increased linearly ($P<0.05$). This is in contrast with previous research (Jittakhot et al., 2004; Ram et al., 1998) in which greater dietary K concentration reduced Mg absorption. Leonhard-Marek and Martens (1996) reported that in isolated ruminal epithelium the relationship between ruminal K concentration and the mucosal to serosal Mg flux reach a plateau at high ruminal K concentrations, resulting in decreased suppression of Mg absorption. Jittakhot et al. (2004) reported that the plateau of mucosal to serosal Mg flux did not decrease suppression of Mg absorption at high K levels in nonpregnant, dry cows. Dietary K concentration in that study was considerably lower than that in the present study. Ruminal K concentration for the KNa5 ratio may have been sufficiently high to reach the plateau and the suppression in Mg absorption was alleviated. Serum Mg increased to 2.65 mg/dl for KNa5 from 2.44 and 2.40 mg/dl for KNa2 and KNa3, respectively.

A significant quadratic K:Na ratio effect was noted for BUN ($P<0.10$) (Table 3.6). The BUN concentration for KNa3 (14.9 mg/dl) was lower than that observed for KNa5 (16.7 mg/dl). No difference was observed between KNa2 and the other K:Na ratios. Greater BUN for KNa5 may be explained by the relationship between dietary K and intracellular AA concentration (Arnauld and Lachance, 1980; Austic and Calvert, 1981). Greater dietary K in KNa5 may have

led to increased availability of free AA. If available in excess, these amino acids may be catabolized as an energy source, resulting in greater BUN. A linear increase ($P<0.05$) for serum K concentration with increasing K:Na ratio was also observed, probably a result of increasing dietary K concentration (Table 3.2).

There was a trend for an interaction of DCAD x K:Na ratio ($P<0.10$) for fractional excretion of K (Table 3.7). The increase in K excretion between 2:1 and 3.5:1 K:Na ratio was similar for DCAD concentrations (17.4 and 16.1 % increase for DCAD45 and DCAD60, respectively), but the increase in K excretion between 3.5:1 and 5:1 K:Na ratio was considerably larger for DCAD60 (26.9%) compared with DCAD45 (10.5%). This is consistent with the increase in dietary K for these treatments (Table 3.2). Fractional excretion of Na declined linearly ($P<0.001$) as K:Na ratio increased because dietary Na decreased with increasing K:Na ratio (Table 3.2); resulting in decreased Na fractional excretion.

CONCLUSIONS

The quadratic response in MY and ECM for K:Na ratio suggests that increasing DCAD primarily with Na or K may have greater effects on milk yield. The absence of a DCAD effect for DMI, MY, ECM, and blood bicarbonate along with increased fractional excretion of bicarbonate with increased DCAD concentration suggests adequate blood buffering at DCAD concentrations in the previously determined optimum range of 25 to 50 meq / 100 g feed DM. Lower BUN in cows fed a high DCAD concentration suggests an effect on ruminal protein metabolism. The mechanism behind these changes in protein metabolism is unclear and warrants further investigation. By elucidating these effects, the efficiency with which protein is fed can be improved.

Table 3.1. Ingredient composition of diets formulated to contain a DCAD of 45 or 60 meq/100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

DCAD	45			60		
K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1
	% of DM					
Corn silage	51.58	51.58	51.58	51.58	51.58	51.58
Cottonseed hulls	7.22	7.22	7.22	7.22	7.22	7.22
Ground corn	17.37	17.37	17.37	17.37	17.37	17.37
Base concentrate[1]	16.92	16.92	16.92	16.92	16.92	16.92
Mineral premix[2]	2.07	2.07	2.07	2.07	2.07	2.07
Ground corn[3]	2.48	2.50	2.58	1.22	1.36	1.44
$NaHCO_3$[3]	1.82	1.20	0.83	2.41	1.61	1.16
K_2CO_3[3]	0.02	0.58	0.87	0.63	1.29	1.66
$MgSO_4$[3]	0.52	0.56	0.56	0.58	0.58	0.58

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

[2] Mineral premix contained 64.58% limestone, 10.42% salt, 10.42% distiller's grain, 8.33% magnesium oxide, and 6.25% vitamin-trace mineral premix.

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

Table 3.2. Chemical composition[1] of diets formulated to contain DCAD concentration of 45 or 60 meq/100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

Formulated DCAD	45			60		
Formulated K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1
	%					
DM	55.00 ± 3.59	55.13 ± 3.29	55.52 ± 3.31	54.78 ± 3.22	54.52 ± 3.25	54.98 ± 3.11
	% of DM					
CP	17.42 ± 0.69	17.29 ± 1.43	17.64 ± 1.08	17.83 ± 1.00	16.90 ± 1.05	18.08 ± 1.87
NDF	31.05 ± 1.88	29.79 ± 1.99	29.38 ± 1.75	29.03 ± 2.14	30.49 ± 2.09	30.23 ± 1.91
ADF	17.66 ± 1.14	17.43 ± 1.48	16.58 ± 1.66	16.65 ± 1.44	17.61 ± 1.06	17.53 ± 1.64
Ca	0.87 ± 0.10	0.90 ± 0.22	0.94 ± 0.16	0.87 ± 0.15	0.89 ± 0.09	0.98 ± 0.18
P	0.36 ± 0.02	0.38 ± 0.05	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.38 ± 0.01
Mg	0.36 ± 0.02	0.40 ± 0.05	0.37 ± 0.02	0.36 ± 0.04	0.37 ± 0.02	0.37 ± 0.04
K	1.03 ± 0.09	1.42 ± 0.21	1.45 ± 0.04	1.38 ± 0.11	1.76 ± 0.08	1.95 ± 0.12
Na	0.50 ± 0.08	0.46 ± 0.06	0.35 ± 0.04	0.74 ± 0.10	0.57 ± 0.06	0.45 ± 0.05
Cl	0.41 ± 0.03	0.39 ± 0.04	0.40 ± 0.01	0.36 ± 0.05	0.38 ± 0.02	0.38 ± 0.02
S	0.28 ± 0.01	0.29 ± 0.02	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.30 ± 0.01
Calculated DCAD, Meq Na + K – Cl / 100 g DM	36.5	45.1	40.8	56.9	58.8	58.5
Calculated K:Na Ratio	2.1:1	3.1:1	4.1:1	1.9:1	3.1:1	4.3:1

[1]Mean value ± standard deviation.

Table 3.3. Environmental conditions for the trial.

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

[1] Week 1 and 2 were the standardization period, while weeks 3 through 10 were the treatment period.

[2] Relative humidity.

[3] Temperature-humidity index = ^{o} F – (0.55 – 0.55 * relative humidity) * (^{o} F – 58). (NOAA, 1976).

Table 3.4. Mean body weight, respiration rate, and milk temperature for cows fed diets formulated to contain a DCAD of 45 or 60 meq Na + K – Cl / 100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

DCAD	45			60				*P*		
K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1	SEM	DCAD	Ratio	DCAD x Ratio
Initial BW, kg	587.5	597.9	609.5	623.6	662.3	548.5	29.9	NS[1]	NS	NS
BW change, kg	8.1	4.5	1.1	-1.2	6.0	1.4	3.6	NS	NS	NS
Respiration rate, breaths/min	81	83	77	83	73	82	4	NS	NS	NS
Milk temperature, ^{o} C										
AM	38.7	38.5	38.3	38.6	38.6	38.5	0.1	NS	L*	NS
PM	38.8	38.8	38.6	38.8	38.7	38.7	0.1	NS	NS	NS
Change[2]	0.1	0.2	0.3	0.2	0.1	0.3	0.1	NS	L†	NS

[1]NS = Not significant ($P > 0.10$).

[2]Calculated as (PM Temperature – AM Temperature).

L = Linear effect

* $P < 0.05$

† $P < 0.10$

Table 3.5. Dry matter intake, milk and component yield, and milk composition for cows fed diets for cows fed diets containing a DCAD of 45 or 60 meq/100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

DCAD	45			60				*P*		
K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1	SEM	DCAD	Ratio	DCAD x Ratio
DMI										
kg/d	22.4	22.3	23.8	23.0	23.5	22.2	0.94	NS[1]	NS	NS
kg/100 kg BW	3.84	3.82	3.92	3.73	3.66	4.07	0.17	NS	NS	NS
Yield, kg/d										
Milk	27.5	26.1	28.8	27.9	26.4	27.4	0.87	NS	Q[2]	NS
ECM[2]	28.8	26.4	29.7	29.8	27.7	27.6	1.04	NS	Q	NS
Fat	1.07	0.98	1.11	1.12	1.07	1.03	0.06	NS	NS	NS
Protein	0.83	0.79	0.86	0.83	0.81	0.80	0.03	NS	NS	NS
Composition, %										
Fat	3.85	3.82	3.90	3.91	3.84	3.92	0.08	NS	NS	NS
Protein	3.03	3.16	3.01	3.02	3.08	2.95	0.06	NS	Q	NS

[1] NS = Not significant ($P > 0.10$)

[2] Q = Quadratic effect ($P < 0.05$)

[3] ECM = 0.3246 * (kg milk) + 12.86 * (kg fat) + 7.04 * (kg protein). (Tyrrell and Reid, 1965)

Table 3.6. Serum mineral and metabolite concentrations for cows fed diets formulated to contain a DCAD of 45 or 60 meq/100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

DCAD	45			60				*P*		
K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1	SEM	DCAD	Ratio	DCAD x Ratio
Ca, mg/dl	10.19	10.03	9.93	9.92	9.87	10.07	0.12	NS[1]	NS	NS
Mg, mg/dl	2.51	2.42	2.59	2.37	2.38	2.70	0.08	NS	L[2]*	NS
Na, mmol/L	144.8	144.6	144.6	143.9	144.4	144.7	0.4	NS	NS	NS
K, mmol/L	4.67	4.83	4.78	4.58	4.64	4.82	0.09	NS	L†	NS
Cl, mmol/L	102.5	102.4	101.9	101.2	102.1	101.7	0.6	NS	NS	NS
BUN, mg/dl	16.9	15.8	18.2	14.4	13.9	15.2	0.8	***	Q[3]†	NS
Bicarbonate, mmol/L	22.1	21.9	22.8	22.9	22.5	22.8	0.5	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

[2] L = Linear Effect

[3] Q = Quadratic Effect

† $P < 0.10$

* $P < 0.05$

*** $P < 0.001$

Table 3.7. Urinary excretion of mineral and metabolites for cows fed diets formulated to contain a DCAD of 45 or 60 meq/100 g DM and K:Na ratio of 2:1, 3.5:1, or 5:1.

DCAD	45			60				*P*		
K:Na Ratio	2:1	3.5:1	5:1	2:1	3.5:1	5:1	SEM	DCAD	Ratio	DCAD x Ratio
Fractional excretion[1], %										
Ca	0.26	0.23	0.35	0.26	0.20	0.28	0.08	NS[2]	NS	NS
Mg	12.6	10.1	16.4	10.9	7.4	14.7	2.2	NS	Q[3]*	NS
Na	1.80	1.45	1.12	2.16	1.38	1.32	0.19	NS	L[4]***	NS
K	42.9	60.3	70.8	52.6	68.7	95.6	3.9	***	L***	†
Cl	1.17	1.23	1.27	1.29	1.16	1.50	0.11	NS	NS	NS
Urine metabolite / urine creatinine[5]										
Urea N	10.6	10.3	10.7	10.1	10.2	10.6	0.4	NS	NS	NS
Bicarbonate	2.38	2.72	2.54	3.52	3.06	3.70	0.34	**	NS	NS

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

[2] NS = Not significant ($P > 0.10$)

[3] Q = Quadratic Effect

[4] L = Linear Effect

[5] Expressed as (milligrams of urine metabolite / 100 ml urine) / (milligrams of urine creatinine / 100 ml of urine)

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

CHAPTER 4

EFFECT OF DIETARY CATION-ANION DIFFERENCE AND DIETARY CRUDE PROTEIN ON PERFORMANCE OF LACTATING DAIRY COWS DURING HEAT STRESS[1]

[1] Wildman, C. D, J. W. West, and J. K. Bernard. 2005. To be submitted to Journal of Dairy Science.

ABSTRACT

Thirty-two lactating Holstein cows, 225 ± 63 days in milk, were used in a 6 wk trial to determine the response to dietary cation-anion difference (**DCAD**) and dietary crude protein (**CP**) concentration fed during hot weather. Treatments were arranged as a 2 x 2 factorial within a randomized complete block design to provide 15 or 17 % CP and DCAD of 25 or 50 meq (Na + K – Cl) / 100g DM. A DCAD by CP interaction was detected for milk yield because milk yield was lower for high DCAD than low DCAD on the high CP diets. No differences were noted at low CP. Milk fat percentage was higher with high DCAD than low DCAD and high CP diets supported higher milk fat percentage than low CP diets. No differences were observed among treatments for DMI or milk protein percentage. Serum total amino acid and essential amino acid concentrations and ratio of essential amino acid:total amino acids were greater with high DCAD. These results suggest that raising DCAD improves amino acid availability for protein synthesis by sparing amino acids for maintaining acid-base balance. A better understanding of the mechanisms behind this amino acid sparing effect will improve management of protein nutrition in the lactating dairy cow.

(**Key words:** dietary cation-anion difference, dietary crude protein, sodium, potassium)

Abbreviation key: CP15 = low dietary crude protein treatment, **CP17** = high dietary crude protein treatment, **DCAD** = dietary cation-anion difference, **DCAD25** = low dietary cation-anion difference treatment, **DCAD50** = high dietary cation-anion difference treatment, **RH** = relative humidity, **SP** = standardization period, **THI** = temperature-humidity index, **TP** = treatment period**.**

INTRODUCTION

Acid-base chemistry affects many aspects of metabolism. It has direct effects on mineral (Block, 1984), AA (Haydon and West, 1990), and vitamin metabolism (Reddy et al., 1982). An interaction between AA and maintenance of acid-base chemistry has long been established. Van Slyke (1943) reported the role of glutamine in acid-base chemistry via its use in ammoniagenesis. Method of N excretion is dependent on acid-base chemistry. Cai and Zimmerman (1995) reported increased plasma urea N concentration when pigs were fed cationic diets, and increased urine ammonia concentration using anionic diets. While the method of N excretion is dependent on acid-base chemistry, the effect on overall N excretion is unclear. Cai and Zimmerman (1995) reported that while method of N excretion was altered with changes in acid-base chemistry, total N excretion did not change. Welbourne et al. (1986), however, reported greater total N excretion during acidosis. May et al. (1986) also noted increased protein degradation and decreased N retention during metabolic acidosis. The primary interaction between AA and acid-base chemistry is the role of glutamine in ammoniagenesis. The body can supply increasing quantities of glutamine or glutamine precursor AA as required for excretion (Vinay et al., 1978). Under conditions of normal acid-base chemistry, sufficient glutamine is present in the diet or can be generated from other AA to meet ammoniagenesis needs. However, Heitmann and Bergman (1978) observed that skeletal tissue in sheep was sacrificed to release glutamine when dietary supply was inadequate. In the lactating dairy cow, skeletal tissue and milk production could be sacrificed in order to supply adequate glutamine for ammoniagenesis to maintain acid-base homeostasis.

Another facet of the interaction between acid-base chemistry and AA metabolism is the contribution of sulfur-containing AA to the acid load of the animal. When fed in excess of that

needed for protein synthesis, these AA are metabolized for energy. Patience (1990) calculated the contribution of sulfur-containing AA to total acid production in swine. Growing swine consuming 2 kg per day of a 16% CP corn-soybean meal diet produce approximately 26 meq of acid per day from the catabolism of sulfur-containing AA. Total dietary acid production is 100 to 200 meq per day in swine. Although not the predominant source of acid production, metabolism of these AA contributes a considerable amount of acid under normal conditions.

Higher DCAD results in greater blood bicarbonate concentration, increasing blood alkalinity (Tucker et al., 1988a; West et al., 1991). Because DCAD alters acid-base chemistry, it may also impact AA metabolism. O'Dell and Savage (1966) reported a reduction in the growth-inhibiting antagonism between lysine and arginine by feeding potassium acetate. Patience (1990) reported inconsistent results from previous research to determine if a DCAD sparing effect on lysine existed. The author reported greater improvements in BW gain in swine when larger quantities of alkaline salts were fed. The author also noted that the positive effect of alkaline salt on BW gain increased as lysine deficiency became more severe. The author proposed that the differences in response to alkaline salts might be due to a buffering effect because diets were deficient in lysine, instead of a lysine-sparing effect. Sulfur-containing AA, although fed at recommended levels, were actually in excess due to the lysine deficiency. Excess acid produced from the metabolism of the sulfur containing AA increased acid load which was buffered by the cationic salts. Patience (1990) suggested that improved performance resulted from the alleviation of the excess acid load instead of a lysine-sparing effect.

While much research has been conducted to determine the relationship between acid-base chemistry and protein metabolism in other species, limited data exist for the lactating dairy cow. Metabolic challenges faced by the lactating dairy cow are unique, especially under heat stress

conditions. Heat stress in dairy cows results in reduced DMI (Her et al., 1988; West et al., 2003), which can result in reduced MY. The objective of this study was to determine effects of low or high DCAD on N utilization of lactating dairy cows fed low or high concentrations of CP. Results of this research will lead to further understanding of the mechanism by which Na and K effect affect N utilization in the lactating dairy cow.

MATERIALS AND METHODS

Thirty-two multiparous lactating Holstein cows averaging 255 $\pm$ 63 DIM were used in a randomized complete block design with a 2 x 2 factorial treatment arrangement. The trial was conducted from July 8 to August 27, 2002 and consisted of a 9 d standardization period (**SP**) followed by a 6 wk treatment period (**TP**). During the SP cows were offered the standard herd diet and baseline DMI, MY, BW, milk composition, and serum and urinary electrolyte concentration was determined. Data collected during the SP were used as a covariate in the statistical analysis. Cows were ranked by DMI per BW (kg/100 kg) during the SP and were blocked into groups of 4 by rank for random assignment to treatment within block.

Dietary treatments were formulated to contain DCAD of 25 (**DCAD25**) or 50 (**DCAD50**) meq Na + K – Cl / 100 g DM and CP concentration of 15 (**CP15**) or 17% (**CP17**) within each DCAD treatment. Low protein diets contained 39.5 % corn silage, 8.8 % alfalfa hay, and 51.7 % concentrate and high protein diets contained 37.7 % corn silage, 8.4 % alfalfa hay, and 53.9 % concentrate (dry basis). Low and high DCAD diets contained 58.0% DM. All diets were formulated to meet or exceed NRC (2001) requirements. Sodium bicarbonate and K_2CO_3 were used to alter DCAD and were carried in experimental premixes using ground corn as a carrier. Blood meal, fishmeal, urea, and soybean meal were used in an experimental premix for adjustment of CP. Composition of each treatment mix is summarized in Table 4.1.

Prior to beginning the trial, all cows were trained to eat behind electronic gates (American Calan, Inc., Northwood, NH). Cows were fed once daily (0800 h), and the amount of feed offered and refused was recorded. The amount of feed offered was adjusted daily based on the previous day's consumption to maintain approximately 10% orts. The TMR was pushed up at least four times daily. Ingredient DM content for adjustment of ration components was determined weekly by drying in a forced air oven at 60^{o} C for 72 h. Cows were housed in a free stall barn with fans activated by thermostat when the ambient temperature exceeded 24^{o}C. Cows were milked twice daily at approximately 0400 and 1500 h.

Sampling

Forage, concentrate, treatment mixes, and experimental TMR were sampled three times weekly, dried at 60^{o} C for 72 h, and composited by week for later analysis. Samples were ground to pass through a 1-mm screen using a Wiley Mill (Arthur B. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltec System (Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as the percentage N x 6.25 (AOAC, 1990). Acid detergent fiber and NDF were determined by the method of Van Soest et al. (1991). Chloride was extracted using a combination of nitric and acetic acids and concentration determined using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) using the method of Cotlove et al. (1958). Phosphorus concentration was determined by colorimetry (Beckman DU Series 500 Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) following wet ashing (AOAC, 1990). Sodium, K, Mg, and Ca were measured by atomic absorption spectrophotometry (Perkin-Elmer Model Number 3030, Norwalk, CT) following wet ashing (AOAC, 1990).

Milk yield was measured using electronic milk meters (DeLaval, Kansas City, MO). Milk samples were collected from consecutive p.m. and a.m. milkings during each week of the

trial. Samples were shipped to Southeast Milk Inc. (Belleview, FL) for analyses of fat and protein using infrared analysis (B2000; Bentley Instruments Inc., Chaska, MN).

Whole blood samples were collected by coccygeal venipuncture into evacuated tubes at the end of the SP and three times during the TP at 1300 h. Blood was centrifuged at 3300 rpm for 20 min for separation of serum. Serum was harvested and analyzed immediately for Ca, P, Mg, Na, K, Cl, bicarbonate, urea N, creatinine, and glucose using a Bodhringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN) at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Plasma was analyzed for pH, pCO_2, and ionized Na, K, and Ca using an AVL OPTI Critical Care Analyzer (OSMETECH, Inc., Roswell, GA) at the University of Georgia Veterinary Diagnostic Laboratory. Serum from the SP collection and final TP collection was analyzed for free AA using a Beckman 6300 Amino Acid Analyzer (Beckman Coulter, Inc., Fullerton, CA) (Slocum and Lee, 1983) at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO. Urine samples were collected via manual stimulation at the same time as blood collection for analysis of Ca, Mg, Na, K, Cl, bicarbonate, urea N, and creatinine concentration.

Respiratory rates were measured at 1300 h once during the SP and three times during the TP. Two individuals measured the respiration rate of each cow for 1 minute. Counts exceeding 10% error were recounted until a difference of less than 10% was obtained. Body weights were recorded once weekly immediately following the 1500 h milking.

Milk temperature, an indicator of body temperature, was measured at each milking using a thermocouple in one short milk tube of each milker (Temp-Sense, Udder Health Systems, Bellingham, WA). Ambient temperature and relative humidity (**RH**) were recorded hourly using a HOBO Pro RH/Temp Data Logger (Onset Computer Corp., Bourne, MA). Temperature-

humidity index (**THI**) was calculated using the equation, THI = db – (0.55 – 0.55 RH) x (db – 58), where db was the dry bulb temperature in degrees Fahrenheit and RH was expressed in decimals as described by NOAA (1976).

Statistical Analysis

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

$$Y_{ijklm} = \mu + I + B_{i+} D_j + P_k + DP_{jk} + W_l + DW_{jl} + PW_{kl+} C_{m(ijk)} + e_{ijklm}$$

where

μ = the mean intercept;

I = the covariate;

B_i = the effect due to the ith block;

D_j = the effect due to the jth DCAD;

P_k = the effect due to the kth CP concentration;

W_l = the effect due to the lth week;

e_{ijklm} = residual error associated with each $Y_{ijklm.}$

Treatment means were separated by least square means in the presence of a significant F-test ($P < 0.10$) using pdiff.

RESULTS AND DISCUSSION

Chemical Composition of Diets

Dietary CP concentrations were slightly lower than originally formulated (Table 4.2). The difference, however, between low and high CP was large enough to preserve the potential for treatment differences. The DCAD was as formulated, representing the high and low end of the optimal range reported by Sanchez and Beede (1996). Low and high DCAD diets also met or exceeded DCAD (27 meq / 100 g DM) that would result from current NRC (2001) guidelines for Na, K, and Cl (0.19, 1.02, 0.25%, respectively).

Environmental Conditions

The environmental conditions during the study are presented in Table 4.3. Mean maximum and minimum temperatures were similar across the SP and TP and averaged 31.7 and 22.3^{o}C, respectively. Mean maximum and minimum THI (81.3 and 71.7, respectively), as well as mean daily THI (76.7) were similar across SP and TP. Johnson (1987) reported that Holstein cows become less productive due to heat stress when daily mean THI exceeds 72. Mean daily THI exceeded 72 across all periods, causing heat stress conditions for the duration of the trial.

Dry Matter Intake

No differences in DMI or DMI/100 kg BW were observed among treatments (Table 4.4). Previous research has reported positive responses in DMI when DCAD was increased. Tucker et al.(1988a) reported an 11% increase in DMI when DCAD was increased from –10 to +20 meq (Na + K – Cl) / 100 g DM. The DCAD values in that research were lower than those observed in the present study. West et al. (1991) reported a quadratic response for DMI when DCAD ranged from –12 to 31 meq / 100 g DM during hot weather. These researchers reported a plateau for DMI at approximately 20 meq / 100 g DM, which is consistent with the absence of a DCAD

effect in the present study. In later work, however, West et al. (1992) observed a linear improvement in DMI with increasing DCAD ranging from 12 to 46 meq / 100 g DM. The lack of a DCAD effect in the present study may be explained by stage of lactation. Delaquis and Block (1995b) reported improved DMI and milk yield for cows in early and midlactation when DCAD was increased, but no difference for cows in late lactation. Erdman et al. (1982) reported that as lactation progresses, blood pH and bicarbonate concentration increases, perhaps lessening the need for highly positive DCAD diets. The cows in the present study were 255 $\pm$ 63 DIM, which may have been sufficiently late in lactation to reduce the need for high DCAD.

Milk Yield

A DCAD x CP interaction was observed for MY ($P < 0.10$) (Table 4.4) due to a negative response to DCAD50 for CP17 treatments. No differences for DCAD were observed for low CP15 diets. These results are inconsistent with earlier DCAD work (Tucker et al., 1988a; West et al., 1991) in which DCAD improved MY. As with DMI, much of the previous work has been conducted with early and midlactation cows. Meta-analyses established optimal DCAD ranges for MY and DMI (Hu and Murphy, 2004; Sanchez and Beede, 1996). In each of these studies, a quadratic increase was observed for MY, with decreased MY when DCAD exceeded the maximum optimal value of 50 meq Na + K – Cl / 100 g DM. Much of the data included in this work was for early and midlactation cows, with little data available on late lactation cows. Because the need for additional dietary buffer decreases as stage of lactation increases (Erdman et al., 1982), optimal DCAD levels would decrease. The high DCAD treatment in the present study was at the high end of the optimal range established using early and midlactation cows (Sanchez and Beede, 1996) and may have been too high to elicit a response in late lactation cows. The absence of a DCAD response suggests that adequate buffering is provided by a

DCAD on the low end of the optimal range for late lactation cows. Additional bicarbonate that resulting from increased DCAD above the low end of the optimal range is excreted via the urine. In high CP diets, dietary CP may meet buffering needs in late lactation cows. Consequently, providing additional buffering by feeding supplemental DCAD may reduce MY similar to the quadratic DCAD response observed for DCAD outside of the optimal range in early and mid-lactation cows. (Hu and Murphy, 2004; Sanchez and Beede, 1996).

Milk fat percentage was higher for DCAD50, in agreement with previous reports (Tucker et al., 1991a; West et al., 1991) (Table 4.4). Higher milk fat percentage in the present study may result from additional ruminal buffering provided by DCAD50 diets. Increased milk fat percentage ($P < 0.10$) was observed for CP17 diets. No treatment differences were observed among treatments for fat or energy-corrected milk yield or milk protein percentage. Protein yield was lower ($P < 0.10$) for DCAD50, which may have been due to differences in DMI between DCAD25 and DCAD50, especially within CP17 treatments.

Body Weight and Temperature

Similar initial BW and BW gains were observed among treatments and averaged 669.9 and 9.8 kg, respectively (Table 4.5). Milk temperature (AM and PM) and change in milk temperature were not affected by treatment, and were near normal (38.6^{o}C). Respiration rates, although elevated due to high THI, were similar across treatments.

Blood and Urinary Minerals and Metabolites

An interaction between DCAD and CP was observed for serum Na, Cl, and glucose concentrations (Table 4.6), and for fractional excretion of Na (Table 4.7). Within CP15 treatments, serum Na concentration decreased for DCAD 50 compared with DCAD25. Serum Na was higher for DCAD50 within CP17 treatments. Serum Cl concentration decreased for

DCAD50 compared with DCAD25 within CP15 treatments. No differences were noted within CP17 diets. Fractional excretion of Na and Cl was higher for DCAD50 diets across CP treatments (Table 4.7). This increase in urinary Na excretion is consistent with greater Na intake from the DCAD50 diets. In earlier work, greater urinary Cl was associated with increased blood bicarbonate (Yen et al., 1981). Serum bicarbonate concentrations were higher ($P < 0.01$) for DCAD50 in the current study, and may have contributed to the increase in urinary Cl excretion.

Serum bicarbonate concentration and urinary bicarbonate excretion were higher for DCAD50 diets and is consistent with previous research (Tucker et al., 1988a; West et al., 1991). Greater bicarbonate excretion at high DCAD suggests that the buffering needs of the cow are being met at low DCAD. High DCAD increased pCO_2 ($P < 0.10$), which is a response to increased serum bicarbonate. Greater pCO_2 concentration is caused by decreased expiration of CO_2, which is inconsistent with the absence of a DCAD response for respiration rate (Table 4.5). Serum DCAD was also greater ($P < 0.01$) for DCAD50 diets across CP treatments. This difference is associated with increased serum bicarbonate and is indicative of improved blood buffering capacity.

Reductions in both serum Mg concentration ($P < 0.01$) and Mg fractional excretion ($P < 0.01$) were observed for DCAD50. Increased dietary K concentrations reduce Mg absorption (Jittakhot et al., 2004; Ram et al., 1998), which is consistent with the results of the present study. Lower absorption would reduce Mg available for excretion, resulting in a decline in urinary Mg excretion.

No differences in BUN or urine urea N were observed for DCAD treatments. Earlier work (Cai and Zimmerman, 1995) reported increased plasma urea N and urinary urea N

excretion in swine when DCAD was elevated. Escobosa et al. (1984) reported increased BUN in lactating dairy cows when DCAD was elevated from –19 to 17 meq Na + K – Cl / 100 g DM. No change was observed when DCAD was increased to 32 meq / 100 g DM. The results of the current research are consistent with these findings and suggest that the mechanism behind N excretion in ruminants may be different from those observed in other species.

Blood Amino Acids

Concentrations of total serum AA, essential AA, and essential:total AA ratio were greater for high DCAD. Increased Lys, Arg, Val, Ile, and Leu concentrations were also observed for DCAD50 treatments. These differences may be attributed to a decreased role of these AA in acid-base chemistry in high DCAD diets. Van Slyke et al. (1943) and May et al. (1986) demonstrated greater glutamine metabolism and protein degradation during acidosis. May et al. (1987) also reported that administration of $NaHCO_3$ reduced proteolysis in acidotic rats. The animals in the present study were not as acidotic as were the animals in these previous studies, however, because some enzymes are sensitive to changes in pH, altering the acid-base status of the animal may alter AA metabolism. Differences in serum AA concentrations may be attributed to greater AA absorption in high DCAD diets. Amino acids are absorbed via a Na co-transport mechanism (Guyton, 1981). Increasing DCAD with dietary sodium concentration could lead to enhanced AA absorption. Improved serum AA concentrations for DCAD50 could also be explained by a decreased role for AA in blood buffering in high DCAD diets, increasing availability of AA for protein synthesis.

CONCLUSIONS

The absence of a response to DCAD for MY and DMI in late lactation cows suggests that adequate blood buffering is supplied in diets with DCAD concentrations near NRC (2001)

recommendations for Na, K, and Cl. Increased blood concentration of essential AA suggests that the higher DCAD reduced the need for AA degradation to maintain acid-base balance sparing additional AA. Whether the mechanisms behind these changes in AA metabolism are a result of changes in ruminal protein metabolism or post-ruminal utilization is unclear. A better understanding of these mechanisms, however, offers the opportunity to improve the efficiency of protein use by the lactating dairy cow.

Table 4.1. Ingredient composition of treatment premixes for diets containing a low or high DCAD and low or high CP.

Dietary CP %	15		17	
DCAD, meq / 100 g DM	25	50	25	50
	---------- % of TMR DM ----------			
Protein Treatment Mix				
Soybean Meal	4.17	4.17	6.92	6.92
Fish Meal	1.23	1.23	2.06	2.06
Blood Meal	0.05	0.05	0.09	0.09
Urea	0.02	0.02	0.03	0.03
% of TMR DM	5.47	5.47	9.10	9.10
DCAD Treatment Mix				
Ground Corn	3.30	1.36	3.30	1.36
$NaHCO_3$	0.35	1.32	0.35	1.32
K_2CO_3	0.00	0.96	0.00	0.96
% of TMR DM	3.65	3.64	3.65	3.64

Table 4.2. Chemical composition[1] of diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17	
DCAD	25	50	25	50
	%			
DM, %	57.45 ± 3.14	57.84 ± 2.53	58.52 ± 2.75	58.16 ± 3.32
	% of DM			
CP, % of DM	14.60 ± 0.50	14.80 ± 1.07	16.54 ± 0.38	16.77 ± 1.09
NDF, % of DM	31.65 ± 2.61	31.99 ± 2.50	28.25 ± 1.34	28.12 ± 1.72
ADF, % of DM	17.19 ± 0.56	16.39 ± 1.63	15.23 ± 0.43	14.81 ± 1.46
Ca	0.97 ± 0.05	0.97 ± 0.13	0.97 ± 0.12	0.96 ± 0.17
P	0.48 ± 0.02	0.49 ± 0.05	0.52 ± 0.02	0.49 ± 0.04
Mg	0.30 ± 0.00	0.29 ± 0.02	0.29 ± 0.02	0.27 ± 0.02
K	1.00 ± 0.08	1.65 ± 0.18	1.09 ± 0.03	1.80 ± 0.11
Na	0.24 ± 0.01	0.52 ± 0.07	0.24 ± 0.02	0.50 ± 0.09
Cl	0.32 ± 0.00	0.30 ± 0.01	0.30 ± 0.02	0.29 ± 0.03
DCAD[2]	26.8	56.1	29.7	59.3

[1]Mean value ± standard deviation

[2]meq Na + K – Cl / 100 g DM

Table 4.3. Environmental conditions for the study.

	Week							
Item	SP	1	2	3	4	5	6	Mean ± SD
Maximum Temp, ^{o} C	31.67	33.03	30.98	32.09	30.45	32.05	31.87	31.73 ± 0.83
Minimum Temp, ^{o} C	22.58	22.81	22.37	22.26	20.08	22.59	23.16	22.26 ± 1.01
Maximum RH,[1] %	96.84	95.19	98.59	96.49	93.58	97.96	96.26	96.42 ± 1.68
Minimum RH, %	55.18	50.12	61.97	52.30	44.04	54.86	57.83	53.76 ± 5.73
Maximum Daily THI [2]	81.47	82.35	81.64	81.59	78.15	81.97	82.00	81.31 ± 1.43
Minimum Daily THI	72.36	72.52	72.10	71.74	67.36	72.37	73.29	71.68 ± 1.96
Mean Daily THI	76.96	77.36	76.56	76.81	74.01	77.18	77.67	76.65 ± 1.22

[1] Relative humidity.

[2] Temperature-humidity index = ^{o} F – (0.55 – 0.55 * relative humidity) * (^{o} F – 58). (NOAA, 1976).

Table 4.4. Dry matter intake, milk and component yield, and milk composition for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
DMI								
kg/d	22.5	22.0	22.6	21.6	0.8	NS[1]	NS	NS
kg/100 kg BW	3.24	3.39	3.24	3.18	0.09	NS	NS	NS
Yield, kg/d								
Milk	29.0	28.7	31.4	27.8	0.8	*	NS	†
ECM[2]	27.6	28.5	30.8	28.7	1.0	NS	NS	NS
Fat	0.92	0.99	1.05	1.01	0.04	NS	NS	NS
Protein	0.93	0.92	1.01	0.92	0.03	†	NS	NS
Composition, %								
Fat	3.16	3.51	3.39	3.70	0.11	**	†	NS
Protein	3.21	3.27	3.19	3.30	0.05	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

[2] ECM = 0.3246 * (kg milk) + 12.86 * (kg fat) + 7.04 * (kg protein). (Tyrrell and Reid, 1965).

† $P \leq 0.10$

* $P \leq 0.05$

** $P \leq 0.01$

Table 4.5. Mean body weight, respiration rate, and milk temperature for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Initial BW, kg	685.6	639.1	703.8	653.1	35.7	NS[1]	NS	NS
BW gain[2], kg	10.6	7.3	9.2	11.9	2.9	NS	NS	NS
Respiration rate, breaths/min	66.7	67.0	70.6	66.0	3.8	NS	NS	NS
Milk temperature, ^{o} C								
AM	38.2	38.2	38.3	38.3	0.04	NS	NS	NS
PM	38.3	38.3	38.4	38.4	0.04	NS	NS	NS
Change[3]	0.08	0.11	0.08	0.13	0.02	NS	NS	NS

[1] Not significant ($P > 0.10$)

[2] Weight gain over the duration of the trial

[3] Calculated as (PM temperature – AM Temperature)

Table 4.6. Serum mineral and metabolite concentrations for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Na, mmol/L	146	145	144	145	0.3	NS[1]	**	**
K, mmol/L	4.5	4.5	4.5	4.6	0.1	NS	NS	NS
Cl, mmol/L	103	100	102	102	1	†	NS	**
DCAD, meq Na + K – Cl / L	46.6	48.8	46.3	47.3	0.5	**	†	NS
Ca, mg/dl	10.5	10.5	10.4	10.3	0.1	NS	NS	NS
Mg, mg/dl	2.4	2.2	2.4	2.1	0.1	**	NS	NS
Glucose, mg/dl	75	70	74	72	1	**	NS	†
Bicarbonate, mmol/L	22.2	23.8	22.1	23.0	0.5	**	NS	NS
BUN, mg/dl	8.8	9.1	13.6	13.4	0.6	NS	**	NS
pH	7.424	7.437	7.424	7.428	0.009	NS	NS	NS
pCO_2[2], mmHg	45.2	47.9	43.3	47.6	1.9	†	NS	NS

[1] NS = Not significant ($P > 0.10$)

[2] Partial pressure of CO_2

† $P \leq 0.10$

* $P \leq 0.05$

** $P \leq 0.01$

Table 4.7. Urinary excretion of mineral and metabolites for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Fractional excretion[1], %								
Ca	0.45	0.14	0.33	0.08	0.09	**	NS[2]	NS
Mg	4.87	3.73	6.10	2.37	0.84	**	NS	NS
Na	0.49	1.61	0.51	1.13	0.12	**	†	*
K	38.4	65.7	36.4	58.8	2.5	**	NS	NS
Cl	0.84	1.54	0.85	1.26	0.10	**	NS	NS
P	1.40	3.45	2.70	3.14	0.69	†	NS	NS
Urine metabolite / urine creatinine[2]								
Urea N	6.02	6.23	8.94	9.84	0.54	NS	**	NS
Bicarbonate	0.79	2.72	0.71	2.33	0.25	**	NS	NS

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

[2] NS = Not significant ($P > 0.10$)

[3] Expressed as (milligrams of urine metabolite / 100 ml urine) / (milligrams of urine creatinine / 100 ml of urine)

† $P \leq 0.10$

* $P \leq 0.05$

** $P \leq 0.01$

Table 4.8. Serum AA concentration for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% dietary CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Essential AA, µg/ml								
Lys	19.69	20.86	18.88	22.63	1.11	*	NS[1]	NS
Arg	22.11	25.31	21.73	29.64	3.08	†	NS	NS
Met	2.81	2.67	3.76	3.72	0.33	NS	**	NS
Phe	13.39	13.74	11.80	13.74	0.74	NS	NS	NS
Val	28.87	31.16	31.13	35.82	1.85	†	†	NS
Trp	7.65	7.01	7.70	7.75	0.33	NS	NS	NS
Thr	17.41	16.73	16.72	18.57	0.92	NS	NS	NS
Ile	11.72	13.44	12.27	14.66	0.77	*	NS	NS
His	11.05	10.38	12.27	12.30	0.73	NS	*	NS
Leu	28.13	30.62	29.40	34.31	1.45	*	†	NS
Total Essential AA, µg/ml	160.69	172.65	165.85	194.18	7.17	*	†	NS
Total AA, µg/ml	367.77	378.81	370.83	407.56	13.50	†	NS	NS
Essential AA:Total AA	0.44	0.45	0.45	0.47	0.01	*	NS	NS

[1] NS = Not significant ($P > 0.10$)

† $P \leq 0.10$

* $P \leq 0.05$

** $P \leq 0.01$

CHAPTER 5

DIETARY CATION-ANION DIFFERENCE AND DIETARY CRUDE PROTEIN EFFECTS ON LACTATION PERFORMANCE, ACID-BASE CHEMISTRY, AND RUMINAL FERMENTATION[1]

[1] Wildman, C. D, J. W. West, and J. K. Bernard. 2005. To be submitted to Journal of Dairy Science.

ABSTRACT

Eight primiparous lactating Holstein cows fitted with ruminal cannulae were used to determine the effect of dietary cation-anion difference (**DCAD**) and dietary crude protein (**CP**) concentration on milk yield and composition, acid-base chemistry, and N metabolism parameters in lactating dairy cows. Treatments were arranged as a 2 x 2 factorial in a replicated Latin Square design to provide 15 or 17 % CP and DCAD of 25 or 50 meq (Na + K – Cl) / 100 g DM. High DCAD improved dry matter intake, milk yield, and concentrations of milk fat and protein. An interaction of DCAD and CP was observed for uric acid excretion, an indicator of microbial protein yield. Microbial protein yield was higher for high DCAD than low DCAD in low CP diets and was similar for low and high DCAD with high CP. Serum bicarbonate concentration, urinary bicarbonate excretion, blood pH, and serum Na were elevated with high DCAD compared with low DCAD. Fractional excretion of Na, K, Cl, and Ca increased for high DCAD. Blood urea N and urinary urea N were greater for high than low CP diets. No DCAD differences were observed for these parameters. Results of this study suggest that in early lactation cows, blood acid-base chemistry is altered by differences in DCAD that range between the high and low end of the optimal DCAD range. Modifications of acid-base chemistry and the corresponding changes in protein metabolism may allow for more efficient feeding of protein and better nutritional management of the lactating dairy cow.

(**Key words:** dietary cation-anion difference, dietary crude protein, sodium, potassium)

Abbreviation key: **BUN** = blood urea N, **CP15** = low dietary crude protein treatment, **CP17** = high dietary crude protein treatment, **DCAD** = dietary cation-anion difference, **DCAD25** = low

dietary cation-anion difference treatment, **DCAD50** = high dietary cation-anion difference treatment, **MY** = milk yield**.**

INTRODUCTION

The interaction between acid-base chemistry and N metabolism has been established. Van Slyke (1943) first identified the role of glutamine as a precursor for ammoniagenesis. Ammonia serves as a major systemic buffer during metabolic acidosis (Heitmann and Bergman, 1978). Acid load stimulates renal ammoniagenesis (Madias and Zelman, 1986) and inhibits hepatic ureagenesis (Kashiwagura et al., 1984), while alkaline load stimulates urea production (Guder, 1987; Waring et al., 1974). Although Cai and Zimmerman (1995) did not observe any difference in total N excretion when acid-base chemistry was altered, other researchers have reported greater total N excretion (Welbourne et al., 1986) and decreased N retention (May et al., 1987) during acidosis. Under conditions of normal acid-base chemistry and dietary conditions, adequate glutamine is available in the diet or can be generated from other amino acids to meet the need for ammoniagenesis (Patience, 1990). When dietary supply is inadequate or acid-base chemistry is unbalanced, skeletal tissue may be sacrificed to release glutamine (Heitmann and Bergman, 1978). Oxidation of excess sulfur-containing amino acids also contribute to overall acid load (Patience, 1990).

The influence of acid-base chemistry on N metabolism coupled with the effect of dietary cation-anion difference (**DCAD**) on acid-base chemistry suggests that opportunities exist to alter N metabolism by altering DCAD. Increased blood urea N (**BUN**) concentrations were reported for castrated male pigs (Cai and Zimmerman, 1995) fed high DCAD diets. Golz and Crenshaw (1991) also reported greater ammonium excretion in pigs fed low DCAD diets. In lactating dairy cows the effect of DCAD for BUN is not as clear. Wang and Beede (1990) observed greater

urinary ammonium excretion for low DCAD diets. Escobosa et al. (1984), however, reported higher BUN when DCAD was elevated from –19 to 17 meq Na + K – Cl / 100 g DM, but BUN was similar when DCAD was raised to 32 meq / 100 g DM. This suggests that the effect of DCAD on N metabolism in ruminants may differ compared with other species.

Total N retention was similar for lactating (Delaquis and Block, 1995b) or dry (Delaquis and Block, 1995c) dairy cows when DCAD was changed. Sheep fed low CP diets had improved N retention and digestibility when $NaHCO_3$ was included in the diet, increasing the DCAD (Phillip, 1983). Patience et al. (1986) reported no difference in total N digestibility in high DCAD diets fed to swine, whereas, Haydon and West (1990) noted increased N retention when DCAD was elevated.

Dietary K concentration appears to have an effect on the metabolism of lysine and arginine. Free lysine and arginine concentrations increased in K-depleted rat muscle (Arnauld and Lachance, 1980). Robbins et al. (1982) observed higher free muscle lysine, arginine, and glutamine concentrations when dietary K was elevated from 0.12 to 0.18%. However, concentration of these AA decreased when dietary K exceeded 0.18%. Alleviation of the growth-depressing effects of lysine-arginine antagonism was also noted in poultry with the addition of dietary K (O'Dell and Savage, 1966).

While an interaction between acid-base chemistry and N metabolism is evident, the mechanisms are unclear and limited research has been conducted in ruminants, especially the lactating dairy cow. The objective of this study was to determine the effects of low or high concentrations of dietary CP and low or high DCAD on nutrient digestibility, rumen function, and AA metabolism of lactating dairy cows in early lactation.

MATERIALS AND METHODS

Eight primiparous lactating Holstein cows fitted with ruminal cannulae and averaging 47 ± 10 DIM were used in a 4 x 4 replicated Latin Square design. The trial was conducted from March 28 to June 19, 2003 and consisted of four 3 wk treatment periods. The first two weeks of each TP were used for acclimation to treatments with data collection occurring during week 3 of each TP. Two cows were assigned to each treatment combination, with cows being paired for no more than one treatment period.

Dietary treatments were arranged as a 2 x 2 factorial and were formulated to provide a DCAD of 25 (**DCAD25**) or 50 (**DCAD50**) meq Na + K – Cl / 100 g DM and CP concentrations of 15 (**CP15**) or 17% (**CP17)** (DM basis). All diets were formulated to meet or exceed NRC (2001) requirements (Table 5.1). Sodium bicarbonate, K_2CO_3, Prolak®, urea, and soybean meal were used in an experimental premix for adjustment of DCAD and CP.

Prior to the start of the study cows were trained to eat behind electronic gates (American Calan, Inc., Northwood, NH). Cows were fed experimental diets once daily (0800 h) as a TMR, with the amount of feed offered and refused recorded and adjusted daily based on the previous day's consumption to allow for 10% orts. The TMR was pushed up at least four times daily. Feed DM content was determined weekly by drying at 60^{o} C for 72 h in a forced air oven and was used for adjustment of ration components. Cows were housed in a free stall barn with fans activated by thermostat when the ambient temperature exceeded 24^{o}C and high-pressure misters activated by a humidistat when fans were activated and relative humidity was below 85%. Cows were milked twice daily at approximately 0400 and 1500 h.

Sampling

Forage, concentrate, treatment mixes, and TMR were sampled three times weekly, dried at 60^{o} C for 72 h, and composited by week for later analysis. Samples were ground to pass through a 1-mm screen using a Wiley Mill (Arthur B. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltec System (Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as the percentage N x 6.25 (AOAC, 1990). Acid detergent fiber and NDF were determined according to Van Soest et al. (1991) and indigestible acid detergent fiber was determined using the method of Cochran et al. (1986). Apparent DM and nutrient digestibilities were calculated as outlined by Goering and Van Soest (1970). Intake data from wk 3 of each TP was used for calculation of nutrient digestibility. Samples were extracted using a combination of nitric and acetic acids and chloride was determined using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) as defined by Cotlove et al. (1958). Phosphorus concentration was determined colorimetry (Beckman DU Series 500 Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) following wet ashing (AOAC, 1990). Other minerals (Na, K, Cl, Ca, Mg) were measured by atomic absorption spectrophotometry (Perkin-Elmer Model Number 3030, Norwalk, CT) following wet ashing (AOAC, 1990).

Milk yield was measured using the Alpro electronic weight system (DeLaval, Kansas City, MO). Milk samples were collected from consecutive p.m. and a.m. milkings and shipped to Southeast Milk Inc. (Belleview, FL) for analyses of fat and protein using infrared analysis (B2000; Bentley Instruments Inc., Chaska, MN).

Blood was collected by jugular venipuncture into evacuated tubes at 0, 2, 4, 6, and 10h post-feeding. Blood was put on ice, serum separated by centrifugation at 3300 rpm for 20 min, then analyzed for pH, Ca, P, Mg, Na, K, Cl, bicarbonate, BUN, creatinine, glucose, and uric acid

using a Bodhringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN) at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Serum from the 6 h post-feeding collection was analyzed for free AA using a Beckman 6300 Amino Acid Analyzer (Beckman Coulter, Inc., Fullerton, CA) (Slocum and Lee, 1983) at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO. Urine samples were collected via manual stimulation at 1300 h for analysis of pH, Ca, P, Mg, Na, K, Cl, bicarbonate, urea N, creatinine, glucose, and uric acid analysis at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Fractional excretion was calculated as ([Urinary Mineral] / [Serum Mineral])*([Serum Creatinine] / [Urinary Creatinine])*100 (Vander, 1991).

Fecal grab samples were collected on four consecutive days at 12-h intervals with sampling time advancing by 3 h each day. Samples were dried in a forced-air oven at 60°C, ground to pass through a 1-mm screen, and composited by cow within treatment. Fecal samples were analyzed for DM, CP, ADF, NDF, and indigestible ADF, as outlined previously.

Ruminal fluid samples were collected at 0, 2, 4, 6, 8, 10, and 12 h post-feeding. Samples were filtered through two layers of cheesecloth mixed with 25% metaphosphoric acid in a 1:4 acid:rumen fluid ratio prior to freezing for later VFA analysis (Erwin et al., 1961). Volatile fatty acid concentrations were determined using liquid gas chromatography (Hewlett-Packard 5890A Gas Chromatograph; Hewlett-Packard Company, Avondale, PA).

Statistical Analysis

Means for DMI, MY and composition, serum AA concentration, and fractional excretion data were analyzed using the PROC GLM procedure of SAS (1999). Sums of squares were partitioned to DCAD, CP, TP, cow within treatment, and DCAD x CP. Serum mineral and metabolite data and rumen parameters included repeated measures and were analyzed using the

mixed procedure of SAS (1999). Sums of squares in these models were partitioned to DCAD, CP, TP, cow within treatment, time post-feeding, DCAD x CP, DCAD x time, and CP x time. Cow within treatment was the random variable with time post-feeding as the repeated measure. Treatment means were separated by least square means in the presence of a significant F-test ($P < 0.10$) using pdiff.

RESULTS AND DISCUSSION

Chemical Composition of Diets

The DCAD was higher than originally formulated for all diets (Table 5.2) because of higher Na and lower Cl concentrations than expected. All diets met or exceeded NRC (2001) guidelines for Na, K, and Cl (0.19, 1.02, 0.25%, respectively) and the calculated DCAD using these values (27 meq Na + K – Cl / 100 g DM). The DCAD was formulated to represent the high (50 meq Na + K – Cl / 100 g DM) and low (25 meq / 100 g DM) end of the optimal range reported by Sanchez and Beede (1996). The dietary CP concentration was formulated to meet NRC (2001) guidelines (17%) or be lower (15%).

Intake and Nutrient Digestibility

A positive DCAD effect ($P < 0.01$) was observed for DMI, DMI/100kg BW, and CP intake (Table 5.3). This is consistent with previous research by Tucker et al. (1988a) who reported an 11% increase in DMI as DCAD increased from –10 to +20 meq (Na + K – Cl) / 100 g DM. West et al. (1992) reported a linear improvement in DMI as DCAD increased from 12 to 46 meq / 100 g DM. Although DMI was greater for DCAD50, an interaction of DCAD and CP was observed for intake of NDF ($P < 0.05$) and ADF ($P < 0.10$) due to differences in NDF and ADF concentrations between treatments (Table 5.2).

An improvement in ADF digestibility was observed for DCAD50 compared with DCAD25 diets (Table 5.3). Addition of dietary buffers, the equivalent of increasing DCAD, improves fiber digestibility which creates space for more DMI (Erdman et al., 1982). This is consistent with increased DMI observed in the present study. No DCAD effects were noted for apparent digestibility of DM or CP. A DCAD and CP interaction ($P < 0.10$) was observed for NDF digestibility because NDF digestibility was higher for DCAD50 in CP15 diets and lower for DCAD50 in CP17 diets. Apparent digestibility of CP was greater ($P < 0.01$) for CP17 compared with CP15 diets.

Milk Yield and Composition

Yield of milk (**MY**), energy-corrected milk, milk fat, and milk protein, as well as milk fat and protein percentage were higher for DCAD50 versus DCAD25 (Table 5.4). Milk yield increased from 23.9 to 26.2 kg/d for low versus high DCAD. Milk yield has responded positively when DCAD was increased in earlier work (Tucker et al., 1988a; West et al., 1991), especially in early and mid-lactation cows. Although milk fat percentage was low across treatments, it increased from 2.44 to 2.92% with high DCAD. Others researchers have reported improved milk fat percentage for diets high in DCAD (Tucker et al., 1991a; West et al., 1991). Greater MY and milk fat percentage resulted in a 0.15 kg/d improvement in fat yield. An interaction of DCAD and CP ($P < 0.01$) was observed for milk protein percentage (Table 5.4). Higher milk protein percentage was observed for DCAD50 in CP15 diets, consistent with earlier work in which West et al. (1991) reported that DCAD elicited a positive linear response for milk protein percentage. Milk protein percentage was similar between DCAD treatments for CP17 treatments. This suggests that higher DCAD in diets with limited CP increases the availability of CP for protein synthesis. In diets where CP is not limiting, the effect on protein synthesis may

not be reflected in milk protein. Delaquis and Block (1995b) reported similar results for cows fed diets with CP concentrations similar to the high CP diets fed in the current study. Greater milk protein yield was noted for high DCAD diets. Improved milk protein may result from a protein-sparing effect caused by increased DCAD. Additional buffering provided by DCAD may reduce the role of protein in blood buffering, resulting in greater proportions of protein available for protein synthesis.

Blood and Urinary Mineral and Metabolites

Serum Na concentration (Table 5.5) and fractional excretion of Na (Table 5.6) was elevated by high DCAD. Higher dietary Na concentrations in high DCAD treatments contributed to greater serum concentration and fractional excretion of Na. Greene et al. (1983) reported increased absorption and excretion of Na when dietary K concentration was elevated, as K was for high DCAD treatments in the current study. A decrease in both serum Mg concentration and Mg fractional excretion was observed with high DCAD. Jittakhot et al. (2004) reported depressed Mg absorption when dietary K was elevated. Decreased absorption would reduce serum Mg concentration with less Mg available for excretion. Blood bicarbonate concentration and excretion were both elevated by high DCAD. West et al. (1991) and Tucker et al. (1988a) reported similar relationships between DCAD and blood bicarbonate concentration and urinary excretion. Serum DCAD and pH were also greater for high DCAD diets (Table 5.5). Higher serum pH is related to greater serum DCAD and bicarbonate concentration (Tucker et al., 1988a), and is indicative of improved blood buffering. Because of improved blood buffering, the need for dietary CP in blood buffering is reduced. Therefore, the possibility exists for improved utilization of CP for protein synthesis. Additional blood buffering was also associated with improved DMI (Delaquis and Block, 1995b), especially in early and mid-lactation cows.

High DCAD resulted in greater fractional excretion of Cl and K, and reduced urinary Ca excretion (Table 5.6). Greater fractional excretion of Ca for high DCAD diets is inconsistent with earlier work, where Ca excretion was greatest when low DCAD diets were fed (Wang and Beede, 1992a). Urinary excretion of Cl is related to serum bicarbonate concentration and excretion. In an effort to maintain electroneutrality, excretion of urinary Cl increases due to elevated levels of serum bicarbonate.

High DCAD, which included higher dietary K, increased urinary K excretion. West et al. (1992) reported higher urinary K excretion as DCAD increased, especially in treatments in which DCAD was raised using dietary K. There was no effect of DCAD on BUN or urinary urea N excretion. Increased BUN in lactating cows (Escobosa et al., 1984) was reported when DCAD was elevated from –19.9 to 16.8 meq Na + K – Cl / 100 g DM. No difference was observed, however, when DCAD was further elevated to 32 meq / 100 g DM. Differences in BUN observed by these authors may have been the result of increased DMI when DCAD was raised. Acid-base effects on form of N excretion may be minimal in ruminants.

A DCAD and CP interaction occurred for urinary uric acid:urinary creatinine ratio. A much larger effect for DCAD was observed in low CP diets compared with high CP diets. Uric acid excretion is an indicator of microbial protein flow to the duodenum (Johnson et al., 1998). Therefore, DCAD appears to have a greater effect on ruminal protein metabolism in low CP diets. Changes in ruminal pH may allow greater availability of rumen ammonia for uptake by the microbial population.. In high CP diets, ruminal ammonia is more plentiful; therefore greater availability from high DCAD would not necessarily result in improved microbial protein yield. Greater microbial protein yield may also contribute to the improvement in milk protein yield observed in low CP diets when high DCAD is fed (Table 5.4).

Blood Amino Acids

With the exception of isoleucine, there were no DCAD effects for serum concentration of any individual essential AA, total essential AA, or total AA (Table 5.7). A DCAD and CP interaction was noted for isoleucine ($P < 0.05$) and leucine ($P < 0.10$). For CP15 treatments, DCAD50 resulted in decreased leucine concentration. Leucine concentrations increased for DCAD50 in CP17 diets. Crude protein treatment effects were observed for lysine ($P < 0.10$), valine ($P < 0.05$), and tryptophan ($P < 0.10$), as well as for total essential amino acids ($P < 0.05$). For each of these parameters, serum concentrations were higher for CP15 compared with CP17. These differences could have been caused by the relative availability of each AA in post-ruminal CP. Microbial crude protein may have contained higher concentrations of Lys, Val,Trp, and total essential AA than dietary CP.

Rumen Volatile Fatty Acids

No DCAD or CP effects were observed for ruminal acetate or propionate molar proportion. A numeric trend for greater acetate:propionate ratio for DCAD was observed. No interactions with main effects or time post-feeding were observed. Molar proportion of butyrate was noted for DCAD50. In earlier work, Tucker et al. (1988a) reported no difference in acetate, propionate, or butyrate concentration, or for acetate:propionate ratio for DCAD. Both the low and high DCAD in the current study were greater than those used by Tucker et al. (1988a). The presence of a trend towards a DCAD effect for acetate:propionate ratio in the current study is consistent with greater milk fat percentage for high DCAD diets (Table 5.4).

CONCLUSIONS

Greater DMI and MY, as well as blood bicarbonate and pH further for high DCAD solidifies the role of DCAD in altering acid-base chemistry and production in early-lactation

dairy cows. Increased milk protein percentage suggests that increasing DCAD improves protein utilization. Improved microbial crude protein synthesis for low dietary CP concentrations may offer the opportunity for more efficient N feeding. If efficiency of N utilization is improved using DCAD, there is an opportunity to reduce N waste by feeding less CP. While a relationship between DCAD and N metabolism may exist, a greater understanding of the mechanisms involved is needed to better manage the DCAD and protein nutrition of the lactating dairy cow.

Table 5.1. Ingredient composition of diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17	
DCAD	25	50	25	50
	---------- % of DM ----------			
Corn silage	40.98	37.29	38.79	36.09
Alfalfa hay	10.47	9.81	10.82	9.79
Whole cottonseed	9.30	9.56	8.84	9.28
Steam-flaked corn	30.93	32.60	29.22	29.75
Trace mineral-vitamin premix	2.98	2.98	2.98	2.98
Soybean meal[1]	3.53	3.82	6.96	7.62
Urea[1]	0.22	0.22	0.40	0.40
Prolak®[1,2]	1.59	1.77	1.99	2.14
K_2CO_3[1]	0.00	0.97	0.00	0.97
$NaHCO_3$[1]	0.00	0.97	0.00	0.97

[1]These ingredients were mixed in a treatment premix for inclusion in each respective diet.

[2] Protein supplement manufactured by H. J. Baker & Bro., Inc., Westport, CT

Table 5.2. Chemical composition[1] of diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17	
DCAD	25	50	25	50
	%			
DM, %	60.1 ± 2.7	61.8 ± 2.6	61.7 ± 2.6	62.5 ± 2.5
	% of DM			
CP, % of DM	14.43 ± 0.56	14.93 ± 0.41	17.07 ± 0.70	17.53 ± 0.37
NDF, % of DM	29.65 ± 2.04	30.39 ± 1.82	29.77 ± 1.36	28.29 ± 1.83
ADF, % of DM	16.62 ± 1.84	16.52 ± 1.37	16.44 ± 0.61	16.40 ± 1.80
Ca	0.99 ± 0.12	0.99 ± 0.09	1.01 ± 0.03	0.99 ± 0.07
P	0.43 ± 0.01	0.45 ± 0.02	0.44 ± 0.03	0.45 ± 0.01
Mg	0.22 ± 0.00	0.23 ± 0.02	0.23 ± 0.01	0.24 ± 0.01
K	1.04 ± 0.11	1.54 ± 0.09	1.06 ± 0.08	1.58 ± 0.13
Na	0.24 ± 0.03	0.51 ± 0.03	0.27 ± 0.02	0.51 ± 0.02
Cl	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.01	0.27 ± 0.01
DCAD[2]	29.1	53.7	31.0	55.0

[1]Mean value ± standard deviation

[2]meq Na + K – Cl / 100 g DM

Table 5.3. Nutrient intake and digestibility in cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Intake								
DM, kg/d	17.8	19.2	17.7	19.0	0.35	**	NS[1]	NS
DM, kg/100 kg BW	3.8	4.1	3.8	4.1	0.08	**	NS	NS
CP, kg/d	2.6	2.9	3.0	3.3	0.05	**	**	NS
NDF, kg/d	5.2	5.8	5.3	5.4	0.10	**	†	*
ADF, kg/d	2.9	3.1	2.9	2.9	0.06	*	†	†
Digestibility, %								
DM	60.6	62.2	62.6	61.7	1.7	NS	NS	NS
CP	57.2	58.5	65.3	64.5	2.0	NS	**	NS
NDF	28.7	32.7	29.8	24.8	2.1	NS	NS	†
ADF	23.5	27.0	19.5	27.9	2.9	†	NS	NS

[1] NS = Not significant ($P > 0.10$)

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 5.4. Milk yield, composition, and component yield for cows fed diets for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Yield, kg/d								
Milk	24.0	25.7	23.8	26.6	0.4	**	NS [1]	NS
ECM[2]	20.5	23.4	20.6	24.1	0.4	**	NS	NS
Fat	0.60	0.76	0.61	0.77	0.01	**	NS	NS
Protein	0.70	0.76	0.71	0.79	0.01	**	NS	NS
Composition, %								
Fat	2.44	2.95	2.44	2.89	0.03	**	NS	NS
Protein	2.89	2.94	2.99	2.98	0.01	*	**	**

[1] NS = Not significant ($P > 0.10$)

[2] ECM = 0.3246 * (kg milk) + 12.86 * (kg fat) + 7.04 * (kg protein). (Tyrrell and Reid, 1965)

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 5.5. Blood serum mineral and metabolite concentrations at 0, 2, 4, 6, and 10 h post-feeding for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Na, meq/L	142.92	143.53	142.08	144.50	0.58	*	NS [1]	NS
K, meq/L	4.08	4.22	4.14	4.24	0.09	NS	NS	NS
Cl, meq/L	101.10	99.53	101.05	100.18	0.94	NS	NS	NS
DCAD, Na + K – Cl, meq/L	45.90	48.22	45.16	48.57	0.93	**	NS	NS
Ca, mg/dl	10.06	9.93	10.28	9.99	0.17	NS	NS	NS
Mg, mg/dl	2.53	2.27	2.55	2.16	0.08	**	NS	NS
Glucose, mg/dl	68.43	67.80	68.10	66.45	2.03	NS	NS	NS
Bicarbonate, mmol/L	22.54	24.46	22.06	24.57	0.73	**	NS	NS
BUN, mg/dl	8.68	8.35	12.48	12.73	0.73	NS	**	NS
pH	7.468	7.497	7.456	7.495	0.009	**	NS	NS

[1] NS = Not significant ($P > 0.10$)

* $P < 0.05$

** $P < 0.01$

Table 5.6. Urinary excretion of mineral and metabolites for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Fractional excretion[1], %								
Ca	1.08	0.22	0.71	0.50	0.21	*	NS[2]	NS
Mg	13.44	9.93	12.33	9.54	1.62	†	NS	NS
Na	0.41	2.04	0.53	1.48	0.20	**	NS	NS
K	37.17	78.00	39.63	77.84	4.74	**	NS	NS
Cl	0.28	1.08	0.33	0.82	0.10	**	NS	NS
Urine metabolite / urine creatinine[2]								
Urea N	4.13	4.56	7.87	8.09	0.47	NS	**	NS
Bicarbonate	0.38	2.67	0.73	2.44	0.26	**	NS	NS
Uric Acid	0.68	0.84	0.73	0.74	0.04	*	NS	*

[1] Fractional Excretion = ([urinary mineral] / [plasma mineral]) * ([plasma creatinine] / [urinary creatinine]) * 100 (Vander, 1991).

[2] NS = Not significant ($P > 0.10$)

[3] Expressed as (milligrams of urine metabolite / 100 ml urine) / (milligrams of urine creatinine / 100 ml of urine).

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 5.7. Serum AA concentration for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
Essential AA, µg/ml								
Lys	25.84	27.52	23.57	24.13	1.47	NS[1]	†	NS
Arg	29.17	24.92	27.12	22.80	2.52	NS	NS	NS
Met	33.72	35.19	32.65	33.94	1.83	NS	NS	NS
Phe	0.45	0.18	0.46	0.21	0.17	NS	NS	NS
Val	1.12	1.05	0.59	0.87	0.15	NS	*	NS
Trp	7.08	7.38	6.18	6.51	0.41	NS	†	NS
Thr	22.80	23.72	21.88	21.00	1.03	NS	NS	NS
Ile	0.01	0.01	0.00	0.03	0.00	†	NS	*
His	13.94	13.56	13.02	12.95	0.56	NS	NS	NS
Leu	13.63	12.05	11.63	12.81	0.74	NS	NS	†
Total Essential AA, µg/ml	147.76	145.59	137.10	135.24	4.16	NS	*	NS
Total Amino Acids, µg/ml	403.20	412.61	392.28	391.71	15.24	NS	NS	NS
Essential AA:Total AA	0.37	0.35	0.35	0.35	0.01	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

† $P < 0.10$

* $P < 0.05$

Table 5.8. Acetate, propionate, and butyrate concentration and acetate:propionate ratio over 12 h post-feeding for cows fed diets formulated to contain a DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and 15 or 17% CP.

Dietary CP %	15		17			*P*		
DCAD	25	50	25	50	SEM	DCAD	CP	DCAD x CP
VFA, mole/100 mole								
Acetate	55.24	56.24	54.00	54.89	1.34	NS[1]	NS	NS
Propionate	30.98	29.16	31.07	29.95	1.42	NS	NS	NS
Butyrate	9.56	10.79	10.06	10.77	0.38	*	NS	NS
Acetate:Propionate Ratio	1.82	2.03	1.81	1.87	0.14	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

* $P < 0.05$

CHAPTER 6

DIETARY CATION-ANION DIFFERENCE AND RUMINAL DEGRADABLE PROTEIN EFFECTS ON COW PERFORMANCE, ACID-BASE CHEMISTRY, AND RUMINAL FERMENTATION[1]

[1] Wildman, C. D., J. W. West, and J. K. Bernard. 2005. To be submitted to Journal of Dairy Science.

ABSTRACT

Eight primiparous lactating Holstein cows fitted with ruminal cannulae were used to determine the effect of dietary cation-anion difference (**DCAD**) and ruminal degradable protein (**RDP**) concentration on milk yield, blood acid-base chemistry, amino acid metabolism, and rumen fermentation. Treatments were arranged as a 2 x 2 factorial in a replicated Latin Square design to provide DCAD of 25 or 50 meq (Na + K – Cl) / 100 g of dry matter and RDP of 59 or 67% of dietary crude protein. An interaction of DCAD and RDP occurred for DMI, yield of milk, fat, and protein. With high DCAD, the magnitude of improvement in DMI was greater for low RDP diets compared to that observed for high RDP diets. Yield of milk, fat, and protein were greater with high DCAD for low RDP diets. Serum K and bicarbonate concentrations and pH were higher and serum Cl and Mg were lower for high DCAD. Fractional excretion of Ca and Mg decreased for high DCAD while fractional excretion of Na, K, and Cl increased. In high RDP diets, urinary uric acid:creatinine ratio decreased when DCAD was increased. No DCAD differences were noted in low RDP diets. These results suggest that in mid-lactation cows, greater improvements in N metabolism are expected when low RDP diets are fed. These improvements could improve the efficiency with which we feed protein to the lactating dairy cow.

(**Key words:** dietary cation-anion difference, rumen degradable protein, sodium, potassium)

Abbreviation key: **DCAD** = dietary cation-anion difference, **DCAD25** = low dietary cation-anion treatment, **DCAD50** = high dietary cation-anion treatment, **ECM** = energy-corrected milk yield, **HRDP** = diet high in rumen degradable protein, **LRDP** = low rumen degradable protein treatment, **MCP** = microbial crude protein yield, **MY** = milk yield**.**

INTRODUCTION

Acid-base chemistry of livestock influences N utilization (Haydon and West, 1990; Patience, 1988; Patience, 1990; Patience et al., 1986), and can alter the method by which N is excreted. Acid load stimulates renal ammoniagenesis (Madias and Zelman, 1986) and inhibits hepatic ureagenesis (Kashiwagura et al., 1984). Alkaline load stimulates ureagenesis (Guder, 1987; Waring et al., 1974) while decreasing ammoniagenesis (Cai and Zimmerman, 1995). Metabolism of glutamine, the primary AA utilized for ammoniagenesis, is increased during acidosis (Van Slyke et al., 1943). Greater N excretion (Welbourne et al., 1986) and increased protein degradation (May et al., 1986) were observed during metabolic acidosis, which may be due to an inadequate supply of glutamine for ammoniagenesis. Ammonia serves as a major systemic buffer during metabolic acidosis (Heitmann and Bergman, 1978). Under conditions of normal acid-base chemistry and dietary conditions, adequate glutamine or glutamine precursors are available for ammoniagenesis (Patience, 1990). However, during extreme acidosis or when dietary supply is inadequate, body mass is mobilized for release of glutamine (Heitmann and Bergman, 1978). Amino acids fed in excess of dietary requirements may contribute to the animal's acid load. Patience (1990) reported that the oxidation of sulfur-containing AA contributes 13 to 26% of the total acid production in swine.

The influence of dietary cation-anion difference (**DCAD**) on AA metabolism has been reported for several species including chicks (Austic and Calvert, 1981; O'Dell and Savage, 1966) and swine (Patience, 1990). Much of the research with ruminants and the interaction of DCAD with N metabolism has centered on the effect of DCAD on N balance. In lambs, $NaHCO_3$ improved N retention was greater when dietary CP concentrations were low (Phillip, 1983; Rogers and Phillip, 1986). The effect of $NaHCO_3$ on N balance was greater when CP was

less degradable in the rumen (Phillip, 1983). High CP diets, especially those high in RDP, may generate excess ammonia in the rumen to support systemic buffering. No change in N retention was reported for either dry (Delaquis and Block, 1995a) or lactating (Delaquis and Block, 1995b) dairy cows. Limited data are available regarding the effect of DCAD on N metabolism in dairy cows fed low CP diets or diets varying in RDP. The objective of this research was to determine the effects of diets low in CP with high or low DCAD and high or low RDP on milk yield and composition, nutrient digestibility, serum AA concentration, and ruminal fermentation of lactating dairy cows.

MATERIALS AND METHODS

Eight primiparous ruminally-cannulated lactating Holstein cows averaging 180 $\pm$ 10 DIM were used in a 4 x 4 replicated Latin Square design with a 2 x 2 factorial treatment arrangement. The trial was conducted from August 15 to November 6, 2003 and consisted of four 3 wk periods. The first two weeks of each period were used for acclimation to treatment diets with data collection occurring during wk 3. Two cows were assigned to each treatment combination, with cows being paired for no more than one treatment period.

Dietary treatments were formulated to contain DCAD of 25 (**DCAD25**) or 50 (**DCAD50**) meq Na + K – Cl / 100 g DM and 59 (**LRDP**) or 67 (**HRDP**) % RDP (% of CP). All diets were formulated to meet or exceed NRC (2001) requirements (Table 6.1).

Prior to the start of the study cows were trained to eat behind electronic gates (American Calan, Inc., Northwood, NH). Cows were fed experimental diets once daily (0800 h) as a TMR, with the amount of feed offered recorded and adjusted daily based on the previous day's consumption to allow for 10% orts. The TMR was pushed up at least four times daily. Feed DM content for adjustment of ration components was determined weekly by drying at 60^{o} C for 72 h

in a forced air oven. Cows were housed in a free stall barn with fans activated by thermostat when the ambient temperature exceeded 24°C and high-pressure misters activated by a humidistat when fans were activated and relative humidity was below 90%. Cows were milked twice daily at approximately 0400 and 1500 h.

Sampling

Ingredients and TMR were sampled three times weekly, dried at 60° C for 72 h, and composited by week for later analysis. Feed samples were ground to pass through a 1-mm screen using a Wiley Mill (Arthur B. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltec System (Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as the percentage N x 6.25 (AOAC, 1990). Rumen degradability of CP was determined using 24 h degradation by *Streptomyces griseus* as described by Krishnamoorthy et al. (1983). Acid detergent fiber and NDF were determined according to Van Soest et al. (1991) and indigestible acid detergent fiber (**IADF**) was determined using the method of Cochran et al. (1986). Apparent nutrient digestibilities were calculated as outlined by Goering and Van Soest (1970). Feed samples were extracted using a combination of nitric and acetic acids and chloride was determined using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) as defined by Cotlove et al. (1958). Following wet ashing (AOAC, 1990), P concentration was determined by colorimetry (Beckman DU Series 500 Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA) and Na, K, Ca, and Mg were measured by atomic absorption spectrophotometry (Perkin-Elmer Model Number 3030, Norwalk, CT).

Milk yield was measured using electronic meters (DeLaval, Kansas City, MO). Milk samples were collected from four consecutive p.m. and a.m. milkings. Samples were shipped to

Southeast Milk Inc. (Belleview, FL) for analyses of fat and protein using infrared analysis (B2000; Bentley Instruments Inc., Chaska, MN).

Blood was collected by jugular venipuncture into evacuated tubes during wk 3 of each TP at 0, 2, 4, 6, and 10h post-feeding. Blood was placed on ice, centrifuged at 3300 rpm for 20 min for serum separation, serum harvested, and analyzed immediately for pH, Ca, P, Mg, Na, K, Cl, bicarbonate, blood urea N (**BUN**), creatinine, glucose, and uric acid using a Bodhringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN) at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Serum from the collection at 6 h post-feeding was analyzed for AA concentration using a Beckman 6300 Amino Acid Analyzer (Beckman Coulter, Inc., Fullerton, CA) at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO. Urine samples were collected via manual stimulation approximately 6 h post-feeding and analyzed for pH, Ca, P, Mg, Na, K, Cl, bicarbonate, urea N, creatinine, glucose, and uric acid similarly to blood serum samples. These values were used for calculation of fractional excretion, calculated as ([urinary mineral] / [plasma mineral]) * ([plasma creatinine] / [urinary creatinine]) * 100, as described by Vander (1991).

Fecal grab samples were collected for 4 consecutive days at 12-h intervals, with sampling time advanced by 3 h each day. Samples were dried in a forced-air oven at 60°C, ground to pass through a 1-mm screen, and composited by cow within treatment. Fecal samples were analyzed as described previously.

Rumen fluid samples were collected at 0, 2, 4, 6, 8, 10, and 12 h post-feeding. Samples were filtered through 2 layers of cheesecloth and further microbial action was stopped with the addition of 25% metaphosphoric acid in a 1:4 acid:rumen fluid ratio before freezing for later

VFA analysis (Erwin et al., 1961). Volatile fatty acid concentrations were determined using liquid gas chromatography (Hewlett-Packard 5890A Gas Chromatograph; Hewlett-Packard Company, Avondale, PA).

Statistical Analysis

Nutrient intake, milk yield and composition, serum AA concentration, and fractional excretion data were analyzed using PROC GLM procedure of SAS (1999). Sums of squares were partitioned to DCAD, RDP, TP, cow within treatment, and DCAD x RDP. Serum mineral and metabolite data and rumen parameters included repeated measures and were analyzed using the mixed procedure of SAS (1999). Sums of squares in these models were partitioned to DCAD, RDP, TP, cow within treatment, time post-feeding, DCAD x RDP, DCAD x time, and RDP x time. Cow within treatment was the random variable with time post-feeding as the repeated measure. Differences among treatment means were determined using least squares means, with significance determined at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Diets

The nutrient concentrations of experimental diets are presented in Table 6.2. Dietary CP concentrations averaged 15.29% across treatment combinations and were formulated to be slightly lower than NRC (2001) recommendations. Mean values for DCAD25 (23 meq / 100 g DM) and DCAD50 (44.3 meq / 100 g DM) DCAD were close to formulated values and a large enough difference was maintained to show any treatment differences that might exist. Ruminal degradability of dietary CP was formulated to represent differences in RDP that might be observed in typical dairy production rations. All other nutrients met or exceeded NRC recommendations (2001).

Nutrient Intake and Digestibility

Significant DCAD and RDP interactions were observed for DMI ($P < 0.01$) and intake of CP ($P < 0.01$), NDF ($P < 0.01$), and ADF ($P < 0.01$) (Table 6.3). Intake of each of these components was higher for DCAD50 compared with DCAD25 within LRDP treatments. No DCAD response was observed for HRDP treatments. Similar DCAD responses to those observed within the LRDP treatments for greater DMI have been reported (Tucker et al., 1988a; West et al., 1992; West et al., 1991) due to enhanced blood buffering from feeding diets higher in DCAD. The absence of a DCAD effect for HRDP diets may be a result of excess ammonia available for systemic buffering compared to what might be available in LRDP diets. Differences in CP intake were similar to those for DMI. The DCAD and RDP interaction for NDF and ADF intake were affected by DMI. For LRDP diets, the larger increase in DMI caused an increase in NDF and ADF intake. For HRDP diets, the increase in intake was not as significant and dietary concentrations of NDF and ADF were slightly lower for DCAD50 versus DCAD25 diets. Consequently, the combination of these factors resulted in reduced NDF and ADF intake.

A DCAD and RDP interaction was observed for DM digestibility ($P < 0.01$) (Table 6.3). No difference in DM digestibility was observed for DCAD50 versus DCAD25 diets within the HRDP treatment; however, apparent digestibility of DM for LRDP was greatly enhanced by DCAD50 compared with DCAD25. Digestibility of NDF was greater ($P < 0.05$) for DCAD50 across RDP treatments. Improved fiber digestibility has been reported with the addition of buffers to lactating cow diets (Erdman et al., 1982). Greater NDF ($P < 0.01$) and ADF ($P < 0.01$) digestibility was observed for LRDP compared with HRDP diets. These results are inconsistent with earlier work (Christensen et al., 1993) in which no difference in NDF or ADF

digestibility was reported for diets differing in RDP. The authors did report greater total tract N digestibility with higher RDP concentration, consistent with the results of the present study.

Milk Yield and Composition

Greater yield of milk (**MY**) and energy-corrected milk (**ECM**) was observed for DCAD50 within LRDP diets while a decrease was noted for DCAD50 within HRDP diets resulting in a DCAD and RDP interaction ($P < 0.01$) for both MY and ECM (Table 6.4). This suggests that in low CP diets, the role of DCAD may be greater in diets with lower RDP because of the potential reduction in the amount of ruminal ammonia available for absorption and ultimately systemic buffering.

Within LRDP, greater milk fat and protein percentage were observed for DCAD50 causing a DCAD and RDP interaction ($P < 0.05$ and $P < 0.01$, respectively). Improved milk fat and protein percentage within LRDP, coupled with greater MY, resulted in a DCAD and RDP interaction for fat ($P < 0.01$) and protein ($P < 0.01$) yield. Greater milk fat and protein percentage was reported by West et al. (1991) for high DCAD. However, Tucker et al. (1988a) reported no effect of DCAD on milk fat percentage and reduced milk protein percentage when DCAD was raised from 0 to 20 meq Na + K – Cl / 100 g DM. The DCAD values were lower than those in the present study, suggesting that DCAD effects on protein metabolism are not reflected in milk protein production at low DCAD.

Milk fat percentage increased for DCAD50 within HRDP treatments. The magnitude of the increase in milk fat percentage, however, was similar to the magnitude of the decrease in MY. Therefore, improved milk fat percentage for DCAD50 within HRDP diets may be a dilution effect and not an effect of DCAD and there was no difference in fat yield between DCAD50 and DCAD25 within the HRDP diets. No change in milk protein percentage was

observed for DCAD50 compared with DCAD25 within HRDP treatments. Even though no effect of DCAD was observed for milk protein percentage within HRDP diets, the slight decrease in MY resulted in a depression in protein yield for DCAD50 compared with DCAD25 within HRDP diets.

Serum, Urinary, and Rumen Parameters

Serum K increased ($P < 0.10$) for DCAD50 whereas serum Cl ($P < 0.10$) and Mg ($P < 0.10$) concentration declined (Table 6.5). Changes in K and Cl resulted in higher serum DCAD ($P < 0.01$) for the DCAD50 treatments. Lower serum Mg concentration may be due to reduced Mg absorption because of high dietary K concentrations in DCAD50 diets (Jittakhot et al., 2004). Serum Cl is the electrolyte most affected by changes in DCAD (Tucker et al., 1988a). Renal excretion of Na and K must be accompanied by a concomitant cation such as Cl to maintain electroneutrality. Greater urinary excretion of both Na and K for DCAD50 caused greater fractional excretion of Cl (Table 6.6), resulting in lower serum Cl concentration. Blood pH increased for DCAD50. Higher blood pH and serum bicarbonate is indicative of improved blood buffering, are correlated with serum DCAD (Tucker et al., 1988a). In the current study, correlation coefficients were 0.78 and 0.60 between serum DCAD and serum bicarbonate and pH, respectively. Improved blood buffering results in enhanced animal performance through increased DMI (Tucker et al., 1988a; West et al., 1992; West et al., 1991).

No difference in BUN was noted among treatments, although a numerical trend for decreased BUN was noted for DCAD50. Greater BUN concentrations have been reported in lactating dairy cows (Escobosa et al., 1984) when DCAD was increased from –19.9 to 16.8 meq Na + K – Cl / 100 g DM. No additional increase in BUN was observed when DCAD was raised to 32 meq / 100 g DM (Escobosa et al., 1984). This suggests that the BUN differences may have

been caused by a depression in DMI associated with the negative DCAD. The DCAD treatments in the current study were all above the DCAD at which Escobosa et al. (1984) no longer observed a response.

Fractional excretion of Ca and Mg decreased ($P < 0.01$) for DCAD50. Reduced excretion of Ca with DCAD50 is consistent with earlier work in which urinary Ca concentration decreased when higher DCAD diets were fed (Fredeen et al., 1988a). Dietary absorption of Ca is reduced in livestock experiencing metabolic or respiratory alkalosis (Fredeen et al., 1988a). The Ca requirement is thus more dependent on bone resorption. Because cows receiving the DCAD50 present study were alkalotic as evidenced by higher serum bicarbonate concentration and pH, less dietary Ca may have been absorbed. Reduced fractional excretion of Ca may also be caused by a reduction in the concentration of ionized Ca. The exact mechanism is unclear. The reduction in fractional excretion of Mg is probably associated with impaired Mg absorption in diets with high dietary K content, as were the DCAD50 diets in the current study. Impaired Mg absorption leads to lower serum Mg concentration (Table 6.4), and reduced urinary excretion. Elevated dietary K and Na concentration increased urinary K and Na excretion. West et al. (1992) reported greater urinary K excretion as DCAD increased, especially when dietary K was used to elevate DCAD. As expected, urinary bicarbonate excretion increased ($P < 0.01$) as DCAD was elevated. Greater urinary excretion of bicarbonate is reflective of greater serum concentrations and enhanced blood buffering. Tucker et al. (1988a) and West et al. (1991) reported a similar relationship between DCAD and blood bicarbonate concentration and urinary excretion.

Urinary acid excretion is an indicator of microbial protein yield (**MP**) (Johnson et al., 1998). A DCAD and RDP interaction ($P < 0.05$) was observed for urinary uric acid excretion

(Table 6.6). In HRDP diets, urinary uric acid:creatinine ratio decreased for DCAD50, reflecting a possible decrease in MCP. Reduced MCP may have contributed to the lower milk and protein yield reported for the DCAD50:HRDP treatment combination.

Greater serum total essential AA concentration was observed for DCAD50 compared with DCAD25 in LRDP diets, resulting in a DCAD and RDP interaction ($P < 0.05$) (Table 6.7). A DCAD and RDP interaction was also observed for serum Lys, Phe, Val, Thr, Ile, and Leu, total AA and total essential AA. In each of these interactions, there was increased AA concentration for DCAD50 compared with DCAD 25 in LRDP diets. Greater serum AA concentration for DCAD50 in LRDP diets may be related to a systemic buffering effect. Austic and Calvert (1981) reported increased muscle concentrations of free AA in chicks fed low DCAD diets. This was due to the increased intracellular buffering role of AA in low DCAD diets. Therefore, when DCAD is increased, the buffering role of AA is decreased, and greater quantities of AA are available for protein synthesis.

Ruminal fluid VFA profile was unaffected by DCAD or RDP (Table 6.8). These results are consistent with earlier work (Tucker et al., 1988a) where no differences in ruminal fermentation were observed when DCAD was elevated from 0 to 20 meq Na + K – Cl / 100 g DM.

CONCLUSIONS

Increased DMI and changes in acid-base parameters such as blood bicarbonate and pH were consistent with earlier studies in which the relationship between DCAD and acid-base chemistry was established, and their effect on DMI. Differences in the response to DCAD for MY as well as milk fat and protein percentage suggests that DCAD effects are not consistent across feeding regimens, and are dependent on other factors such as RDP. In diets with low

RDP, higher DCAD improved production parameters such as MY, milk fat, and milk protein percentage. Increasing the DCAD in diets with CP below NRC (2001) guidelines that are low in RDP could increase the availability of essential AA for protein synthesis, as the demand for use in systemic buffering is reduced. Further understanding of the role of AA, especially essential AA, in systemic buffering for the lactating dairy cow should improve the understanding of interactions between DCAD and N metabolism and will enhance the ability to feed N more efficiently, improving the nutritional management of the lactating dairy cow.

Table 6.1. Ingredient composition of diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP	
DCAD	DCAD25	DCAD50	DCAD25	DCAD50
	---------- % of DM ----------			
Corn silage	43.16	42.41	43.53	42.41
Alfalfa hay	10.28	10.09	10.37	10.09
Whole cottonseed	9.61	9.43	9.71	9.43
Steam-flaked corn	25.58	24.05	27.52	27.04
Wet brewers grain	6.46	7.42	4.66	4.36
Trace mineral-vitamin premix	2.28	2.26	2.28	2.26
Soybean meal[1]	0.00	0.00	1.14	1.90
Urea[1]	0.34	0.34	0.80	0.80
Prolak[1,2]	2.28	2.28	0.00	0.00
K_2CO_3[1]	0.00	1.14	0.00	1.14
$NaHCO_3$[1]	0.00	0.57	0.00	0.57

[1]These ingredients were mixed in a treatment premix for inclusion in each respective diet.

[2] Protein supplement manufactured by H. J. Baker & Bros., Inc., Westport, CT

Table 6.2. Chemical composition[1] of diets formulated formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP	
DCAD	DCAD25	DCAD50	DCAD25	DCAD50
	---------- % ----------			
DM, %	46.0 ± 2.4	46.7 ± 2.8	46.4 ± 2.1	47.5 ± 2.7
	---------- % of DM ----------			
CP	15.25 ± 0.68	15.32 ± 0.21	15.30 ± 0.65	15.29 ± 0.56
RDP, % of CP[2]	61.55 ± 1.01	60.69 ± 1.20	69.80 ± 1.69	68.74 ± 1.40
NDF	34.29 ± 1.36	34.35 ± 1.09	35.30 ± 0.59	33.52 ± 1.24
ADF	21.34 ± 0.91	19.27 ± 0.90	21.15 ± 0.47	19.17 ± 1.23
Ca	0.84 ± 0.08	0.84 ± 0.03	0.80 ± 0.10	0.81 ± 0.10
P	0.39 ± 0.01	0.41 ± 0.00	0.39 ± 0.01	0.40 ± 0.01
Mg	0.21 ± 0.02	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.03
K	0.89 ± 0.08	1.51 ± 0.07	0.93 ± 0.11	1.50 ± 0.11
Na	0.18 ± 0.03	0.32 ± 0.02	0.17 ± 0.03	0.31 ± 0.05
Cl	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.28 ± 0.01
DCAD[3]	22.70	44.64	23.28	43.95

[1]Mean value ± SD

[2]24 h degradation by *Streptomyces griseus* (Krishnamoorthy et al., 1983)

[3]calculated as meq Na + K – Cl / 100 g DM

Table 6.3. Dry matter, CP, NDF, and ADF intake and digestibility in cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
Intake								
DM, kg/d	16.8	18.4	17.2	17.6	0.20	**	NS[1]	**
DM, kg/100 kg BW	3.6	3.9	3.7	3.8	0.04	**	NS	**
CP, kg/d	2.5	2.8	2.7	2.7	0.03	**	NS	**
NDF, kg/d	5.7	6.3	6.1	5.7	0.07	*	NS	**
ADF, kg/d	3.3	3.6	3.6	3.3	0.04	NS	†	**
Digestibility, %								
DM	59.9	64.3	59.1	58.3	0.83	*	**	**
CP	56.2	56.7	63.0	64.7	1.34	NS	**	NS
NDF	34.7	38.3	31.3	32.6	1.06	*	**	NS
ADF	33.5	35.2	26.4	27.8	1.09	NS	**	NS

[1] NS = Not significant ($P > 0.10$)

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 6.4. Milk yield, composition, and component yield for cows fed diets for cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
Yield, kg/d								
Milk	19.7	21.2	20.8	20.0	0.32	NS[1]	NS	**
ECM[2]	18.8	21.0	20.1	19.7	0.32	**	NS	**
Fat	0.61	0.70	0.67	0.67	0.01	**	NS	**
Protein	0.65	0.72	0.68	0.65	0.01	†	NS	**
Composition, %								
Fat	3.05	3.30	3.14	3.27	0.03	**	NS	*
Protein	3.31	3.38	3.28	3.27	0.01	†	**	**

[1] NS = Not significant ($P > 0.10$)

[2] ECM = 0.3246 * (kg milk) + 12.86 * (kg fat) + 7.04 * (kg protein). (Tyrrell and Reid, 1965)

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 6.5. Blood serum mineral and metabolite concentrations at 0, 2, 4, 6, and 10 h post-feeding for cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
Na, meq/L	142.5	143.5	142.7	142.8	0.49	NS[1]	NS	NS
K, meq/L	4.0	4.2	4.0	4.1	0.09	†	NS	NS
Cl, meq/L	100.3	99.4	100.3	98.4	0.81	†	NS	NS
DCAD, Na + K – Cl, meq/L	46.2	48.3	46.3	48.5	0.70	**	NS	NS
Ca, mg/dl	10.2	10.4	10.3	10.2	0.15	NS	NS	NS
Mg, mg/dl	2.4	2.3	2.4	2.3	0.07	†	NS	NS
Glucose, mg/dl	65.0	65.6	68.0	66.8	1.31	NS	NS	NS
Bicarbonate, mmol/L	22.5	24.1	22.6	24.7	0.50	**	NS	NS
BUN, mg/dl	12.3	10.9	13.0	12.4	0.66	NS	NS	NS
pH	7.40	7.43	7.41	7.43	0.005	**	NS	NS

[1] NS = Not significant ($P > 0.10$)

† $P < 0.10$

** $P < 0.01$

Table 6.6. Urinary excretion of mineral and metabolites for cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
Fractional excretion[1], %								
Ca	1.26	0.23	1.54	0.44	0.31	**	NS[2]	NS
Mg	12.62	8.71	10.46	6.02	1.16	**	†	NS
Na	0.33	1.01	0.26	0.78	0.12	**	NS	NS
K	39.71	63.44	31.89	59.77	3.27	**	†	NS
Cl	0.30	0.78	0.22	0.73	0.08	**	NS	NS
Urine metabolite / urine creatinine[3]								
Urea N	6.51	6.20	8.00	8.01	0.46	NS	**	NS
Bicarbonate	0.31	1.63	0.26	1.76	0.13	**	NS	NS
Uric Acid	0.42	0.44	0.52	0.41	0.02	†	NS	*

[1]Fractional Excretion = ([urinary mineral] / [plasma mineral]) * ([plasma creatinine] / [urinary creatinine]) * 100 (Vander, 1991).

[2] NS = Not significant ($P > 0.10$)

[3] Expressed as (milligrams of urine metabolite / 100 ml urine) / (milligrams of urine creatinine / 100 ml of urine).

† $P < 0.10$

* $P < 0.05$

** $P < 0.01$

Table 6.7. Serum amino acid concentration for cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
Essential AA, µg/ml								
Lys	25.8	27.6	26.5	24.4	1.09	NS[1]	NS	†
Arg	30.4	33.9	32.2	31.0	2.32	NS	NS	NS
Met	3.8	3.2	2.9	3.1	0.40	NS	NS	NS
Phe	17.5	19.0	18.4	17.2	0.67	NS	NS	†
Val	36.1	40.1	32.8	30.1	1.48	NS	**	*
Trp	7.5	8.4	7.8	7.7	0.34	NS	NS	NS
Thr	20.3	22.5	21.5	19.0	0.96	NS	NS	*
Ile	13.7	15.8	13.8	12.4	0.77	NS	*	*
His	14.8	15.0	13.7	12.7	0.51	NS	**	NS
Leu	34.3	38.5	34.0	31.3	1.56	NS	*	*
Total Essential AA, µg/ml	204.0	224.1	203.6	189.0	7.55	NS	*	*
Total AA, µg/ml	437.0	465.8	440.5	406.8	14.61	NS	†	**
Essential AA:Total AA	0.5	0.5	0.5	0.5	0.01	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

† $P < 0.10$

* $P < 0.05$

Table 6.8. Rumen VFA concentration and acetate:propionate ratio over 12 h post-feeding for cows fed diets formulated to contain DCAD of 25 or 50 meq Na + K – Cl / 100 g DM and RDP of 59 or 67% of dietary CP.

RDP	LRDP		HRDP			*P*		
DCAD	DCAD25	DCAD50	DCAD25	DCAD50	SEM	DCAD	RDP	DCAD x RDP
VFA, mol/100mol								
Acetate	53.7	55.0	55.3	56.4	1.32	NS[1]	NS	NS
Propionate	31.6	30.2	30.4	28.9	1.40	NS	NS	NS
Butyrate	9.9	10.8	10.0	10.5	0.41	NS	NS	NS
Total VFA, mmol/L	56.5	53.8	53.4	55.7	2.69	NS	NS	NS
Acetate:Propionate Ratio	1.7	1.9	1.9	2.1	0.14	NS	NS	NS

[1] NS = Not significant ($P > 0.10$)

CHAPTER 7

CONCLUSIONS

Dietary cation-anion difference (**DCAD**) is useful in ration formulation for lactating dairy cows because of its potential for improvement of animal performance. The effect of DCAD is mediated through changes in acid-base chemistry, ruminal buffering, and changes in N metabolism. Feeding positive DCAD diets to lactating cows improves acid-base status by increasing blood bicarbonate concentration and can result in improved DMI, milk yield, and milk component yield, especially in early and mid-lactation cows when acid-base challenges are greatest. Feeding early and mid-lactation dairy cows a DCAD on the high end of the proposed 25 to 50 meq Na + K – Cl / 100 g DM optimal range maximizes the benefit of DCAD on DMI and milk production. As cows progress in lactation, DCAD levels high in this optimal range are excessive, and can depress intake and production.

The interaction between DCAD and N metabolism seems to be dependent on level of dietary crude protein (**CP**) and rumen degradability of the protein (**RDP**). A greater impact of DCAD was observed in diets in which CP was lower than typically fed. Greater improvements from DCAD for DMI and milk yield were observed in diets with 15% CP compared with 17% CP. The interaction between DCAD and CP may result from changes in systemic buffering caused by greater DCAD. Higher DCAD improves blood buffering by increasing serum bicarbonate concentration and blood pH. The need for AA in systemic buffering would be reduced, increasing their availability for protein synthesis. Greater response for DMI and milk and milk component yield to DCAD was also noted for diets low in RDP compared with higher RDP. This may also result from a decreased role of AA in blood buffering. In high RDP diets, greater ammonia concentrations are normally produced in the rumen and are available for

systemic buffering. In low RDP diets, ruminal ammonia production is reduced, possibly resulting in a greater need for the additional buffering from other sources such as AA. High DCAD diets increase blood buffering through greater blood bicarbonate concentration thereby reducing the need for AA as systemic buffers. Availability of AA for protein synthesis is therefore increased.

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