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THE ROLE OF DIETARY CATION-ANION DIFFERENCE (DCAD) ON THE BLOOD BUFFERING CAPACITY AND THE SUSCEPTIBILITY OF DAIRY CATTLE TO INDUCED KETOACIDOSIS

By

John D. Fletcher

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Dietary cation-anion difference and induced ketosis in dairy heifers.

Contribution of Authors

Chapters 3 and 5 are papers in preparation for submittal. The authors of these two papers are J.D. Fletcher (graduate student) and E. Block (graduate supervisor).

The study design and preparation was conceived and developed by J.D. Fletcher and E. Block. The author of this thesis, J.D. Fletcher, carried out the following duties; fed and cared for study animals throughout the course of the experiments, collected feed and blood samples, performed laboratory analysis of blood samples, performed statistical analysis of the data, and wrote Chapters 3 and 5. Dr. E. Block provided expert advice throughout the project as well as review and editing of the two papers. Feed samples were analyzed by the Ralston Purina Canada Central Laboratory Services (Strathroy, Ontario).

Abstract

Master of Science Animal Science (nutrition)

John D. Fletcher

The role of dietary cation-anion difference (DCAD) on the blood buffering capacity and the susceptibility of dairy cattle to induced ketoacidosis.

Two experiments were conducted to investigate the effects of metabolic acid-base balance on feed intake and susceptibility of dairy heifers to ketoacidosis. Animals were fed two dietary cation-anion difference diets [dietary cation-anion difference is defined as mEq $(Na^{+}+K^{+})-(Cl^{-}+S^{2-})$ kg^{-1} dietary dry matter], a high dietary cation-anion difference (positive) and a low dietary cation-anion difference (negative). The heifers fed the positive dietary cation-anion difference had significantly higher blood pH, blood bicarbonate and dry matter intake than heifers fed the negative dietary cation-anion difference. There was an initial decrease in dry matter intake by the heifers fed the negative dietary cation-anion difference. However this difference was not significant by the end of the two week period. It is unclear whether the reduced dry matter intake in the heifers fed the negative dietary cation-anion difference is the result of metabolic-acidosis caused by the anionic salts, poor palatability of the anionic salts or a combination of both. Heifers were induced into a nervous ketosis by feeding 1,3-butanediol. There was no difference in the incidence of ketosis between animals fed positive and negative dietary cation-anion difference. Animals fed the positive diet tended to be more susceptible to anorexia.

Résumé

Maîtrise en Science — Science animales (nutrition)

John D. Fletcher

Rôle de la différence alimentaire cation-anion sur la capacité tampon du sang et sur la susceptibilité des vaches laitières à l'acétonémie induite.

Deux expériences ont été menées pour étudier les effets métaboliques de la différence alimentaire cation-anion sur la consommation volontaire de matière sèche et la susceptibilité des taures à l'acétonémie. Les animaux ont été alimentés avec deux rations dont la différence anion-cation différait. La différence anion-cation est définie par : $(Na^+ + K^+) - (Cl^- + S^{2-})$ meq kg^{-1} de matière sèche. La première ration avait une différence anion-cation élevée et la deuxième avait une différence anion-cation faible. Les taures nourries avec la différence anion-cation élevée ont présenté un pH sanguin, une concentration sérique en bicarbonate et une consommation volontaire de matière sèche significativement supérieures aux taures alimentées avec la différence anion-cation faible. La diminution de consommation volontaire, observée au départ pour le groupe différence anion-cation faible, n'était pas significative à la fin de la période d'observation. Les résultas ne permettent pas d'établir si la réduction de consommation volontaire de matière sèche des taures alimentées avec la différence anion-cation faible est le résultat de l'acidose métabolique causée par les sels anioniques, la mauvaise palatabilité des sels anioniques ou d'une conbinaison de ces deux facteurs. Une acétonémie nerveuse a été induite chez les taures par l'absorption de 1,3-butanediol. Aucune différence n'a été observée sur l'incidence d'acétonémie induite entre les deux groupes d'alimentation. Nous avons observés une tendance vers une susceptibilité plus grande à l'anorexie pour le groupe nourri avec la ration différence anion-cation élevée.

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I. General Introduction

Ketosis is a metabolic disease of nutritional origin that affects high producing dairy cows in early lactation. There have been major reviews and symposia on the subject and although progress has been made in our understanding of the disease some questions still remain (Shaw,1956; Bergman, 1971; Shultz, 1971; Littledike, Young and Beintz, 1981; Baird, 1982; Kronfeld, 1982; Zamit, 1990; Grummer, 1993). The major factor contributing to ketosis in early lactation is a reduction in plasma glucose. The measures required to maintain or improve glucose status in the dairy cow have not been fully elucidated. As well, all high producing dairy cows in early lactation are to some degree ketotic, but it is not known why some animals become clinically ketotic while others do not.

Dietary cation-anion difference (DCAD) has been used to influence acid-base balance in the dairy cow (Tucker et al., 1988b; Delaquis and Block, 1995c). Formulating diets for DCAD can also influence feed intake (Tucker et al., 1988b; Tucker et al., 1991a; Delaquis and Block, 1995c) and DCAD could, therefore, be used to improve feed intake and improve glucose status of the animal. Since Manipulation of DCAD could also be used to alter blood acid-base balance in early lactation animals and it could potentially be used to alleviate metabolic stress (acidosis).

Bovine ketosis will continue to plague dairy producers in part due to their perceived need to push the modern dairy cow to its biological limits for milk production. Dietary cation-anion difference may be a potential feeding strategy to minimize bovine ketosis with improved management of cows in the periparturient period.

II. Literature Review

2.1 Dietary Cation-Anion Difference

2.1.1 Dietary cation-anion difference defined

Dietary cation-anion difference (DCAD) is based on the concepts of Mongin (1981) for poultry and Dishington (1975) for dairy cows. The work by Block (1984) renewed interest in cation-anion difference, acid-base balance and parturient paresis. The theory behind DCAD is that by balancing select cations [sodium (Na^+) and potassium (K^+)] against select anions [chloride (Cl^-) and sulfate (SO_4^{2-})] in the diet, it is possible to influence certain physiological functions in animals. The "fixed ions" are Na^+, K^+ and Cl^- which are bioavailable ions that are not metabolized and thus play a major role in determining acid-base balance in biological fluids (Stewart, 1978). Sulfur (S), in the form of sulfate (SO_4^{2-}), is included in the equation because of its ability to acidify biological fluids when incorporated into diets in high concentrations (Whiting and Draper, 1981; Cole and Zlotkin, 1983). Feeding a more positive DCAD (cationic) diet leads to metabolic alkalosis and feeding a low or more negative DCAD (anionic) diet produces a metabolic acidosis (Block 1994).

Attempts have been made to define and describe DCAD (Oetzel, 1991; Tucker et al., 1991a; Block, 1994; Horst et al., 1997). Two key issues remain in defining the equation for DCAD. They are: 1) which ions should be included and 2) the absorption coefficients of these ions. The list below represents four equations that have been used or proposed for the calculation of DCAD:

mEq $(Na^+ + K^+)-(Cl^- + SO_4^{2-})$ kg^{-1} dietary DM [Eq.1]

mEq $(Na^+ + K^+)-(Cl^-)$ kg^{-1} dietary DM [Eq.2]

mEq $(0.38\ Ca^{2+}+0.3\ Mg^{2+}+ Na^+ + K^+)-(Cl^-+0.6\ SO_4^{2-})$ kg^{-1} dietary DM [Eq.3]

mEq $(Ca^{2+}+Mg^{2+}+ Na^+ + K^+)-(Cl^- + SO_4^{2-} + PO_4^{2-})$ kg^{-1} dietary DM [Eq.4]

The most commonly used equation for the calculation of DCAD is equation one (Horst et al., 1997). The major drawback of this equation is that it omits some of the other major dietary cations and ions such as Ca^{2+}, Mg^{2+} and PO_4^{2-} that may influence acid-base balance (Oetzel, 1991; Horst et al., 1997). Another criticism is that it is oversimplified in its assumption that sulfate and chloride have the same acidifying ability (Tucker et al., 1991a). However, Tucker et al. (1991a) were unable to show differences in acid-base status of lactating dairy cows using diets containing chloride or sulfate.

Equation two defines DCAD as the mEq $(Na^+ + K^+)-(Cl^-)$ kg^{-1} dietary dry matter (DM) (Gaynor et al., 1989; Tucker et al., 1988a; Tucker et al., 1988b). To save on the cost of analysis sulfate can be excluded from the equation when calculating DCAD in rations with the same sample sulfate content, usually within the same trial. The obvious drawback is in comparing these results with those from other research. This makes it difficult to develop recommendations on DCAD values for various physiological states (early, mid-, and late lactation and dry period). Another obvious reason to include sulfate in the DCAD equation is its use in the form of anionic salts [$MgSO_4$, $CaSO_4$, and $(NH_4)_2SO_4$] in dry cow rations.

Equation three has been proposed by Horst et al. (1997) to include all the major dietary cations and anions with their average efficiency of absorption (NRC, 1989). Sodium, potassium and chloride are considered to be completely absorbed. Sulfate is given a modifying coefficient of 0.6 as suggested by Tucker et al. (1991a). As mentioned earlier, dietary sulfur and chloride have been shown to have the same acidogenic effect over the DCAD range of 0 to 300 mEq kg^{-1} (Tucker et al., 1991a). Horst et al. (1997) suggest that the efficiency of the absorption of sulfate may be closer to 20% in late gestation. Delaquis and Block (1995a,b,c) have shown apparent absorption of sulfate to be around 60% in early, mid- and late lactation as well as during the dry period. They also reported that the apparent absorption of sulfate was closer to 80% when $MgSO_4$ was used

to lower DCAD (Delaquis and Block, 1995c). Absorption of sulfate may be decreased in late gestation, but absorption may also be greater when sulfate is included in higher concentrations in dry cow rations. Further research is required before any coefficient (efficiency of absorption) is assigned to cations and anions.

Equation four has not been accepted because little evidence exists to prove that Ca or Mg contribute to the DCAD concept of altering blood acid-base status. Horst et al. (1997) state that phosphate (PO_4^{3-}) should not be included in the DCAD equation because of its poor acidifying ability and one would not like to use it because high concentrations of blood phosphate can inhibit production of vitamin D resulting in an increased risk of milk fever.

Table 2.1 DCAD values, calculated using different equations.

	DCAD (mEq kg^{-1}) Diet (control with added salts)						
	Control	$MgCl_2$	$MgSO_4$	$CaCl_2$	$CaSO_4$	NH_4Cl	$(NH_4)_2SO_4$
Eq.1	-4	-172	-171	-170	-171	-173	-175
Eq.2	295	121	290	124	290	121	293
Eq.3	322	199	268	202	266	149	216

Table 2.1 is a comparison of the first three DCAD equations using data from Oetzel et al. (1991). Rations were formulated by Oetzel et al. (1991) according to the equation DCAD = mEq $(Na^+ + K^+)-(Cl^- + SO_4^{2-})$ kg^{-1} dietary DM (Equation 1). Their trial evaluated various anionic salts and their effects on acid-base balance and calcium metabolism as they related to milk fever. There were no differences in DMI, blood pH and HCO_3 among six anionic diets (DCAD approx. = -170 mEq kg^{-1}) and a control diet (DCAD = 0 mEq kg^{-1}). There were differences among the anionic diets for urinary pH and urinary calcium excretion. Table 2.1 also demonstrates the wide range of DCAD values depending on what equation is used. It is difficult to make recommendations for DCAD based on one

formula and even more difficult when DCAD values are reported using different equations. DCAD formulation should be clearly defined by the industry (NRC, feed manufacturers and producers). Recommendations could then be defined accordingly.

2.1.2 Strong Ion Difference (SID)

One of the mechanisms by which DCAD changes acid-base balance is through the absorption of strong ions (Na^+, K^+ and Cl^-) (Stewart, 1983). Stewart (1983) explains quantitative acid-base chemistry in biological fluids in terms of independent and dependent variable concentrations. The independent variables are; i) the partial pressure of carbon dioxide (pCO_2); ii) net strong ion difference (SID); and iii) total weak acids (usually proteins, P_{TOT}). Strong ions are Na^+, K^+, Cl^-, lactate and ketone bodies (acetoacetate and β-hydroxybutyrate) (Stewart, 1983; Jones, 1990). Dependent variables are bicarbonate (HCO_3^-), weak acids (HA), weak acid ions (A^-), carbonate ion (CO_3^{2-}), hydroxyl ion (OH^-) and hydrogen ion (H^+). Whole body acid-base status is controlled by three independent variables and not the dependent variables. The lungs, kidneys, gut and liver regulate the independent variables.

Hydrogen ion movement between compartments in a biological system does not change hydrogen ion concentrations. Change occurs as a result of movement of strong ions between compartment membranes (Stewart, 1983). Strong ion difference ($Na^+ + K^+ - Cl^-$) is cation-anion difference (CAD), and one of the principle theories associated with CAD is the movement of strong ions in the gut which change systemic acid-base status. Systemic acid-base balance is controlled in the short term (constantly and rapidly) by pCO_2 production by tissue and pCO_2 removal by the lung. Strong ions are absorbed by the gut and excreted by the kidney and change occurs more slowly over a period of hours. Proteins are produced by the liver and their effect on acid-base balance occurs over a period of days.

2.1.3 DCAD and acid-base balance

Blood acid-base status has been manipulated by DCAD. Decreasing DCAD has lowered blood pH and HCO_3 and increasing DCAD has raised blood pH and HCO_3 in dairy calves (Jackson et al. 1992), dairy heifers (Tucker et al., 1991b), dry dairy cows (Block, 1984; Oetzel et al., 1988; Gaynor et al. 1989; Goff et al., 1991; Joyce et al., 1997;) lactating dairy cattle (Tucker et al., 1988b) and growing and finishing steers (Ross et al., 1994a,b). Small or no differences in blood acid-base status in lactating dairy cattle (Delaquis and Block, 1995b; Sanchez et al., 1997) and dry cows (Oetzel et al., 1991; Delaquis and Block, 1995a) have been observed. The lack of difference was likely due to small differences in DCAD. Examples are 327 to 481 mEq kg^{-1} (Delaquis and Block, 1995a), 55 to 375 mEq kg^{-1} (Delaquis and Block, 1995c) and 250 to 400 mEq $(Na^+ + K^+)-(Cl^-)$ kg^{-1} (Sanchez et al., 1997).

2.1.4 Milk Fever

Lowering DCAD by feeding anionic salts in the dry period has proven to be an effective tool in the prevention of periparturient hypocalcemia (Block, 1984; Oetzel et al., 1988; Gaynor et al., 1989; Goff et al., 1991). Based on these studies Horst and Goff (1997) suggest that a DCAD of -50 to -100 mEq kg^{-1} be used for the prevention of milk fever. Goff and Horst (1997) have also recently shown that feeding diets lower in potassium and sodium is effective in reducing the risk of milk fever. Prepartum diets that have a relatively high DCAD (>250 mEq kg^{-1}) may require excessive amounts of anionic salts making them unpalatable. Unpalatability may then result in lower feed intake, which could lead to a host of postpartum metabolic disorders. It is recommended that forages with lower potassium contents be fed.

2.1.5 Dry Matter Intake

Feeding positive DCAD improves dry matter intake because the cationic

salt (i.e. sodium bicarbonate ($NaHCO_3$)) acts as a buffer. Dietary buffers have been broken up into buffering and non-buffering effects; that is to say the buffering effect of the bicarbonate and the non-buffering effect of the cation [sodium] (Schneider et al., 1986). Schneider et al. (1986) hypothesized an effect of both HCO_3^- (buffering effect) and Na^+ (solute effect). Rumen buffering reduces rumen acidity and improves systemic acid-base status (Erdman, 1988). Non-buffering effects are due to solute action. Solute action increases rumen osmotic pressure and liquid dilution rate (Rogers, et al., 1979, 1982). Sanchez et al. (1994) demonstrated maximum dry matter intake between 300-500 mEq kg^{-1} ($Na^+ + K^+ - Cl^-$) kg^{-1} dietary DM or 240-360 mEq kg^{-1} ($Na^+ + K^+$)-($Cl^- + SO_4^{2-}$)). Delaquis and Block (1995c) showed that increasing DCAD increased DMI and milk production in early and midlactation but not late lactation. They also showed that increasing DCAD increased free and total water consumption for dairy cows in early and midlactation. Improvements in dry matter intake have been attributed to buffering and non-buffering effects. Sanchez et al. (1994), using a model estimating the effects of modifying DCAD, showed a curvilinear effect of DCAD and DMI that differed between winter and summer. Differences in feed intake in lactating dairy cattle were demonstrated in diets with a DCAD of -268 and -168 mEq kg^{-1} but not between diets with DCADs of -168, -67, and +32, mEq kg^{-1} (Tucker et al., 1988).

A disadvantage of using anionic salts (negative DCAD) to prevent periparturient hypocalcemia in dry cows is that it can reduce DMI. Vagnoni and Oetzel (1998) suggested an alternative explanation for the reduction of DMI caused by lowering DCAD; the reduction in DMI may be the result of a metabolic acidosis caused by the anionic salts and not the salt's palatability. Their conclusions are based on studies of anionic salts in which $MgSO_4$ resulted in the lowest reduction in dry matter intake (Oetzel and Barmore, 1993). $MgSO_4$ was the least acidogenic of the anionic salts used (Oetzel et al., 1991).

2.2 Ketosis

2.2.1 Ketosis Defined

Ketosis can be defined as abnormally high concentrations of ketone bodies in body tissues and fluids. Ketone bodies are acetoacetate (ACAC), acetone (AC) and β-hydroxybutyrate (BHBA). Kronfeld (1982) has defined four types of ketosis: 1) primary underfeeding, 2) secondary, 3) alimentary, and 4) spontaneous. Primary underfeeding ketosis occurs when a cow is not offered enough acceptable feed. Secondary ketosis occurs when voluntary feed intake is reduced due to disease. Alimentary ketosis, also referred to as ketogenic ketosis, is the result of animals consuming large quantities of a ketogenic feed. Finally spontaneous ketosis takes place when the cow seems to be consuming sufficient amounts of nutritionally adequate feed. A number of terms are used to describe spontaneous ketosis (an early lactation metabolic event that effects high producing dairy cattle.) These terms include bovine, lactation and primary ketosis. Acetonemia has been used to describe bovine ketosis; however, this term suggests only an elevation of acetone, which is incorrect. It can be confusing to use the term primary ketosis when referring to spontaneous ketosis because Kronfeld (1982) uses primary ketosis to define "primary underfeeding ketosis".

A case can be made that primary underfeeding ketosis and spontaneous ketosis are the same because an animal in negative energy balance, as seen in early lactation, is unable to consume sufficient dietary energy. However, some authors suggest that there is a difference between underfeeding ketosis and spontaneous ketosis (Baird, 1982; Kronfeld, 1982; Zammit, 1990; Emery et al., 1992). Lactating cows show a greater ketogenic response than nonlactating cows when both are subjected to several days of starvation (Baird, 1979). In this thesis, spontaneous ketosis (early lactation metabolic event) and primary underfeeding ketosis (starvation ketosis) are referred to as two separate and

distinct metabolic events.

2.2.2 Bovine Ketosis

The major factor causing ketosis in early lactation dairy cows is hypoglycemia although other minor factors may contribute to the development of this metabolic disorder. The incidence of clinical ketosis in North America and Western Europe is in the range of 4-15% (Baird, 1982). Shultz (1988) reports that one-third of all cases of ketosis are secondary and are attributable to other problems such as retained placentas, metritis, hardware, displaced abomasums etc. Incidence is higher in older cows. Primary ketosis occurs in the first eight weeks postpartum with week three being the most critical period.

Ketosis can occur in clinical and subclinical forms (Baird, 1982). Clinical signs include decreased appetite, decreased milk yield, loss of body weight, hypoglycemia, hyperketonemia, elevated concentrations of nonesterified fatty acids (NEFA) in the blood, fatty infiltration of the liver and decreased liver glycogen (Baird, 1982). Baird (1982) suggested that all cows in negative energy balance in early lactation are subclinically ketotic and clinical and subclinical ketosis may have long term adverse effects on production. Cows rarely die of ketosis. Death is usually due to fatty liver, which can be a consequence of severe and prolonged ketosis. Ketotic animals, if left alone, will generally recover on their own.

Spontaneous bovine ketosis can be broken down into wasting and nervous ketosis (Radostits et al., 1994). Wasting is the more common of the two forms. Wasting ketosis is characterized by a gradual loss of appetite and a decrease in milk production over several days. Loss of appetite follows an orderly fashion with the refusal of grains, then silage and finally forages (Fox, 1971). As feed intake decreases there is a rapid loss in body weight and milk production. Physical findings include normal vital signs, firm dry feces, moderate

depression and a reluctance to move. Transient nervous signs such as staggering and blindness may occur and last a short period of time.

Fox (1971) and Radostits et al. (1994) have described nervous ketosis in great detail. Nervous ketosis is characterized by an acute onset of bizarre neurological signs which may include circling, proprioceptive deficits, head pressing, apparent blindness, wandering, excessive grooming behavior, pica and excessive salivation. Animals may also show hyperesthesia, bellows, moderate tremors and tetany. Animals can show aggressive behavior towards humans or inanimate objects. Nervous episodes last from 1 to 2 hours and reoccur at 10 to 12 hour intervals (Radostits et al., 1994).

Acetone is produced from acetoacetate at approximately 5% per hour (Bergman et al., 1963). It is associated with the distinctive odor in breath, milk and urine of ketotic animals (Fox, 1971). In normal animals the ratio of BHBA to ACAC plus acetone is 7:1 but this ratio decreases as the animal becomes ketotic. In non-ruminants the reverse is true (the ratio increases as ketosis persists). It is not known whether ketone bodies are responsible for the symptoms of clinical ketosis (Bergman, 1971). It has been suggested that high levels of acetoacetate and acetone are responsible for the symptoms of ketosis (Bergman, 1970). High concentrations of acetoacetate and acetone can depress central nervous system activity (Bergman, 1970).

2.2.3 Clinical and Biochemical Aspects

The blood of dairy cattle in early lactation shows three characteristics, a decrease in glucose, an increase in nonesterified fatty acids and an increase in ketone bodies (Acetoacetate, β-hydroxybutyrate and acetone). By definition ketone bodies are elevated. Normal levels for the total of ketone bodies are less than 10 mg dL^{-1} (Shultz, 1988). Ketone body concentration in milk is about half the amount of blood, and urine concentrations can be four times greater than

that of blood. Although ketosis is defined as elevated ketone bodies in bodily fluids it is most likely the initial drop in glucose that causes the rest of the metabolic changes (Shultz, 1988). Blood glucose concentrations below 40 mg dL^{-1} are subnormal and severely ketotic animals may have blood glucose concentrations as low as 25 mg dL^{-1}. The third major blood change characterizing ketosis is a rise in plasma concentration of NEFA. Mobilization of body fat allows dairy cattle to produce quantities of milk greater than that supported by the diet. Animals are in negative energy balance in early lactation because energy requirements for milk production are greater than energy intake. Negative energy balance is exaggerated in early lactation due to decreased feed intake. Decreased feed intake is one of the great mysteries of early lactation and one of the key areas of interest in feeding the transition (periparturient) cow. Reduced blood glucose causes a mobilization of adipose tissue in the form of NEFA. NEFA are membrane bound to albumin in the plasma, and uptake of NEFA by the liver is concentration dependent. NEFA are then converted to ketone bodies in the liver. In normal cows, 40% of ketone bodies are from NEFA. In the ketotic cow, 100% of ketone bodies are from NEFA because the animals become anorexic.

Baird et al. (1968) showed significantly higher liver concentrations of BHBA and ACAC and lower oxaloacetate in ketotic vs non-ketotic cows in early lactation. The ratio of BHBA to ACAC is higher in ketotic cows than in other ketotic mammals (rat). Liver fat content was seven times higher in ketotic compared to normal cows in early lactation. Liver carbohydrate content was 75 percent lower in the livers of ketotic cows. There were no significant differences in protein or nitrogen content. Concentrations of ketone bodies were 13 times higher in the ketotic cows' blood than in the blood of normal cows.

Ketotic animals are in a negative energy balance. Blood glucose decreases from 50-60 mg dL^{-1} to as low as 25 mg dL^{-1}. There is a marked increase in blood, urine and milk ketone bodies. Normal blood ketones are below

10 mg dL^{-1} and may increase to 50 mg dL^{-1} in ketotic animals. (Bergman, 1971; Littledike et al., 1981). Grohn et al. (1983) suggested the threshold for mildly and severely ketotic cows for blood concentrations of 1.0 and 3.0 mM of BHBA and 0.35 and 1.0, mM of ACAC. Emery et al. (1988) suggested that ketosis may be self-regulating in that it protects against excess NEFA uptake by the liver. That is to say that high concentrations of BHBA inhibit lypolysis, which may be partly due to BHBA stimulation of insulin (Heitmann and Fernandez, 1986).

2.2.4 Ketosis and Fatty Liver

Fatty liver and ketosis are related metabolic disorders in early lactation. Cows suffering from clinical ketosis usually have a high concentration of liver lipids (Grohn et al., 1983). It is uncertain which takes place first; fatty liver as the result of the decreased feed intake associated with clinical ketosis or does fatty liver precede ketosis? Grummer (1993) presents two lines of evidence that suggest that fatty liver precedes clinical ketosis. Cows that have triglyceride (TAG) to glycogen (GLY) ratios greater than 2.3 at 14 day postpartum will develop clinical ketosis. Those with ratios below 0.5 do not become ketotic (Mills et al., 1986; Veenhuizen et al., 1991; Drackley et al., 1992). However, Smith et al. (1997) had different TAG to GLY ratios between ketotic and non-ketotic cows. They concluded that the precise ratio of TAG to GLY that makes cows susceptible to ketosis is undefined. Secondly, fatty liver impairs conversion of propionate to glucose making cows more prone to ketosis (Mills et al., 1986; Veenhuizen et al., 1991; Cadorniga-Valino et al., 1997). Therefore, animals experience fatty infiltration during the early postpartum period before the onset of ketosis. Fatty liver can also impair carbohydrate status leaving cows more susceptible to ketosis.

2.2.5 Ketogenesis

McGarry and Foster's (1980) bihormonal model suggests that the mobilization of NEFA from adipose tissue to liver is primarily caused by a

decrease in insulin and an activation of hepatic fatty acid oxidation. Ketogenesis is caused by an elevation of the glucagon:insulin ratio. The antilipolytic effect of insulin is greater than the lipolytic effect of glucagon. Reduction of insulin and increased glucagon levels result in mobilization of NEFA from adipose tissue. This helps to explain why cows in early lactation are more susceptible to starvation ketosis than non-lactating cows, their insulin levels are severely reduced in the periparturient period. Uptake of NEFA by the liver is dependent on its plasma concentration. In the liver, under conditions of elevated glucagon, acetyl CoA carboxylase is in the phosphorylated and inactive state. Acetyl CoA does not proceed to malonyl CoA; therefore it does not enter fatty acid synthesis. Since malonyl CoA is an inhibitor of carnitine palmitoyltransferase I (CPT I) and fatty acyl CoA production, the inhibition is removed. Fatty acids are activated to form fatty acyl CoA and attach to carnitine. Conditions that stimulate ketogenesis also stimulate an increase in hepatic carnitine concentrations. Fatty acyl CoA is then transported into the mitochondria by facilitated diffusion and CoA is carried back outside the mitochondria by carnitine. Acetyl CoA is formed via β-oxidation, which is used for the formation of ketone bodies.

It has been suggested that CPT I is a possible regulation site for ketogenesis, whereby CPT I regulates the entry of long chain fatty acids into the mitochondria for β-oxidation (McGarry and Foster, 1980). McGarry and Foster (1980) proposed the bihormonal model. Under the influence of an increased ratio of glycogen:insulin the production of malonyl CoA is inhibited. Malonyl CoA is a strong inhibitor of CTP I activity. Methylmalonyl-CoA, an intermediate in the breakdown of propionate, has also been show to inhibit CPT I (Brindle and Zammit, 1985). However, under conditions of reversal of a ketogenic state (insulin treatment in diabetic ketosis and refeeding in starvation) the CPT I activity level is not decreased in the short term even though ketogenesis has been stopped (Grantham and Zammit 1986,1988). This suggests that CPT I plays an important role only in the onset of ketogenesis.

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase catalyses the reaction of acetyl-CoA and acetoacetyl-CoA to HMG-CoA. HMG-CoA synthase is one of a number of mitochondrial enzymes that are involved in the production of ketone bodies from acetyl-CoA, which is the product of β-oxidation. Evidence suggests that HMG-CoA synthase contributes to the regulation of ketogenesis. Lowe and Tubbs (1985) showed that succinyl-CoA inactivates mitochondrial HMG-CoA synthase in the liver of oxen. The succinylation of HMG-CoA synthase prevents the binding of acetoacetyl-CoA and is desuccinylated over time and by acetyl-CoA. It has been shown that desuccinylation is glycogen induced (Quant et al., 1990). Casals et al. (1992) found that HMG-CoA synthase mRNA control is exerted by cyclic AMP, insulin, fasting and refeeding conditions and diabetes. Conditions such as diabetes, starvation, and cyclic AMP lead to an increase in gene expression; refeeding and insulin lead to a decrease in gene expression. Figure 2.1 shows the mitochondrial pathway of ketone body production from fatty acids to BHBA (Guzman and Geelen, 1993).

2.2.6 Prevention

A number of measures can be taken to reduce the risk of ketosis in the early postpartum. They include avoiding overconditioned cattle at calving, due to overfeeding during the early part of the dry period. Propylene glycol can be fed prepartum to reduce fat mobilization at calving (Struder et. al., 1993). Feeding niacin at 6g/day during the prepartum period (Dufva et al., 1993) can also reduce ketosis. Using Monensin Controlled Release Capsule (Rumensin CRC) has been shown to reduce the incidence of subclinical ketosis (Duffield et al., 1998).

2.2.7 Treatment

i. Intravenous Glucose

Treatment for ketosis is directed towards increasing blood glucose.

Treatment effectiveness is dependent on the ability to maintain blood glucose at acceptable concentrations. The most common therapy is intravenous injection of 500 mL of 50% glucose (dextrose). This produces a transient hyperglycemia, after which glucose levels return to preinjection levels. Ketone body levels then decrease and clinical signs disappear. Milk production will then increase for the next milking, although the animal may then relapse. Repeated administration of glucose will likely be required to prevent relapses. Slow continuous intravenous drips of glucose (2000 mL) are effective but impractical under normal field conditions.

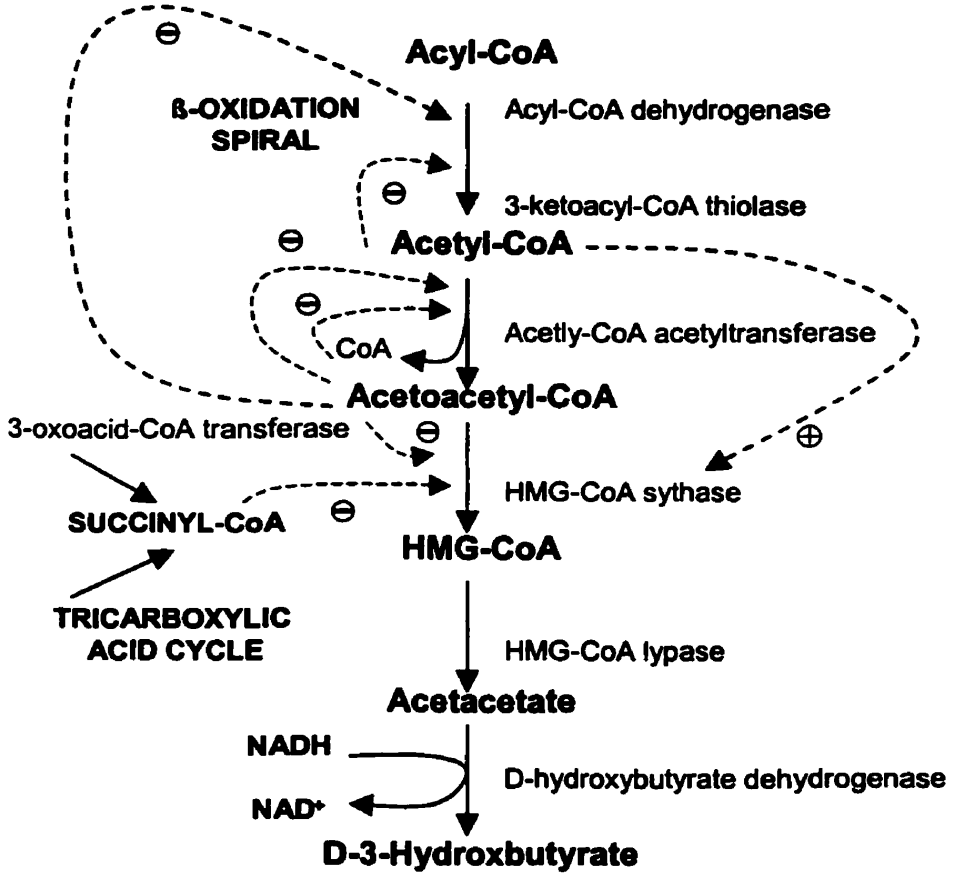

Figure 2.1 Control of the intramitochondrial reactions of ketogenesis by CoA and its derivatives. (Guzmán and Geelen, 1993). Conversion of ACAC to BHBA is in the cytisol in ruminants.

ii. Oral Glucose Precursors

Glucose precursors can be given orally in the feed or as a drench. The compounds include propylene glycol, sodium propionate, ammonium lactate, and sodium lactate (converted in the liver to glucose via pyruvate and oxaloacetate). Due to the danger of aspiration when drenching the animals, it is advisable to give these compounds in the feed. Oral glucose precursors tend to reduce milk fat and prolonged use may lead to digestive disruption. Propylene glycol is an effective treatment when given along with intravenous glucose.

iii. Hormones

Hormone therapy has been used alone or in conjunction with glucocorticoids. However they are not generally effective over the long term. It has been shown that cortisol production is insufficient. Glucocorticoids increase blood glucose by stimulation of gluconeogenesis from amino acids.

iv. Others

Pharmacological doses of niacin (nicotinic acid) have been used in treatment of ketosis. Cobalt and cobalt containing B_{12} deficiencies have been suggested as possible causes of ketosis. The dietary supplementation of cobalt and B_{12} has not shown any benefits (Croom et al., 1981). Chloral hydrate is a traditional treatment for ketosis which increases rumen breakdown of starch and influences production of propionate (Radostits et al., 1994). It has also been effective as a sedative in the treatment of recurring nervous ketosis. Choline, cysteamine and l-methionine have all been suggested because of their lipotrophic effects.

2.3 1,3-Butanediol (BD)

2.3.1 1,3-Butanediol Defined

1,3-Butanediol (BD) is a colorless odorless glycol that has been used as

an energy source in pigs (Romos et al., 1972), chickens (Davenport and Griffith, 1969), cattle (Hess and Young, 1972; Bonner et al., 1975; Bonner, Young and Berger, 1976) and humans (Tobin et al., 1975). Butanediol has a high metabolic energy value of 6 kcal/g in rats (Miller and Dymsza, 1967) and 6.5 kcals/g in chicks (Davenport and Griffith, 1969). It is an industrial solvent, fungicide and food additive. Recently BD has been investigated as a potential parenteral and enteral nutrition feed for humans (Desrochers et al., 1995). Butanediol has been used as a ketogenic agent in the investigation of bovine ketosis (Mills et al. 1986; Veenhuizen et al. 1991; Drackley et al. 1991; Drackley et al. 1992; Smith et al. 1997).

2.3.2 Biochemical Aspects

The liver is the primary site of metabolism of 1,3-butanediol to D-ß-hydroxybutyrate (BHBA) in dairy cattle. Conversion of BD to BHBA by bovine liver in vitro occurred in appreciable amounts when compared to production by kidney and rumen mucosa (Drackley et al., 1990). Drackley et al. (1990) calculated that the liver of a mature Holstein dairy cow could metabolize 1 Kg of BD daily. Conversion of BD to BHBA occurs in the liver by means of cytosolic alcohol and aldehyde dehydrogenases (Tate et al., 1971). Desrochers et al. (1992) demonstrated in rats that R- and S-1,3-butanediol are taken up equally. They also showed that the majority of R-BD is converted to physiological ketone bodies while S-BD is metabolized to S-BHB, lipids and CO_2 .

2.3.3 Ketosis Induction Protocol

A ketosis induction protocol (KIP) has been developed to mimic spontaneous bovine ketosis (Mills et al., 1986a; Veenhuizen et al., 1991; Drackley et al., 1992; Smith et al., 1997). This protocol is based on minimal feed restriction plus the feeding of a 1,3-butanediol (ketogenic agent) starting at two weeks postpartum and continuing until about seven weeks postpartum. In studies with either feed restriction (de Boer, Trenkle and Young, 1985; Drackley

et al., 1991) or BD alone (Drackley et al., 1991) clinical ketosis was not shown. Mills et al. (1986) obtained 50% induced ketosis by overconditioning the cows prepartum which may have led to a reduced feed intake in the early postpartum period. Smith et al. (1997) were unable to show a difference in induced ketosis between fat and normal cows. They reported two hyperketonemic cows and one clinically ketotic cow in both fat and normal cow groups (six cows per group). Veenhuizen (1991) reported that five of six cows on the KIP protocol developed clinical ketosis. Drackley et al. (1992) produced ketonemia in all treatment animals, although only one of the seven cows became clinically ketotic. Drackley et al. (1992) suggest that the differences between their results and those of Veenhuizen et al. (1991) were due to a lower protein and higher energy diet during treatment and lower average age at calving. In the four KIP studies 12 of 30 cows developed clinical ketosis. Drackley et al. (1992) have suggested that a liver triglyceride:glycogen ratio (as a percent of wet weight) of greater than two during the early postpartum period may be an indicator of predisposition to fatty liver and ketosis.

Feeding BD at 6% of the total ration DM to ruminating calves caused hyperketonemia, hyperactivity, muscular tremors, and reduced weight gain in a study carried out by Hess and Young (1972). They suggested that calves showed toxicity at 6-8% BD while rats display symptoms at 18-20% of dietary intake. Differences in metabolism account for this. Dymsza (1975) reports that large doses of BD depress the central nervous system of dogs and this is common with all glycols. He also reports that dogs were observed to lack coordination after eating diets where carbohydrate had been replaced by BD. Bonner and Young (1975) fed BD to Holstein heifers weighing between 180 to 300 kg and found that feeding BD above 0.8 kg per day caused hyperactivity, nervousness, excessive urination, muscular tremors, hyperketonimia and ketonuria. Symptoms were diagnosed as a type of ketosis. Animals became ketotic after five days at levels of BD above 0.8kg.

2.4 Acidosis

Kronfeld (1971) suggests that the severity of acidosis in early lactation cows is not on the same magnitude as that of diabetes in humans and pregnancy toxemia in sheep. Clinically observable manifestations are therefore not comparable. This statement is correct but are there comparable degrees of acidosis. Perhaps the mild acidosis associated with early lactation is sufficient to decrease glucose tolerance (Bigner et al., 1997) and reduce feed intake (Vagnoni and Oetzel, 1998). This may then further exacerbate the acidosis. Bigner et al. (1996) demonstrated that cows in mild metabolic acidosis (7.33 vs 7.40, blood pH and 17.18 vs 24.90 mEq L^{-1}, blood HCO_3) had higher plasma glucose and lower insulin after a glucose tolerance test. Treatment of the acidosis with a sodium bicarbonate drench (Bigner et al., 1996,1997) and sodium propionate (Bigner et al., 1997) corrected the acidosis. It also partially improved glucose status after animals were challenged with a glucose tolerance test. They concluded that glucose utilization is altered in cows with metabolic acidosis and that animals with ketosis may benefit from an oral drench of sodium bicarbonate or sodium propionate prior to treatment of ketosis with glucose solution.

Based on the above literature review the following experiments were carried out to test the hypothesis that dairy cattle with metabolic acidosis are more susceptible to clinical ketoacidosis than animals with metabolic alkalosis. Non-pregnant, non-lactating dairy heifers were used as a model in order to avoided different levels of milk production in lactating cows. The first experiment was conducted to demonstrate differences in blood pH, blood bicarbonate concentrations and dry matter intake in dairy heifers fed different DCAD. The second experiment investigated the effects of different blood pH and bicarbonate concentration, caused by altering DCAD, on the incidence of induced clinical ketoacidosis caused by 1,3-Butanediol in dairy heifers.

III. The role of dietary cation-anion difference (DCAD) on blood acid-base balance, feed intake and plasma metabolites in dairy heifers.

3.0 Abstract

The objective of the study was to investigate the effects of two dietary cation-anion differences on blood acid-base balance and feed intake of dairy heifers. Blood acid-base balance was manipulated by altering the dietary cation-anion difference [mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dry matter]. Twelve Holstein heifers between 180-300 kgs were paired according to body weight and fed a negative (-183 mEq kg^{-1}) or a positive dietary cation-anion difference (363 mEq kg^{-1}) for a 13 day period. Heifers fed the negative diet had a lower daily dry matter intake (4.9 vs. 6.0 kgs, p=.02) during week one. There was no difference in daily dry matter intake (5.8 vs. 6.2 kgs, p=.29) between the negative and positive diets during the second week. This resulted in an overall lower daily dry matter intake (5.3 vs. 6.1, p<.001) among the heifers fed the negative diet. Blood pH and HCO_3^- but not pCO_2 were lower for the heifers fed the negative diet at hour 0 (prefeeding), and hours 1, 2 and 4 postfeeding. The largest changes in blood acid-base balance occurred during the first week. Plasma acetoacetate concentrations were lower for the negative diet prefeeding but not at hour 4 postfeeding and conversely β-hydroxybutyrate concentrations showed no differences prefeeding but the positive diet was high four hours postfeeding. There were no differences in plasma concentration of glucose, insulin and nonesterified fatty acids. Heifers fed the negative ration had lower plasma sodium and higher plasma sulfate and chloride compared to the positive ration. Results indicate that changes in dry matter intake for animals on the negative diet corresponded to changes in systemic acid-base balance.

3.1 Introduction

Dietary cation-anion difference (DCAD) can be defined as milliequivalents of $(Na^+ + K^+)-(Cl^- + SO_4^{2-})$ per kilogram of dietary dry matter (DM). DCAD is based on the concepts of Mongin (1981) for poultry and Dishington (1975) for dairy cows. The theory behind DCAD is that by balancing select cations (Na^+ and K^+) against select anions (Cl^- and SO_4^{2-}) in the diet it is possible to influence certain physiological functions in animals. These functions include acid-base balance (blood buffering capacity), osmotic pressure, cell membrane integrity and the sodium-potassium pump (Block, 1994). Feeding a more positive DCAD (cations) diet will lead to metabolic alkalosis and feeding a low or more negative DCAD (anions) diet, produces a metabolic acidosis (Block, 1994).

Manipulating DCAD with mineral salts has been used in dairy rations to prevent milk fever (Block, 1984), reduce heat stress, increase dry matter intake, and buffer the rumen. Most notably, DCAD has been used as a means of reducing the incidence of milk fever. Recently, considerable attention has been placed on the transition period of cows (3 weeks prepartum) and diets to reduce the incidence of milk fever. Anionic salts (reduce DCAD) cause metabolic acidosis, which eventually leads to increased blood levels of ionized calcium at the time of parturition and the onset of lactation thus preventing a fatal drain of blood calcium.

The objective of the trial was to show differences in acid-base balance by feeding different DCAD. Additionally we wished to investigate the effects of different acid-base balances on dry matter intake and blood metabolites in dairy heifers.

3.2 Materials and Methods

3.2.1 Experimental design and housing

Twelve Holstein heifers (180-300 kgs) were paired according to body weight and randomly assigned to two different DCADs. One heifer per pair received a negative DCAD (-183 mEq kg^{-1} DM) and the other a positive DCAD (363 mEq kg^{-1} DM). Heifers were housed in metabolic stalls in the Macdonald Campus Dairy Research Complex of McGill University. The experiment was carried out in two replicates, each involving three separate pairs of heifers. The first replicate (three pairs) of heifers were fed the two different DCADs for 14 days followed by the second replicate (three pairs), which was fed the two different DCAD for 13 days. The second replicate was fed for one day less due to logistical problems. For the purpose of statistical analysis day 14 in replicate 1 was dropped.

3.2.2 Diets and Feeding schedule

Animals were fed a total mixed ration (TMR). The dry matter composition of the diet is listed in Table 3.1. The vitamin mineral premix mix was Prime Time Dry Cow Mineral Checkers (Ralson Purina Canada Inc., Lasalle, Quebec). Ammonia sulfate was fed at 1.8 % of TMR DM in the anionic diet to lower the DCAD to -183 mEq kg^{-1} DM and 1.8% sodium bicarbonate was fed in the cationic diet to raise the DCAD to +363 mEq kg^{-1} DM. The diets were made isonitrogenous by including 0.8% urea in the diet with the positive DCAD. Diets were formulated to meet or exceed NRC (1989) requirements. Animals were fed once daily between 11h30 and 12h45 and rations were fed to allow for approximately 10% refusal the next day.

Table 3.1 Ingredient composition of basal diet.

Feed ingredient	% of DM
corn silage	64.2
alfalfa-grass haylage	20.0
soybean meal	12.0
mineral salts	1.8
Urea	0.8
Limestone	0.5
vitamin premix [1]	0.5
NaCl	0.2

[1] The dry matter composition of the vitamin mineral mix was: 12% P, 2.5% Mg, 1% S, 1% K, 145 mg kg^{-1} I, 7500 mg kg^{-1} Fe, 2400 mg kg^{-1} Cu, 7200 mg kg^{-1} Mn, 7200 mg kg^{-1} Zn, 25 mg kg^{-1} Co, 500 mg kg^{-1} F, 410000 I.U. kg^{-1} Vitamin A, 123000 I.U. kg^{-1} Vitamin D_3 and 1640 I.U. kg^{-1} Vitamin E.

3.2.3 Sampling Schedule and Procedure

Feeds: Feed was sampled weekly for each diet. Daily feed refusals were weighed and 10% of the weighbacks were pooled for a weekly sample. Feeds and refusals were frozen at - 40° C for later analysis.

Blood Sampling: The day before the start of the trial a 30.5 cm, 16 G catheter (Intercath®, Becton Dickinson Vascular Access; Sandy, Utah) was inserted into the jugular vein of each heifer. Catheters were installed by a modified method. A 5.1 cm 14 G needle was inserted into the jugular vein. A stylet (guitar string, diameter approximately 1.0 mm.) was then inserted into the needle and jugular vein. The 14 G needle was then removed from the animal leaving the stylet in the jugular vein. The catheter was filled with heparinized saline and slipped over the stylet and the stylet was then pulled out through the catheter. The catheter was secured to the animal with a small butterfly (medical tap) at the hub of the catheter and sutured on both sides of the catheters hub. An 89 cm venous extension set (Baxter; Deerfield, IL) was attached to the catheter and sutured to the animal's withers. Elastoplast bandages (Smith and Nephew, Lachine, Quebec) were wrapped around the animal's neck to keep the catheter and extension in place. Extensions were stoppered with a three-way stopcock (Argyle EZ-Flo, Sherwood Medical, St. Louis, MO). A preliminary trial was conducted to investigate the feasibility of long-term (30-day) jugular catheterization on dairy heifers in tie-stalls. This preliminary study showed that catheters remained both in place and functioning for 6 to 14 days. Catheters usually became blocked at the hub of the catheter when a "kink" developed. Elastoplast bandages were necessary because of the rubbing and licking (heifers, unlike mature cows, are more agile, tend to get extremely bored and are intolerant of the jugular catheter).

Blood samples were collected four times daily at 0, 1, 2 and 4 hours postfeeding for the 13 day period. Catheters and extensions (internal volume of

approximately six mL) were flushed into the vein with 20 mL of heparinized saline at hour 0 every day. This helped keep the catheter open and clean for an extended length of time. At each bleeding the first 10 mL of blood-saline was discarded. Four, seven mL vacutainers (Becton Dickinson Vacutainer Systems; Franklin Lakes, NJ) (two containing lithium heparin and two containing fluoride-oxalate) were collected per sampling. The catheters were then flushed with heparinized saline. Whole blood was stored on ice and analyzed for blood gasses within two hours of collection then centrifuged. Lithium heparin tubes were used for determination of blood gasses and plasma sodium and potassium. The fluoride oxalate tubes were used for the determinations of plasma ACAC, BHBA, chloride, glucose, insulin, NEFA, and sulfate. Plasma was transferred to seven mL plastic scintillation vials and frozen at - 40° C until analysis.

3.2.4 Analytical Methods

Feeds: Weekly feed samples were analyzed using wet chemistry by the Ralston Purina Canada Central Laboratory Services (Strathroy, Ontario). Samples were analyzed for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), and sulfur (S).

Blood samples: Blood samples were collected daily for 13 days at hour 0, 1, 2, and 4 postfeeding and analyzed within two hours for pH, HCO_3^- and pCO_2 using a blood gas analyzer (IL1306, Instrument Lab., Milan Italy).

Plasma determination of ACAC, BHBA, glucose, insulin and NEFA were performed on daily plasma samples for hour 0 and hour 4. Plasma analysis for ACAC, BHBA, glucose and NEFA were done on a discrete clinical analyzer (VP Super System; Abbott Laboratories, Mississauga, ON., Canada). Commercial kits were used to determine glucose (Kit Quickstart #5A15, Abbott Laboratories, Abbott Park, IL.) and BHBA (Kit #310-A, Sigma Chemical Co., St. Louis, MO.).

ACAC was determined by a modified method described by Gau (1986) using procedure No. 67-UV (Sigma Chemical Co.) with the following changes; dilution ratio 1:11, low and high standard 0.1 mmol L^{-1} and an initial absorption difference (Ad) of 0.63. Plasma NEFA concentrations were determined using a NEFA-C kit (WAKO Pure Chemical Industries LTD., Osaka, Japan) and a modified procedure by McCutcheon and Bauman (1986). McCutcheon and Bauman (1986) found the assay to be linear between 125 to 2000 μeq L^{-1}, which was evident in our assays. All NEFA values below 125 μeq L^{-1} were given the value of 125 μeq L^{-1}. Insulin was determined using a commercial radioimmunoassay kit (Immunocorp, Montreal, Quebec, Canada).

Plasma mineral concentrations of Na, K, Cl and S were determined for daily hour 4 samples. Chloride was determined using a clinical analyzer (Abbott Laboratories) and a commercial kit (Kit # 461, Sigma Chemicals Co.). Plasma sulfate (SO_4^{2-}) was determined by the turbidimetric method of Bergland and Sorbo (1960) modified by Krijgsheld et al. (1979). To 0.5 mL of plasma 2.0 mL of 5% (w/v) TCA solution was added. Samples were vortexed and allowed to stand for 10 min at room temperature. The mixture was centrifuged at 4,400 X G for 15 minutes at room temperature. One mL of clear supernatant was added to 0.25 mL $BaCl_2$ reagent (20 g $BaCl_2$ and 10 g dextran per liter H_2O). The absorbance was read after precisely 35 min at 360 nm against a sample background (1 mL of supernatant added to 0.25 mL reagent containing 10 g dextran in 1 liter H_2O). A standard curve was constructed with Na_2SO_4 using SO_4^{2-} concentrations of 0, 1, 2, 3, 4 mmols. Plasma Na and K were determined using an atomic absorption spectrophotometer (Perkins-Elmer 360: Perkins-Elmer, Norwalk, CT). One-half milliliter of plasma sample was added to 2 mL of HPLC water and vortexed to make a 1 in 5 dilution. Dilutions were made to achieve a 1:250 dilution for potassium where the final dilution was made in an excess of Na (2 mL L^{-1} of a 1000 ppm Na standard). Dilutions were made to achieve a 1 in 5000 dilution for Na where the final dilution was made in an excess of K (2 mL L^{-1} of a 1000 ppm K standard).

3.2.5 Statistical Analysis

The data were analyzed using the mixed model procedure SAS (Littell et al. 1996). The model used for the analysis is described by the following equation;

$$Y_{ijkl} = \mu + Diet_i + Block_j + Pair_K(Block_j) + Cow_m(Block_j*Pair_k*Diet_i) + Day_l + Diet_i* Day_l + E_{ijklm}$$

Were: Y_{ijkl} = dependent variable

μ = overall mean

$Diet_i$ = effect of i^{th} diet, i=1,2;

$Block_j$ = effect of j^{th} block, j=1,2;

$Pair_K(block_j)$ = effect of k^{th} pair within i^{th} block, k=1,2,3;

Cow_m(Blockj*Pairk*Dieti) = effect of m^{th} cow within the k^{th} pair, within the j^{th} block, within the i^{th} diet, m=1,2;

Day_l = effect of the l^{th} day, l=1,...13;

$Diet_i* Day_l$ = effect of i^{th} diet and the l^{th} day;

E_{ijkm} = random error associated with the ijklmth observation.

A mixed model analysis of variance was performed and differences between diets were estimated using least square means. Since there were repeated measurements, over the thirteen days for each cow, the repeated measures facility of PROC MIXED was used as per Chapter 3 of Littell et al. (1986). An autoregressive (AR1) and compound symmetry (CS) covariance structure was tested for each trait to determine the most appropriated (best fitting) model in each case. Compound symmetry was found to be the most appropriate model and all subsequent results reported use compound symmetry structure for the repeated measurements across days. Probability levels appear in the tables and text as they were reported by the SAS PROC MIXED procedure. Standard error of the difference is reported. The criterion for declaring

and considering an effect to be statistically significant remained the predetermined 5% level. Data were excluded for two heifers in the same pair from day 10 to 13 because of infection (fever) and the animals became anorexic on days 10 and 11. The six heifers in the block were given an intramuscular antibiotic injection (10-12 mL) of Penlong XL® (Rogar/STB Inc; London, Ont.) after hour 4 on day 10 as a prophylactic measure.

3.3 Results

Diet Composition: The average DM composition of the two diets is shown in Table 3.2. There were no differences in DM, CP, ADF, NDF, Ca, P, Mg, K, TDN, NE_L, NE_M, NE_G and Cl. The negative (NEG) diet had higher S, and the POS diet had higher Na. The standard deviation for Na was high for the POS diet, which made the standard deviation for the DCAD large. DCAD were lower than expected for both diets. However there was a large difference of 546 mEq kg^{-1} (-183 vs. +363, mEq kg^{-1}) between the two diets.

Dry Matter Intake: The average DMI of the two diets is shown in Table 3.3 and average daily DMI are shown in Figure 3.1. There was a difference (P=0.05) between NEG and POS diets (5.3 kg d^{-1} vs 6.1 kg d^{-1}, respectively) over the 13 day period. There was also an effect of day (P=0.001) and a diet by day interaction (P=0.001). During the first week of feeding there were differences (P=0.02) in DMI between NEG and POS diets (4.9 kg d^{-1} vs 6.0 kg d^{-1}, respectively) with an effect of day (P=0.02) and a diet-by-day interaction (P=0.003). There was no effect of diet on feed intake during the second week of feeding (days 8-13) although there was an effect of day (P=0.004). The largest difference in DMI between NEG and POS diets was on day 4 when the difference was 1.7 kg d^{-1}.

Acid-Base Balance: The average blood acid-base parameters (pH, HCO_3^- and CO_2) for hours 0 (prefeeding), 1,2 and 4 postfeeding are in Table 3.4.

Table 3.2 Average dry matter (DM) composition of diets differing in dietary cation-anion difference (DCAD) offered to heifers over a 13 day period.[1]

Analyte	NEG[2]	SD[3]	POS[4]	SD[3]
DM %	43.6	1.5	45.3	1.2
CP %	18.1	1.3	17.6	0.4
ADF %	23.7	1.3	23.7	1.9
NDF %	36.9	1.5	38.5	1.9
Ca %	0.59	0.06	0.54	0.04
P %	0.35	0.07	0.34	0.06
Mg %	0.21	0.01	0.21	0.02
K %	1.29	0.13	1.29	0.06
TDN %	67.9	0.9	67.9	1.2
NE_L	1.54	0.02	1.54	0.03
NE_M	1.57	0.03	1.57	0.04
NE_G	0.97	0.02	0.97	0.03
Sodium %	0.09	0.00	0.59	0.10
Chloride %	0.359	0.022	0.361	0.018
Sulfur %	0.72	0.05	0.19	0.01
DCAD[1]	**-183.4**	**4.8**	**363.1**	**47.2**

[1] DCAD : mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -183 mEq kg^{-1} DM, n=4
[3] Standard deviation
[4] Ration with a DCAD of 363 mEq kg^{-1} DM, n=4

Table 3.3 Average daily dry matter intake (DMI) of heifers fed different DCADs over a 13 day period. [1]

			P>F			
	NEG[2]	POS[3]	TRT	DAY	TRT*DAY	SED[4]
	Average DMI (kg/d)					
day 1-7	4.9	6.0	0.02	0.003	0.02	0.3
day 8-13	5.8	6.2	0.29	0.004	0.65	0.3
day 1-13	5.3	6.1	0.05	<.001	0.001	0.3

[1] DCAD : mEq $(Na^{+}+K^{+})-(Cl^{-}+S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -183 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +363 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference

Figure 3.1. Average daily dry matter intake with SE bars of heifers fed different dietary cation-anion differences (DCAD) over a 13 day period.

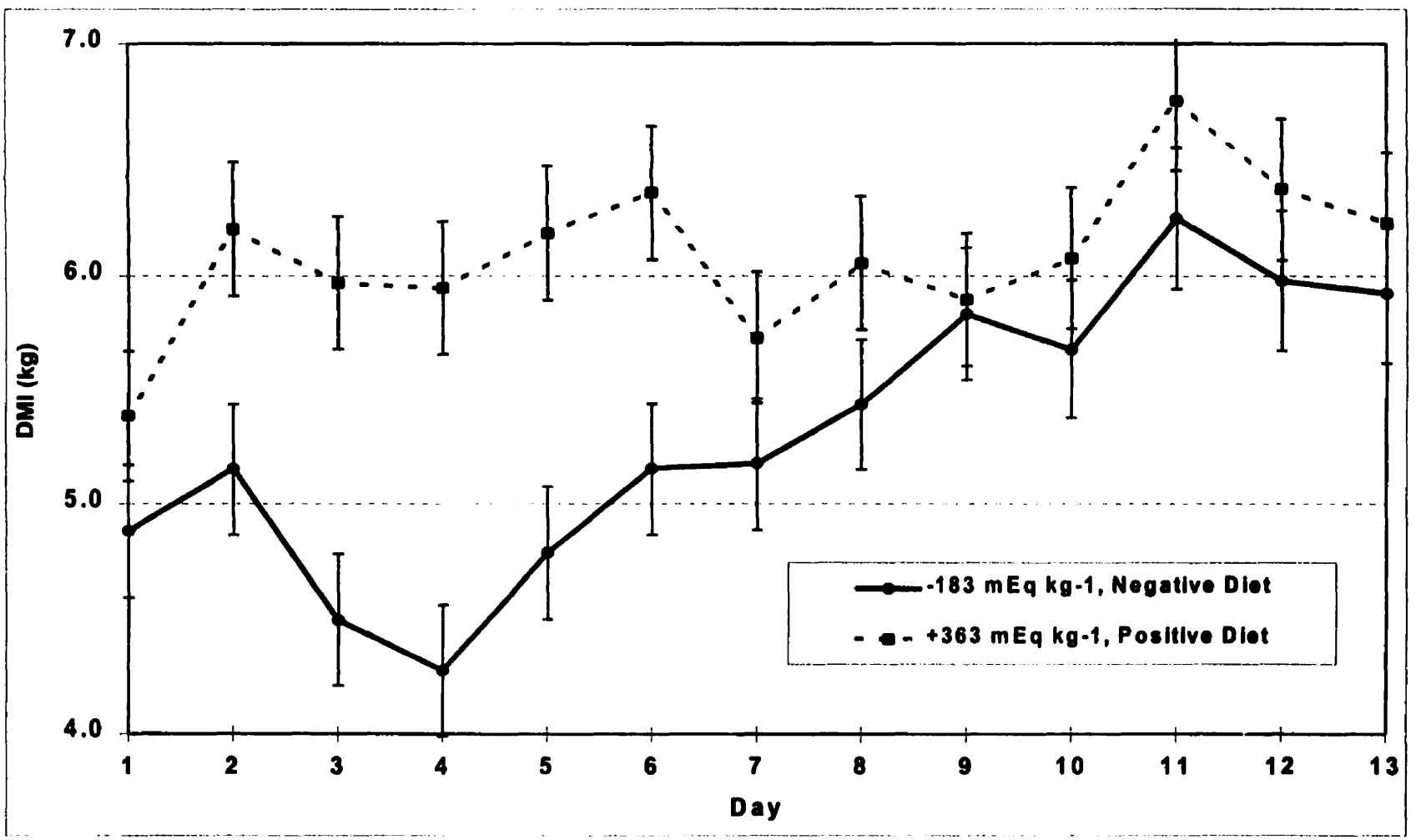

Table 3.4 Average blood pH, bicarbonate ion concentration (HCO_3^-) and pCO_2 in heifers fed different DCADs, prefeeding, 1, 2 and 4 hours postfeeding over a 13 day period.[1]

			P>F			
	NEG[2]	POS[3]	TRT	DAY	TRT*DAY	SED[4]
Prefeeding						
pH	7.346	7.394	<.001	<.001	<.002	0.004
HCO_3^-, mmol L^{-1}	26.7	29.5	0.01	<.001	<.001	0.6
pCO_2, mm Hg	48.1	47.7	0.70	0.73	0.35	1.0
1 h postfeeding						
pH	7.347	7.396	<.001	<.001	0.001	0.004
HCO_3^-, mmol L^{-1}	26.8	29.9	0.002	<.001	0.002	0.5
pCO_2, mm Hg	48.3	48.3	0.98	0.05	0.13	0.8
2 h postfeeding						
pH	7.342	7.396	<.001	<.001	0.04	0.006
HCO_3^-, mmol L^{-1}	26.3	30.3	0.002	<.001	0.02	0.7
pCO_2, mm Hg	47.9	48.8	0.40	0.11	0.61	1.0
4 h postfeeding						
pH	7.345	7.405	<.001	<.001	<.001	0.006
HCO_3^-, mmol L^{-1}	26.2	30.2	0.002	<.001	0.04	0.7
pCO_2, mm Hg	47.3	47.7	0.76	0.02	0.83	1.1

[1] DCAD : mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -183 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +363 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference

Average daily blood pH and HCO3- are shown in Figures 3.2 and 3.3. There were differences (P=0.01) between diets in blood pH and HCO_3^- at all four hours and there was an effect of day and a diet-by-day interaction. The NEG diet consistently produced an acid-base profile that was more acidic. There was no difference in blood CO_2.

Plasma Metabolites: Average daily blood metabolites at prefeeding and at hour 4 postfeeding are shown in Table 3.5. There were no differences (P=0.07) in blood BHBA between NEG and POS diets prefeeding (3.6 mg dL^{-1} vs 4.0 mg dL^{-1}, respectively) but there were differences (P=0.01) in BHBA at hour 4 (4.7 mg dL^{-1} vs 6.1 mg dL^{-1} for NEG and POS, respectively). There was an effect of day prefeeding and a day and diet-by-day effect at hour 4 postfeeding. There was a difference (P=0.04) in ACAC plasma concentrations between NEG and POS diets prefeeding (30.2 μmol L^{-1} vs 35.0 μmol L^{-1} for NEG and POS, respectively) and no difference (P=0.06) between diets at hour 4 (28.9 μmol L^{-1} vs 35.3 μmol L^{-1} for NEG and POS, respectively). There was an effect of day for ACAC but no diet by day interaction. There were no differences between diets for plasma glucose, insulin and NEFA prefeeding and at hour 4 postfeeding.

Plasma Minerals: Average concentrations of plasma sodium, potassium, chloride and sulfate of the NEG and POS diets are shown in Table 3.6. Average plasma mineral concentrations were taken at hour 4 and averaged over the 13 days in the period. There was a difference in plasma sodium concentrations between the NEG and POS diets (140.4 mmol L^{-1} vs 143.4 mmol L^{-1} for NEG and POS, respectively). There were no differences in potassium concentrations between the NEG and POS diets. There was a difference (P=0.02) between NEG and POS diets for plasma chloride (95.5 mmol L^{-1} vs 93.7 mmol L^{-1} for NEG and POS, respectively). There was also an effect of day and a diet by day interaction. There was a difference (P=.003) in plasma sulfate concentrations between diets (2.6 mmol L^{-1} vs 1.9 mmol L^{-1} for NEG and POS, respectively) and there was a day effect.

Figure 3.2. Average daily blood pH of heifers fed different dietary cation-anion differences (DCAD) over a 13 day period.

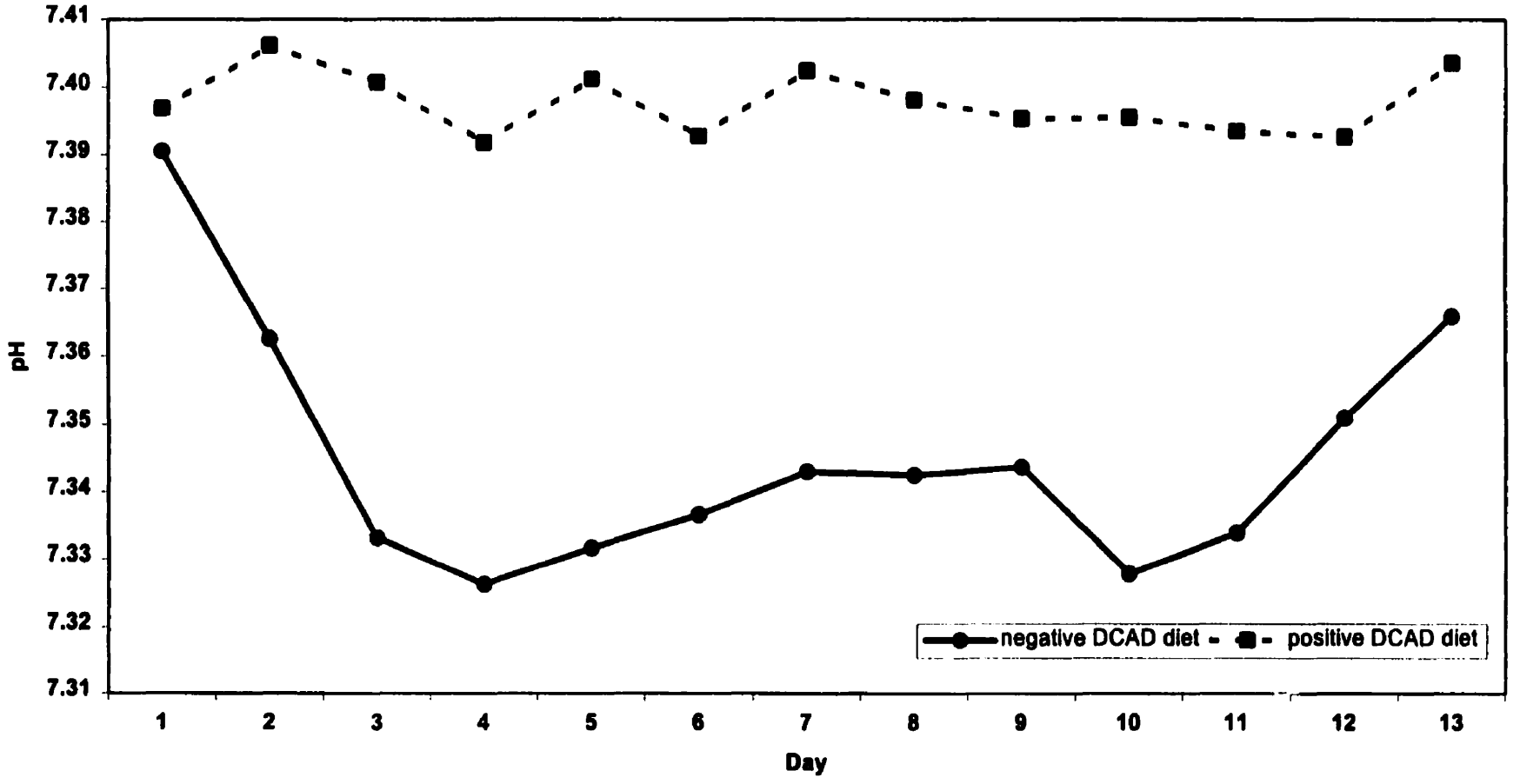

Figure 3.3. Average daily blood bicarbonate ion concentration (HCO_3^-) of heifers fed different dietary cation-anion differences (DCAD) over a 13 day period.

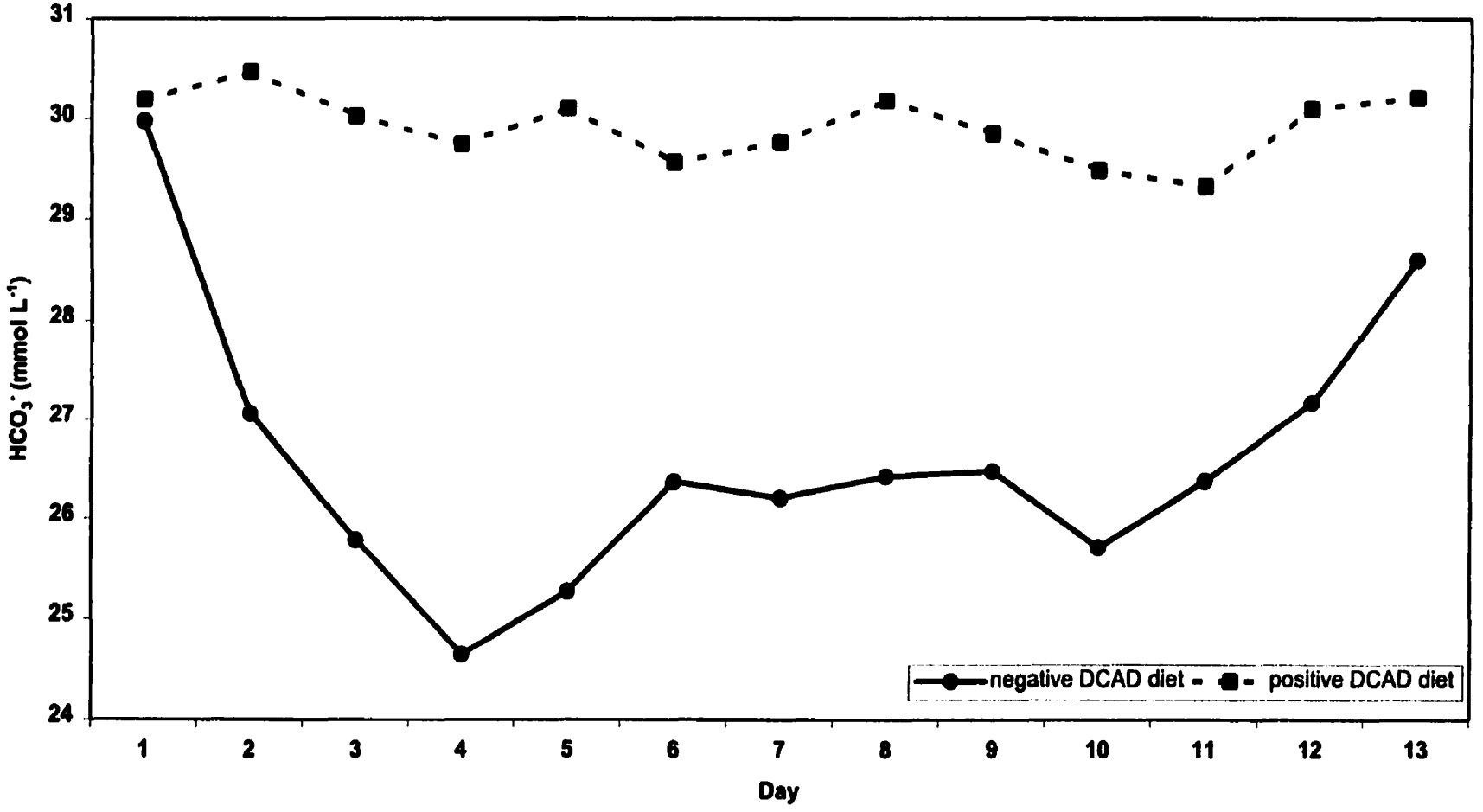

Table 3.5 Average plasma concentrations of metabolites for heifers fed different DCADs, prefeeding, 1, 2 and 4 hours postfeeding over a 13 day period.[1]

	NEG[2]	POS[3]	P>F TRT	P>F DAY	P>F TRT*DAY	SED[4]
Prefeeding						
ACAC[5], μmol L^{-1}	30.2	35.0	0.04	0.09	0.51	1.8
BHBA, mg dL^{-1}	3.6	4.0	0.07	0.002	0.13	0.2
glucose, mg dL^{-1}	87.4	87.3	0.95	<.001	0.47	2.2
Insulin, pmol L^{-1}	191.1	204.8	0.64	0.01	0.18	27.3
NEFA[6], mEq dL^{-1}	140.9	149.0	0.42	<.001	0.85	9.3
4 h postfeeding						
ACAC[5], μmol L^{-1}	28.9	35.3	0.06	<.001	0.99	2.5
BHBA, mg dL^{-1}	4.7	6.1	0.01	0.08	0.05	0.4
glucose, mg dL^{-1}	87.1	86.8	0.87	0.003	0.08	2.2
Insulin, pmol L^{-1}	242.0	276.1	0.43	0.01	0.14	40.0
NEFA[6], mEq dL^{-1}	132.7	129.0	0.21	0.56	0.49	2.6

[1] DCAD : mEq $(Na^+ + K^+) - (Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +372 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference
[5] Acetoacetate
[6] Non-esterified fatty acids

Table 3.6 Average plasma mineral concentrations of heifers fed different DCADs over a 13 day period.[1]

	NEG[2]	POS[3]	P>F TRT	P>F DAY	P>F TRT*DAY	SED[4]
Prefeeding						
Sodium, mmol L^{-1}	140.4	143.4	<.001	0.52	0.82	0.2
Potassium, mmol L^{-1}	4.4	4.5	0.18	0.12	0.29	0.1
Chloride, mmol L^{-1}	95.5	93.7	0.02	0.01	0.07	0.5
Sulfate, mmol L^{-1}	2.6	1.9	0.003	0.02	0.54	0.1

[1] DCAD : mEq $(Na^+ + K^+) - (Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -183 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +363 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference

3.4 Discussion

Diet Composition: The DCAD calculated for the rations was not identical to the amount obtained in the actual rations themselves. Rations were formulated to obtain a DCAD of 0 mEq kg^{-1} (NEG) and 450 mEq kg^{-1} (POS). The DCAD in the actual rations were –183 (NEG) and 363 mEq kg^{-1} (POS). Differences between calculated and actual DCAD were due to the ration formulation program (Spartan). We overlooked the fact that Spartan does not include the sulfate concentrations from forage in the calculations of the DCAD. Despite this omission the desired difference (400-500 mEq kg^{-1}) was obtained between the two diets.

Sulfur concentration in the NEG diet was 0.72%. This exceeds the 0.4% sulfate level set by the National Research Council's Mineral Tolerance of Domestic Animals (1980) which is the maximum tolerable dietary level for cattle. Feeding high levels of sulfur has been shown to cause reduced feed intake, anorexia, weight loss, constipation, diarrhea and depression (NRC, 1980). Reduced feed intake is in agreement with our observations. It is unclear if the reduced DMI is the result of palatability, metabolic acidosis or some other metabolic stress associated with feeding high sulfur concentrations. High concentration of sulfur (0.61%) have been fed to dry cows (Joyce et al., 1997) and lactating dairy cows (0.54% and 0.78%) to investigate the effects of sulfur on DCAD (Tucker et al., 1991a).

Dry Matter Intake: The differences between the diets were evident over the first week and the overall two week period but there were no differences in feed intake between NEG and POS diets during week 2. Mixed results were seen in other studies. Jackson et al. (1991) were unable to show a difference ($p=.09$) in DMI between dairy calves fed four DCADs between –179 and 383 mEq kg^{-1} although there were differences in blood pH (7.34 vs. 7.38) and HCO_3 (24.6 vs 29.3, mmol L^{-1}). Ross, Spears and Garlich (1994) showed an effect of

increasing DCAD on DMI of growing (1994b) and finishing steers (1994a). Decreases in DMI for dry cows have been nonsignificant (Oetzel et al., 1988; Oetzel et al., 1991) and significant (Tucker et al., 1988; Gaynor et al., 1989; Tucker et. al., 1991).

This study showed that heifers change their feeding behavior when fed NEG vs. POS. It was observed that animals fed the NEG diet consumed their feed more slowly over a 24 hour period. While the heifers fed the POS diet consumed a large part of their daily ration (75%) within the first two hours of its introduction, heifers fed the NEG diet may require about 7 days to overcome either the palatability and/or metabolic perturbation elicited by low or negative DCAD. However, as previously stated this was only an observation and was not measured or recorded. It presents an interesting possibility for the study of feed intake and the effects on metabolic events.

Acid-Base Balance: Vagnoni and Oetzel (1998) suggest that metabolic acidosis and not palatability may be the cause of reduced ad libitum DMI. They found that using $MgSO_4$ as an anionic salt resulted in a less severe DMI depression compared to other anionic salts (Oetzel and Barmore, 1993) and animals had less metabolic acidosis (Oetzel et al., 1991). Likewise Tucker et al. (1991) suggest that systemic acidosis due to the feeding of anions is likely the cause of the reduction in DMI although they feel that palatability may play a role.

Palatability vs. Metabolic Acidosis in Reduction of Dry Matter Intake: An interesting question arises. Do animals reduce DMI because of feed palatability or does the feed cause them to become acidotic resulting in a reduced DMI? If a high concentration of anions cause the taste of the feed to become less palatable the animal may eat less. As the animal adapts to the taste it may then resume eating more feed over time. Blood pH initially decreases because of the quantity of anions consumed. As the animal eats less, because of reduced feed palatability, the animal receives less anions and blood

pH rises. If the animal then eats more, due to taste adaptation, the blood pH should drop again. If an animal eats an anionic diet and becomes acidotic, this may result in a reduction of DMI. As the animal compensates for metabolic acidosis through the kidneys (long term), it will again increase its intake. However, this does not explain why some commercial products are reportedly well tolerated in terms of intake quantity but still result in a reduction in systemic pH. One explanation may be that the flavoring promotes an adequate DMI. Reduced DMI may also be a combination of changes in palatability and induced acidosis.

Plasma Metabolites: Ketone bodies were reduced for the heifers consuming the NEG diet. ACAC was also lower and BHBA tended (P=.07) to be lower prefeeding for the animals fed NEG. Similarly ACAC tended to be lower at 4 hours post feeding (P=.06) and BHBA was lower. This could have been a result of the amount of DMI. Under normal conditions (non-ketotic) rumen ketone body production is comparable to that of the liver (Heitmann, Dawes and Sensenig, 1987). Since the animals fed the POS consumed greater amounts of feed, it is likely that they had higher plasma concentration of ketone bodies because of production by the rumen epithelium. It has been shown in humans that decreases in blood pH lead to decreases in ketone body production by the liver (Hood et al., 1982; Hood et al., 1990). According to Hood et al. (1990) decreases in the production of ketone bodies are the result of decreased plasma concentrations of NEFA. In our studies there were no differences in plasma concentrations of NEFA pre or post feeding between diets. However, in human studies where subjects have been fasted overnight, plasma NEFA levels are not comparable. Hood et al. (1990) make the point that acidosis could act as a negative feedback mechanism in a ketogenic diet to help prevent severe ketoacidosis. Numerous other mechanisms have been cited as potential feedback mechanisms in the regulation of ketogenesis. They include inhibition of lypolysis at the adipose tissue level by ketone bodies (Bjorntorp, 1966). Stimulation of insulin production from the pancreas by ketone bodies inhibits

lypolysis (Heitmann and Fernandez, 1986).

Plasma Minerals: Sulfate and sodium in the plasma were most affected by the diet because they were used to alter DCAD. Sulfate in the form of ammonia sulfate was used in the NEG diet to lower DCAD and sodium in the form of sodium bicarbonate was used in the POS diet to increase DCAD. Heifers fed NEG had higher sulfate concentrations in their diet (0.72%) compared to heifers fed POS (0.19%). Greater intakes of sulfate resulted in higher concentrations of plasma sulfur. These results are comparable to those found by Delaquis and Block (1995c) and Takagi and Block (1991).

3.5 Conclusion

Heifers fed rations with differing DCADs demonstrated differences in DMI. Feed intake decreased during the first week and increased over the second week in the NEG DCAD group indicating an adaptation. Decreases and increases in DMI tended to correspond with fluctuations in blood pH and bicarbonate. The exact mechanism, which causes a decrease in DMI when feeding anionic salts could not be determined from this study.

IV. Joining Statement

Chapter 3 is part of a preliminary trial investigating acid-base balance and induced ketoacidosis. The trial was conducted to induce metabolic acidosis in a group of heifers and a metabolic alkalosis in an other group of heifers. It was also conducted to determine dry matter intake in the two groups.

Chapter 5 reports a continuation of the previous trial. The trial was conducted to investigate acid-base balance and induced ketocidosis.

V. The role of dietary cation-anion difference (DCAD) on blood buffering capacity and the susceptibility of dairy heifers to induced ketoacidosis.

5.0 Abstract

The objective of this study was to investigate the effects of different blood pH and bicarbonate ion concentrations on the incidence of induced ketoacidosis in dairy heifers. Acid-base balance was manipulated by means of dietary cation-anion difference [mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dry matter] and ketosis was induced by the inclusion of 1,3-butanediol at 15% of dry matter in the diet. Twelve Holstein heifers between 180-300 kgs were paired according to body weight and fed a negative (-161 mEq kg^{-1}) or a positive dietary cation-anion difference (372 mEq kg^{-1}). Heifers were fed the diets for 13 days with no butanediol then given the same positive and negative dietary cation-anion difference plus butanediol for another 13 days. There were differences between blood pH prefeeding and at all sampling times postfeeding. There were differences in blood bicarbonate at one, two, and four hours postfeeding but not prefeeding. Differences in plasma concentrations of nonesterified fatty acids were only seen at four hours postfeeding. There were no differences in plasma concentrations of acetoacetate, β-hydroxybutyrate, glucose and insulin. Two thirds of the heifers became anorexic at least once (50% of the animals on the negative diet and 83% on the positive diet). Three heifers were removed from the trial due to nervous ketosis (two of the animals on the positive and one on the negative diet). While it appears that dietary cation-anion difference directly affects blood acid-base parameters, dietary cation-anion difference does not seem to directly influence the development of clinical ketoacidosis.

5.1 Introduction

Bovine ketosis is a frequently encountered metabolic disease of nutritional origin in dairy cows during early lactation. Negative energy balance, due to high-energy requirements for milk production, combined with decreased feed intake in early lactation, predisposes cows to ketosis. Negative energy balance brings about lipid mobilization in the form of nonesterified fatty acids (NEFA) from adipocytes. Nonesterified fatty acids are taken up by the liver and oxidized for energy or re-esterified to triglycerides and accumulate in hepatocytes. Uptake of NEFA by the liver is concentration dependent. Ketone bodies, β-hydroxybutyrate (BHBA) and acetoacetate (ACAC) are also formed in hepatocytes as a result of incomplete oxidation of fatty acids. Ketone bodies can be removed from the body in a number of ways. Acetoacetate is spontaneously decarboxylated to acetone at the rate of 5% (Bergman et al., 1963). Acetone is then expired from the lungs, excreted in urine and secreted in milk. The peripheral tissues are able to utilize ketone bodies (ACAC and BHBA) as an energy source (Heitmann, Dawes and Sensenig, 1987).

Because ACAC and BHBA are acids, a metabolic acidosis can occur when production is greater than utilization and excretion. Blood bicarbonate buffers the body from ketoacidosis. However, the buffering system could be depleted and intracellular protein buffering may be recruited to buffer some of the acid load. Theoretically, bicarbonate depletion and protein buffering could affect enzyme systems and compromise normal metabolic pathways.

A protocol to induce ketosis in early lactation dairy cows was developed at Iowa State University (Mills et al., 1986; Drackley et al., 1991; Veenhuizen et al., 1991; Drackley et al., 1992; Smith et al., 1997). The protocol involves moderate feed restriction (80% of ad libitum feed intake) plus the inclusion of 7% 1,3-butanediol (BD) in the diet (Mills et al., 1986; Drackley et al., 1991; Veenhuizen et al., 1991; Drackley et al., 1992; Smith et al., 1997). Butanediol is a ketone

body precursor and is converted to BHBA by the liver (Tate, Mehlman and Tobin, 1971; Drackley, Richard and Young, 1990; Desrochers et al. 1992). BD has been shown to induce ketosis in dairy calves (Hess and Young, 1972) and heifers (Bonner et al., 1975).

DCAD has been defined as the milliequivalents of $(Na^{+}+K^{+})-(Cl^{-}+S^{2-})$ per kilogram of dietary dry matter. DCAD has been shown to alter blood acid-base balance. Feeding a diet with a positive DCAD will increase the blood buffering capacity of the cow (Tucker et al., 1988b). Therefore, feeding dairy cattle a diet with a positive DCAD should alleviate the metabolic acidosis associated with ketosis and reduce the occurrence of clinical ketosis.

Our objective was to investigate the effects of different blood pH and bicarbonate concentrations caused by altering DCAD, on the incidence of induced clinical ketoacidosis caused by BD in dairy heifers.

5.2 Materials and Methods

5.2.1 Experimental design and housing

Prior to this (experiment in Chapter 3), twelve Holstein heifers (180-300 kgs) were paired according to body weight and randomly assigned to two different DCADs. One heifer per pair received a negative DCAD (-183 mEq kg^{-1} DM) and the other a positive DCAD (363 mEq kg^{-1} DM). The experiment was carried out in two replicates, each involving three separate pairs of heifers.

The first replicate (three pairs) were fed for 14 days to determine dry matter intake and to develop different acid-base metabolic conditions in the paired animals. The animals on the negative DCAD diet had metabolic acidosis and the heifers on the positive DCAD diet had metabolic alkalosis. The same animals were subsequently used for this experiment and they were fed for an

additional 13 days with a different diet (current experiment, Chapter 5). Paired heifers were fed diets differing in DCAD (-161 vs. 372 mEq kg^{-1} DM) and BD.

Similarily the second replicate (three pairs) were fed for 13 days (experiment in Chapter 3) to determine dry matter intake and to develop different acid-base metabolic conditions in the paired animals. These heifers were also then fed diets differing in DCAD (-161 vs. 372 mEq kg^{-1} DM) and 1,3-butanediol for an additional 13 days (current experiment, Chapter 5). Heifers were housed in metabolic stalls in the Macdonald Campus Dairy Research Complex of McGill University.

5.2.2 Diets and feeding schedule

Rations were similar to those in Chapter 3 (Table 3.1) except that an amount of corn silage was replaced with 1,3-butanediol (Hoechst Celanese Chemical Group, Etobicoke, Ontario). BD was incorporated into the ration gradually over a five day period to avoid feed refusal (personal communication with J.W. Young). The first two days, 5% of the corn silage (dry matter basis) was replaced with BD, day 3-4 10% and day 5-13 the heifers were receiving 15% BD. Butanediol was fed at 15% to insure that the heifers would become ketotic within a week (personal communication with J.W. Young). Final TMRs were corn silage (49% of diet DM), alfalfa-grass haylage, (20% of diet DM), BD (15% of diet DM), soybean meal (12% of diet DM), limestone (0.5% of diet DM), 0.5% vitamin premix (0.5% of diet DM) and 0.2% NaCl (0.2% of diet DM). The NEG diet had 1.8 % ammonium sulfate and 0.4% urea. The POS diet had 1.8% sodium bicarbonate and 1.2% urea. Urea was added to make the rations isonitrogenous with the diets in Chapter 3.

Paired heifers were fed the lower DMI of the two from the previous day. The heifer with the lowest DMI from the previous day was offered 110 percent of its previous day's intake. When animals became anorexic and their DMI dropped

below about 1% of their body weight then both animals within the pair were no longer feed restricted.

Three heifers became clinically ketotic and were removed (two heifers in the first block were diagnosed as clinically ketotic by the farm veterinarian, one heifer in second block was removed from the trial when she showed similar signs). One heifer was removed from the trial due to a jugular abscess. The feeding schedule, sampling schedule, analytical methods and statistical analyses were the same as those described in Chapter 3.

5.3 Results

Diet Composition: The mean dry matter composition of the two diets is shown in Table 5.1. There were no differences in DM, CP, ADF, NDF, Ca, P, Mg, K, and Cl. The NEG diet had higher S and the POS diet had higher Na.

Acid-Base Balance: Table 5.2 shows blood gas data for prefeeding, 1, 2 and 4 hours postfeeding for animals fed NEG and POS diets. There were differences in blood pH between NEG and POS prefeeding (7.367 vs. 7.389, P=0.004), and at hour 1 (7.373 vs. 7.390, P=0.05), hour 2 (7.368 vs. 7.396, P=0.01) and hour 4 (7.368 vs. 7.396, P<0.001) postfeeding. There were also effects of day and diet-by-day interactions for prefeeding, 1, 2 and 4 hours postfeeding. There were differences in blood bicarbonate at hour 1 (28.0 vs. 29.6, mmol L^{-1}, P=0.04), hour 2 (27.4 vs. 29.7, mmol L^{-1}, P=0.02) and hour 4 (27.3 vs. 29.4, mmol L^{-1}, P=0.01) but not prefeeding. There were no differences in pCO_2 between the two diets.

Plasma Metabolites: Results for plasma ACAC, BHBA, glucose and insulin are presented in Table 5.3. There were no differences in plasma concentrations of ACAC, BHBA, glucose or insulin between NEG and POS diets at prefeeding and at hour 4 postfeeding. There was a difference (P=0.04) in

Table 5.1 Average dry matter composition of diets differing in dietary cation-anion difference plus 15% 1,3-Butanediol offered to heifers over a 13 day period. [1]

Analyte	NEG[2]	SD[3]	POS[4]	SD[3]
DM %	47.4	1.7	47.4	1.3
CP %	17.2	1.2	16.0	1.3
ADF %	20.6	1.8	21.8	1.6
NDF %	33.2	2.3	35.1	1.1
Ca %	0.52	0.02	0.53	0.03
P %	0.36	0.10	0.32	0.02
Mg %	0.18	0.03	0.19	0.00
K %	1.19	0.12	1.21	0.04
TDN %	69.92	1.14	69.14	1.06
NE_L	1.59	0.03	1.57	0.03
NE_M	1.63	0.04	1.61	0.03
NE_G	1.02	0.03	1.00	0.03
Sodium %	0.10	0.01	0.62	0.05
Chloride %	0.332	0.006	0.333	0.011
Sulfur %	0.66	0.05	0.18	0.01
DCAD[1]	**-161.0**	**9.3**	**371.8**	**31.6**

[1] DCAD : mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=4
[3] Standard deviation
[4] Ration with a DCAD of +372 mEq kg^{-1} DM, n=4

Table 5.2 Average blood pH, HCO_3^- and pCO_2 of heifers fed diets with 15% 1,3-butanediol and different DCADs prefeeding, 1, 2 and 4 hours postfeeding over a 13 day period. [1]

	NEG[2]	POS[3]	P>F TRT	P>F DAY	P>F TRT*DAY	SED[4]
Prefeeding						
pH	7.367	7.389	0.004	0.01	0.02	0.004
HCO_3^-, mmol L^{-1}	27.7	29.2	0.11	0.33	0.08	0.7
pCO_2, mm Hg	47.8	47.8	0.94	0.85	0.69	1.0
1 h postfeeding						
pH	7.373	7.390	0.05	0.01	0.03	0.007
HCO_3^-, mmol L^{-1}	28.0	29.6	0.04	0.65	0.00	0.6
pCO_2, mm Hg	47.6	48.3	0.52	0.03	0.67	1.0
2 h postfeeding						
pH	7.368	0.396	0.01	0.03	0.03	0.006
HCO_3^-, mmol L^{-1}	27.4	29.7	0.02	0.10	0.01	0.7
pCO_2, mm Hg	47.1	47.9	0.50	<.001	0.17	1.1
4 h postfeeding						
pH	7.368	7.396	<.001	0.002	0.002	0.004
HCO_3^-, mmol L^{-1}	27.3	29.4	0.01	0.01	<.001	0.5
pCO_2, mm Hg	46.9	47.5	0.56	0.10	0.32	0.9

[1] DCAD : mEq $(Na^+ + K^+) - (Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +372 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference

Table 5.3 Average plasma concentrations of metabolites for heifers fed diets with 15% 1,3-butanediol and different DCADs prefeeding and 4 hours postfeeding over a 13 day period. [1]

	NEG[2]	POS[3]	P>F			SED[4]
			TRT	DAY	TRT*DAY	
Prefeeding						
ACAC[5], μmol L^{-1}	24.7	30.6	0.07	<.001	0.63	2.6
BHBA, mg dL^{-1}	7.0	6.5	0.37	<.001	0.31	0.5
glucose, mg dL^{-1}	81.1	82.8	0.50	<.001	0.46	2.5
Insulin, pmol L^{-1}	180.1	174.3	0.85	<.001	0.70	29.4
NEFA[6], mEq dL^{-1}	190.9	257.3	0.04	<.001	0.30	23.2
4 h postfeeding						
ACAC[5], μmol L^{-1}	23.0	25.6	0.23	<.001	0.94	2.1
BHBA, mg dL^{-1}	9.2	11.3	0.14	<.001	0.11	1.2
glucose, mg dL^{-1}	78.8	78.0	0.76	<.001	0.25	2.5
Insulin, pmol L^{-1}	193.3	210.6	0.64	<.001	0.66	34.7
NEFA[6], mEq dL^{-1}	163.5	217.0	0.09	0.03	0.16	25.4

[1] DCAD : mEq $(Na^+ + K^+) - (Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +372 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference
[5] Acetoacetate
[6] Non-esterified fatty acids

plasma NEFA concentrations between NEG and POS diets (190.9 vs 257.3, mEq d L^{-1}) prefeeding but not at hour 4 postfeeding. There was also an effect of day for ACAC, BHBA, glucose, insulin and NEFA for both prefeeding and at hour 4 postfeeding.

Plasma Minerals: Mean plasma concentrations of sodium, potassium, chloride and sulfate of the NEG and POS diets are shown in Table 5.4. The mean plasma mineral concentrations are for hour 4 over the 13 day period. There was a difference (P=0.01) in plasma sulfate concentrations between NEG and POS diets (2.2 vs 1.7, mmol L^{-1}). There were no differences in sodium, potassium and chloride concentrations between the NEG and POS diets. There was, however, an effect of day for potassium and chloride.

Ketosis and anorexia: The incidences of anorexia and ketosis are expressed in Table 5.5. Three of the six heifers on the NEG diet and five of the six heifers on the POS diet became anorexic at least once over the 13 day period. All the animals showed signs of a nervous ketosis at some stage of the BD treatment. One heifer on the NEG diet and two heifers on POS diets became ketotic and were removed from the trial. The animals removed from treatment showed signs of severe nervous ketosis and were deemed unfit to continue.

5.4 Discussion

We hypothesized that if the heifers consumed a positive DCAD diet they would have a more favorable blood acid-base balance. High blood pH and bicarbonate would buffer the blood against a metabolic acidosis induced by ketosis. This would make them less susceptible to clinical ketosis. However this was not the case. Two of the three clinical cases of ketosis were in the POS DCAD group. These ketotic animals were diagnosed by the farm veterinarian as being clinically ketotic and showed classical signs of nervous ketosis (Fleming, 1996). Eight of the twelve animals became anorexic over the 13 day period with

Table 5.4 Average plasma mineral concentrations of heifers fed diets with 15% 1,3-butanediol and different DCADs over a 13 day period. [1]

	NEG[2]	POS[3]	P>F			SED[4]
			TRT	DAY	TRT*DAY	
Prefeeding						
Sodium, mmol L^{-1}	137.3	139.7	0.45	0.24	0.94	0.2
Potassium, mmol L^{-1}	4.2	4.3	0.16	<.001	0.39	0.1
Chloride, mmol L^{-1}	94.4	93.3	0.18	0.06	0.17	0.7
Sulfate, mmol L^{-1}	2.2	1.7	0.01	0.58	0.27	0.1

[1] DCAD : mEq $(Na^+ + K^+)-(Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +372 mEq kg^{-1} DM, n=6
[4] Standard error of the estimated difference

Table 5.5 Incidence of anorexia and induced clinical ketosis in heifers fed diets with 15% 1,3-butanediol and different DCADs over a 13 day period. [1]

	NEG[2]	POS[3]	Total
Anorexia			
# heifers	3/6	5/6	8/12
%	50.0	83.3	66.7
Ketosis			
# heifers	1/6	2/6	3/12
%	16.7	33.3	25.0

[1] DCAD : mEq $(Na^+ + K^+) - (Cl^- + S^{2-})$ kg^{-1} dietary dry matter (DM)
[2] Ration with a DCAD of -161 mEq kg^{-1} DM, n=6
[3] Ration with a DCAD of +372 mEq kg^{-1} DM, n=6

the larger proportion from the POS DCAD (five of six on POS DCAD and three of six on NEG DCAD). This is contrary to what we had hypothesized. The lack of difference between the two diets may have been due to a butanediol-DCAD interaction, however, the study was not designed to investigate this interaction.

The physical behavior (bizarre neurological signs) of the heifers fed BD was consistent with that of nervous ketosis (Fleming, 1996). Observed clinical signs were acute and consisted of excessive grooming, pica, excessive salivation, bellowing, and moderate to severe tremors. These clinical signs were similar to those described by Hess and Young (1972) who fed greater than 6% BD to ruminating calves and observed hyperactivity, muscle tremors and reduced gain. Bonner et al. (1975) reported that five calves fed 15 to 20% of their grain with BD (estimated about 8-10% BD on DM basis) were hyperactive, nervous, urinated profusely and exhibited muscle tremors after a week.

Blood glucose concentrations in heifers on NEG and POS diets were 81.1 and 82.8 mg dL^{-1}, respectively, values which are higher than the 45-60 mg dL^{-1} reported by Bonner et al. (1975). In their study the animals had high acetoacetate + acetone concentrations (24-97 mg dL^{-1}) and low BHBA concentrations (4-6 mg dL^{-1}) when fed the high BD diets (10-20% BD in the grain). Animals fed BD at 5 and 10 % of grain had much lower acetoacetatae + acetone levels (4-22 mg dL^{-1}) and higher BHBA concentrations (9-27 mg dL^{-1}). Total ketone body concentration was consistent with the diet (high BD in diet had high total ketones) but the shift in acetoacetate + acetone to BHBA is unexplained. Total ketone bodies are not comparable in our study because we did not measure acetone. The distinctive ketone breath was not apparent in our animals, and ketone sticks (Chemstrip®, Boehringer Mannheim, Canada) were negative except for a brief period between day four and six.

Animals were introduced to BD over a five day period (two days at 5%, then two days at 10% and by day five, 15% of dry matter intake was BD). Heifers

were gradually introduced to the BD because of the potential for feed refusal (personal communication with J.W. Young). The heifers also consumed diets high in anionic salts (1.8% DM). The animals did not have a problem with palatability because they readily consumed the ration with 5 and 10% BD. However at 15% BD the animals did become anorexic. A number of factors may have contributed to this. Two thirds of the animals became anorexic during the trial. Three animals were diagnosed as clinically ketotic (nervous ketosis) and went off feed. These animals were removed from trial. The remaining five animals were anorexic and may have gone off feed due to the induced ketosis.

A likely possibility is that a portion of the animals reduced feed intake because of the high energy content of the feed. BD has a high metabolizable energy of 6 to 6.6 kcal/g (Miller and Dymsza, 1967; Davenport and Griffith, 1969) and animals may have eaten to fulfill energy requirements. We had anticipated that this might occur and hoped that the animals would become ketotic before this happened (Bonner et al., 1975; personal communication with J.W. Young). However we then had the problem of animals consuming inadequate quantities of feed. This prevented us from maintaining the acid-base profiles that we had originally established. The animals were also not consuming sufficient BD to cause clinical ketosis. Therefore, a number of animals were in subclinical ketosis (chronic metabolic stress).

Interestingly, as well as showing signs of nervous ketosis, the three animals that were removed from trial appeared to be severely anorexic. The anorexia may have been due to a lack of ability to physically coordinate the effort required to consume feed. One animal had severe signs of nervous ketosis but did not go off feed. Three heifers had severe episodes of nervous ketosis yet managed to recover within an 8 to12 hour period. They showed mild to moderate tremors but went back on feed allowing us to keep them on trial. An attempt was made to take weekly weights but this became unmanageable with nervous animals. We also wished to avoid putting them into a ketotic state simply through

handling.

A number of things could be done to improve similar trials in the future. Animals could be pretreated for five to seven days to establish the appropriate acid-base profile. Inclusion of BD into the ration could be done more quickly (5%, 10%, and 15% by day three). Alternatively animals could receive 10% BD throughout the whole BD trial period. Energy requirements of the BD rations could also be better matched to the animal's needs. This would allow them to consume a sufficient quantity of mineral salts to maintain differences in acid-base balance and have an adequate intake of BD to maintain higher levels of ketone bodies.

5.5 Conclusion

Heifers became subclinically and clinically ketotic after five days of consuming a diet containing 1,3-butanediol. Animals demonstrated signs of nervous ketosis most notably hyperactivity and moderate to severe muscle tremors. Feeding a positive (POS) DCAD and having a higher blood pH and bicarbonate did not prevent heifers from becoming clinically ketotic. In fact it may have made them more prone to ketosis because they consumed feed at a faster rate as compared to heifers fed a negative (NEG) DCAD. This may have given them a higher plasma concentration of ketone bodies.

VI. General Conclusion

Dairy heifers fed rations with differing DCAD demonstrated differences in DMI. Feed intake decreased during the first week and increased over the second week in the NEG DCAD group indicating an adaptation. Decreases and increases in DMI tended to correspond with fluctuations in blood pH and bicarbonate. Feed palatability or metabolic acidosis or a combination of the two may influence dry matter intake in dairy heifers when feeding anionic salts.

Dairy heifers became subclinically and clinically ketotic after five days of consuming a diet containing 1,3-butanediol. Animals demonstrated signs of nervous ketosis most notably hyperactivity and moderate to severe muscle tremors. Feeding a positive (POS) DCAD and having a higher blood pH and bicarbonate did not prevent heifers from becoming clinically ketotic. In fact it may have made them more prone to ketosis because they consumed feed at a faster rate as compared to heifers fed a negative (NEG) DCAD. This may have given them a higher plasma concentration of ketone bodies and led to subclinical and clinical ketosis.

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