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THE EFFECTS OF DIETARY CATION-ANION BALANCE, STAGE OF LACTATION AND RATION INGREDIENTS ON ACID BASE METABOLISM AND PRODUCTIVITY OF DAIRY COWS.

by

ANNICK MARIE DELAQUIS

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SUGGESTED SHORT TITLE: Dietary cation-anion balance and acid-base status of dairy cows

Dedicated to:

My fiancé, Daniel,
and my parents, Marie and Roger ,
for their love and encouragement.

Abstract

Doctor of Philosophy Animal Science (nutrition)

Annick Delaquis

THE EFFECTS OF DIETARY CATION-ANION BALANCE, STAGE OF LACTATION AND RATION INGREDIENTS ON ACID BASE METABOLISM AND PRODUCTIVITY OF DAIRY COWS.

Experiments were conducted to investigate the effects of formulating rations using different ingredients and of manipulating the cation-anion balance of the rations on production, acid-base status, metabolism of macrominerals and renal function of dairy cows. The effects of dietary cation-anion balance were studied during early, mid and late lactation as well as during the dry period. Feeding an alfalfa-haylage based diet *vs* a corn silage based diet, both having the same cation-anion balance did not affect the voluntary consumption, milk yield or milk composition of cows in early lactation. The acid-base status of the animals was not affected by dietary treatment. The lower urinary bicarbonate concentration observed with the alfalfa haylage-based diet was not associated with a lower total urinary bicarbonate excretion since urine volume was significantly higher than when cow were fed the corn silage-based diet. Manipulating dietary cation-anion balance , however, resulted in changes in acid-base status at all stages of lactation studied. Urinary bicarbonate concentration and total daily excretion were increased by a higher dietary cation-anion balance at all stages of lactation. Similar effects of dietary cation-anion balance on urinary bicarbonate did not, however, translate into similar changes in production or intake by cows at differing stages of lactation. Cows in early and mid lactation seemed to have benefited more from a highly positive dietary cation-anion balance than cows in late lactation or dry period.

Résumé

Docteur en Philosophie Zootechnie (nutrition)

Annick Delaquis

EFFETS DE LA BALANCE ALIMENTAIRE CATION-ANION, DU STADE DE LACTATION ET DES INGREDIENTS DE LA RATION SUR LE METABOLISME ACIDO-BASIQUE ET LA PRODUCTIVITE DES VACHES LAITIERES

Cinq expériences ont été conduites pour étudier les effets des ingrédients utilisés pour formuler les rations et les effets de la balance alimentaire cation-anion sur la production, l'équilibre acido-basique, le métabolisme des macrominéraux et la fonction rénale des vaches laitières. Les effets de la balance cation-anion ont été étudiés en début, milieu et fin de lactation ainsi que durant la période de tarissement. Lorsqu'une ration à base d'ensilage de maïs fût comparée à une ration à base d'ensilage de luzerne aucune différence ne fût observée en termes de consommation alimentaire, production ou composition du lait. La plus faible cocentration urinaire de bicarbonate observée avec la ration à base d'ensilage de luzerne ne fût pas accompagnée par une plus faible excrétion totale car le volume urinaire total fût augmenté par cette ration. La manipulation de la balance alimentaire cation-anion a affecté l'équilibre acido-basique des vaches à tous les stades de production. A tous les stades de lactation la balance alimentaire cation-anion a eu des effets similaires sur l'excrétion de bicarbonate. Ces-derniers n'ont pas toujours résultés en des effets similaires sur les paramètres de production pour tous les stades de lactation. Les vaches en début et milieu de lactation ont plus bénéficié d'une balance plus positive que les vaches en fin de lactation ou les vaches en période de tarissement.

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TABLE OF CONTENTS

LIST OF TABLES

I. GENERAL INTRODUCTION

Dietary cation-anion balance (DCAB) is defined as the summation of the milliequivalents of sodium (Na^+) and potassium (K^+) minus the milliequivalents of chloride (Cl^-) and sulfur (S^{-2}) per kilogram (kg) of dry matter (DM) in the ration. (DCAB= $mEq(Na^+ + K^+ - Cl^- - S^{-2})\ kg\ DM^{-1}$).

Since the early 1970's the concept of controlling the cation-anion balance (CAB) of rations used in animal production has gained more and more interest and has generated a number of studies in ruminant, poultry and swine nutrition. The modification of the concentration of a cation in a ration implies the simultaneous modification of the concentration of at least one anion so that electrical neutrality of an organism is maintained (Block, 1988a). When a living organism absorbs a cation it must absorb an anion with it or excrete a cation in exchange to maintain equality between the number of positive charges and the the number of negative charges. This principle does not imply that local potential differences cannot be created but, taken as a whole, an organism is electrically neutral. In theory DCAB could include the summation of all cations minus the summation of all anions (Mongin 1981). However the 4 minerals mentioned above have been selected because of their direct involvement in the maintenance of acid-base and osmotic balance (Mongin 1981, Block 1988) and the determination of membrane potentials. The maintenance of acid-base balance is important for every cell's metabolism since each enzyme has an optimal pH for maximal activity. The maintenance of osmolarity and fluid volume are also critical since they influence the concentrations of metabolites important for enzymatic activity.

Ion transport systems, used by epithelial cells in particular, further support the idea that these minerals cannot be studied independently from one another. For example in the absorptive intestinal epithelial cell, a model for the electroneutral transport of NaCl has been proposed to involve a Na^+/H^+ antiport and a Cl^-/HCO_3^- antiport coupled by intracellular pH (Murer et al., 1976; Reuss, 1984; Liedke and Hopfer, 1982); the absorption of $S^=$ by intestinal cells and its reabsorption by renal cells occurs by a $Na^+/SO_4^=$ cotransport system (Conway et al., 1989, Renfro et al.,

1989); the basolateral membranes of certain epithelial cells also contain a bicarbonate anion exchanger which can, in certain circumstances, carry Cl^- or $SO_4^=$ (Pritchard and Renfro 1983).

To date, the manipulation of DCAB for ruminants has focused on the determination of levels that will reduce the incidence of milk fever and on how it affects calcium metabolism of the periparturient cows (Block, 1984, Dishington, 1975, Wang and Beede, 1992, Gaynor et al., 1989). Studies in lactating animals have recently been reported and have shown promising results in terms of feed consumption and milk production.

The work presented in this thesis was directed towards gaining more information on important dietary determinants of acid-base balance and on the effects of DCAB on renal function, production and mineral metabolism of dairy cows at different stages of production.

II. LITERATURE REVIEW

1. PREVENTION OF MILK FEVER

A. Milk fever

The first studies published on the potential importance of considering DCAB when formulating rations for dairy cows have focused on the role this ratio could play in the etiology of hypocalcemic parturient paresis (milk fever). At the onset of lactation, cows have to face an important drain of calcium (Ca). The secretion of 23g of Ca in 10 L of colostrum represents six times the amount of Ca present in the extracellular pool (Goff et al. 1987). It has been estimated that, as a result of producing 25 kg d^{-1}, an animal's blood Ca pool must be replaced every hour (Horst, 1986). The onset of lactation is invariably associated with some degree of hypocalcemia and negative Ca balance as the demand for Ca changes from fetal development to milk production, which is of greater significance in view of the selection and management programs designed to maximize production (Naito et al., 1990; Ramberg et al., 1984).

Concentration of blood Ca being tightly regulated, animals will usually adapt to the periparturient hypocalcemia caused by the increased demand for Ca secretion in the milk (Ramberg et al., 1984). Some cows, however, will not adapt, not because of greater amounts of Ca secreted in the colostrum but probably because of inadequate compensating mechanisms (Goff et al., 1989; Horst 1986; Ramberg et al., 1984). In these cows the hypocalcemia will be severe and they will develop milk fever. Since milk fever affects about 5% of the cows in the United States (Horst, 1986) and since it leads to economic losses in the short (subsequent lactation) (Block, 1984) and long terms (shortened productive life) (Curtis et al., 1983) much research has been directed towards the development of prophylactic management practices. Among those are the feeding of a diet low in Ca prepartum (Goings et al., 1974; Wiggers et al., 1975; Kichura et al., 1982), injections of various vitamin D (vit D) metabolites prepartum (Hodnett et al., 1992; Gast et al., 1979, 1977, Jorgensen, 1974) and the reduction of the DCAB (Dishington, 1975; Block, 1984; Oetzel et al., 1988; Gaynor et al., 1989).

B. Low calcium diets

High dietary concentrations or intakes of Ca and P during the dry period increases the risks of milk fever (Boda and Cole, 1954; Goings et al., 1974, Beitz et al., 1974; Wiggers et al., 1975). Green et al. (1981) studied the effects of feeding two levels of dietary Ca , 8 or 80 g d^{-1}, to dry cows beginning at least 14 d before expected calving. The cows that had consumed the low Ca diet for a few days had increased plasma concentrations of 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) and decreased concentrations of Ca and P. At parturition, blood Ca fell to lower values for the cows that had consumed the high Ca diet prepartum; this explains why they had higher plasma concentrations of 1,25-$(OH)_2D$ the first 2 d postpartum. Just prior to parturition, cows on the low Ca diet were also observed to have lower plasma concentrations of 24,25-dihydroxyvitamin D (24,25-$(OH)_2D$) compared to the animals on the high Ca diet. The latter could have resulted from an increased activity of the 1-alpha-hydroxylase of the kidneys converting 25-hydroxyvitamin D (25-(OH)D) to 1,25-$(OH)_2D$ diminishing the quantity of substrate available to the other vit D hydroxylating systems (Goff et al., 1991). From these observations, Green et al. (1981) concluded that a low Ca intake prepartum reduces the incidence of milk fever by turning on the Ca homeostatic machinery before parturition and thus before the onset of the high Ca demand for lactation. Kichura et al. (1982) came to the same conclusions in terms of the mode of action of low Ca diets prepartum despite different levels of P inclusions. Low dietary P levels could have some beneficial effects when prepartal diets contain high levels of Ca. Low dietary P has been suggested to cause an increase in 1,25-$(OH)_2D$ in the intestinal mucosa in the chick without altering the plasma concentration of the hormone (Sommerville et al., 1978).

The suggestion that reduced dietary Ca helps reduce the risks of milk fever by activating the vit D homeostatic control mechanism is supported by results of Shappell et al. (1987) who observed beneficial effects of low dietary Ca prepartum in cows with 3 or more pregnancies but not in heifers. These young animals almost never develop the disease because they adapt more quickly to changes in Ca demand (Goff et al., 1991). The more rapid adaptation by heifers to hypocalcemia may be related to the fact that since they are still growing, their homeostatic mechanism for Ca is already stimulated or, because renal tissues are more sensitive to stimulation by

parathyroid hormone (PTH) at a younger age, as has been observed in rats and humans (Fox and Mathew, 1991; Marcus et al., 1984). When injected with physiological levels of PTH, older adult rats had a significantly lower response in terms of 1,25-$(OH)_2D_3$ and plasma concentration of Ca compared to young animals probably due to failure of the renal 1-hydroxylase to respond to PTH stimulation (Fox and Mathew, 1991). The inability of PTH and vit D system to restore the concentration of Ca in the blood of older animals may explain the increases in plasma concentrations of these hormones with age (Fox and Mathew, 1991). These authors did not observed a reduction of the metabolic clearance rate of PTH, thus elevated levels can be attributed to increased synthesis.

Feeding diets low in Ca to older cows may help circumvent their decreased ability to cope with rapid changes in Ca metabolism probably not so much by increasing bone resorption which does not appear to contribute much to Ca homeostasis around parturition (Van de Braak and Van't Klooster, 1987) but by increasing their capacity for intestinal Ca absorption via stimulation of 1,25-$(OH)_2D$ production (Fox et al., 1990). Note that both chronic infusions of 1,25-$(OH)_2D$ and low Ca diets appear to increase the metabolic clearance rate of 1,25-$(OH)_2D$ but not as much as they increase its production resulting in higher serum concentrations of 1,25$(OH)_2D$ (Fox et al., 1990, Halloran and Castro, 1989). It can also be hypothesized that a diet low in Ca prepartum could help prevent milk fever by increasing the clearance rates of 24,25 and 25,26-$(OH)_2D$ proportionally more than their production rates resulting in lowered plasma concentrations of these vit D metabolites (Fox et al., 1990). The latter can have detrimental effects as will be mentioned later. Although effective in preventing milk fever, the feeding of a diet low in Ca prepartum may not always be practical because of the high Ca content of legume forage and some grass hays, which normally constitute a major fraction of the dry cow ration (Oetzel et al., 1991).

C. Vitamin D metabolites

The use of vit D has been successful in preventing milk fever. However, toxicity remains a major risk factor of repeated injections (Littledike and Horst, 1982). Blood concentrations of 1,25-$(OH)_2D_3$ have been reported to reach 1000 pg

mL^{-1} 24 h after intramuscular injections and to fall to undetectable levels 3 d after injection (Hove et al., 1983). This implies that the injection must be administered within the four days preceding calving and thus requires accurate prediction of the calving date (Hoffis et al., 1978). In addition to this uncertainty, the use of 1,25-$(OH)_2D_3$ can affect adversely renal function and cause severe hypercalcemia (Naito et al., 1989; Goff et al., 1986) since it promotes intestinal Ca absorption and bone resorption before calving thus prior to the actual increase in Ca demand has occurred (Costa et al., 1985, Naito et al., 1989, Goff and Horst, 1990). It has been observed in pigs that intravenous administration of 1,25-$(OH)_2D_3$ causes increased plasma concentration of calcium- binding- protein (CaBP) correlated with intestinal levels of CaBP (Maunder et al., 1987). CaBP is thought to facilitate the intracellular transport of Ca from the luminal membrane to the basolateral membranes of the intestinal epithelial cells thus explaining some of effects of vit D on intestinal Ca (Horst, 1986). Alteration of membrane lipid composition of epithelial intestinal cells by 1,25-$(OH)_2D_3$ has also been proposed as a mechanism by which vit D can modify Ca transport (Bellido et al., 1987, Matsumoto et al., 1981).

Although promising results have been obtained recently with metabolites of vit D_3 namely 24F-1,25-dihydroxyvitamin D_3 (Goff and Horst, 1990) and a combination of 1 alpha hydroxyvitamin D_3 and 25-OH-D_3 (Hodnett et al., 1992) in terms of avoiding toxic effects while reducing the degree of hypocalcemia associated with the onset of lactation, the problem of delayed hypocalcemia once the injected vit D has been metabolized still remains. Hodnett et al. (1992) observed that 14 d after parturition ,treated cows had lower plasma concentration of 1,25-$(OH)_2$-D_3 than the untreated cows, and they attributed this to a depression in the activity of the renal 1-alpha-hydroxylase enzyme which is responsible for the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3. The effectiveness of the vit D metabolites also appears to be dependent upon the Ca content of the ration being more effective at higher concentrations (Hodnett et al., 1992).

Even though vit D injections appear to provide some degree of protection against milk fever the disease is not associated with reduced plasma concentrations of 1,25-$(OH)_2$-D_3, or PTH in the periparturient period (Horst et al., 1979; Kichura et al., 1982; Shappell et al., 1987); conversely paretic animals tend to have elevated plasma concentrations of these two calcitropic hormones (Horst et al., 1978). The

only exceptions are the cows who have relapses and show an impaired capacity to synthesize 1,25-$(OH)_2$-D_3 (Goff et al., 1989). In addition to being hydroxylated at the number 1 and 25 positions, vit D can also undergo hydroxylation at positions 23,24 and 26. These pathways appear to be of greater importance when the stimulation of the 1-alpha-hydroxylase enzyme of the kidneys is minimal (Goff et al., 1991). Although these metabolites have not always been measured in plasma of cows injected with 1,25-$(OH)_2$-D_3 increased concentrations can result (DeLuca, 1981) and could have adverse effects. In fact Barton et al. (1984) observed an increase incidence of milk fever in animals treated with 24,25-$(OH)_2D_3$. In rats large doses of 25,26-$(OH)_2D_3$ can reduce the serum concentrations of 1,25-$(OH)_2D_3$ (Zerwekh et al., 1987). Until better understanding of the vit D hormonal system is gained and until full characterization of the effects of injecting vit D metabolites is obtained the widespread use of such injections to prevent milk fever appears uncertain specially since deficiencies in 1,25-$(OH)_2D_3$ or PTH synthesis are not the cause of milk fever. The problem rather appears to be one of end-organ resistance to stimulation by these hormones.

Typically, older cows are at more risks of developing milk fever. These animals have fewer intestinal and bone cell receptors for 1,25$(OH)_2D_3$ which impairs\ their ability to respond to an increased demand for Ca by increasing intestinal absorption and bone mobilization (Horst et al., 1990). Abnormality in PTH-related peptide (PTH-RP) which interacts with PTH receptors cannot be disregarded (Horst et al., 1991). The discovery of this hormone which is synthesized in association with pregnancy and lactation (Horst et al., 1991, Turner et al., 1988, Thiede and Rodan, 1988) is recent, and all of its physiological roles have not been established. It has however been associated with cancer hypercalcemia but it may also play an important role in normal homeostatic control of Ca metabolism associated with the reproductive cycle (Goltzman et al., 1981, Moseley et al., 1987). The implication of PTH-RP in the etiology of milk fever is still purely hypothetical.

The results obtained suggest a definite decreased sensitivity to Ca regulating hormones in paretic animals and not a deficiency in hormone synthesis in response to hypocalcemia. Thus the method of choice to reduce the incidence of milk fever would be one that can counteract resistance to this hormone in milk fever. This method could be the manipulation of the DCAB of the ration prepartum.

Manipulating the DCAB of the prepartum diet to reduce the incidence of milk fever offers the advantage that it is effective even with diets high in Ca, thus permitting the utilization of legume forages (Gaynor et al., 1989, Oetzel et al., 1988) and it reduces the hormonal resistance to PTH observed precalving without requiring the injections of supraphysiological doses of vit D or its metabolites (Goff et al., 1991).

D. Dietary cation-anion balance (DCAB)

The first studies which stimulated interest in this area were those of Ender et al. (1971) , Dishington (1975) who observed reductions in the incidence of milk fever when cows were fed a ration with a negative DCAB prepartum. Subsequently, several other researchers have confirmed the results (Goff et al., 1991; Gaynor et al. 1989; Oetzel et al., 1988). A recent analysis of all studies having examined the nutritional factors involved in the etiology of milk fever established the DCAB and the S content of the ration as the two most important nutritional elements; note that since they are correlated it is difficult to establish if their effects are independent (Oetzel 1991).

Feeding a diet prepartum with a negative DCAB reduces the severity of the hypocalcemia associated with the initiation of lactation in terms of length of time during which blood Ca is depressed, the minimal concentration of Ca reached in the blood, as well as the time at which this minimal concentration is reached postpartum. Cows offered a diet with a positive DCAB prepartum (+300 mEq kg^{-1}) had depressed blood concentrations of Ca for at least 3 d following parturition and lower minimal concentrations compared with cows which had been consuming a ration with a negative DCAB prepartum (-129 mEq kg^{-1}) (Block 1984). Similar observations were made by Leclerc and Block (1989) and Goff et al. (1991).

A negative DCAB prepartum seems to prevent drastic changes in normocalcemia even when the prepartum ration has a high concentration of Ca. Oetzel et al. (1989) concluded that the DCAB of the ration of the dry cow was more important than its Ca content in determining the concentration of Ca in the blood close to parturition. There are three major ways by which the negative DCAB could help maintain blood Ca concentrations: 1- by increasing the intestinal absorption of

Ca; 2- by increasing bone resorption (or decreasing bone accretion) rate; 3- by reducing urinary excretion of Ca (Goff et al., 1991). The third possibility can be eliminated as a source of Ca since an increase, rather than a decrease, in urinary Ca excretion was observed with a reduction in DCAB in cows, sheep and goats (Takagi and Block, 1991a, Gaynor et al., 1989, Fredeen et al., 1988a). The effects on intestinal Ca absorption are still unclear since decreases in apparent absorption, no effect and increases have been observed with a reduction in DCAB by different authors (Leclerc and Block, 1989, Takagi and Block, 1991a). However, in sheep and goats, reductions in DCAB have been demonstrated to increase the true Ca intestinal absorption (Takagi and Block 1991c, Fredeen et al., 1988b). DCAB may also affect bone metabolism. A reduction in DCAB causes: increases in plasma levels of hydroxyproline which indicatives a higher rate of bone mobilization (Block, 1984, Leclerc and Block, 1989, Goff et al., 1991, Gaynor et al., 1989); decreased time of recovery from a short term infusion of EDTA infusion, suggesting that the homeostatic mechanisms are effective and responsive to a drop in blood Ca (Takagi and Block 1991b); increased rates of bone mobilization and decreased rate of bone accretion measured with infusions of ^{45}Ca and EGTA (Takagi and Block 1991c).

The increased rates of bone mobilization and decreased rate of bone accretion observed when DCAB was reduced do not appear to result from an enhanced production of PTH in response to hypocalcemia but rather from an increased production of 1,25-$(OH)_2D$ per unit of PTH secreted (Goff et al., 1991). The manipulation of the DCAB may affect Ca homeostasis by overcoming the tissue resistance to PTH observed in early lactation as discussed before. Fredeen et al. (1988), working with pregnant or lactating does suggested that the effects of the DCAB on Ca metabolism were mediated by alterations of the acid-base status of the animals.

A more positive DCAB (higher than 85 mEq $100g^{-1}$ calculated as mEq Na + mEq K - mEq Cl) offered to female goats caused a metabolic alkalosis and a reduction in the rate of bone mobilization whereas a diet with a DCAB of less than 10 mEq/kg was associated with a metabolic acidosis, hypercalciuria and increased bone Ca mobilization (Fredeen et al., 1988). The supplementation of the ration of non-pregnant non-lactating cows with different anionic salts ($MgCl_2$, $MgSO_4$, $CaCl_2$, NH_4Cl or $(NH_4)_2SO_4$) reduced their blood bicarbonate and base excess levels

indicative of a mild compensated metabolic acidosis (Oetzel et al., 1991). Tucker et al. (1991b) incorporating increasing amounts of $CaCl_2$ in the ration of heifers while keeping the dietary Ca concentration constant, found a linear increase in blood H^+ and decrease in blood bicarbonate (HCO_3^-) and an increase in urinary free H^+ and Ca excretions. Working with rats fed different Cl^- and $SO_4^=$ salts Whiting and Cole (1986) observed a decrease in urine pH, increased ammonium (NH_4^+) excretion and increased net acid excretion when animals were offered these salts instead of some carbonate salts. When considering the different rates of absorption of Cl^- vs $S^=$ both these minerals were found to have the same impact on urinary Ca excretion; both caused hypercalciuria to a degree related to their effect on acid base status.

An acid stress affects Ca metabolism, and especially urinary Ca excretion. The inclusion of NH_4Cl in the ration of sheep (which would cause a reduction of the DCAB), increased their intestinal Ca absorption as well as their urinary Ca excretion (Brathwaite 1972). The same salt given to rats increased bone mobilization and urinary Ca excretion (Barzel and Jowsey, 1969). The effects of acidosis on bone resorption are supported by *in vitro* results which demonstrated an enhanced activity of osteoclasts as the pH of the medium was reduced from 7.4 to 6.8 causing a 14 fold increase in bone resorption (Arnett and Dempster, 1986). Acidosis or a reduction in blood pH also causes a release of Ca from the amorphous minerals (Ca carbonates and Ca phosphates) on bone surfaces (Bushinsky et al., 1985). Although bone mobilization may not play an important role in buffering the acid load it certainly can help maintain normocalcemia.

The effect of acidosis on renal handling of Ca is still not well understood. Some authors proposed that acidosis augments the effect of PTH on renal Ca reabsorption but not enough to compensate for the independently increased excretion (Beck and Webster, 1976). Since serum Ca is increased during acidosis presumably the amount of filtered Ca should increase provided no changes in renal blood flow occur. An increased filtered load may contribute to the increased excretion (Webster and Beck, 1976). However it has been demonstrated in dogs that renal distal tubules have a PTH independent mechanism of Ca reabsorption which is stimulated by acidosis and inhibited by alkalosis (Sutton et al., 1979). It was observed that a reduction in the DCAB of the ration of nonpregnant non-lactating animals alters not only their acid-base status; it also seems to affect renal function as

suggested by increased and decreased fractional excretion of Ca and Mg with a reduction in the DCAB (Wang and Beede, 1992). However, these animals and those of Tucker et al. (1991), were not pregnant as would normally be the case in dry dairy cows. Additionally their dry matter intake (DMI) was restricted to a fixed amount, thereby eliminating some potential effect of DCAB on DMI and subsequently on acid-base balance and renal function. Neither water absorption nor urine volume were monitored. If renal handling of Ca and Mg are altered so may some other minerals affected, known to play a role in acid-base and fluid homeostasis.

Pregnancy also affects renal function by significantly altering blood volume and renal glomerular filtration rates (Lindheimer and Katz, 1985). Fetal demands being greatest at the end of pregnancy, will in themselves affect Ca homeostasis and possibly the homeostasis of other minerals. Thus, it has been demonstrated that modification of the DCAB of the ration of dry cows will alter: the sensitivity of different organs to the regulators of Ca homeostasis; renal handling of different minerals and acid-base status. It is likely that the latter plays a role in mediating the changes observed in Ca and mineral metabolism and perhaps some of the changes observed in renal function since the kidneys are known to be directly involved in the metabolic regulation of acid-base equilibrium.

2. LACTATING COWS AND ACID-BASE DISTURBANCES

A. Dietary buffers

The maximization of milk production by the dairy cow requires the inclusion of feed ingredients with high energy densities and high digestibilities in order to meet the energy requirements of the animals. This has lead to the inclusion of increased proportions of concentrates and silages in the rations. These types of feed have an inherently lower buffering capacity and generally result in higher volatile fatty acid concentrations in the rumen, lower acetate-to-propionate ratio and reduced rumen pH postfeeding (Hogue et al., 1991; Arambel et al., 1988; Jasaitis et al., 1987; Donker and Marx 1985) and thus an altered acid-base status of the rumen and possibly of the host. To alleviate the acidosis-related problems including milk fat depression, several researchers have studied the effect of adding dietary buffers,

such as $NaHCO_3$ or $KHCO_3$, to the rations of dairy cowson their performance in terms of milk production, milk composition and dry matter intake (DMI).

The results obtained using rumen buffers are controversial. Vicini et al. (1988) observed an increase in DMI but no change in milk yield when 1 % $NaHCO_3$ was added to a corn silage-based ration offered to multiparous cows in a switchback design starting 70 d postpartum. Improvements in DMI with no change in milk production have also been reported by Escobosa et al. (1984) when 1.7% $NaHCO_3$ was added to a ration based on corn, cottonseed and corn silage offered to cows within their first trimester of lactation at the beginning of the experiment. However, Erdman et al. (1982) did not find any improvement in DMI (or milk production) when 1 % $NaHCO_3$ was added to a 40 % corn silage, 60 % concentrate ration offered immediately after calving for 12 wk. Results of Arambel et al. (1988) were similar to those of Erdman (1982) except that he used an alfalfa-based ration. Using corn silage based rations Rogers et al. (1985) demonstrated increases in DMI and milk production when 1.2% $NaHCO_3$ was supplemented to the diet of cows from parturition to 8 wk postpartum supporting results of Kilmer et al. (1980). Canale and Stokes (1988) observed increases in milk production when 1.25% $NaHCO_3$ was added to a hay crop-silage based ration (30% silage, 70% concentrate) but trends towards decreases were found when $NaHCO_3$ was added to a corn silage-based ration (40% corn silage, 60 % concentrate) as was observed by Erdman et al. (1982).

The lack of consensus among all these results could be related to several factors. The three most important ones appear to be the DCAB of the ration used, which were different among studies, the stage of lactation of the animals used in the different experiments, which would affect their response and the ingredients of the basal ration. Although these factors may have independent effects on the response of the cows to dietary buffers, interactions cannot be disproven at this point.

B. Dietary cation-anion balance

The lack of consensus among results from studies on the supplementation of $NaHCO_3$ to the ration of dairy cows could be explained in part by the differences in DCAB caused by such addition as well as the different DCAB of the control rations. used usedused. When studying the addition of dietary buffers one encounters a

Although the effects observed with the supplementation of $NaHCO_3$ were attributed to HCO_3^- buffering the rumen and reducing the variations in rumen pH and volatile fatty acid concentrations (Hogue et al., 1991, Aslam et al., 1991), the simultaneous addition of Na cannot be overlooked and could be more important metabolically when added to diets which result in normal milk fat in which case the need for supplemental rumen buffering is less (Block, 1988a). The DCAB of the controls could also influence the response via the initial acid-base status of the animals. Although initial studies focusing on the effects of dietary buffers on parameters of acid-base balance in ruminants (Rogers and Phillip 1986, Escobosa et al., 1984, Kilmer et al., 1981, James and Wohlt, 1985), or on milk production and DMI as previously mentioned have never considered their results as being related to DCAB per se, this possibility cannot be ruled out.

Supplementation of $NaHCO_3$ (4% of DM) compared to NaCl (2.8% of DM) resulted in significantly higher organic matter intake by sheep (Rogers and Phillip 1986). Similarly, the equivalent addition of Na in the form of $NaHCO_3$ (.75%) *versus* NaCl (.52%) resulted in increased DMI, crude protein digestibility (CPD) , acid detergent fiber digestibility (ADFD), urine pH and P and N retentions in growing Dorset lambs (James and Wohlt, 1985). For both of these studies, the effects could relate to the fact that adding $NaHCO_3$ causes an increase in DCAB whereas adding NaCl does not. Sodium bicarbonate has a DCAB close to 12 Eq kg^{-1} whereas the DCAB of NaCl is zero. An increase in DCAB translates into an alkali stress or a reduction of an acid stress (Block. 1988).

Similarly, Escobosa et al. (1984) compared a high Na diet (1.7% $NaHCO_3$), a high Cl diet (2.28% $CaCl_2$) and a control diet. Significant increases in DMI, body weightand percentages of milk fat and protein as well as trends toward increased milk production for dairy cows on the high Na diet compared with those on the high Cl^- or on the control diet. Since the high Na diet had a DCAB of +320 mEq kg^{-1}, the control diet a DCAB of +168 mEq kg^{-1} and the high Cl diet a DCAB of -191 mEq kg^{-1} the results could have resulted from an increase in DCAB. Note that for that study DCAB was calculated as mEq ($Na^+ + K^+ - Cl^-$) kg^{-1} the S content of the diet was not mentioned. These results disagreed with those of Fettman et al. (1984) who observed decreases in DMI, body weight and milk production for cows fed a low Cl

diet (DCAB of + 277 mEq kg^{-1} calculated without S) *versus* a medium Cl (DCAB +237 mEq kg^{-1}) or a high Cl (DCAB +177 mEq kg^{-1}) diet with no consistent differences between the medium and high Cl diets. Although in both studies cows in early lactation were used, there were differences between the control diets. Escobosa et al. (1984) used a diet based on corn grain, cottonseed meal and corn silage and Fettman et al. (1984) used a diet based on corn silage, shelled corn and soybean meal. More importantly, cows fed the low Cl diet (high DCAB) in the study of Fettman et al. (1984) were probably suffering from a Cl deficiency. This would override the effect of altering DCAB (Block 1988) and may explain why cows performed better as the Cl content of the diet was increased.

It is also interesting to note that in the study of Escobosa et al. (1984), the high Na diet (highest DCAB) resulted in higher blood pH, HCO_3 and pCO_2 than obtained with the high Cl diet suggesting that buffers or DCAB can alter the blood acid-base parameters of lactating cows as well as dry cows. Studies with rats have also demonstrated the importance of considering DCAB when studying the effects of different inorganic salts on mineral and acid-base balances (Greger et al., 1991). The addition of different salts having a DCAB of 0 (NaCl, Na_2SO_4) to the diet of rats caused no change in urine pH and urinary NH_4^+ excretion compared to rats for whom no salt was added to the diet. The addition of $NaCO_3$ or $NaHCO_3$ (salts having a positive DCAB) resulted in a higher urinary pH and lower urinary NH_4^+ excretion compared to controls or animals consuming the salts having a CAB of 0. The same differences were observed with K salts. These results also suggest that Na and K salts or Cl and S salts have a similar impact on acid-base status supporting the greater importance of the DCAB *versus* the individual ions for that physiological function provided none of these mineral is present in deficient or toxic amount which will override this effect (Block, 1988). It can also be hypothesized that certain metabolic functions may be more affected by the amounts of Na and K or their ratio than by DCAB, however, in terms of acid-base balance in dairy animals fed usual diets, DCAB appears very important.

Researchers have reported some effects of NaCl on urinary Ca and PTH function in rats (Goulding and Gold, 1986, Goulding and McIntosh, 1986) but these results were obtained with a diet containing 8% NaCl, therefore 3 % added Na, levels which are rarely encountered in ruminant rations . Although the National

Research Council (NRC 1988) does not set any level of Na toxicity probably because of the difficulty in defining signs of toxicity for that mineral, 3% is several times higher than the minimal requirements which range from 0.10 to 0.18 %, depending on the physiological status of the animal. Although not as numerous as studies looking at the effect of DCAB on dry cow metabolism, a few studies have been published on the effects of altering DCAB for lactating animals. All demonstrated effects on the acid-base status of the animals (Tucker et al., 1991, 1988a,b; West et al., 1991).

West et al. (1991) observed a linear and quadratic effects in milk yield and DMI, respectively, of increasing DCAB regardless of environmental temperature. Four DCAB calculated as mEq(Na^++ K^+- Cl^-) kg^{-1} DM ranging from -79 to +324 were included in the experiment . The basal diet used was based on corn silage and animals had been in lactation for at least 87 d. Blood pH and HCO_3 concentration were also increased by higher DCAB, however, there was no mention as to when the samples were taken in relation to feeding. Results of Tucker et al. (1991) confirmed an increase in milk production as DCAB was increased from 0 to +300 mEq (Na^++ K^+- Cl^-- S^{-2}) kg^{-1} DM in a corn silage or sorghum silage based ration offered to cows in mid-lactation at the beginning of the experiment. Changes in urine pH were also reported (Tucker et al., 1991a; Tucker et al., 1988a) which increased with increasing DCAB but results were based on only one urine sample taken 4 h postfeeding. There are as yet no measurements of total H^+ excretion in the urine or in the milk of cows consuming different DCAB. Milk, having an acidic pH could contribute significantly to the acid excretion of lactating animals (Anderson 1992) but there is no information available on the possibility of altering milk H^+ secretion of dairy cows by dietary manipulations (Anderson 1992).

Dietary buffers and DCAB could, in addition to altering the acid base status of the cows, have some hemodynamic effects. Increases in water intake and urine volume have been observed with $NaHCO_3$ supplementation or higher DCAB (Escobosa et al., 1984; Erdman et al., 1982; Kilmer et al., 1981). Escobosa et al. (1984) have also reported a lower count of red blood cells and lower hemoglobin concentration for cows offered the diet with a high Na concentration or the diet with a positive DCAB *versus* cows fed the diet high in Cl or the diet having the negative DCAB. These observations suggest that a more positive DCAB can cause some

degree of hemodilution. However, no water balance trials have been conducted to study the effects of DCAB on fluid retention by dairy cows. Changes in water consumption and urine volume are indicators of possible effects on renal function with the kidneys playing a major role in volume and osmolarity regulation. Boisclair et al. (1987) reported that supplementation of .75% $NaHCO_3$ reduced the time required to excrete a dose of phenolsulfophtalein (PSP) indicative of altered renal function and probably increased renal effective plasma flow. Since some of the dye remains in the blood after a single circulation through the kidneys (some remaining bound to proteins) the clearance of PSP is not as exact as the clearance of paraaminohippuric acid to estimate effective renal plasma flow. However, it is a valid comparative tool and has been validated in cattle as a measure of renal function (Valtin, 1983, Mixner and Anderson, 1958). The effect of DCAB on renal function could be direct or mediated by changes in acid-base balance. Indeed, metabolic acidosis induced by the oral administration of NH_4Cl in rats resulted in a progressive decline in cardiac output and renal and hepatic blood flows (Yudkin et al., 1976).

As was the case for dry cows, DCAB has been demonstrated to affect the metabolism and performance of lactating dairy cows. Manipulating the DCAB of the diet of lactating animals also alters their acid-base balance and renal function. It is not known, however, if the effects of DCAB would be similar for cows in early mid and late lactation. Indeed results obtained with dietary buffers indicate that the response could differ according to the stage of lactation.

C. Stage of lactation

It is apparent from the preceding discussion that dry cows benefit from a negative balance whereas lactating cows studied so far seem to benefit from a positive balance. Since metabolism and diet change through lactation and pregnancy it can be hypothesized that the response to DCAB will also differ.

The importance of considering the stage of production when studying dietary buffers or DCAB is well illustrated by results of Block (1988b) who observed that the beneficial effects of adding $NaHCO_3$ to a haylage based diet offered to cows in early lactation were maintained in mid lactation but eliminated and sometimes reversed

when the buffer was added to the ration in late lactation. The parameters affected were: milk yield, weight gain and gross efficiency of 4% fat-corrected milk production. The adverse effects of supplementing $NaHCO_3$ in late lactation may indicate that at that point cows were not subjected to any acid stress and the addition of the buffer resulted in an alkali stress . It is also possible that cows in late lactation benefit from an acid stress as do dry cows and that the buffering of this acid impairs production performance. In any case these results indicate that the response to $NaHCO_3$ supplementation depends on the stage of production and since such supplementation is equivalent to an increase in the DCAB response to the latter is also likely to differ depending on the stage of production. Rogers et al. (1985) obtained results slightly different from those of Block (1988) in that milk production and DMI were improved by $NaHCO_3$ for the first 8 wk of lactation and these effects were eliminated from wk 9 to 16. The apparent discrepancy may be related to the composition of the control diet since Rogers et al. (1985) used a corn silage based diet. The beneficial effects of the buffer still showed a dependence on the stage of lactation.

Interestingly, the effect of stage of lactation could be related to changes in acid-base metabolism as lactation and/or pregnancy progresses. Increases in blood pH, HCO_3^- concentration and pCO_2 were shown to occur as weeks postpartum went from 1 to 12 with very sharp changes between wk 9 to 12 (Erdman et al., 1982). Note that the basal diet was based on 40% corn silage as was the ration of Rogers et al. (1985). As lactation progressed animals progressively moved from a relative acidotic state (blood pH < 7.4 and HCO_3^- around 26 mEq/L) to an alkalotic state (blood pH > 7.41 and HCO_3^- > 34 mEq/L). Obviously an alkalotic animal is not as likely to benefit from a higher DCAB which would only worsen the situation. The time at which the acidotic stress of early lactation declines appears to depend on the diet composition if we consider the different time frame obtained by Block (1988) and Rogers et al. (1985).

With current management practices mid and late lactation cows are typically pregnant. The stage of pregnancy could also affect the response to DCAB since pregnancy is known to induce acid-base disturbances reflected in blood parameters of the mother. The blood pH of women increases by 2 to 4 nmol very early in pregnancy and remains elevated until term. In addition, the blood pH triggering

HCO_3 regeneration appears to be higher in pregnant than non-pregnant women suggesting a better ability to cope with an acid stress and probably a greater susceptibility to alkalosis (Lindheimer and Katz, 1985). This has never been considered in dairy cows.

D. Ingredients of the ration

The observation that the effects of dietary buffers depend on the type of diet (NRC, 1989, Erdman, 1988) is not surprising since buffering capacity will vary with each ingredient and so will the final ruminal and metabolic productions of protons. If the cow is unable to compensate for changes in H^+ production and absorption then her acid-base status will be modified and signs of acidosis or alkalosis will develop. Jasaitis et al. (1987) studied the buffering capacity of a wide variety of feed ingredients *in vitro* which were grouped into 5 categories; 1- energy feeds including grains and by-products with a CP level of less than 15%), 2- low protein feeds including feeds having 15 to 35 % CP, 3-high protein feeds those having a CP content above 35 %, 4- forages, 5-feeds which were fermented or combined with fermented ingredients during processing having an initial pH below 4.5. The energy and fermented feeds were found to have a lower titratable acidity than did the high and low protein feeds and the forages. But in terms of buffering capacity, energy feeds were found to have the lowest index, low protein and grass forages were intermediate and highest were legume forages and high protein feeds. The authors were also able to formulate diets for which buffering capacity was equivalent to the one predicted from their ingredient composition demonstrating that the measurements were also valid for complete diets. From these it is easy to see that the acid or alkali stress caused to the animal by the diet it is consuming can depend on the ingredients of the ration. A higher buffering capacity does not necessarily imply a higher pH in the rumen and/or blood but rather a better ability to resist changes in pH. Unfortunately no comparison between the buffering capacity of corn silage and haylage was made, therefore, better responses observed when dietary buffers are added to a corn silage based diet *versus* a haylage based diet cannot be attributed to the different buffering capacity of the forage source used (Kilmer et al., 1980; Chase et al., 1981; NRC, 1988; Erdman, 1988).

Supporting further the potential for the ingredients of the ration to affect acid-base status are results obtained with sheep by Asplund et al. (1980) who observed a better response to $NaHCO_3$ in terms of growth rate when the diet contained 9% instead of 12% protein. Furthermore, although they did not show significant interactions between the type of protein and $NaHCO_3$ supplementation, the latter tended to affect N retention of lambs to a greater extent when fed brewers dry grain than with linseed meal, with the effects on blood HCO_3 being greater the diet containing brewers grain (Rogers and Phillip, 1986). NaCl supplementation did not have the same effects on acid-base parameters as did $NaHCO_3$ probably because its DCAB is 0. A relationship between the acid-base status of the animal and the protein in the diet has also been shown by Thomas et al. (1980) where lactic acid addition to a silage based diet depressed DMI and urine pH of calves; this effect was inhibited by a fishmeal supplement.

3. Acid-Base perturbations and metabolism

Briefly, results obtained in humans indicate that blood pH is normally maintained at 7.40 ± 0.04, blood HCO_3 at 25 ± 1 mmol L^{-1}, and pCO_2 at 40 ± 4 mmHg (Emmett and Seldin, 1989). Metabolic acidosis is characterized by a decreased HCO_3^{-1} concentration and a fall in blood pH, metabolic alkalosis by an increase in HCO_3^{-1} and an increase in blood pH, respiratory acidosis results from an increased CO_2 tension and a drop in blood pH and, respiratory alkalosis from a reduction in CO_2 and an increase in pH (Emmett and Seldin 1989). However because blood indicators of acid-base status are so tightly regulated mild perturbations will be compensated by cellular and blood buffers as well as by renal and respiratory functions making the diagnosis more difficult. To evaluate the renal response to an acid or alkali load urine analysis should be performed (Laski and Kurtzman, 1989).

Changes in extracellular fluid or blood acid base parameters can alter intracellular pH which can cause, depending on the severity and cells involved, changes in cellular metabolism since every enzyme has an optimal pH (Boron, 1985). Consequently perturbations of acid-base equilibrium can alter almost any metabolic

reaction. It is also known that certain enzymes are more sensitive to changes in HCO_3 than to changes in pH (Wanders et al., 1983).

The effects of acidosis on Ca metabolism have already been discussed in a previous section, acidosis resulting in increased bone mobilization and increased urinary Ca excretion. This could in part be related to the hemodymanic changes occurring in acidosis (Yudkin et al., 1976). Disturbances in acid-base balance have also been shown to affect elements of protein or N metabolism. In skeletal muscles, acidosis has been shown to increase the rates of protein breakdown probably via an increase in glucocorticoid production (May et al., 1987, 1986, Hara et al., 1987, Hannaford et al., 1982). Changes in protein turnover rates *in vitro* and degree of response to insulin are also dependent on extracellular pH (England et al., 1991). The latter studies used a range of extracellular pH which is physiological: 7.2 - 7.6. Rates of ammonia and urea production and excretion can be increased and decreased respectively in presence of a metabolic acidosis (Welbourne et al., 1988, Cheema-Dhadli et al., 1987, Halperin et al., 1985, Phromphetcharat et al., 1981).

Protein metabolism is affected by changes in acid-base parameters and so are fat and carbohydrate metabolisms. Rates of lipolysis and ketone body production are dependent on pH, ketogenesis and urinary excretion of ketone bodies, lipolysis in fat cells being reduced in acidosis (Hood et al., 1990, 1982, Fafournoux et al, 1987, Vega et al., 1974). Acidosis also causes insulin resistance and thus impaired tolerance to glucose in humans (DeFronzo and Beckles, 1979). The effects mentioned are not necessarily independent. For example interactions between ketone body and branched-chain amino acids are known to occur (Wu and Thomson 1988). From this succinct look at some consequences of changes in acid-base parameters it becomes apparent that even small perturbations can affect metabolism greatly and in a variety of manners.

Based on the reviewed literature, the following hypotheses were formulated: -DCAB will affect acid-base status and production of cows and the response will vary according to the stage of production; -the ingredient profile of a ration will affect the acid-base status and performance of cows in early lactation. The experiments described below were designed to test the above hypotheses.

III. EFFECTS OF MANIPULATING DIETARY CATION-ANION BALANCE ON ACID-BASE BALANCE, RENAL FUNCTION AND MACROMINERAL METABOLISM OF DRY COWS.

Abstract

The dietary cation-anion balance (DCAB) was defined as the summation of the mEq of Na^+ and K^+ minus the sum of the mEq of Cl^- and $S^=$ per kg dry matter (DM). Twelve Holstein cows were used in a cross-over experiment to compare the effects of changing the cation-anion balance (CAB) of a hay-haylage based diet. Two DCAB were compared 481.8 mEq kg^{-1} (DA) and 327.2 mEq kg^{-1} (DB). Increasing DCAB had no significant effects on body weight , DM , ADF, NDF and N intakes and digestibilities. The DA ration resulted in a higher apparent water absorption and a larger urine volume. Fecal Na excretion and absorption and urinary excretion of S were increased by a lower DCAB. Except for S, plasma macromineral concentrations were not affected by DCAB. Although blood H^+ and HCO_3^- concentrations were not significantly affected, a reduction of the DCAB resulted in lower H^+ and HCO_3^- urinary concentrations and lower total urinary excretion of HCO_3^-. Plasma volume, packed cell volume, glomerular filtration rate and effective renal plasma flow were not significantly affected by DCAB. The fractional excretion of S tended to be increased by DB *vs* DA, but DCAB had no significant effect on the fractional excretion of the other macrominerals. These results demonstrated that small changes in DCAB, even in the positive range, does affect the acid-base status of dry pregnant cows as well as their water metabolism without affecting renal function ,plasma concentration of macrominerals or blood volume.

1. Introduction

Studies to design prophylactic measures to reduce the incidence of milk fever at the onset of lactation have established that manipulation of the DCAB for the dry cow can be as effective as vit D therapy before calving and probably in most cases more practical than feeding a low Ca diet prepartum (Gaynor et al., 1989; Block, 1984). A recent study conducted by Oetzel (1991) has established that S and DCAB, which are not independent, are the 2 more important nutritional risk factors for milk fever. A negative DCAB prepartum will reduce the severity and duration of the hypocalcemia associated with parturition (Goff et al., 1991). Increases in the intestinal true Ca absorption, increases in urinary Ca excretion and increases in the rate of bone mobilization have all been observed with decreases in DCAB in the ration of dry cows , goats and sheep (Takagi and Block, 1991c; Leclerc and Block, 1989; Fredeen et al., 1988b). A reduction in DCAB increases the production of 1,25-$(OH)_2$ D per unit of PTH and thus reverses the tissue resistance to PTH that develops at the end of pregnancy and onset of lactation (Goff et al., 1991).

Results of Fredeen et al. (1988) obtained with does and those of Wang and Beede (1992) obtained in dry non-pregnant cows suggest that the effects of DCAB on Ca metabolism are mediated by changes in the acid-base status and renal function of the animals. One should be careful in extrapolating these results to pregnant dry cows since pregnancy will affect renal function and hemodynamics significantly (Lindheimer and Katz 1985). Furthermore, changes in acid-base status and renal function could affect water and balances of minerals (other than Ca) as well as protein, fat and carbohydrate metabolism (Takagi and Block, 1991a, May et al., 1987, 1986, Hood et al., 1982, 1990, Vega et al., 1974). Such effects of DCAB have not been investigated in dry pregnant cows to date.

The objectives of the following study were: 1- to determine the effects of DCAB on blood and urine acid-base parameters; 2- to determine the effects of DCAB on Na, K, Cl, S, Ca, Mg and P balances; 3- to determine the effects of DCAB on plasma and urine indicators of protein, fat and carbohydrate metabolism; 4- to establish the effects of DCAB on water intake and excretion and plasma volume; 5- to determine the effects of DCAB on indicators of renal function.

2. Material and methods

A. Experimental design and housing.

Twelve dry pregnant Holstein cows were used in a cross-over design during which two diets were compared. The animals were selected on the basis of their expected calving date and had to be dry for at least 60 d before their expected calving date. Two cows were of first parity, 6 of second parity and 6 of third parity or higher. Since not all cows were available at the same time animals were paired by expected calving date and members of one pair had to be both first calf heifers or both of higher parity. Within each pair of animals random allocation of the 2 diets was performed. This eliminated the effect of time because both diets were represented equally at each time period. Each cow was studied through two successive experimental periods of 28 d each.

The first 2 wk of each period were allowed for adaptation to the experimental ration. During these 2 wk animals were housed in the main dairy barn of Macdonald College. The first day of the wk 3, which will be referred to as d 1 of the period, cows were brought into the environmentally controlled metabolic room of the dairy barn and housed in individual stainless steel metabolism stalls. The temperature of the room was maintained between 18 and 20 °C. Lights were turned on at 0500 h and turned off at 1730 h. Each stall was equipped with a water bowl and a water meter as well as a plastic container for feces collection. Days 1 to 3 were allowed for adaptation to the metabolic room and d 4 to 12 were used for data collection. On d 12 or 13, animals were weighed and returned to the dairy barn at which time their diet was changed and the next experimental period started.

B. Diets and Feeding schedule

One basal diet was formulated using chopped timothy hay (26% of diet DM), alfalfa haylage (70% of diet DM) and a mineral and vitamin and supplement (.74 % of diet DM). The DM composition of the mineral and vitamin supplement was: 36.6 % NaCl, .95% $MnCO_3$, .95% $ZnSO_4$, .4% $CoCO_3$ and 61% of a vitamin supplement from Novy (Laurel, Quebec) supplying 8,000,000 IU of vitamin A,

800,000 IU of vitamin D and 8,000 IU of vitamin E. The remaining 3 % of the diet DM were reserved for the addition of the mineral salts used to manipulate the DCAB. Minerals were added to the basal diet to create the 2 experimental diets; one was formulated to have a CAB close to 0 mEq kg^{-1} (DB) and the other to have a CAB of +200 mEq kg^{-1} (DA). To determine the amount of mineral salts that had to be added to the basal diet a mineral and DM analyses were performed on the hay and haylage every 2 wk during the trial. Based on these results, the addition of mineral salts was modified to try to maintain the DCAB constant throughout the experiment. As will be seen in the results, this was quite a difficult task since the DM and mineral content of the feed ingredients and especially haylage varies greatly with time. Trying to alleviate this problem Tagaki and Block (1991a) had frozen the feed required for the entire experiment but this is an impossible solution when working with dairy cattle. When necessary, the DCAB was made more positive by the addition of K_2CO_3 or more negative by the addition of $MgSO_4$. When the latter was added to a diet $MgCO_3$ was added to the other one to maintain the Mg content constant between diets. Mineral salts and vitamin supplements were obtained from BASF (Montréal, Québec). The end result of these manipulations was that DCAB was altered by changing the S and K contents of the diet. If the amounts of salts added were not sufficient to increase the DM content of the diet to 100% or if more had to be added to one diet *vs* the other the difference was compensated for by addition of starch. The latter, however, never represented more than 1.0 % of the diet DM.

Animals were offered their respective diets twice daily between 0750h and 0830h and between 1430h and 1530h in amounts sufficient to allow 10% refusals.

C. Sampling Schedule and Procedure

Feeds and refusals: Voluntary DMI of each cow was measured from d 4 to 8 (inclusively) of each experimental period, thus samples of the feed and refusals were collected daily during this period. One feed sample per ration was obtained by mixing an equal amount of the morning and afternoon rations. Refusals were weighed and sampled before the morning feeding. Feed samples were kept as daily samples and pooled at the end of each period according to each cow's individual daily

consumption so that one feed sample per cow per period was obtained. Within one period, feed samples were kept in a refrigerator at 4°C and after pooling they were dried in a forced air oven (Despatch Industries Inc., Mississauga, Ontario) at 60°C, ground through a 2 mm screen using a hammer mill grinder (Thomas Scientific, U.S.A.) and stored until further analysis in 100 mL plastic bottles (Fischer Scientific, Montréal, Québec). Individual daily refusal samples collected as a percentage of the daily feed offered were subjected to the same procedure as the feed samples.

Water: Water consumption was measured daily for each cow using Volumag 15 water meters (C.G.E., Montréal, Québec). A water sample (200 mL) was obtained randomly from 3 meters per day. The samples were collected in sterile containers and stored frozen until further analysis.

Feces: Total fecal excretion was measured from d 4 to d 8 inclusively. Feces were collected in a plastic tray placed under the cages and weighed once a day immediately after the morning feeding. Daily samples were taken as a fixed proportion of the daily excretion. The samples were then stored in the refrigerator at 4°C . At the end of the 5 d collection the samples were pooled per cow, dried in a forced air oven at 60°C (Despatch Industries, Mississauga, Ontario) and ground through a 2 mm screen in a hammer mill (Thomas Scientific, U.S.A.) They were then stored until further analysis in small plastic containers.

Urine: The morning of d 3, after feeding, Bardex Foley catheters were inserted into the bladder and held in place by a 50mL balloon. The catheters were 28 mm in diameter and were connected to a polyethylene bottle (capacity 25 L) using PVC tubing. To avoid having to take the cows from the metabolism stalls to a holding gate to restrain them they were given 0.2 mL of Rompun (100 mg ml^{-1}, Haver, Etobicoke, Ontario) prior to the insertion of the catheters. The tranquilizer made the insertion easier and reduced the time required and thus the risks of infection. The cows were given one day of adaptation to the catheters. The morning of d 4, 50 mL of light mineral oil (ICN biochemicals, Mississauga, Ontario) were added to each bottle as well as approximately 1 g of thymol (ICN Biochemicals, Mississauga, Ontario). The oil was used to trap urine gases (CO_2 and NH_3) and thymol was used as an antibacterial agent (Phillip 1990). The interest in Cl and S

precluded the use of acidifying agents such as HCl and H_2SO_4. Urine volume was measured twice daily after the two feedings and samples proportional to the excretion were obtained each time. One sample was collected in a plastic container and acidified with HCl (5 drops of concentrated acid, Anachemia, Montréal, Québec) to ensure that no losses of NH_3 would occur during the storage period. This was purely for security reasons because it does not appear to be necessary when mineral oil is used. One unacidified sample was collected for immediate measurement of pH and pCO_2 and stored frozen. At the end of a collection period urine samples, were pooled by cow and approximately 100 mL of acidified and unacidified urine were kept per cow per period and frozen until further analysis.

Some cows lost their catheter before the end of d 5. If 3 d of collection had been completed the catheters were not reinserted to avoid increasing the risks of infection.

Blood sampling: On d 9 of each collection period blood samples were obtained by puncture of the coccygeal vein using 20 G, 3.8 cm needles. Twenty eight mL of blood were collected in 4 vacutainers (2 containing lithium heparin and 2 containing Na-ethylenedinitrilotetraacetic acid, Na-EDTA) at 3 different times: before the morning feeding and at 2 and 4 h after the morning feeding. Whole blood samples were first analyzed for acid-base parameters and then centrifuged at 800 x *g* for 20 min at 4°C in a Sorvall RC-3 centrifuge. Plasma samples were transferred to plastic scintillation vials and frozen until analysis.

On the morning of d 10, 11 or 12, (only 3 cows could be done in anyone d), jugular catheters were inserted with 12 G 5 cm needles. The tubing was Intramedic polyethylene tubing with an internal diameter of 1.14 mm and an outer diameter of 1.57 mm (Fischer Scientific, Montréal, Québec). The catheters were filled with heparinized saline and stoppered with 3 way stopcock obtained from CDMV (St-Hyacinthe, Québec). After insertion of the catheters cows were prevented from drinking and eating and a blood sample was obtained. The animals were then given via the catheter 50 mL of a solution containing 10g of phenolsulfonphtalein (PSP, Sigma Chemical Co., St. Louis, Missouri) in 500 mL of physiological saline. After the single injection, the catheters were rinsed with 30 ml of saline. Blood samples were

obtained at 5, 10, 20 and 30 min after the injection as described by Kaneko (1980). From the first sample, an aliquot of whole blood was taken to measure hematocrit (Critocaps, Fisher Scientific, Montréal, Québec). The blood samples were then centrifuged at 800 x *g* and plasma was transferred to 7 mL scintillation vials. The samples were kept in the refrigerator and analyzed for their PSP content the same day. When obtaining a sample the first few mL which were the heparinized saline used to fill the catheters were discarded, then blood was collected in a heparin containing vacutainer and an amount of heparinized saline equivalent to the volume of blood withdrawn was infused. In addition the exact volume given to each cow was calculated from the difference in weight of the syringe used before and after infusion. This was done to correct for the fluid remaining in the syringe. Immediately after the 30 min sample for PSP was obtained a sample was taken corresponding to the initial sample for the next infusion. The next injection consisted of 50 mL of a solution made of 6 g of Evans blue (Sigma chemical Co., St. Louis, Missouri) in 500 mL of physiological saline. Blood samples were obtained 5, 15, 25, 35, 45, 60, 90, 120 and 150 min after the infusion according to the method described for PSP. Blood samples were processed in the same manner as samples obtained after the PSP injection.

D. Analytical methods

Feeds and refusals: Absolute DM content was determined on each feed and refusal sample by drying approximately 1 g in a vacuum oven (National Appliance Co., Portland, Oregon) at 100°C. Samples were also analyzed for their CP content using a N analyzer (Leco Corporation, St. Joseph, Missouri), and for their acid- and neutral detergent fiber according to the procedure of Van Soest(1967). A wet digestion (A.O.A.C., 1984) with 15 mL concentrated nitric acid and 2 mL of concentrated perchloric acid (Anachemia, Montréal, Québec) was done to prepare the samples for analysis of Na, K, Ca, Mg, P and S. The digested samples were transferred to 10 mL volumetric flasks and volume was made with deionized distilled water after which they were rapidly transferred to acid washed plastic test tubes to avoid contamination by Na leaching out of the glass volumetric flasks. Subsequently, all manipulations to prepare the samples for Na analysis were done using acid washed polyethylene tubes. The final dilutions required for Mg and Ca analysis were done using a .25% (wt/vol) solution of lanthanum oxide (Anachemia, Montréal,

Québec). The last dilution for Na analysis was done with a solution containing 2 mL L^{-1} of a 1000 ppm K standard (BDH, Toronto, Ontario). A similar procedure was used to prepare the samples for K analysis except that an excess of Na was added. After these preparations Ca, Mg, Na and K concentrations were determined using an atomic absorption spectrophotometer (Perkin Elmer 360, Norwalk, Connecticut). Concentrations of P and S were determined on the wet digests using the alkalimeter ammonium molybdate method (A.O.A.C., 1984) and the turbidimetric method of Berglund and Sorbo (1960) respectively. For both analyses a Beckman (DU-20) spectrophotometer was used (Beckman Instrument Inc., California, U.S.A.). Measurements of Cl were done on nitric acid suspensions of the sample according to the potentiometric titration procedure (Lacroix et al., 1970) using a Cl ion specific electrode (Orion model 94-17A, Orion Research Inc., Boston, Massassuchett). Dilutions that were not done with volumetric flasks were done using a MicroLab dilutor (Hamilton, Bonaduz, Switzerland).

Consumption figures were calculated by subtracting concentrations in the refusals from concentrations in the feed samples. Averages were obtained per cow within each experimental period.

Water Samples: Daily water samples were analyzed for Ca, Mg, Na and K as described for the feed samples except that no wet digestion was required. The Cl, SO_4 and PO_4 contents were determined using an ion chromatograph model DX-100 (Dionex, Brossard, Quebec) equipped with a guard column (Dionex AG4A) and an anion column (Dionex AS4A). Using the daily water analysis and daily water consumption, average mineral intakes from water were calculated for each cow-experimental period unit. These values were added to the mineral intakes from the diet to obtain the total mineral intake of the animals.

Feces Samples: Feces samples were processed as were feed and refusal samples. Apparent absorption of the seven macrominerals was calculated by subtracting the quantities excreted in the feces from the quantities consumed. Apparent absorption was also expressed as a percentage of intake.

Urine Samples: Fresh urine samples collected under oil twice daily were

analyzed for pH immediately after collection using a Corning pH meter model 120 (Corning, New York,U.S.A.) and a Cole Parmer pH electrode (Chicago, Illinois, U.S.A.). The pCO_2 content was determined using a gas analyzer (Instrument Lab. Spa, Italy). The concentration of HCO_3 was calculated from the Henderson Hasselback equation using a pK value for each sample corrected for ionic strength (Laski and Kurtzman, 1989) and a solubility for CO_2 of 0.03. Before any calculation was made with pH the values were converted to H^+ concentration. The concentration obtained for each sample was multiplied by the corresponding urine volume excreted at the time of collection. Total daily excretions and concentrations were then calculated and averaged per cow and collection period for statistical analysis.

The samples of urine stored without acid were used for mineral analysis. The same methods were used as for the samples of feeds and refusals and averages of daily excretions by cow and experimental period were calculated. The same samples were analyzed for creatinine and urea with kits from Abbott laboratories (kit #6222 and # 6098) adapted to use with a clinical analyzer (VP Super System, Abbott Laboratories, Mississauga, Ontario). Acidified urine samples were analyzed with the clinical analyzer for their ammonia concentration using a kit from Sigma Chemicals (St. Louis, Missouri, kit # 170B) and for their total N content using the same procedure as for the samples of feeds. Total daily excretion and concentration of minerals were calculated and averaged per cow and collection period for statistical analysis.

Values for apparent mineral retentions were calculated by subtracting the amount excreted in the urine from the amount absorbed. The values were also expressed as a percentage of intake.

Blood samples: The samples collected on d 9 were immediately analyzed for pH, HCO_3, pCO_2 using a blood gas analyzer (IL1306, Instrument Lab., Milan, Italy).

The plasma samples obtained on that day with lithium heparin were analyzed for their mineral content. The methods were the same as those described for samples of feeds except for Cl. The latter was analyzed using the clinical analyzer using a kit

from Sigma Chemicals (St. Louis, Missouri, kit # 461-M). Samples that had been collected with Na-EDTA were analyzed for urea, ammonia and creatinine as were the urine samples. In addition, determinations of glucose (kit 6082, Abbott Laboratories, Mississauga, Ontario), non-esterified fatty acids (NEFA,Wako Pure Chemicals, Osaka, Japan) and ß-hydroxybutyrate (Sigma Chemicals, St-Louis, Missouri) were conducted using the clinical analyzer.

The concentrations of PSP were determined using the method described by Kaneko (1980). From the decline in plasma concentration , the clearance of PSP was calculated using the following equation:

$$Cl = \frac{Db}{a},$$

where: Cl is the clearance,
D is the dose injected,
b is the slope of the single exponential term,
a is the intersection with the ordinate.

The equation was described by Bianchi (1980). The clearance of PSP was used as an estimate of effective renal plasma flow (ERPF). Since the hematocrit content of the samples was also measured as mentioned previously it was possible to calculate the effective renal blood flow (ERBF) according to the following equation:

$$ERBF = \frac{ERPF}{(1-Ht)},$$

where: Ht is the hematocrit volume (Vander 1991).

Evans blue was determined using the procedure of Holmes and Weiskopf (1987) with the correction equation developed by Hamilton (1958). The concentrations were transformed to natural logarithms and plotted *vs* time. By extrapolating to time 0 the initial concentration in plasma was obtained and knowing the dose given the volume of distribution was calculated. This volume is an estimate of plasma volume. As for the estimation of ERBF from ERPF, the blood volume was calculated knowing the level of hematocrit.

Since plasma creatinine concentration, urine creatinine concentration and urine volume were measured, glomerular filtration rate could be estimated from the clearance of creatinine which was calculated as described by Duarte et al. (1980):

$$\text{Creatinine clearance} = \frac{Uc \cdot V}{Pc},$$

where: Uc is concentration of creatinine in urine,
V is the volume of urine per unit time,
Pc is the plasma creatinine concentration.

For this calculation the plasma creatinine concentration before feeding was used.

From these measurements the plasma filtration fraction and fractional excretion of different minerals were calculated (Vander,1991). The filtration fraction (FF) was calculated as follows: FF = GFR/ ERPF and the fractional excretion (FE) was calculated as follows: FE = (Ux)(V)/GFR (Px) where Ux is the urine concentration, V urine volume, Px plasma concentration. The plasma concentrations used were those measured before feeding.

Metabolic proton flux was calculated for different time intervals after the morning feeding from blood pH and HCO_3^- measurements. This value represents the amount of H^+ ions entering the blood compartment from non volatile acids. The equation used was obtained from McDonald et al. (1980) and is presented below:

$$H^+ \text{ production} = [HCO_3^-]_1 - [HCO_3^-]_2 - B(pH_1 - pH_2)$$

where B is the slope of the non-bicarbonate buffer line in the blood (ratio of bicarbonate change per unit of pH change). The two concentrations of HCO_3^- represent the concentrations at two different times and the two pH values correspond to the pH at the two time points studied.

To obtain the B values the hemoglobin concentrations were first calculated from the hematocrit concentrations and the mean corpuscular hemoglobin

concentration for cow blood. The latter was obtained by averaging reported values (Coppock et al., 1982, Kilmer et al., 1981 and Escobosa et al., 1984) and 35 g dL^{-1} was used. Knowing the hemoglobin concentration, B can be calculated with the following equation (Turner et al., 1983):

$$B = -1.073[Hb] - 2.48$$

where [Hb] is the hemoglobin concentration in g 100 mL^{-1}

E. Statistical Analysis

The data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, North Carolina, U.S.A.) according to the model for a cross-over design design (Lucas, 1983). The linear model used is described by the following equation:

$$Y_{ijk} = \mu + A_{ij} + B_k + T_h + E_{ijk}$$

where: Y_{ijk}=variable studied during the k^{th} period (k=1,2), of the j^{th} cow (j=1,...12), in the i^{th} group (i=1,2).
μ = population mean
A_{ij} = effect of the j^{th} cow in the i^{th} group
B_k = effect of the k^{th} period
T_h = effect of the h^{th} treatment
E_{ijk} = random error

An analysis of variance was performed and the difference between the treatment means was estimated using contrast. The standard error of the difference is reported. Statistical significance was declared when the probability of a greater F was less than 5%.

In addition, when thought necessary certain variables were included in the model as a linear regression term. When such analyses were done there is mention of them in the text.

3.Results

Three cows calved before the expected date and thus did not complete their second period. Based on their actual calving dates, cows were between 144 and 250 d in calf at the beginning of the experiment.

Diet Composition: The average composition of the 2 diets is shown in Table 3.1 where DA represents the more positive diet and DB the less positive one. There were no differences between the two diets for CP, ADF, NDF, Na, P, Ca and Mg contents. The more positive diet had a greater concentration of K whereas DB had a higher concentration of S. The standard deviations illustrate the variability encountered especially in K and shows the difficulty of obtaining constancy in the DCAB formulated. Both diets had DCAB higher than expected. Even though diets did not have the exact DCAB for which they were formulated the major objective was attained in that diets differed only in DCAB.

Dry matter, ADF, NDF and N intakes and digestibilities: Body weight (BW), DMI (g d^{-1}), DM digested and DM digestibility were not affected by dietary treatments (Table 3.2). Measures of ADF and NDF intakes and digestibilities also did not change due to differences in DCAB. In most instances there was a tendency for digestibility coefficients to be numerically higher with the more positive diet however in view of the large SE obtained relative to the estimated difference between the two diets no statistical significance was achieved. Since different cows were at different stages of pregnancy, days in calf was used as a covariate in the model but was found to have no significant effect and thus was dropped out. Body weight used as a covariate was also not significant.

Intake, apparent absorption and apparent retention of N were not affected by dietary treatment. A trend of higher absorption of N with DA was counteracted by a trend towards increased urinary excretion resulting in no change in total retention expressed in g d^{-1}, or as percentage of N intake (Table 3.3). Correcting the data for BW by fitting this parameter as a covariate in the model did not change the results , it had no significant effect.

Table 3.1. Mean dry matter composition of diets differing in dietary cation-anion balance (DCAB)[1] offered to dry cows.

Analyte	DA[2]	SD[3]	DB[4]	SD[3]
CP,%	13.6	1.96	13.7	1.49
NDF, %	55.4	4.13	55.6	3.69
ADF, %	42.1	2.04	41.9	2.25
Calcium, %	0.61	0.17	0.62	0.14
Magnesium, %	0.24	0.04	0.25	0.05
Phosphorus, %	0.19	0.05	0.18	0.05
Sodium, %	0.15	0.02	0.15	0.01
Potassium, %	2.61	0.4	2.29	0.37
Chlorine, %	0.50	0.13	0.46	0.11
Sulfur, %	0.19	0.03	0.31	0.12
DCAB[1]	481.8	73.8	327.2	144.8

[1] DCAB: mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of 481.2 mEq kg^{-1} DM, n=11

[3] Standard deviation

[4] Ration with a DCAB of 327.2 mEq kg^{-1} DM, n=10

Table 3.2. Mean body weight (BW) dry matter (DM), ADF and NDF intake and digestibilities of diets differing in dietary cation-anion balance (DCAB)[1] offered to dry cows.

Item	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
BW kg	723.3	719.9	0.621	3.4	6.5
DM Intake, kg d^{-1}	10.2	10.1	0.872	0.2	0.9
DM Digested, kg d^{-1}	6.5	6.5	0.901	0.1	0.8
DM Digested, % intake	63	61	0.632	2	4
ADF Intake, g d^{-1}	4329	4247	0.831	82	369
ADF Digested, g d^{-1}	2660	2598	0.867	63	358
ADF Digested, %intake	61	58	0.544	3	5
NDF Intake, g d^{-1}	5725	5679	0.925	46	468
NDF Digested, g d^{-1}	3630	3620	0.985	9.5	470
NDF, % of intake	63	61	0.648	2	4

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=11

[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimated difference

Table 3.3. Mean nitrogen (N) balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Item	DA[2]	DB[3]	P[4]	EH-EC[5]	SED[6]
N intake, g d^{-1}	232	222	0.94	2	20
N absorption					
Fecal N, %	1.9	1.9	0.88	0.02	0.1
Fecal N, g d^{-1}	73	75	0.97	0.3	8
N absorbed, g d^{-1}	159	147	0.94	1	15
N absorption, % of intake	68	65.	0.74	1	3
N retention					
Urine N, %	5.7	6.1	0.09	-0.04	0.02
Urine N, g d^{-1}	113	47	0.21	4	3
N retained, g d^{-1}	101	97	0.97	-1	13
N retained %intake	43	42	0.90	1	4

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Water intake and absorption: Cows consuming DA drank more water than cows consuming DB (P=0.088) leading to a trend in overall water consumption calculated from the amount drunk and the amount obtained from the feed (Table 3.4). Increasing DCAB significantly increased apparent water absorption expressed as a percentage of water consumed or in total liters of water absorbed daily. Cows offered DA absorbed 4.65 L more than cows offered DB (65% of the water consumed *vs* 61%).

Mineral Balances:

Calcium, Magnesium and Phosphorus: Since both diets had the same Ca concentration and since DMI did not differ between the diets, Ca intake was also similar between treatments (Table 3.5). No differences between the two diets were observed in terms of absorption or retention of Ca. Cows consuming the diet with the lower DCAB tended to have a higher urinary Ca concentration but a lower total daily excretion resulting in the same overall balance. As can be noted the percentage of Ca intake which was absorbed was almost the same as the percentage retained because of the very low urinary concentrations, thus explaining why the higher urinary concentration for the diet with the lower DCAB did not have any impact on the balance. Results of Mg balance are presented in Table 3.6. As was the case for the Ca balance, DCAB had no effect on Mg balance. Results of P balance are presented in Table 3.7. Phosphorous balance followed the same tends as did Ca and Mg balances; means being numerically but not statistically higher for the more positive diet.

Table 3.4. Mean water intake and apparent absorption of dry cows fed diets with different dietary cation-anion balance[1].

Item	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Free water consumed, L d^{-1}	51.8	47.4	0.09	4.0	2.0
Feed water, L d^{-1}	7.7	7.7	0.88	-0.1	0.8
Total water consumed, L d^{-1}	59	55	0.16	4	2
Water absorbed, L d^{-1}	39	34	0.08	5	2
Water absorbed ,% of intake	65	61	0.05	5	2

1 mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
2 Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=11
3 Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
4 Probability associated with the F test
5 Estimate of the difference between treatment means
6 Standard error of the estimated difference

Table 3.5. Mean calcium balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Calcium	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	65.1	66.6	0.86	-1.7	9.3
Fecal concentration, %	0.99	0.95	0.72	0.04	0.11
Fecal excretion, g d^{-1}	36.9	37.0	0.99	0.06	6.03
Apparent absorption, g d^{-1}	28.2	28.5	0.78	-1.8	6.1
Apparent absorption ,% of intake	40.5	40.0	0.99	-0.14	7.1
Urinary concentration, ppm	10	36	0.26	-31	25
Urinary excretion, mg d^{-1}	282	180	0.20	150	104
Apparent retention, g d^{-1}	29.9	28.3	0.80	-1.6	6.0
Apparent retention, % intake	42.0	39.3	0.95	0.5	7.3

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.6. Mean magnesium balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Magnesium	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	26.0	25.1	0.85	0.5	2.8
Fecal concentration, %	0.44	0.44	0.93	0.003	0.03
Fecal excretion, g d^{-1}	17.0	17.4	0.92	-0.2	2.0
Apparent absorption, g d^{-1}	9.0	7.7	0.73	0.8	2.1
Apparent absorption, % of intake	35.4	30.6	0.70	3.3	8.2
Urinary concentration, ppm	176	215	0.17	-39	24
Urinary excretion, mg d^{-1}	3115	3116	0.99	-0.001	0.1
Apparent retention, g d^{-1}	7.4	6.1	0.74	0.7	2.1
Apparent retention ,% of intake	30.2	24.2	0.70	3.6	8.9

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.7. Mean phosphorus balance of dry ccws fed diets with different dietary cation-anion balance (DCAB)[1].

Phosphorous	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	19.7	19.0	0.92	0.3	2.3
Fecal concentration, %	0.45	0.41	0.31	0.03	0.03
Fecal excretion, g d^{-1}	16.7	15.8	0.58	1.2	2.0
Apparent absorption, g d^{-1}	3.0	3.1	0.48	-0.9	1.2
Apparent absorption, % of intake	10.3	5.7	0.90	0.9	7.7
Urinary concentration ,ppm	1.5	1.9	0.31	-0.3	0.3
Urinary excretion, mg d^{-1}	16.8	13.8	0.68	0.7	1.6
Apparent retention, g d^{-1}	3.4	3.1	0.48	0.9	1.2
Apparent retention ,% of intake	12.0	5.7	0.91	6.9	7.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Mineral Balances: Sodium, potassium, chloride and sulfur.

Intake of sodium was similar between diets again to similar DMI and mineral concentrations in the diets (Table 3.8). Fecal excretions were very low explaining the high apparent absorption of Na obtained (75 % for DB and 83 % for DA). DA decreased Na fecal concentration and daily fecal excretion significantly but there was only a trend towards improved absorption when expressed as a percentage of Na intake. This trend was increased was paralleled by a significant increase in total daily urinary excretion (P= 0.08). Overall Na balance was positive and similar for both diets. The small differences observed in terms of dietary concentration of K did not affect K intake or balance significantly (Table 3.9). Chloride metabolism was not affected by dietary manipulation (Table 3.10). No shift between urinary and fecal excretions was observed as appeared to be the case for Na. A small increase in Cl balance was observed with a higher DCAB paralleled by a slightly elevated intake which compensated only partly by increased urinary excretion.

Reducing DCAB by increasing the S content of the diet resulted in a higher S intake for cows consuming the less positive diet (DB) (Table 3.11). These animals consumed on average 10 more g of S per day (P=0.01). Since fecal excretion expressed in g d^{-1} or as a percentage of S intake remained unchanged the reduced DCAB resulted in a significantly higher total S apparent absorption: 20.8 g d^{-1} *vs* 11.5 g d^{-1} for DB and DA, respectively. However, urinary concentration and total excretion were both increased (P=0.09 and P=0.08) by DB resulting in similar S balances expressed in g d^{-1} for cows consuming either diet.

Table 3.8. Mean sodium balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Sodium	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	16.6	15.8	0.96	-0.1	1.8
Fecal concentration ,%	0.07	0.10	0.03	-0.03	0.01
Fecal excretion, g d^{-1}	2.8	3.8	0.04	-1.0	0.4
Apparent absorption, g d^{-1}	13.8	12.0	0.61	0.9	1.7
Apparent absorption, % of intake	83.1	75.2	0.14	7.1	4.2
Urinary concentration ,ppm	629	521	0.22	212	153
Urinary excretion, mg d^{-1}	8355	3206	0.08	2966	1410
Apparent retention, g d^{-1}	7.6	8.5	0.40	-2.1	2.3
Apparent retention ,% of intake	46.1	49.5	0.57	-6.7	11.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.9. Potassium balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Potassium	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	280.2	235.9	0.23	39.6	29.3
Fecal concentration, %	0.35	0.37	0.58	-0.01	0.01
Fecal excretion, g d^{-1}	13.1	14.1	0.94	0.1	0.7
Apparent absorption, g d^{-1}	267.1	221.8	0.22	39.5	28.9
Apparent absorption, % of intake	95.2	93.5	0.18	1.4	0.9
Urinary concentration, ppm	1715	1859	0.62	-105	200
Urinary excretion, mg d^{-1}	15115	14570	0.75	858	2578
Apparent Retention, g d^{-1}	254.5	206.4	0.23	38.7	28.6
Apparent Retention, % of intake	88.9	86.5	0.20	2.4	1.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.10. Mean chloride balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Chloride	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	53.8	47.9	0.74	4.1	11.8
Fecal concentration, %	0.26	0.29	0.24	-0.029	0.02
Fecal excretion, g d^{-1}	9.8	11.4	0.51	-1.0	1.4
Apparent absorption, g d^{-1}	44.0	36.5	0.65	5.0	10.5
Apparent absorption, % of intake	80.7	74.9	0.16	3.3	2.1
Urinary concentration, ppm	1772	1969	0.42	-338	386
Urinary excretion, mg d^{-1}	18014	15983	0.70	-1718	4319
Apparent retention, g d^{-1}	27.6	18.6	0.38	6.8	7.2
Apparent retention, % of intake	48.0	38.0	0.20	8.5	5.9

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.11. Mean sulfur balance of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Sulfur	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Intake, g d^{-1}	20.2	30.6	0.01	-6.2	1.7
Fecal concentration, %	0.23	0.25	0.38	-0.02	0.02
Fecal excretion, g d^{-1}	8.7	9.8	0.47	-0.7	0.3
Apparent absorption, g d^{-1}	11.5	20.8	0.02	-5.5	1.8
Apparent absorption, % intake	57.0	65.7	0.19	-5.9	3.9
Urinary concentration, ppm	348	2241	0.09	-1232	604
Urinary excretion, mg d^{-1}	3059	15017	0.08	-7486	3529
Apparent retention, g d^{-1}	8.4	7.0	0.47	2.0	2.6
Apparent retention, % intake	40.7	25.2	0.18	13.4	9.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Plasma Minerals: The plasma concentrations of the macrominerals measured at different times relative to the morning feeding are presented in Table 3.12. Concentrations of plasma Ca, Mg and P were within normal ranges reported for these minerals (Kaneko, 1980). Reducing the DCAB from +481.8 to +327.2 mEq kg^{-1} did not affect the plasma concentration of these 3 minerals at any sampling time. For each diet a transient increase in plasma Ca was observed at 2 h postfeeding but concentrations were back to their prefeeding concentratin at 4 h. Plasma concentrations of Na, K and Cl remained unaffected by DCAB. Prefeeding concentration of plasma S was identical for cows consuming either diet. However, at 2 h, cows offered the reduced DCAB had higher plasma S concentrations ($P = 0.12$); the difference between the two diets was significant 4 h postfeeding.

Blood and urine acid-base parameters: Blood pH, pCO_2, HCO_3^- and free H^+ concentrations measured at different times postfeeding are presented in Table 3.13. In addition to the statistical analysis performed directly on pH values, results of the analysis done on free H^+ concentration are also presented. No statistically significant effect due to manipulation of the DCAB was observed for these parameters. However, interestingly, blood H^+ concentration and pCO_2 tended to be higher prefeeding and 2 h after for cows consuming the diet with the highest DCAB.

Urinary concentration of free H^+ was significantly lower forDA, contrary to the situation in blood. The significantly higher urine volume for cows fed the highest DCAB eliminated the effect of diet on urinary free H^+ when expressed as total moles excreted daily (Table 3.14); cows consuming the higher DCAB still tended to excrete less free H^+ daily in the urine but because of the variations in these measurements the difference was not statistically significant. Note that for both diets urinary H^+ concentrations were relatively low (pH = 8.68 for cows eating DA and pH = 8.51 for cows eating DB). However results of HCO_3^- excretion indicate a definite effect of diet on acid-base status. Both concentration and total excretion of HCO_3^- via the urine were significantly reduced by a reduction in DCAB. Cows fed DB excreted 43 % less bicarbonate daily and had urinary concentration 34 % lower than cows fed DA.

Blood volume and parameters of ren function: No difference in the mean

plasma volumes was found between diets (Table 3.15). Days in calf and BW as covariates in the model were found to have no significant effect on parameters presented in Table 3.15. Similarly, expressing the parameters per unit of BW did not alter the results. Despite the large difference in plasma volumes, estimates of glomerular filtration rate (GFR) and renal plasma flow (RPF) were similar for both diets. Renal plasma flow tended to be higher but GFR tended to be lower for cows consuming the diet with the highest DCAB. The greater difference in GFR *vs* RPF resulted in a small elevation of the filtration fraction (FF) for cows consuming DA meaning that a larger fraction of the blood entering the kidneys was filtered. The difference in the FF was not significant. Fractional excretions of the minerals were not significantly affected by DCAB as can be seen from Table 3.16. Sulfur fractional excretion was almost 7 times higher when cows were consuming the ration with the lower DCAB (P= 0.12).

Metabolic parameters: A small difference in plasma concentration of ß-hydroxybutyrate 2 h postfeeding was observed but no dietary effect was observed for plasma urea, ammonia, creatinine, glucose and non-esterified fatty acids at the various sampling times (Table 3.17). Urinary urea and ammonia concentrations tended to be lower and higher respectively as DCAB increased (Table 3.18) but because of the large SE the differences were not significant. However, total excretion of these metabolites were similar between diets.

Metabolic proton flux: Metabolic proton flux were calculated for different time intervals and results are presented in Table 3.19. Although no significant differences due to diet were found, it is interesting to note that for both diets H^+ productions were positive postfeeding 0 to 4 h postfeeding and became negative later on. This indicates that animals went from a point where protons were entering the blood to a point where protons were leaving.

Table 3.12. Mean plasma mineral concentration of dry cows fed diets with different dietary cation-anion balance (DCAB)[1], prefeeding, 2 and 4 h postfeeding.

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Prefeeding					
Ca, mg dL^{-1}	9.3	9.5	0.64	-0.3	0.5
Mg, mg dL^{-1}	2.1	2.1	0.80	-0.1	0.2
P, mg dL^{-1}	4.1	4.1	0.84	-0..04	0.2
Na, mmol L^{-1}	116.5	119.0	0.70	-1.8	4.5
K, mmol L^{-1}	3.8	3.7	0.70	0.1	0.2
Cl, mmol L^{-1}	102.4	104.1	0.37	-2.8	2.8
S, mmol L^{-1}	1.1	1.1	0.34	0.2	0.2
2 h postfeeding					
Ca, mg dL^{-1}	10.1	9.6	0.94	0.02	0.3
Mg, mg dL^{-1}	2.2	2.2	0.91	0.02	0.1
P, mg dL^{-1}	4.5	4.4	0.85	0.07	0.4
Na, mmol L^{-1}	122.5	122.2	0.85	-0.8	3.8
K, mmol L^{-1}	3.9	3.5	0.55	0.1	0.2
Cl, mmol L^{-1}	103.2	105.5	0.3	-3.4	3.1
S, mmol L^{-1}	1.5	1.9	0.12	-0.4	0.2
4 h postfeeding					
Ca, mg dL^{-1}	9.7	9.8	0.56	-0.3	0.5
Mg, mg dL^{-1}	2.2	2.2	0.75	-0.05	0.1
P, mg dL^{-1}	4.2	4.3	0.32	-0.3	0.3
Na, mmol L^{-1}	124.4	118.9	0.28	6.0	5.1
K, mmol L^{-1}	3.7	3.8	0.91	-0.03	0.2
Cl, mmol L^{-1}	101.0	102.7	0.53	-1.1	1.7
S, mmol L^{-1}	1.5	2.3	0.002	-0.9	0.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM , n=11

[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimate difference

Table 3.13. Mean blood pH, free hydrogen ion (H^+) and bicarbonate ion (HCO_3^-) concentration and pCO_2 of dry cows fed diets with different dietary cation-anion balance (DCAB)[1], pre-feeding, 2 and 4 h postfeeding.

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Prefeeding					
pH	7.385	7.402	0.264	-0.021	0.018
H^+, mol L^{-1}	4.18×10^{-5}	3.99×10^{-5}	0.271	0.15×10^{-5}	0.18×10^{-5}
HCO_3^-, mmol L^{-1}	28.14	27.97	0.929	0.065	0.699
pCO_2, mm Hg	46.71	44.83	0.337	2.14	2.08
2 h postfeeding					
pH	7.394	7.42	0.207	-0.03	0.02
H^+, mol L^{-1}	4.05×10^{-5}	3.83×10^{-5}	0.217	0.20×10^{-5}	0.23×10^{-5}
HCO_3^-, mmol L^{-1}	27.73	27.75	0.544	-0.248	0.389
pCO_2, mm Hg	45.14	42.74	0.377	2.42	2.57
4 h postfeeding					
pH	7.391	7.392	0.971	0.001	0.029
H^+, mol L^{-1}	4.09×10^{-5}	4.09×10^{-5}	0.982	0.01×10^{-5}	0.02×10^{-5}
HCO_3^-, mmol L^{-1}	27.26	27.31	0.77	0.24	0.781
pCO_2, mm Hg	44.75	44.75	0.871	0.575	3.402

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=11
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.14. Mean urine volume, free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (CO_2) excretion by dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Volume, L	18.9	15.9	0.0048	3.0	0.7
Total free H^+, mol d^{-1}	3.9×10^{-8}	4.7×10^{-8}	0.281	-1×10^{-8}	1.2×10^{-8}
Free H^+, mol L^{-1}	2.1×10^{-9}	3.1×10^{-9}	0.035	-1.1×10^{-9}	0.3×10^{-9}
Total HCO_3^-, mmol d^{-1}	14794	8375	0.011	6418	1754
HCO_3^-, mmol L^{-1}	771	508	0.030	263	93
pCO_2, mm Hg	63.6	56.5	.339	7.1	22.0

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter. (DM)
[2] Ration with a DCAB of 481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6]. Standard error of the estimated difference.

Table 3.15. Mean plasma volume, packed cell volume and renal function parameters of dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Plasma volume , L	60.1	57.3	0.35	2.7	2.5
Packed cell volume, %	29.1	28.2	0.59	0.6	1.1
Blood volume ,L	85.5	74.4	0.35	14.2	16.2
Effective renal plasma flow, L min^{-1}	4.01	4.67	0.60	-0.85	1.39
Effective renal blood flow, L min^{-1}	5.71	6.52	0.61	1.35	2.25
Glomerular filtration rate, L d^{-1}	800	717	0.57	77	115
Filtration fraction	0.153	0.108	0.33	0.039	0.029

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of +327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.16. Fractional excretion (FE) of minerals by dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

Macromineral	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Na	0.003	0.002	0.299	0.001	0.0009
K	0.163	0.189	0.313	-0.054	0.048
Cl	0.239	0.253	0.404	-0.053	0.058
S	0.111	0.721	0.116	-0.244	0.129
P	0.0004	0.0005	0.831	-7×10^{-6}	0.00005
Ca	0.001	0.005	0.34	-0.004	0.004
Mg	0.088	0.109	0.65	-0.01	0.021

[1] mEq $(Na^+ + K^+ - Cl^- - S^{--})$ kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 3.17 Plasma concentration of metabolites of dry cows fed diets with different dietary cation-anion balance (DCAB)[1], prefeeding, 2 and 4 h postfeeding.

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Prefeeding					
ß-OHButyrate, mg dl^{-1}	5.69	5.22	0.79	-0.19	0.66
Urea N[7], mg dl^{-1}	10.34	10.27	0.91	0.13	1.08
Ammonia, μg ml^{-1}	2.94	2.96	0.51	-0.20	0.29
Creatinine, mg dl^{-1}	1.05	1.09	0.55	-0.03	0.05
Glucose, mg dl^{-1}	56.71	48.97	0.27	9.67	7.91
NEFA[8], mEq ml^{-1}	0.31	0.45	0.59	-0.09	0.16
2 h postfeeding					
ß-OHButyrate, mg dl^{-1}	6.05	6.51	0.03	-1.75	0.65
Urea N, mg dl^{-1}	12.56	13.52	0.54	-0.82	1.27
Ammonia, μg ml^{-1}	3.08	3.26	0.30	-0.35	0.31
Creatinine, mg dl^{-1}	1.05	1.10	0.21	-0.10	0.08
Glucose, mg dl^{-1}	52.03	51.62	0.76	1.97	6.25
NEFA, mEq ml^{-1}	0.25	0.27	0.44	-0.04	0.05
4 h postfeeding					
ß-OHButyrate, mg dl^{-1}	7.44	6.42	0.51	0.59	0.86
Urea N, mg dl^{-1}	12.95	13.36	0.75	-0.44	1.33
Ammonia, μg ml^{-1}	2.85	3.25	0.17	-0.53	0.34
Creatinine, mg dl^{-1}	1.01	1.05	0.56	-0.05	0.072
Glucose, mg dl^{-1}	51.05	51.24	0.62	-2.74	5.31
NEFA, mEq ml^{-1}	0.22	0.24	0.59	0.02	0.04

1 mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
2 Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=11
3 Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
4 Probability associated with the F test
5 Estimate of the difference between treatment means
6 Standard error of the estimated difference
7. Nitrogen
8. Non-esterified fatty acids

Table 3.18. Mean urinary excretion of Nitrogenous compounds by dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
Urea N[7], mg dl^{-1}	369.8	434.4	0.414	-26.9	30.2
Urea N, g d^{-1}	35.6	34.1	0.097	3.8	1.9
Ammonia, μg ml^{-1}	140.0	94.7	0.694	59.6	142.7
Ammonia, g d^{-1}	8.0	7.9	0.530	1.1	1.6
Creatinine, mg dl^{-1}	75.1	93.2	0.370	-7.9	7.9
Creatinine, g d^{-1}	16.4	7.0	0.442	0.5	0.6

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=10
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen

Table 3.19. Mean metabolic proton flux production (mmol L^{-1}) by dry cows fed diets with different dietary cation-anion balance (DCAB)[1].

	DA[2]	DB[3]	P[4]	DA-DB[5]	SED[6]
β[7]	-13.79	-13.31	0.58	-0.24	0.42
Pre to 2h postfeeding	0.251	-0.004	0.63	0.36	0.72
2 to 4h postfeeding	0.516	0.820	0.25	-0.89	0.70
Pre to 4h postfeeding	0.767	0.815	0.62	-0.52	1.01
4hrpost to prefeeding	-0.767	-0.815	0.62	0.52	1.01

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +481.2 mEq kg^{-1} DM, n=11
[3] Ration with a DCAB of+327.2 mEq kg^{-1} DM, n=10
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
7. Slope of the non-bicarbonate blood buffer line

4. Discussion

As mentioned the DCAB obtained were not exactly the DCAB for which rations were formulated. The discrepancy is very difficult to avoid especially when working with forages because of their highly variable content of minerals. The same problem was encountered by Leclerc and Block (1989). It is difficult to compare the variation obtained in the present experiment to variations obtained by others since in most instances these figures are not reported. Most of the previous research has focused on the comparison between positive and negative DCAB since the latter was proven efficient in the prevention of milk fever (Wang and Beede, 1992; Oetzel et al., 1988; Block, 1984). But, some authors have studied rations for dry cows with DCAB similar to the ones used in the present experiment (Goff et al., 1991; Gaynor et al., 1989). In the present study, both rations had DCAB within the same range as DCAB used later for the rations of lactating cows so that the response of the animals during the dry period could be compared to the response of the lactating cows used in later experiments.

The lack of effect of DCAB on DMI of dry cows has been observed previously by Oetzel et al. (1988) for DCAB ranging from -75 to +190 mEq kg^{-1} and by Goff et al. (1991) for DCAB of -228 and +978 mEq kg^{-1} . The present experiment support previous results obtained in dry cows (Leclerc and Block 1989) and in sheep (Takagi and Block 1991a) where no effect of DCAB, ranging from -27 to +343 $mEq\ kg^{-1}$, on DM digestibility coefficient was found.

The similarity of ADF and NDF digestibilities between the 2 diets would suggest similar rumen fermentation pattern. Some studies using $NaHCO_3$ supplementation have demonstrated changes in volatile fatty acid (VFA) pattern and rumen pH associated with the addition of the dietary buffer (Rogers et al., 1985, Kilmer et al., 1981). These studies would suggest a beneficial effect on fiber digestion from increasing DCAB. However these results were obtained in lactating animals having much higher DMI and rates of passage through the gastrointestinal tract. Secondly, the addition of 0.75 or 1% $NaHCO_3$ corresponds to an increase of 326 to 435 mEq kg^{-1} DM in DCAB which is much larger then the difference between the diets used in the present experiment. It should also be remembered that some

studies using $NaHCO_3$ to increase DCAB found no effect at all on rumen fermentation (Arambel et al., 1988). Fredeen (1984) found no effect of varying the DCAB of the ration of pregnant does on the total concentration and molar proportions of ruminal VFA. These results, in conjunction with those of Takagi and Block (1991a) and the fact that no effect of DCAB on ADF and NDF digestibilities were observed in the present experiment, it is reasonable to suggest that the 2 dietary treatments resulted in similar rumen fermentation patterns. This does not imply, however, that similar proportions or quantities of VFA were absorbed by the host; this question cannot be answered from these data.

Since cows had similar DMI the amount of water they obtained from their feed was similar between the 2 diets. However, as was reported previously by Escobosa et al. (1984) in lactating cows and by Fredeen (1984) in pregnant does an increase in DCAB caused an increase in total water consumption. The increase in water consumption in the latter experiments could have been related to the increase in Na intake and blood Na concentration as the DCAB of the ration was increased. Blood Na through its effects on osmolarity and possibly peripheral or brain angiotensin or both plays a role in the control of thirst (Blair-West et al., 1992, Robertson and Berl, 1991, Cox et al., 1985). However in the present experiment, the Na content of the two diets and the resulting Na intakes were similar and consequently the increased water consumption cannot be attributed to the intake of Na.

Urinary volume was increased significantly by DA *vs* DB. The same observation was made by Fredeen (1988a) since a diet with a higher DCAB also resulted in a higher urine volume. Interestingly, Fredeen modified the DCAB by adding Cl to reduce the balance instead of S as was done for the present experiment so the effects observed on water consumption and urine volume really appear to be caused by altered DCAB rather than by individual minerals. The similarity of effects of Cl^- and S^{-2} can be explained by the presence in the basolateral membrane of renal epithelial cells of some anion antiport systems that can transport Cl^- or S^{-2} in exchange for HCO_3^- (Pritchard and Renfro 1983). It is impossible to establish from the present study or from the results of Fredeen (1988a) whether the increase in urine volume resulted from the increased water consumption or vice-versa. However, the observation that both water intake and urine volume were increased by

increased DCAB suggests that these changes were not mediated by changes in plasma osmolarity. If that had been the case and DCAB caused, for example, an increase in plasma osmolarity then the secretion of vasopressin by the posterior pituitary should have been triggered and water retention increased leading to a decrease in urine volume (Robertson 1985), which is exactly the opposite of what was observed in this experiment.

Changes in blood or extracellular fluid volume changes are other regulators of water balance and vasopressin secretion (Robertson, 1985, Culpepper et al., 1985) and might have mediated the effects of DCAB on urine volume. It is more likely that DCAB acted by altering fluid volumes (blood, extracellular or both) without changing osmolarities. If it stimulated thirst directly, then the increased water consumption could cause an increase in blood volume reducing vasopressin secretion and renal water reabsorption. Consequently urine volume would increase. A direct effect of DCAB on urine volume would have had the same consequences. The results of water metabolism also could be interpreted as a modification of the cell's sensitivity to vasopressin by the altered acid-base status caused by manipulation of the DCAB. Changes in tissue responsiveness as the acid-base status is altered have been demonstrated for the PTH-vit D- Ca homeostatic system (Goff et al. 1991, Beck and Webster 1976) as discussed in the literature review. It can be hypothesized that the hormonal regulation of fluid volumes and osmolarities could also be modulated or affected by perturbation of the acid-base equilibrium.

Acid stress is known to increase glucocorticoid production (England et al. 1991). It is known that under normal circumstances, glucocorticoids inhibit vasopressin release (Robertson and Berl, 1991) and are required for proper water excretion. In adrenal insufficiency high vasopressin levels are observed and the excretion of a water load is impeded (Ganong, 1989). The increased glucocorticoid secretion of acidosis should reduce vasopressin secretion and increase urine volume. However the opposite occured suggesting that altered acid-base status modified the relationship between glucocorticoid, vasopressin and water excretion. An altered secretion of atrial natriuretic peptide (ANP) could have also been involved. The latter hormone regulates Na and water excretions (Ballermann et al., 1991). However,glucocorticoids have been suggested to increase ANP secretion (Ballermann et al. 1991) which cannot explain why the lower DCAB diet, which

normally should be associated with higher glucocorticoid levels, would have resulted in a decrease urine volume.

Another possibility is that, cows offered DA had a higher true absorption of Na creating a higher gradient for water reabsorption from the gut. The duodenum is a leaky epithelium and absorbs fluid isotonically (Powell, 1987). This increased fluid volume would increase ANP secretion which has been shown to respond to volume expansion in goats (Olsson et al.,1991). The increased ANP secretion would then increase natriuresis and diuresis as observed in our experiment. The trend towards higher GFR observed as DCAB was increased could explain some of the increase in urine volume. However, the elevation in ANP does not explain the increase in the amount of free water consumed in as much as ANP injections in goats have been shown to decrease drinking (Olsson et al.,1991). This scenario also is in conflict with a role of angiotensin reported for drinking behavior in cattle. ANP appears to inhibit the renin angiotensin aldosterone system. The increase in Na absorption with DA *vs* DB is also difficult to reconcile with the fact that SO_4^{-2} absorption is partly Na-dependent (Conway et al., 1989) and thus if any effect was to be observed one might have expected an increased Na absorption with DB and not DA; S absorption was significantly increased with the former diet. The effects of DCAB on water intake and urine volume cannot be explained from the data obtained, but they certainly are consistent across all experiments presented thereafter.

Since urinary Na concentration was not affected by DCAB the trend ($P = 0.08$) towards increased total urinary Na excretion was probably mediated by the increase in total urine volume.

The reduction in DCAB tended to increase urinary concentration of Ca but did not increase total urinary Ca excretion as expected from results of other studies (Takagi and Block, 1991; Gaynor et al., 1989; Fredeen et al., 1988a). This could be attributed to the smaller difference in DCAB between the two rations compared in the present experiment *vs* the ranges of DCAB used in the other studies. The high and low diet differed by 154 mEq kg^{-1} in the present study whereas the difference between the DCAB of the diets altering Ca excretion in the study by Gaynor et al. (1989), for example, was 379 mEq kg^{-1}. The same reasoning can be applied to the

lack of effect of DCAB on Mg urinary excretion in the present study compared to the study of Gaynor et al. (1989) in which urinary excretion of Mg was shown to increase as DCAB (calculated as mEq (Na+ K- Cl) kg^{-1}) was increased from 220 to 500 mEq kg^{-1}. Results of Gaynor et al. (1989) should be interpreted cautiously however since they were obtained from a calculation based on body weight and urine concentration of Na and creatinine measured on one daily sample.

Sulfur was most affected by dietary manipulations in large measure because it was the mineral used to alter the DCAB of the ration. Significant increases in S intakes observed when cows consumed DB *vs* DA, paralleled by similar fecal S concentrations resulted in significantly higher S absorption when cows were offered the diet with the lowest DCAB. These results are comparable to those obtained by Takagi and Block (1991a). These authors reported that as the DCAB was reduced from 281 to 61 mEq kg^{-1} using some S containing salts, sheep consumed more S, excreted similar amounts in the feces resulting in increased S absorption expressed in g d^{-1} and as a percentage of S intake. Similarly, rats administered an oral dose of inorganic sulfate showed rapid increases in plasma concentration of S indicative of rapid absorption (Krijgsheld et al., 1979). Two hours after the rats were given the dose of S, plasma concentration of S increased 3-fold. In the present experiment, increases in plasma S concentration were apparent 2 h postfeeding and were significant 4 h postfeeding. Increases in urinary concentration and total daily excretion of S were observed as a result probably of increased intestinal S absorption and accumulation in the blood as the DCAB was reduced. Increases in urinary S excretion were also observed in rats as intake of S increased (Whiting and Cole, 1986, Krijgsheld et al., 1979). The increased excretion of urinary S was not accompanied by increased urinary excretion of Na or Cl in these rats; this was supported by the results obtained in the present experiment and suggests that HCO_3^- was the anion exchanged for S. Increased urinary excretion of S could result from the acidogenic effects of S. In the gastrointestinal tract, a large fraction of S is absorbed by cotransport with Na as mentioned earlier. The high amount of S in DB *vs* DA (in relation to the other minerals whose dietary concentration did not change) could have limited the amount of Na available for Cl absorption, with Cl also being absorbed in a Na-dependent manner in some areas of the intestine (Powell, 1987). Since intestinal absorption of Cl did not differ between the two diets, some other positively charged element must have been absorbed or some negatively charged

element must have been excreted to maintain electrical neutrality and Cl absorption. Potassium is not a likely candidate since its absorption did not differ between the 2 diets. However, an increased activity of the Cl^-/HCO_3^- or $SO_4^=/HCO_3^-$ exchangers present in the intestinal and renal epithelium could have played a role in the maintenance of Cl absorption (Powell, 1987, Pritchard and Renfro, 1983). Ultimately, increasing HCO_3^- secretion into the gut or absorption of S can lead to a metabolic acidosis because an increase in HCO_3^- excretion has the same metabolic effect as an increase in H^+ absorption.

It can be suggested that the acid stress caused by dietary S resulted in an elevation in glucocorticoids as normally occurs in acidosis. These hormones have been demonstrated to inhibit S reabsorption by the kidneys (Renfro et al., 1989). This would explain the increased urinary concentration of S when DB was fed. It is not known, however, if plasma S in itself can affect the $Na^+/SO_4^=$ transport system or the $HCO_3^-/SO_4^=$ exchanger (Renfro et al., 1989) in the renal tubules. Although the 4-fold increase in the fractional excretion of S could have resulted in part from an increase in renal tubular secretion; it does not contradict an inhibitory effect on some reabsorptive mechanism when DCAB is reduced.

The acidogenic effects of reducing DCAB were demonstrated by changes in urinary bicarbonate concentration and total daily excretion. As DCAB was reduced, both these parameters were significantly reduced. Increased renal HCO_3^- reabsorption is a classical renal compensatory response to metabolic acidosis. The kidneys seem to respond to a decline in blood pH, which stimulates the Na^+/H^+ countertransport system in the renal tubules (Sabatini and Kurtzman, 1989; Baile and Kurtzman, 1985). Interestingly, glucocorticoids may also play a role since they have been demonstrated to increase Na^+/H^+ exchange in renal brush border membrane vesicles (Freiberg et al., 1982). However, from our data it is impossible to conclude if Na^+/H^+ transport system was affected alone or if a modification of the activity of the H^+-ATPase (Alpern et al., 1991) also contributed to the renal response to acidosis. An increase of free H^+ excretion was also demonstrated for cows offered the ration with the lowest DCAB. Even though the acid stress caused by dietary manipulation was evident from urine analysis it was not enough to overload the renal compensatory mechanisms. This explains why the blood acid-base parameters were not different at any time postfeeding. If one calculates the net acid excretion in the

urine for cows offered both diets (using urinary ammonia and bicarbonate excretion) both diets produced negative values because of the high urinary bicarbonate concentrations. However, the absolute value obtained for DB (7849 mmol d^{-1}) is one half the value obtained for DA (14261 mmol d^{-1}. Note that titratable acidity was not included in the equation (Alpern et al., 1991) because urinary pH was always above 7.5 . Increases in urinary concentration of H^+ and decreases in total daily HCO_3^- excretion as DCAB was decreased were also reported in pregnant goats (Fredeen et al., 1988a). It is interesting to note that in the study by Fredeen et al. (1988a) DCAB was manipulated by altering dietary Cl and not S. The similar results obtained in the latter and in the present study supports further an effect of DCAB and not Cl or S, per se. Wang and Beede (1991) observed a decrease in blood pH as DCAB was reduced, however, the difference in DCAB between the two diets used in that study was greater in that DCAB was reduced from 69 to -428 mEq kg^{-1}. The absolute values of the DCAB were also much lower than in the present study. This can explain the uncompensated effect on acid-base status observed by those authors. Their results were obtained with a combination of Cl^- and $SO_4^=$ salts.

The data obtained from calculations of metabolic H^+ production, although not statistically significant, support an acidogenic effect of DB *vs* DA The H^+ input into the blood from 2 to 4 h postfeeding was elevated when the cows were offered DB compared to DA. The drain of H^+ from the blood at later times will include the protons excreted in the urine; because this was shown to be higher for DB, it is expected that the absolute H^+ production between 4 h postfeeding until the next morning was higher for DB *vs* DA.

Although alteration of DCAB affected acid-base status (as illustrated by alterations in urinary HCO_3^-), it did not affect any blood metabolites measured. This probably can be explained by the small difference between the diets and the fact that the reduced DCAB did not result in metabolic acidosis. The studies mentioned earlier which showed effects of acid-base status on N and fat metabolism were all conducted under conditions of metabolic acidosis, thus altered blood pH. This was not the case in the present experiment.

Wang and Beede (1992) reported increase FE of Ca and lowered FE of Mg

which was not observed in the present experiment probably because the plasma concentrations of these minerals were not altered as they were in the study by Wang and Beede (1992). The discrepancy between these studiescould be related to the much larger difference in DCAB between the diets compared by Wang and Beede (1992) (497 meq kg^{-1}) than that between DA and DB (155 meq kg^{-1}) in the present study.

5.Conclusion

This experiment demonstrated that the acid-base status of the dry cows can be modified by smaller changes in DCAB than in other reports. Indeed, both diets used in this experiment had a positive DCAB and yet the diet with the lower DCAB resulted in changes in urinary HCO_3^- excretion and concentration even though it did not cause a metabolic acidosis. These effects were independent of changes in DMI or DM digestibility. A trend towards a reduction in GFR was observed with the reduction of DCAB but the difference between the diets did not reach statistical significance. The lack of effect of DCAB on renal function was accompanied by no effects on Ca, Mg, P, K and Cl metabolism. A trend towards increased intestinal absorption of Na and urinary excretion of Na was observed with a higher DCAB and could explain some of the increases observed in water consumption and urinary excretion as DCAB was increased. The results presented support a role for S in the maintenance of acid-base balance similar to the role of Cl reported by previous authors.

IV. EFFECTS OF CHANGING RATION INGREDIENTS ON ACID-BASE BALANCE, RENAL FUNCTION AND MACROMINERAL METABOLISM OF COWS IN EARLY LACTATION.

Abstract

This experiment was conducted to study the effects of formulating rations to meet NRC requirements but using different ingredients. Ten Holstein and 2 Ayrshire cows were used in a switchback design to compare 2 rations: 1- an alfalfa haylage-soybean based ration (EH) and 2- a corn silage-soybean based ration (EC). Both rations had similar cation-anion balances and contained 1% sodium bicarbonate. Dietary treatment did not affect the intake of dry matter or its digestibility. Ration EH caused an increase in NDF intake but did not affect its digestibility. Intake, absorption and urinary excretion of N were significantly increased by EH . The increased in urinary N excretion eliminated the difference N balance between the 2 diets. Milk production and composition were not affected by dietary treatment. Cows consuming EH absorbed significantly more water than cows consuming EH but also had significantly higher urine volumes . Intakes of Mg, K, Cl and S differed between diets but only K balance was significantly affected. It was higher for EH. The fractional excretions of K, Cl and S were increased by EH, demonstrating that the kidneys reponded to the increased intakes by diminishing the reabsorption or by increasing the secretion of these minerals. Blood, urine and milk acid-base parameters were not affected by dietary treatment. Plasma volume was significantly increased by EH *vs* EC whereas glomerular filtration rate tended to be decreased. The changes in plasma volume demonstrated that the cows did not compensate fully for the increased in apparent water absorption observed with EH. These data demonstrated that an alfalfa haylage-based diet compared to a corn silage-based diet, both having similar cation-anion balances, resulted in different water and mineral metabolism but did not affect significantly the acid-base status of cows in early lactation. Dietary cation-anion balance appears to be more important than the ingredients themselves in determining the acid-base status of dairy cows.

1. Introduction

Effects of the addition of dietary buffers especially ($NaHCO_3$), to the rations of dairy cows are still very controversial in that responses vary from improvements to depressions in the cows' performance. For example, with the addition of $NaHCO_3$ increases in DMI were obtained by Vicini et al. (1988) whereas Erdman et al. (1982) observed no change in voluntary consumption. Reviewing the literature, Erdman (1988) suggested that dietary buffers are more efficient when added to a corn silage-based diet than to a haylage based diet. Since different ingredients will produce different nutrient profiles upon fermentation and digestion and since they have different buffering capacities (Jasaitis et al., 1987) it can be suggested that feed ingredients can alter the acid-base status of the animal. The protein content of the ration, for example, is known to affect acid-base balance partly due to its amino acid profile and renal glomerula filtration rate (Asplund et al., 1980, studies in rats and humans). If the ingredients used have an effect on acid-base status then obviously the response to dietary buffers and to dietary cation-anion balance (DCAB) will be dependent on ration composition. It is impossible to determine if the differential effects of different ingredients on acid-base balance are independent of the DCAB of the rations formulated since no study in that area has been done to address this question.

The objectives of the following experiment were: 1- to determine the effects of feeding haylage-based vs corn silage-based diets, both formulated to meet NRC requirements (1988) and to have similar DCAB on the acid-base status of cows in early lactation; 2- to determine the effects of these diets on milk production and composition; 3- to determine the effects of the 2 diets on renal function and water balance; 4- to determine the effects on macromineral metabolism and 5- to determine the effects on blood metabolic parameters.

2. Materials and methods

A. Experimental design and housing.

Ten Holstein and two Ayrshire cows in early lactation were used in a switchback design during which 2 diets were compared. Animals were selected on the basis of their last calving date, and parity. To correct for the fact that not all cows could be obtained at once they always entered the experiment as pairs with members of each pair assigned to one of the two diets at random. The members of the pair calved within a 2 wk interval and were of similar parity. Cows were all between parity 1 and 3 and were between 22 and 42 d in milk at the beginning of the experiment. This design allowed the elimination of a time effect because both diets were represented equally at each time period.

Each cow was studied through three successive experimental periods of 28 d each. As mentioned, the first diet received by one cow was assigned at random. After having completed one period, the cow received the other diet and for the third period it was given the same ration as it received in the first period. The experimental periods had the same schedule as the experimental period of the experiment presented in Chapter III. Cows were milked twice daily between 0530 h and 0630 h and again between 1530 h and 1630 h. The same schedule was adopted for milking whether the cows were in the dairy barn or in the metabolism stalls.

B. Diets and Feeding schedule

Two diets were formulated to meet NRC requirements (1988). The DM composition of the diet based on corn silage was as follows: 50 % corn silage, 10% haylage, 10% high moisture ear corn, 22% micronized soybeans, 3% Megalac® (Church and Dwight, Inc, Princeton, New Jersey,U.S.A.; a calcium salt of fatty acids designed to be inert in the rumen) 5% mineral and vitamin mix. The DM composition of the alfalfa haylage based diet was: 55% alfalfa haylage, 22% high moisture ear corn, 14% micronized soybeans, 4% Megalac®, 5% vitamin and mineral mix. The vitamin and mineral mix was the same for both diets and its DM composition was: 26.2% $CaCO_3$, 20.2% $NaHCO_3$, 18.1% of a vitamin ADE mix

(same as previous experiment), 10.1% $CaCl_2$, 4% $NaPO_4$, 4% NaCl, 4% $MgSO_4$, .001% $MnCO_3$ and .001% $ZnSO_4$. Samples of the ingredients were taken every second week to correct the ration for changes in DM content. The rations were offered twice daily between 0730 h and 0830 h and between 1430 h and 1530 h. Cows were fed to allow *ad libitum* intake ensuring 5 to 10% refusals.

C. Sampling Schedule and Procedure

The sampling schedule and procedures for feeds, refusals, water, feces, urine and blood were identical to those described in the previous chapter. In addition, milk production was monitored in the present experiment.

Milk: Milk volume was measured using Tru-Test meters (Surge, Mississauga, Ontario) and samples taken at each milking from d 4 to 8. At the initiation of milking, 60 mL were collected by hand into a 100 mL plastic bottle containing 1 mL of light mineral oil (ICN Biochemicals, Mississauga, Ontario). These samples were used for immediate determinations of pH and pCO_2. At the end of each milking the milk was mixed and two proportional samples were taken. Samples taken on the same day were mixed and one was sent to the Dairy Herd Analysis Service (DHAS) for further analysis. The samples kept were pooled by cow at the end of each collection period and stored frozen for further analysis.

D. Analytical methods

The analytical methods were identical to those described in the previous chapter for feeds, refusals, water, feces, urine and blood samples.

Milk Samples: Milk samples were analyzed according to the same procedures as urine samples for determinations of parameters of acid-base balance and minerals. The samples sent to DHAS were analyzed for total protein, fat and lactose content with a Milk-O-Scan 300 (N Foss Electric, Hillerod, Denmark). Note that when calculating N and mineral balances, retentions were calculated first as the intake of the element minus its excretion in the feces and urine to give what will be

referred to as retention and then the secretion of the element in the milk was subtracted from retention to yield the balance.

E. Statistical Analysis

Data were analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, North Carolina, U.S.A.) according to the model for a switchback design (Lucas, 1983). The linear model used is described by the following equation:

$$Y_{ijk} = \mu + A_{ij} + B_{ij} X_k + C_k + T_h + E_{ijk},$$

where Y_{ijk} = variable studied during the k^{th} period (k=1,2,3), of the j^{th} cow (j=1,...12), in the i^{th} group (i=1,2),
μ = population mean,
A_{ij} = effect of the j^{th} cow in the i^{th} group,
B_{ij} = linear time trend of the j^{th} cow in the i^{th} sequence,
X_k = units of time (X=1 in first period, X=0 in second period and x=1 in the third period),
C_k = effect of the k^{th} period,
T_h = effect of the h^{th} treatment and
E_{ijk} = random error.

Analysis of variance was performed and the difference between the treatment means was estimated using contrast. The standard error of the difference is reported. Statistical significance was declared when the probability was less than 5%.

In addition, when thought necessary, certain variables were included in the model as a linear regression term. When such analyses were done, they are mentioned in the text.

3. Results

Diet Composition: The DM composition of the alfalfa haylage based diet (EH) and the corn silage based diet (EC) is shown in Table 4.1.

The ration containing alfalfa haylage had slightly more K , Cl and S than the ration containing corn silage however, both had similar DCAB (+440 for EH and + 412 mEq kg^{-1} for EC). Both rations were similar in their ADF, NDF, Ca, P, Na and S content. Ration EH contained more CP but the difference was small in relation to the standard deviations obtained.

Dry matter, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Nitrogen (N) intakes and digestibilities: Body weight (BW) and dry matter intake (DMI) expressed in kg d^{-1} were not affected by dietary treatment (Table 4.2). The cows consuming EH had significantly higher intakes of ADF and digestible ADF *vs* cows offered EC. However, ADF digestibility did not differ between treatments. No difference between diets was observed for intake and digestibility of NDFexcept. Using DMI as a covariate in the model was significant but did not eliminate the observed dietary effect on ADF and NDF intake and digestibility parameters. Using BW as a covariate had no significant effect.

Significantly higher intake and absorption of N were observed for cows offered EH despite the higher concentration of N in the feces. Nitrogen apparent absorption expressed as a percentage of N intake (N digestibility) did not differ between diets (Table 4.3). Concentration of urinary N was not affected but total daily excretion was increased by EH causing similar retention of N before and after subtracting the N secreted in the milk. Including DMI as a linear regression term in the model was found to have a significant effect (P= 0.001) for N intake, but it did not eliminate the effect of ration. Body weight as a covariate had no significant effect on N intake, absorption or retention.

Table 4.1. Dry matter composition of an alfalfa haylage-based diet (EH) and a corn silage-based-diet (EC) offered to cows in early lactation.

Analyte	EH[3]	SD[1]	EC[4]	SD
CP, %	18.6	1.5	17.0	1.0
NDF, %	35.00	4.20	35.60	3.00
ADF, %	27.80	2.50	25.20	1.70
Calcium, %	1.31	0.31	1.21	0.25
Magnesium, %	0.25	0.02	0.28	0.01
Phosphorus, %	0.26	0.06	0.26	0.07
Sodium, %	0.41	0.05	0.44	0.04
Potassium, %	2.23	0.19	1.86	0.13
Chlorine, %	0.57	0.06	0.49	0.05
Sulfur, %	0.24	0.02	0.19	0.01
Cation-anion balance[2]	440.20	60.60	412.00	37.20

1Standard deviation

2 mEq (Na++ K +- Cl- - S--) kg-1 dry matter. (DM)

3 alfalfa haylage-based diet, n=18

4 corn silage -based diet, n=18

Table 4.2. Mean body weight (BW) dry matter (DM), ADF and NDF intakes and digestibilities of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
BW, kg	517.8	513.7	0.187	4.1	2.9
DM Intake, kg^{-1} d^{-1}	16.70	16.74	0.919	-0.04	0.34
DM Digested, kg d^{-1}	11.27	11.28	0.979	-0.012	0.43
DM Digested, % of intake	67.15	67.54	0.829	-0.386	1.74
ADF Intake, g d^{-1}	4851	4565	0.080	286	147
ADF Digested, g d^{-1}	3219	2908	0.090	311	166
ADF Digested, % of intake	66	64	0.355	2	2
NDF Intake, g d^{-1}	6194	5794	0.022	401	148
NDF Digested, g d^{-1}	4012	3628	0.074	384	193
NDF Digested, % of intake	64	63	0.598	1	2

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.3. Mean nitrogen (N) balance of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
N Intake g d^{-1}	501.7	451.5	0.003	50.2	13.1
Fecal N %	2.45	2.33	0.013	0.15	.051
Fecal N g d^{-1}	128.1	132.1	0.280	-7.8	6.9
N absorption					
N Absorbed g d^{-1}	368.4	324.9	0.017	43.5	15.0
N Absorption % of intake	72.7	71.7	0.647	-0.8	1.6
Urine N, g L-1	6.12	6.38	0.350	.037	.037
Urine N, g d^{-1}	89.5	72.1	0.002	22.5	5.4
N Retention[7]					
N Retention, g d^{-1}	276	255	0.185	21	15
N Retention, % of intake	54	56	0.318	2	2
N Balance[8]					
N Balance, g d^{-1}	173	151	0.159	21	14
N Balance, %of intake	33	33	0.87	0.4	2

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Body weight
[7] Retention= intake- fecal excretion- urine excretion
[8] Balance= retention- milk secretion

Milk yield and composition: Daily milk yield was similar between cows offered the two diets as were yields and percentages of milk components (Table 4.4).

Water intake and absorption: Cows consuming the alfalfa haylage based diet consumed 12 L more water ($P = 0.0005$) but obtained 8 L less from their feed ($P = 0.0001$) than cows offered the corn silage based diet resulting in no significant difference in total water consumed (Table 4.5). The percentage of water consumed that was absorbed and the total quantity absorbed were higher when cows consumed EH instead of EC; the former absorbed 6 L more water.

Mineral Balances

Calcium, magnesium and phosphorous: Calcium intake was not different between the diets; however, it tended to be higher for the EH diet (Table 4.6). Fecal and urinary excretions of Ca were significantly ihigher when cows consumed EH instead of EC. Cows consuming EC tended to absorb a larger fraction of the Ca consumed (48.9 *vs* 42.7 %). Diet had no effect on Ca secreted in milk in terms of total amount and total daily secretion. Calcium balance was not affected by dietary treatment and neither was Ca concentration in milk. The increased intake of Mg when cows were offered EC was counteracted by an increased fecal Mg concentration resulting in no change in Mg apparent absorption expressed as a percentage of Mg eaten or as g absorbed daily (Table 4.7). Similar Mg retentions and secretions in the milk were observed for both diets.

Table 4.8 presents results obtained for parameters of P balance. Dietary treatment had no effect on P intake, absorption or retention. Despite the very low urinary excretions, negative P balances were observed for both diets.

Table 4.4. Mean milk yield and composition of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Yields, kg d^{-1}					
Milk yield	23.36	23.15	0.639	0.211	0.436
Fat yield	1.01	0.99	0.594	0.016	0.029
Protein yield	0.65	0.65	0.84	0.003	0.016
Lactose yield	1.15	1.15	0.96	0.0013	0.025
Concentrations, %					
Fat	4.35	4.31	0.514	0.038	0.056
Protein	2.77	2.8	0.606	-0.026	0.048
Lactose	4.93	4.97	0.21	-0.004	0.003

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.5. Mean intake and apparent absorption of water by cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Free water consumed , L d^{-1}	82.2	74.9	0.0005	12.1	2.4
Feed water, L d^{-1}	15.7	24.7	0.0001	8.0	1.0
Total water consumed, L d^{-1}	97.9	99.7	0.113	1.1	2.4
Apparent absorption, L d^{-1}	73.2	71.2	0.011	6.2	2.0
Apparent absorption, % of intake	74.7	71.7	0.0003	3.0	0.6

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.6. Mean calcium balance of cows in early lactation fed diets differing in ingredient composition.

Calcium	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	213.7	207.0	0.559	-14.3	23.6
Fecal concentration, %	2.12	1.86	0.0001	-0.34	0.04
Fecal excretion, g d^{-1}	135.0	104.5	0.019	-18.7	6.7
Apparent absorption, g d^{-1}	101.2	104.4	0.936	2.2	26.3
Absorption, % of intake	42.7	48.9	0.380	8.8	9.6
Urinary concentration, ppm	35	22	0.078	13	6
Urinary excretion, mg d^{-1}	524	256	0.035	117	-307
Apparent retention[6], g d^{-1}	100.7	103	0.645	-11.7	24.5
Apparent retention, % of intake	42.4	48.2	0.688	3.7	8.9
Milk concentration, ppm	932	953	0.900	4.1	31.6
Milk secretion, g d^{-1}	21.3	22.5	0.990	0.01	0.94
Balance[7], g d^{-1}	79.4	79.8	0.648	-11.6	24.6
Balance, % of intake	31.4	36.7	0.776	3.0	10.3

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Retention = intake - fecal excretion - urine excretion
[7] Balance = retention - milk secretion

Table 4.7. Mean magnesium balance of cows in early lactation diets differing in ingredient composition.

Magnesium	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	40.8	47.5	0.012	-3.99	1.31
Fecal concentration, %	0.51	0.58	0.037	-0.09	0.04
Fecal excretion, g d^{-1}	26.6	32.1	0.141	3.9	2.4
Apparent absorption, g d^{-1}	13.5	15.8	0.100	-4.0	2.2
Absorption, % of intake	32.5	32.8	0.995	-0.02	2.9
Urinary concentration, ppm	69	99	0.194	-16	11
Urinary excretion, mg d^{-1}	1003	1045	0.120	-24	14
Apparent retention[6], g d^{-1}	12.5	14.8	0.740	-2.4	7.1
Apparent retention, % of intake	30.0	30.5	0.990	-2.4	3.3
Milk concentration, ppm	98	119	0.410	13	15
Milk secretion, g d^{-1}	2.3	2.3	0.370	-0.12	1.2
Balance[7], g d^{-1}	10.3	11.9	0.720	-2.7	7.1
Balance, % of intake	24.3	24.5	0.9	-0.43	3.2

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

[6] Retention = intake - fecal excretion - urine excretion

[7] Balance = retention - milk secretion

Table 4.8. Mean phosphorus balance of cows in early lactation fed diets differing in ingredient composition.

Phosphorous	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	45.5	45.0	0.984	0.05	2.4
Fecal concentration, %	0.53	0.55	0.343	0.25	0.03
Fecal excretion, g d^{-1}	27.0	30.7	0.605	1.1	2.0
Apparent absorption, g d^{-1}	14.6	14.5	0.713	-1.4	3.6
Absorption, % of intake	28.0	28.2	0.809	-1.8	7.1
Urinary concentration, ppm	1.7	2.9	0.409	2.8	3.2
Urinary excretion, mg d^{-1}	22.1	31.9	0.510	23.6	33.6
Apparent retention[6], g d^{-1}	14.6	13.6	0.819	-1.0	4.3
Apparent retention, % of intake	27.9	26.1	0.820	-2.0	8.6
Milk concentration, ppm	718.	731	0.049	-19.3	26.7
Milk secretion, g d^{-1}	16.5	17.2	0.430	-0.7	0.8
Balance[7], g d^{-1}	-1.9	-4.1	0.951	0.3	5.1
Balance, % of intake	-14.9	-16.6	0.985	-0.2	12.9

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Retention = intake - fecal excretion - urine excretion
[7] Balance = retention - milk secretion

Sodium, potassium, chloride and sulfur: Cows offered EC tended to eat more Na as illustrated in Table 4.9 although this difference was not significant. Because fecal excretion did not differ between diets, cows consuming EC also tended to absorb more Na expressed per day. The increased urinary concentration of Na observed for EC ($P = 0.002$) did not result in higher total daily excretion of Na and, consequently, EC tended to increase retention calculated as intake-(fecal+urine excretions). Milk Na content and the total amount of Na secreted in the milk daily were not affected by EC therefore, Na balance tended ($P = 0.10$) to increase by 8.3 g d^{-1}.

Potassium intake, apparent absorption (g d^{-1}) and retention (g d^{-1}) were all significantly higher for EH than for EC (Table 4.10). Apparent absorption and retention were not affected by diet when expressed as percentage of K intake. Milk content of K and total quantity of K secreted daily in the milk were similar for EH and EC.

Cows offered EH consumed and absorbed 16.4 g more Cl compared with EC (Table 4.11). Absorption, calculated as percentage of intake, was also higher for EH. However, urinary excretion being increased by EH eliminated the effect of diet on overall Cl retention. There was no difference in Cl content of the milk.

Results of S balances are presented in Table 4.12. Diet had similar effects on S metabolism as it did on Cl, namely, EH increased intake, absorption and urinary excretion resulting in no difference in S balance when compar to EC.

Plasma mineral concentrations: Results of plasma mineral analysis at different sampling times are presented in Table 4.13. The only minerals whose plasma concentrations were affected by diet were P and Cl at 4 h postfeeding. The former was higher for EC and the latter was lower for EC. However, the differences between the diets were small. A difference of 4.1 ppm was observed for P and of 3.7 ppm for Cl.

Table 4.9. Mean sodium balance of cows in early lactation fed diets differing in ingredient composition.

Sodium	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	66.4	75.1	0.129	3.6	2.2
Fecal concentration, %	0.15	0.14	0.436	-0.01	0.02
Fecal excretion, g d^{-1}	8.12	8.21	0.447	-0.78	0.99
Apparent absorption, g d^{-1}	58.4	66.7	0.121	4.3	2.5
Absorption, % of intake	88.0	88.9	0.291	0.5	1.4
Urinary concentration, ppm	1218	1574	0.002	-360	73
Urinary excretion, mg d^{-1}	18656	17828	0.240	4582	3567
Apparent retention[6], g d^{-1}	39.8	49.3	0.094	8.2	4.4
Apparent retention, % of intake	60.6	65.0	0.143	6.6	4.1
Milk concentration, ppm	306	316	0.940	-1.0	13.1
Milk secretion, g d^{-1}	6.6	7.0	0.340	-0.3	0.3
Balance[7], g d^{-1}	32.7	41.7	0.102	-8.3	4.6
Balance, % of intake	49.7	54.9	0.135	7.4	4.5

[1] EH: Alfalfa haylage-based diet
[2] EC: Corn Silage-based diet
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Retention = intake - fecal excretion - urine excretion
[7] Balance = retention - milk secretion

Table 4.10. Mean potassium balance of cows in early lactation fed diets differing in ingredient composition.

Potassium	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	357.9	319.6	0.002	-55.3	9.5
Fecal concentration, %	0.46	0.41	0.120	-0.08	0.05
Fecal excretion, g d^{-1}	66.68	23.74	0.145	-4.83	3.05
Apparent absorption, g d^{-1}	333.2	295.8	0.0004	-51.5	9.8
Absorption, % of intake	93.3	92.6	0.789	-0.2	0.8
Urinary concentration, ppm	1787	1685	0.259	-90	73
Urinary excretion, mg d^{-1}	26456	19134	0.003	-11214	24579
Apparent retention[6], g d^{-1}	306.7	278.0	0.006	-38.6	10.7
Apparent retention, % of intake	85.9	86.5	0.209	1.6	1.2
Milk concentration, ppm	1469	1457	0.490	-27	38
Milk secretion, g d^{-1}	33.7	34.5	0.460	-1.1	1.4
Balance[7], g d^{-1}	273.0	243.4	0.008	-37.5	11.1
Balance, % of intake	76.4	75.6	0.856	0.3	1.7

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Retention = intake - fecal excretion - urine excretion
[7] Balance = retention - milk secretion

Table 4.11. Mean chloride balance of cows in early lactation fed diets differing in ingredient composition.

Chloride	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	92.9	83.3	0.0007	14.8	3.1
Fecal concentration, %	0.30	0.34	0.41	0.02	0.02
Fecal excretion, g d^{-1}	8.1	19.7	0.462	11.3	11.7
Apparent absorption, g d^{-1}	76.7	63.8	0.002	16.4	3.8
Absorption, % of intake	82.4	76.9	0.040	4.6	6.9
Urinary concentration, ppm	2029	1380	0.005	554	139
Urinary excretion, mg d^{-1}	29809	15338	0.001	15878	3303
Apparent retention[6], g d^{-1}	46.8	50.1	0.833	-0.9	4.2
Apparent retention, % of intake	49.9	58.6	0.057	7.7	3.5
Milk concentration, ppm	681	644	0.030	34	32
Milk secretion, g d^{-1}	2.1	1.8	0.480	0.3	0.4
Balance[7], g d^{-1}	31.1	31.4	0.658	-2.2	4.9
Balance, % of intake	32.6	37.1	0.456	-3.7	4.7

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference
[6] Retention = intake - fecal excretion - urine excretion
[7] Balance = retention - milk secretion

Table 4.12. Mean sulfur balance of cows in early lactation fed diets differing in ingredient composition.

Sulfur	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Intake, g d^{-1}	39.1	32.4	0.0001	9.0	1.5
Fecal concentration, %	0.26	0.24	0.325	0.01	0.01
Fecal excretion, g d^{-1}	24.7	13.6	0.382	8.30	9.1
Apparent absorption, g d^{-1}	25.5	19.0	0.0009	8.3	1.8
Absorption, % of intake	64.1	58.3	0.045	6.6	2.8
Urinary concentration, ppm	908	432	0.097	131	68
Urinary excretion, mg d^{-1}	13277	4753	0.002	3703	784
Apparent retention[6], g d^{-1}	13.1	14.4	0.719	2.5	6.9
Apparent retention, % of intake	32.0	43.7	0.307	17.1	15.8
Milk concentration, ppm	91.1	98.3	0.570	3.5	6.1
Milk secretion, g d^{-1}	2.0	2.3	0.5	0.2	0.2
Balance[7], g d^{-1}	11.1	12.1	0.732	2.4	6.9
Balance, % of intake	26.4	36.5	0.354	15.6	16.0

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

[6] Retention = intake - fecal excretion - urine excretion

[7] Balance = retention - milk secretion

Table 4.13. Mean plasma mineral concentrations of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Prefeeding					
Ca, mg dL^{-1}	102	102	0.99	-0.003	0.36
Mg, mg dL^{-1}	2.6	2.5	0.50	0.06	0.09
P, mg dL^{-1}	3.9	3.9	0.84	0.03	0.2
Na, mmol L^{-1}	121.9	118.0	0.60	3.1	5.8
K, mmol L^{-1}	3.7	3.8	0.54	0.09	0.14
Cl, mmol L^{-1}	99.6	97.9	0.22	1.7	1.4
S, mmol L^{-1}	1.7	1.4	0.33	0.2	0.2
2h postfeeding					
Ca, mg dL^{-1}	10.1	10.1	0.69	0.2	0.4
Mg, mg dL^{-1}	2.5	2.3	0.17	0.2	0.1
P, mg dL^{-1}	4.2	4.2	0.77	0.04	0.1
Na, mmol L^{-1}	122.6	119.1	0.47	3.3	4.4
K, mmol L^{-1}	3.6	3.7	0.86	0.03	0.2
Cl, mmol L^{-1}	97.6	98.7	0.58	1.1	1.7
S, mmol L^{-1}	2.1	1.9	0.15	0.2	0.2
4 h postfeeding					
Ca, mg dL^{-1}	10.3	10.2	0.89	-0.05	0.3
Mg, mg dL^{-1}	2.4	2.4	0.46	-0.06	0.09
P, mg dL^{-1}	4.1	4.4	0.0001	0.4	0.07
Na, mmol L^{-1}	123.5	121.7	0.61	-2.9	5.6
K, mmol L^{-1}	3.7	3.7	0.92	-0.01	0.1
Cl, mmol L^{-1}	100.7	97.9	0.02	-3.7	1.4
S, mmol L^{-1}	2.0	2.1	0.48	0.08	0.1

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Blood, urine and milk acid-base parameters: Blood pH and concentrations of free H^+, HCO_3^- and pCO_2 are shown in Table 4.14 for the different sampling times. Analysis of pH and H^+ concentration did not show any difference between the two diets at any sampling time. Blood pCO_2 and HCO_3^- concentration were not affected.

Urine volume was significantly increased by EH (Table 4.15) and since concentration of free H^+ was not altered, cows consuming EH tended to have a greater ($P = 0.12$) total daily excretion of protons in their urine (8.6×10^{-8} *vs* 6.74×10^{-8} mol d^{-1} for EH *vs* EC). The urinary concentration of HCO_3^- was reduced by 134 mmol L^{-1} ($P = 0.08$) when EH was consumed. However the total urinary HCO_3^- excretion via the urine remained unaffected by diet.

Concentration free H^+ in milk tended to be higher when cows consumed EH instead of EC ($P<0.10$). If the figures shown in Table 4.16 were expressed as pH instead of H^+ concentration then the milk of cows consuming EH had a pH of 6.506 whereas the milk of cows consuming EC had a pH of 6.523. Concentration of HCO_3^- and total daily secretion in the milk were not altered by dietary treatment. However, pCO_2 in milk was higher for EH compared to EC.

Blood Volume and Renal Function: The volumes of plasma, the percentage of blood packed cell volume and the calculated blood volume are presented in Table 4.17. Feeding the alfalfa haylage based diet resulted in significantly larger plasma and blood volumes than when feeding the corn silage based diet. When expressed as mL kg^{-1} BW plasma volume was still higher for EH ($P = 0.07$). Using BW as a covariate in the statistical model was not significant. Effective renal plasma and blood flows were not affected significantly but tended to be higher for EH *vs* EC (Table 4.17).

Table 4.14. Mean blood pH, free hydrogen ion (H^+) and bicarbonate ion (HCO_3^-) concentrations and carbon dioxide (pCO_2) of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Prefeeding					
pH	7.359	7.354	0.863	-0.005	0.027
H^+, mol L^{-1}	4.47×10^{-5}	4.41×10^{-5}	0.919	0.05×10^{-5}	0.10×10^{-5}
HCO_3^-, mmol L^{-1}	29.74	29.87	0.857	-0.13	0.70
pCO2, mm Hg	52.83	53.64	0.943	-0.80	3.59
2 h postfeeding					
pH	7.381	7.367	0.535	0.014	0.022
H^+, mol L^{-1}	4.18×10^{-5}	4.34×10^{-5}	0.477	-0.10×10^{-5}	0.14×10^{-5}
HCO_3^-, mmol L^{-1}	30.18	30.35	0.765	-0.17	0.56
pCO2, mm Hg	50.50	52.67	0.388	-2.17	2.40
4 h postfeeding					
pH	7.378	7.396	0.286	-0.018	0.016
H^+, mol L^{-1}	4.21×10^{-5}	4.05×10^{-5}	0.291	0.15×10^{-5}	0.21×10^{-5}
HCO_3^-, mmol L^{-1}	29.31	29.74	0.534	-0.43	0.69
pCO2, mm Hg	49.51	48.18	0.443	1.329	1.66

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.15. Mean urine volume, free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and pCO_2 excretion of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Volume L d^{-1}	28.4	21.5	0.0004	-6.9	1.3
Total free H^+ mol d^{-1}	8.60×10^{-8}	6.74×10^{-8}	0.116	-2.00×10^{-8}	1.01×10^{-8}
Free H^+ mol L^{-1}	3.18×10^{-9}	3.03×10^{-9}	0.712	0.12×10^{-9}	0.10×10^{-9}
Total HCO_3^- mol d^{-1}	17.19	15.33	0.413	-18.62	2.18
HCO_3^- mmol L^{-1}	585.3	719.5	0.077	134.2	68.0
pCO_2 mm Hg	62.1	76.1	0.001	14.0	3.1

[1] EH: Alfalfa haylage-based diet, n=18
[2] EC: Corn Silage-based diet, n=18
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference

Table 4.16. Mean milk free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and pCO_2 secretion of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Total free H^+, mol d^{-1}	7.16×10^{-6}	6.9×10^{-6}	0.361	2.6×10^{-7}	2.80×10^{-7}
Free H^+, mol L^{-1}	3.12×10^{-7}	3.00×10^{-7}	0.104	0.11×10^{-7}	0.10×10^{-7}
Total HCO_3^-, mmol d^{-1}	59.17	60.35	0.584	-1.18	2.08
HCO_3^-, mmol L^{-1}	2.50	2.51	0.826	-0.02	0.07
pCO_2, mm Hg	40.69	39.38	0.024	1.31	0.49

[1] EH: Alfalfa haylage-based diet
[2] EC: Corn Silage-based diet
[3] Probability associated with the F test
[4] Estimate of the difference between treatment means
[5] Standard error of the estimated difference

Table 4.17. Mean plasma volume, packed cell volume and renal function parameters of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Plasma volume, L	43.41	39.34	0.05	7.72	3.48
Packed cell volume, %	28.2	27.5	0.42	0.4	0.5
Blood volume, L	60.5	54.8	0.05	11.0	4.9
Effective renal plasma flow, L min^{-1}	4.62	4.28	0.73	0.20	0.56
Effective renal blood flow, L min^{-1}	6.44	6.04	0.70	0.30	0.75.
Glomerular filtration rate, L d^{-1}	784	860	0.45	-78	99
Filtration fraction	0.119	0.146	0.73	-0.430	1.220

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Glomerular filtration rate tended to be higher for EC (Table 4.17). No significant effect of ration was observed for the filtration fraction; it only tended to be smaller for EH because of the larger difference in GFR (9.7% lower for EH) *vs* RPF (6% higher for EH). Body weight was included in the model as a linear regression term for the analysis of RPF, RBF and GFR but was found to be non-significant.

The fractional excretions of the macrominerals studied are shown in Table 4.18. The fractional excretions of Na, Ca and P were small and were not affected by dietary treatment. Somewhat more than 7% of the Mg filtered was excreted but no difference between diets was noted. However, the fractional excretions of K, Cl and S were all increased when animals consumed EH instead of EC indicating that a larger proportion of the filtered mass was excreted in the urine. Fractional excretions of K and Cl were twice as high for EH and the fractional excretion of S was more than three times higher for EH *vs* EC.

Metabolic parameters: Mean plasma concentrations of ß-hydroxybutyrate, non-esterified fatty acids, NH_3, urea-N, creatinine and glucose are given in Table 4.19 for the different sampling times. Plasma concentration of ß-hydroxybutyrate was not altered by diet at any time postfeeding and neither was plasma glucose concentration. At all times, plasma urea-N was higher for cows consuming EH, but the difference was significant only prefeeding. Creatinine and NH_3 concentrations were affected by diet at different times postfeeding, however, even when significant the differences observed between the 2 treatments were very small. When total plasma content was calculated by multiplying plasma volume and concentration the sdifferences for creatinine concentration disappeared indicating that the small dietary effect on plasma creatinine concentration was due to a hemodilution effect when cows were offered EH.

Urinary creatinine concentration was reduced by 13 mg dL^{-1} when EH was fed but its total daily excretion was not affected by diet (Table 4.20). Urinary ammonia concentration and total daily excretion were both increased when cows consumed EH rather than EC (Table 4.20). Values were at least twice as high for EH vs EC. The urine of cows offered EC had a reduced concentration of urea but the total daily excretion was similar for both diets (Table 4.20).

Metabolic proton flux: Metabolic proton flux are presented in table 4.21 for the different time intervals: from prefeeding to 2 h postfeeding, from 2 to 4 h postfeeding and from 4 h postfeeding to prefeeding values. No significant differences were attributed to diet. Using milk yield as a covariate did not alter the analysis. The cows went from a situation in which protons were drawn from the blood compartment (negative values) from prefeeding to 2 h postfeeding to a situation where protons were entered the blood compartment, from 2 to 4 h postfeeding. The metabolic H^+ production at that time was elevated for EH (0.98 mmol L^{-1} *vs* EC (0.184 mmol L^{-1}). Calculating the total blood H^+ production did not alter the results.

Table 4.18. Mean fractional excretion (FE) of minerals by cows in early lactation fed diets differing in ingredient composition.

Macromineral	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Na	0.011	0.010	0.401	0.003	0.003
K	0.356	0.180	0.047	0.207	0.086
Cl	0.521	0.269	0.054	0.303	0.131
S	0.438	0.132	0.037	0.129	0.05
P	0.001	0.002	0.481	-0.002	0.002
Ca	0.007	0.005	0.314	0.002	0.001
Mg	0.075	0.073	0.377	0.019	0.02

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.19. Mean plasma concentrations of metabolites of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Prefeeding					
ß-OH-Butyrate, mg dl^{-1}	9.85	8.50	0.57	0.88	1.50
Urea N[6], mg dl^{-1}	15.79	13.19	0.02	2.85	1.07
Ammonia, μg ml^{-1}	3.59	3.65	0.91	0.03	0.27
Creatinine, mg dl^{-1}	0.71	0.80	0.06	0.10	0.05
Glucose, mg dl^{-1}	50.62	47.88	0.51	1.92	2.83
NEFA[7], mEq l^{-1}	0.51	0.50	0.76	0.02	0.07
2 h postfeeding					
ß-OH-Butyrate, mg dl^{-1}	10.79	10.48	0.96	0.07	1.19
Urea N, mg dl^{-1}	17.61	16.51	0.32	1.69	1.61
Ammonia, μg dl^{-1}	3.75	3.89	0.63	-0.21	0.41
Creatinine, mg dl^{-1}	0.68	0.82	0.01	-0.16	0.05
Glucose, mg dl^{-1}	50.00	51.91	0.92	-0.47	4.47
NEFA, mEq L^{-1}	0.34	0.35	0.76	-0.01	0.03
4 h postfeeding					
ß-OH-Butyrate, mg dl^{-1}	11.03	11.68	0.55	-1.03	1.67
Urea N, mg dl^{-1}	17.9	16.51	0.14	1.71	1.06
Ammonia, μg dl^{-1}	3.86	3.34	0.05	0.58	0.26
Creatinine, mg dl^{-1}	0.73	0.77	0.67	-0.03	0.06
Glucose, mg dl^{-1}	50.84	50.00	0.74	0.80	2.36
NEFA, mEq Ll^{-1}	0.36	0.29	0.08	0.06	0.03

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

Table 4.20. Mean urinary excretion of nitrogenous compounds in early lactation cows fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
Urea-N[6], mg dl^{-1}	490.9	495.8	0.29	-30.0	27.0
Urea, g d^{-1}	71.0	56.5	0.003	16.2	4.2
Ammonia, μg ml^{-1}	187.3	63.1	0.102	112.5	62.5
Ammonia, g d^{-1}	2.8	0.7	0.079	2.1	1.1
Creatinine, mg dl^{-1}	38.0	47.9	0.0001	-13.1	2.2
Creatinine, g d^{-1}	5.3	5.4	0.604	0.3	0.5

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

[6] Nitrogen

Table 4.21. Mean metabolic proton flux of cows in early lactation fed diets differing in ingredient composition.

	EH[1]	EC[2]	P[3]	EH-EC[4]	SED[5]
β[6]	-13.38	-13.09	.421	0.169	0.20
Pre to 2h postfeeding	-0.822	-0.579	0.89	0.090	0.650
2 to 4h postfeeding	0.982	0.184	0.59	-0.67	1.210
Pre to 4h postfeeding	0.160	-0.395	0.65	-0.58	1.240
4h post to prefeeding	-0.160	0.395	0.65	0.58	1.240

[1] EH: Alfalfa haylage-based diet, n=18

[2] EC: Corn Silage-based diet, n=18

[3] Probability associated with the F test

[4] Estimate of the difference between treatment means

[5] Standard error of the estimated difference

[6] Slope of the non-bicarbonate blood buffer line

4.Discussion

Despite the similarity between the diets in terms of DMI, some significant differences were observed regarding NDF intake and the amount of ADF digested daily with both parameters being higher when cows were offered EH *vs* EC. Using DMI and BW as covariates in the model did not eliminate these effects suggesting that they were probably caused by the higher content of ADF in EH *vs* EC. Despite these small differences in intake the proportion of ADF consumed that was digested was unaffected by dietary treatment suggesting that ruminal fermentation patterns were similar between diets at least for ADF digestion.

As was the case for NDF, EH contained slightly more CP resulting in a higher intake of N when cows were offered EH *vs* EC. The increased absorption was associated with an increase in urinary N excretion and no change in milk N resulting in similar N balances with both diets. The increase in urinary N can be attributed to increases in NH_3 and urea excretion when EH was fed. The origin of these metabolites is difficult to determine from the data collected. They could simply originate from elevated amounts of ammonia and urea absorbed from the rumen or from metabolism of amino acids. The monitoring of the concentration of these metabolites in the plasma postfeeding did not show any rapid or definite increase in these metabolites postfeeding when EH was fed. A significant increase in plasma ammonia was observed 4 h postfeeding when EH was fed but the estimated difference between the treatments was small. The difference in blood urea was significant when expressed as total blood urea content. However, the interpretation of these results is difficult since the increased plasma content could have resulted from an increased absorption from the rumen, an increased production by the cow and/or a decreased excretion. Although the increase in total urinary urea excretion appears related to the volume of urine with its concentration not being affected by dietary treatment; the same cannot be said for urinary ammonia excretion. Canale and Stokes (1988) also studying the effects of forage source on performance of dairy cows in early lactation demonstrated an increase in urinary N excretion expressed in g d^{-1} when the cows were offered a corn silage-based diet *vs* a hay crop-silage based diet in early lactation. The opposite pattern observed in the present experiment can be explained by the fact that in the report by Canale and Stokes (1988) study the

corn silage based diet had a higher CP content *vs* the hay silage based diet (18.1 *vs* 17.1%) whereas in the present experiment the haylage based diet had a higher CP content compared to the corn silage based diet (18.6 *vs* 17.0%). Canale and Stokes (1988) did not demonstrate any effect of forage source on DMI, milk yield or milk composition and this was confirmed in the present study. The difference in CP level was probably too small or of inappropriate quality to improve milk production and or change milk composition.

Since EH differed from EC in its K concentration an effect due to this difference could have been expected. However O'Connor et al. (1988), studying a difference in dietary K comparable to the difference between EH and EC did not demonstrate any effect on milk production or DMI by cows in mid lactation. Since milk production, DMI and diet chemical composition were similar dietary treatment had no effect on BW. A trend for a larger decrease in BW postpartum was observed by Canale and Stokes (1988) when cows were offered a corn silage *vs* a hay silage based diet but only when no $NaHCO_3$ was added to the rations. When both rations were supplemented with 1.25% $NaHCO_3$ the effect of the forage source disappeared. In the present experiment both diets contained 1% $NaHCO_3$ which explains the absence of dietary treatment on BW.

The increase in free water consumed when cows were consuming EH *vs* EC can be explained by the smaller quantity obtained from the feed itself since total water consumption was not different between diets. Water consumption has been demonstrated to be correlated to DMI (Murphy, 1991; Hadjipanayiotou, 1982). The lack of dietary treatment effect on DMI could explain the similar total water consumption.

Cows offered EH had a significantly higher water absorption than cows offered EC the difference (6.2 L) cannot be attributed to an increase in Na intake. Intake of Na tended to be lower for EH. The increased amount of water absorbed appeared to have been completely excreted in the urine since cows consuming EH excreted 6.9 L d^{-1} more urine than cows consuming EC and milk yield did not differ. The difference in water absorption may relate to the increased K, Cl and S absorption observed with EH thereby increasing the osmotic gradient favoring water

absorption (Powell, 1987). This would, in turn, cause a volume expansion inhibiting the secretion of vasopressin (Ganong, 1989) and consequently reducing water reabsorption and increasing urine volume. The volume homeostatic systems were not able to compensate fully for the volume expansion caused by the increase in water absorption since plasma volume was significantly elevated for EH *vs* EC. It is recognized that under most circumstances the kidneys will maintain blood osmolarity and respond to volume changes but 10 to 15% changes in blood volumes are sometimes required to elicit an antidiuretic hormone (ADH) response whereas a 2% change in extracellular fluid osmolarity will cause an ADH response (Culpepper et al.,1985). The cows consuming EH absorbed more K, Cl and S but maintained plasma osmolarity since none of the plasma concentrations were altered. Therefore, changes in plasma volumes could not be entirely compensated. The fact that the differences between water absorption and urine output were similar would leave no water available to explain the increased plasma volume which was estimated to be 7.7 L. This discrepancy could result from variations observed in the water intake and urine volume output measurements and to differences in water obtained from metabolism or lost through evaporation.

The increased absorption of Cl and S may have played a role in the mediation of the volume changes, however, their effect is difficult to verify because SO_4 has been shown to potentiate the activation of the angiotensin-converting enzyme by Cl, but Cl administered as HCl has been demonstrated to inhibit renin secretion in dogs (Bunning and Riordan, 1987, Kotchen et al., 1980). An effect of the increased absorbed K on aldosterone secretion (Ganong, 1989), which would lead to increased Na retention and volume expansion, can be eliminated as a potential effect of EH because Na retention was decreased by EH compared to EC.

The augmentation in the excretion of Ca in the urine when cows were offered EH *vs* EC can be explained by the acid stress imposed by EH *vs* EC as evidenced by its effect on urinary HCO_3^- excretion .

High levels of dietary K have been reported to negatively affect Mg metabolism by several authors (Greene et al., 1983, Poe et al., 1985). The difference in K required to affect Mg absorption appears to be larger then the difference

between EH and EC. Consequently, the small but significant reduction in Mg absorbed in g d^{-1} observed when cows were offered EH must have resulted from the lower Mg consumed with that diet rather then from a higher K content. This is further supported by the fact that the percentage of Mg consumed which was absorbed did not differ between the diets. Dietary treatment did not alter either the Mg balance of the cows or their plasma Mg concentrations which were in the normal range reported for cattle at all times postfeeding (Simesen, 1980).

Plasma P concentrations were low because of the low level of in the diet; plasma concentrations below 4% are considered to be indicate a P deficiency (Georgoevskii, 1982). After accounting for the P secreted in the milk regardless of the diet cows were in negative P balance. Low concentration of P in the urine might however have decreased the buffering capacity of the urine but since urine pH was always maintained well above 7.5 the lower phosphate excretion in the urine can be assumed to have had no interaction with the dietary effect on acid-base parameters. In addition, P has been demonstrated not to affect parathyroid hormone secretion (Sherwood et al., 1968) independently of changes in blood concentration of Ca. Effects of P on renal transport systems mediated by PTH can also be ruled out (Ribeiro and Mandel, 1992, Cohn et al., 1983) because plasma Ca was within the normal range reported for cows (Georgoevskii, 1982) and did not differ between diets. Calcium balance was also similar between diets. Most importantly is the observation that P metabolism did not differ betweendiets and, therefore, the impact of a low dietary P can be considered to have been similar for both diets.

Interestingly, Ca and P appeared to have been equally available from both diets because their apparent absorption expressed as a percentage of intake or in g d^{-1} did not differ between diets. The Ca apparent absorption when cows were offered EH was 42.7 *vs* 48.9% when they were fed EC. Phosphorous absorption was of 27.95% when cows were offered EH vs 28.2% when cows were offered EC. Recently published data by Martz et al. (1990) suggest that Ca and P availabilities from alfalfa are much lower than their availabilities from corn silage. These results, however, were obtained by comparing the true absorptions of Ca and P from a diet composed of 33.7% alfalfa hay, 21.5% corn cobs and 39.1% hominy grits to the absorption from a diet composed of 24 % alfalfa, 41.5% corn silage and 29% hominy grits. The availability of Ca and P from alfalfa hay and corn silage was calculated by difference

between the diets, assuming no intereaction between feed ingredients in terms of mineral absorption. The apparent discrepancy between results of Martz et al. (1990) and the results presented could be related to the fact that in the present study a fraction of the dietary Ca and P was supplied as mineral salts. This was not the case in Martz et al. (1990) study. Since both diets were supplemented with the same mineral salts the Ca and P supplied by these salts might have masked the different availability from the two forage sources. The lack of mineral salts supplementation by Martz et al.(1990) caused the dietary concentrations of Ca and P to be lower than those of EH and EC. This could have led to the discrepancy between the results. It must be emphasized, that from neither study can the availability of Ca and P from the two sources of forages be accurately determined.

Since intakes and absorptions of S, K and Cl were all increased by EH *vs* EC and since their individual metabolism is intimately related to each other in the intestine and kidneys it is very difficult to establish cause and effect relationships (Vander, 1991, Powell, 1987). With the present experimental design it is also impossible to establish if the acid-base status was modified by other elements in the rations resulting in altered mineral metabolism or if such altered mineral metabolism resulted in altered acid-base status. The acidogenic effect of S has been discussed in the previous chapter. Acid stress can also develop from high Cl or K intakes (Tannen, 1991; Batle, 1989; Szylman, 1976). The involvement of K in the development of the acid stress is more likely than the reverse scenario since metabolic acidosis has been demonstrated to lead to K depletion because of the exchange between K and H at the cellular level (Scandlng and Ornt, 1987, Vander, 1991). The increased K retention was probably the cause of the lowered Na retention observed with EH in comparison to EC (Block, 1988a). Increased urinary excretion of Cl could have resulted from a combination of the increased intake of Cl observed with EH and the increased K excretion in the urine reducing the potential gradient driving Cl^- reabsorption in the proximal renal tubules (Vander, 1991). Differences in the metabolism of these minerals were not large enough to affect GFR and RPF despite changes caused in plasma volume. These two parameters are very tightly regulated by the kidneys (Vander, 1991). The increased fractional excretions of S, K and Cl explained why plasma concentrations were maintained constant across diets despite changes in absorptions.

Effects of diets on blood, urine and milk acid-base parameters were not significant. These results demonstrated that a corn silage- based diet is not necessarily more acidogenic than a haylage- based diet, provided the DCAB is kept constant.

5. Conclusion

This experiment demonstrated that the ingredients used in formulating a ration do not necessarily impact on the acid-base status of the animal despite different N, K, Cl and S intakes. The differences in the ingredients were not large enough to affect plasma minerals or plasma indicators of metabolism. Renal function was also maintained but plasma volume was significantly increased by the haylage based diet as were water intake and urine volumes.

The lack of difference between the haylage based diet *vs* the corn silage based diet in terms of acid-base status was surprising because the acidity of corn silage is usually given as an explanation for the greater benefit of supplementing $NaHCO_3^-$ observed when corn silage rather than haylage is used as the forage source. This observation can probably be explained by the fact that normally corn slage based diets have lower DCAB than alfalfa haylage-based diets. Thus the addition of a buffer to the former would increase its DCAB and as will be discussed in the next chapter DCAB has a definite impact on acid-base status.

V. THE EFFECTS OF DIETARY CATION-ANION BALANCE ON ACID-BASE STATUS, MINERAL METABOLISM, RENAL FUNCTION AND PRODUCTION OF COWS IN EARLY, MID AND LATE LACTATION.

Abstract

Three experiments were conducted to study the effects of manipulating the cation-anion balance of the ration at each stage of lactation: early (E), mid (M) and late (L). Each experiment corresponded to a stage of lactation and used 12 Holstein cows in a switchback design to compare 2 rations: ration A had a high dietary cation-anion balance (DCAB) and ration B which had a lower DCAB. Increasing DCAB in early and mid-lactation caused higher intakes and milk production. These effects were not found in late lactation. Milk protein and lactose yields and lactose concentration were all increased by a higher DCAB in early lactation. In mid-lactation the effect on protein yield was reduced but, fat percentage was increased by a lower DCAB. In late lactation none of the milk components were affected by dietary treatment. In early and mid-lactation water apparent absorption and urine volume were increased by a higher DCAB; these effects were not caused by higher Na intakes, or higher glomerular filtration rate. Sulfur intake and balance were increased by a lower DCAB at all stages of lactation. Except for sulfur none of the plasma concentrations of macrominerals were consistently affected by treatment. Urinary bicarbonate excretion was reduced by a reduction of DCAB at all stages of lactation whereas milk proton secretion was reduced by a lower DCAB in late lactation only. The results of these experiments demonstrated that increasing DCAB by reducing the S content of the diet does increase the amount of bicarbonate excreted in the urine of cows at all stages of lactation. Reducing the acid load, by increasing DCAB resulted in higher production and intake in early and mid lactation only; this suggests that the ideal DCAB to maximize performance of cows in early and mid-lactation is higher than the ideal DCAB for cows in late lactation. DCAB affected water consumption throughout lactation without significantly altering renal function and plasma volume at any stage of lactation, .

1. Introduction

Effects of dietary cation-anion balance (DCAB) on blood acid-base parameters have been demonstrated in rats (Greger et al., 1991) goats (Fredeen et al., 1988a), dry cows (Wang and Beede, 1992), and lactating cows (West et al., 1991). Results obtained in lactating animals, however, are confused by the fact that studies conducted so far have used animals that were at different stages of lactation. Studies with $NaHCO_3$ supplementation corresponding to an increase in DCAB suggest that in later stages of lactation negative responses can be observed; that could result from changes in the acid-base status through lactation (Block 1988b, Rogers et al., 1985). As lactation progressed Erdman et al. (1982) observed increases in blood pH and HCO_3^- concentration using diets that either were or were not supplemented with a buffer. Cows in late lactation would appear to be more susceptible to an alkalotic stress whereas cows in early lactation could be more affected by an additional acid stress imposed by the diet. It must not be forgotten that cows in late lactation are also at more advanced stages of gestation. Pregnancy, in itself, can affect acid-base balance and renal function in various species (Lindheimer and Katz, 1985) and could explain, in part, why cows at different stages of lactation, and pregnancy, respond differently to dietary manipulations.

The effects of disturbances in acid-base balance on metabolism are varied. Acidosis has been shown to alter Ca metabolism (Fredeen et al 1988b), N metabolism (May et al., 1987, 1986), fat metabolism (Hood et al., 1990, 1982) and carbohydrate metabolism (DeFronzo and Beckles, 1979). These effects were observed in a variety of species and cells, but indications as to how acid-base disturbances affect the metabolism of lactating cows at different stages of lactation are still unavailable.

The objectives of the following study were: 1- to determine the effects of DCAB on production of cows in early, mid and late lactation; 2- to determine the effects of DCAB on acid-base parameters at each stage of lactation; 3- to determine the effects of DCAB on macromineral metabolism; 4- to determine the effects of DCAB on water balance and renal function; 5- to determine the effects of DCAB on blood metabolic parameters.

2. Materials and methods

A. Experimental design and housing

Three switchback experiments were conducted, one at each stage of lactation. For each switchback, 12 Holstein cows were used. Cows for the early and mid lactation experiments were in parity 1. Cows for the late lactation experiment were in parity 1 or 2. Cows starting the early lactation experiment were between 25 and 55 d in milk and none were pregnant at the end of the experiment. For the mid lactation experiment cows were between 108 and 137 d in milk at the beginning of the experiment, 7 were non-pregnant at first and 3 remained open until the end. The late lactation cows were 162 to 234 d in milk, and between 9 and 172 d in calf at the start of the experiment. Each switchback had the same experimental design as described in the previous chapter.

B. Diets and feeding schedule

Two DCAB were compared at each stage of lactation. Three basal diets were formulated, one for each stage of lactation and their DCAB was manipulated as described in Chapter III for the dry cows. The DM of the basal diet for cows in early lactation was: 51% alfalfa haylage, 7% dry ground corn, 21% high moisture ear corn, 13.7% micronized soybeans, 4% Megalac® (Church and Dwight,Inc., Princeton, New Jersey, U.S.A.; a calcium salt of fatty acids designed to be inert in the rumen) and 1.9% mineral and vitamin mix. The latter was composed of 12.6% NaCl, 52.4% $NaHCO_3$, 1.6% $MgSO_4$, 32.9% vitamin mix (chapter III), .52% $CoCO_3$. The DM composition of the diet offered to mid lactation cows: 49% alfalfa haylage, 11.9% ground corn, 25% high moisture ear corn, 8% micronized soybeans, 2% Megalac®, 1.5% mineral vitamin mix. The latter was composed of: 64.5% $NaHCO_3$, 18.7% $NaPO_4$, 7.1% NaCl, 8.39% vitamin mix (Chapter III), .45% $MnCO_3$, .45% $ZnSO_4$ and .45% $CoCO_3$. The DM composition of the diet offered to cows in late lactation was : 56% alfalfa haylage, 34% dry ground corn, 5.5% micronized soybeans and 1.5% mineral vitamin mix. The latter was composed of 17.9% NaCl, 71.4% $NaHCO_3$ 0.71% $MnCO_3$, 0.71% $ZnSO_4$ and 9.3% of the vitamin mix described in Chapter III. The DCAB of these diets were altered in a manner described in Chapter III by

manipulating dietary K and S. The feeding schedule, sampling schedule and analytical methods were the same as those described in the previous chapter.

3. Results

A. Early lactation experiment

Diet composition: The dry matter composition of the diets is shown in Table 5.1 in which EA represents the ration with the higher DCAB (+258.1 mEq kg^{-1}) and EB the ration with the lower DCAB (+55.5 mEq kg^{-1}). As was the case for the experiment involving dry cows, dietary K was highly variable. Both diets had similar compositions except for their content of S which was higher for EB due to the addition of $MgSO_4$ to modify the DCAB. Magnesium concentration was maintained equivalent between diets.

Dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and nitrogen (N) intakes and digestibilities: Results of DM intake (DMI) and DM digestibility are presented in Table 5.2. Increasing the DCAB of the ration significantly affected DMI (kg d^{-1}) and consequently the amount of DM digested daily but did not alter DM digestibility. Correcting the intakes for BW by fitting the latter as a covariate in the model did not eliminate the effect of ration on DMI meaning that the increased BW caused by the higher DCAB was not the only mediator of the increased DMI observed.

Acid detergent fiber and NDF intakes and digestibilities are presented in Table 5.2. Daily intake of ADF and amount of ADF digested daily were both higher for DA. BW was found to be non-significant when used as a covariate in the model. The inclusion of DMI as a covariate to analyze ADF intake and digestibility was significant ($P = 0.07$ and $P = 0.02$) and eliminated the effect of treatment. For NDF, only the total intake was affected being increased by a higher DCAB. However DMI was significant as a covariate in the analysis and, when included, eliminated the effect of treatment, suggesting that the higher NDF intake was caused by the higher DMI. Digestibilities of ADF and NDF expressed as a percentage of their respective intakes were not affected by dietary manipulation (Table 5.2).

In contrast to ADF and NDF intakes, N intake was not altered by DCAB as illustrated in Table 5.3. Fecal N concentration and total N excretion were similar between diets resulting in equivalent absorption (Table 5.3). Urinary concentration of N was significantly increased by a reduction of DCAB (6.56 g L^{-1} for EA and 8.89 g L^{-1} for EB). However, total urinary N excretion was not different between diets (Table 5.3) and despite a small but significant increase in protein secretion in the milk (Table 5.5), overall N balance was not affected by DCAB. In terms of N intake, absorption and retention, BW was found to have no significant importance when used as a covariate in the statistical model.

Water intake and absorption: An increase in DCAB resulted in a significantly higher water consumption by cows in terms of amount of free water consumed and total quantity consumed (Table 5.4). The latter was increased by 8L d^{-1} when cows were offered EA *vs* EB. Since the same proportion of water consumed was absorbed for both diets (72.6% for EA and 71.0% for EB) the quantity of water absorbed daily was higher for the higher DCAB.

Table 5.1. Mean dry matter composition of rations differing in dietary cation-anion balance (DCAB)[1] offered to cows in early lactation.

Analyte	EA[2]	SD[3]	EB[4]	SD[3]
CP, %	17.9	1.7	17.9	1.8
NDF, %	32.8	3.5	32.8	2.9
ADF, %	26.4	3.0	26.5	2.7
Calcium %	0.86	0.18	0.87	0.18
Magnesium %	0.38	0.09	0.39	0.09
Phosphorus %	0.27	0.03	0.28	0.02
Sodium %	0.39	0.09	0.42	0.14
Potassium %	1.15	0.49	1.09	0.50
Chlorine %	0.3	0.07	0.35	0.11
Sulfur %	0.2	0.04	0.49	0.07
DCAB[1]	258.1	116.5	55.5	126.8

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18

[4] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18

Table 5.2. Mean body weight (BW) dry matter (DM), ADF and NDF intakes and digestibilities for cows in early lactation offered rations differing in dietary cation-anion balance (DCAB).[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
BW, kg	496	477	0.045	19	8
DM Intake, kg^{-1} d^{-1}	16.2	15.2	0.004	1.0	0.3
DM Digested, $kg^{-1}d^{-1}$	11.4	11.0	0.014	0.7	0.2
DM Digested, % of intake	71.1	71.7	0.759	-0.4	1.2
ADF Intake, g d^{-1}	4287	4077	0.101	270	150
ADF Digested, g d^{-1}	2749	2621	0.023	256	94
ADF Digested, % of intake	64.7	64.4	0.584	0.9	1.5
NDF Intake, g d^{-1}	5297	4932	0.017	3645	128
NDF Digested, g d^{-1}	3147	3015	0.142	239	148
NDF Digested, % of intake	60.0	60.4	0.938	-0.2	2.0

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.3. Mean nitrogen (N) balance of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EH-EC[5]	SED[6]
N Intake, g d^{-1}	463.9	439.09	0.220	24.89	19.0
Fecal N, %	2.43	2.35	0.120	0.09	0.05
Fecal N, g d^{-1}	114.4	101.5	0.140	11.7	7.2
N Absorption					
N Absorbed, g d^{-1}	346.0	340.8	0.553	12.7	20.6
N Absorption, % of intake	75.0	76.6	0.407	1.5	1.7
Urine N, g L-1	6.6	8.9	0.001	-0.2	0.1
Urine N, g d^{-1}	82.8	94.6	0.280	-9.3	8.1
N retention[8]					
N Retention, g d^{-1}	264.8	246.2	0.270	22.0	18.7
N Retention, % of intake	56.7	55.3	0.550	1.3	2.0
N balance[9]					
N Balance, g d^{-1}	173.5	163.7	0.427	15.6	18.8
N Balance, %of intake	36.5	36.2	0.683	1.9	2.8

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Body weight
[8] Retention= intake- fecal excretion- urine excretion
[9] Balance= retention- milk secretion

Milk yield and milk composition: Milk yield presented in Table 5.5 responded positively to a higher DCAB. Cows offered EA produced 19.5 kg d^{-1} whereas cows offered EB produced 18.3 kg d^{-1} . Although milk fat concentration was not affected by DCAB, the more positive ration tended to increase the total fat yield ($P = 0.11$, Table 5.5). Protein yield was significantly improved by the higher DCAB as was lactose yield (Table 5.5). The lactose concentration in the milk decreased from 5.08 to 5.01% as DCAB was reduced which was a small but statistically significant difference .

Mineral balances:

Calcium, magnesium and phosphorous: Results of Ca, Mg and P balances are presented in Tables 5.6 to 5.8. Intakes of these minerals were not affected by dietary treatment. Despite the trend towards higher urinary excretion of Ca with a reduction in DCAB, both diets maintained positive and similar Ca balances. Fecal excretion and secretion of Ca in the milk were not altered by ration. The situation was the same for Mg and P, both showing trends towards increased urinary excretions as DCAB was reduced but not enough to affect milk composition or the overall balance. It is interesting to note that for dietary P concentrations that were similar to diets studied in the previous chapter, animals in the present experiment were able to maintain a positive P balance. Such was not the case in the previous experiment.

Table 5.4. Mean milk yield and milk composition of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Yields					
Milk , kg d^{-1}	19.5	18.3	0.008	1.2	0.4
Fat, kg d^{-1}	0.74	0.72	0.110	0.04	0.02
Protein, kg d^{-1}	0.56	0.52	0.018	0.04	0.01
Lactose, kg d^{-1}	0.99	0.91	0.017	0.07	0.03
Concentrations					
Fat %	3.88	3.94	0.458	-0.07	0.07
Protein %	2.89	2.84	0.302	0.03	0.03
Lactose %	5.08	5.01	0.030	0.07	0.03

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18

[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimated difference

Table 5.5. Mean water intake and apparent absorption of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Free water consumed, L d^{-1}	70.6	64.6	0.027	6.9	2.7
Feed water, L d^{-1}	13.1	12.2	0.303	1.2	1.1
Total water intake, L d^{-1}	83.7	76.7	0.003	8.0	2.1
Water absorbed, L	60.8	54.5	0.005	6.6	1.9
Water absorbed, % intake	72.6	71.0	0.328	1.2	1.5

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.6. Mean calcium balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Calcium	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	140.1	136.7	0.589	7.6	13.6
Fecal concentration, %	2.06	2.08	0.645	-0.08	0.18
Fecal excretion, g d^{-1}	97.25	91.17	0.877	1.73	10.92
Apparent absorption, g d^{-1}	45.2	43.2	0.438	6.1	7.5
Apparent absorption, % of intake	31.1	32.0	0.898	0.5	3.8
Urinary concentration, ppm	40.7	102.9	0.190	-105.6	74.3
Urinary excretion, mg d^{-1}	513.3	745.3	0.171	-451.3	300.0
Apparent retention[7], g d^{-1}	44.7	42.4	0.411	6.5	7.6
Apparent retention ,% of intake	30.7	31.3	0.800	1.0	3.8
Milk concentration, ppm	959.6	1005.6	0.750	-28.8	86.4
Milk secretion, g d^{-1}	18.9	18.0	0.680	0.7	1.6
Balance[8], g d^{-1}	25.74	24.41	0.429	5.82	7.07
Balance, % intake	16.23	17.34	0.761	1.26	4.03

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.7. Mean magnesium balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Magnesium	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	61.81	61.91	0.779	1.65	5.73
Fecal concentration, %	0.85	0.92	0.224	-0.10	0.08
Fecal excretion, g d^{-1}	39.98	39.18	0.637	-1.28	2.63
Apparent absorption, g d^{-1}	22.70	21.87	0.59	2.49	4.48
Apparent absorption, % of intake	35.90	34.83	0.46	3.16	4.12
Urinary concentration, ppm	266.80	379.41	0.19	-98.30	69.43
Urinary excretion, mg d^{-1}	3129.9	3956.3	0.54	-399.7	629.0
Apparent retention[7], g d^{-1}	19.57	17.91	0.5	2.92	4.21
Apparent retention ,% of intake	30.68	28.14	0.34	4.07	4.03
Milk concentration, ppm	98.10	95.20	0.76	1.83	5.92
Milk secretion, g d^{-1}	1.94	1.73	0.22	0.14	0.11
Balance[8], g d^{-1}	17.63	16.19	0.53	2.78	4.25
Balance, % intake	27.24	25.13	0.38	3.86	4.22

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Sodium, potassium, chloride and sulfur: The results of the balance studies for these minerals are presented in Tables 5.9 to 5.15. Both treatment resulted in similar fecal, urinary and milk concentrations and total excretions of Na thus similar positive balances. Between 41 and 42% of the Na consumed was retained. Intake of K was significantly higher for EA *vs* EB (184.6 *vs* 172.0 g d^{-1}). Since fecal excretion and the proportion of Na intake that was absorbed were not affected, the higher DCAB resulted in a larger overall apparent absorption of K expressed. The increased urinary total excretion of K was not enough to compensate for the improved absorption since EA resulted in a significantly higher K balance *vs* EB; the difference was 10 g d^{-1}. Despite the similar intake and absorption caused by both diets EB resulted in a higher concentration of Cl in the urine ($P<0.07$), higher urinary excretion ($P<0.12$) and milk secretion ($P<0.09$). Sulfur was the mineral most affected by dietary manipulation. As planned, the intake of S was higher for EB resulting in a lower DCAB. Consequently, fecal and urinary concentrations of S and total excretions were elevated by feeding EB. Even though the output of S was elevated at several points it was not enough to eliminate an effect on total S balance. The later was 13.7 g d^{-1} higher for EB *vs* EA.

Plasma minerals: Table 5.13 shows the plasma mineral concentrations of the seven minerals studied at different sampling times in relation to the morning feeding. Prefeeding, all plasma concentrations were similar for both diets. Plasma concentration of S was increased by EB at 2 and 4 h postfeeding. Plasma Mg was reduced at 4 h postfeeding by the reduction in DCAB. None of the other minerals were affected at 2 and 4 h postfeeding.

Table 5.8. Mean phosphorus balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Phosphorus	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	43.8	42.5	0.319	2.2	2.1
Fecal concentration, %	0.46	0.46	0.824	-0.01	0.02
Fecal excretion, g d^{-1}	21.60	19.37	0.099	1.50	0.83
Apparent absorption, g d^{-1}	22.8	22.5	0.709	0.7	1.8
Apparent absorption, % of intake	51.8	52.8	0.628	-1.2	2.4
Urinary concentration, ppm	4.6	10.4	0.153	-4.9	3.1
Urinary excretion, mg d^{-1}	57.2	103.5	0.312	-34.8	32.2
Apparent retention[7], g d^{-1}	22.7	22.1	0.146	2.1	1.3
Apparent retention ,% of intake	51.6	52.2	0.846	0.4	2.0
Milk concentration, ppm	647	659	0.670	-15.2	34
Milk secretion, g d^{-1}	12.8	12.0	0.580	0.4	0.7
Balance[8], g d^{-1}	10.0	10.1	0.149	1.8	1.1
Balance, % intake	21.4	23.6	0.517	1.4	2.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.9. Mean sodium balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Sodium	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	62.9	64.7	0.790	2.0	7.6
Fecal concentration, %	0.09	0.08	0.24	0.02	0.04
Fecal excretion, g d^{-1}	4.21	3.33	0.138	0.93	0.57
Apparent absorption, g d^{-1}	58.4	61.3	0.869	1.2	7.3
Apparent absorption, % of intake	93.5	94.7	0.234	1.0	0.8
Urinary concentration, ppm	2033	2449	0.341	-298	294
Urinary excretion, mg d^{-1}	25807	28275	0.943	-248	3335
Apparent retention[7], g d^{-1}	32.9	33.1	0.817	1.7	7.3
Apparent retention ,% of intake	52.5	50.6	0.897	0.9	6.4
Milk concentration, ppm	30.7	306.8	0.720	-6.7	18.5
Milk secretion, g d^{-1}	6.1	5.6	0.550	0.2	0.4
Balance[8], g d^{-1}	26.9	27.5	0.840	1.51	7.33
Balance, % intake	42.2	41.1	0.860	1.3	7.3

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.10. Mean potassium balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Potassium	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	184.6	172.0	0.006	16.9	4.9
Fecal concentration, %	0.30	0.27	0.350	0.04	0.04
Fecal excretion, g d^{-1}	14.02	11.94	0.420	1.85	2.21
Apparent absorption, g d^{-1}	170.7	159.9	0.005	15.2	4.2
Apparent absorption, % of intake	90.4	90.8	0.900	0.2	1.5
Urinary concentration, ppm	1445	1460	0.960	4	65
Urinary excretion, mg d^{-1}	17652	15768	0.026	2713	996
Apparent retention[7], g d^{-1}	153.1	144.1	0.009	12.8	4.0
Apparent retention ,% of intake	79.4	80.2	0.924	-0.2	1.7
Milk concentration, ppm	1381.70	1381	0.79	16	58
Milk secretion, g d^{-1}	27.3	24.3	0.14	1.8	1.1
Balance[8], g d^{-1}	125.8	119.8	0.018	10.9	3.9
Balance, % intake	62.0	62.3	0.560	1.8	3.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.11. Mean chloride balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Chloride	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	49.6	54.9	0.467	-5.1	6.8
Fecal concentration, %	0.26	0.26	0.43	-0.02	0.01
Fecal excretion, g d^{-1}	12.11	11.42	0.9	0.15	0.16
Apparent absorption, g d^{-1}	37.8	43.2	0.421	-5.2	6.2
Apparent absorption, % of intake	73.3	77.2	0.368	-3.3	3.5
Urinary concentration, ppm	694	1206	0.072	-591	285
Urinary excretion, mg d^{-1}	7901	11993	0.119	-4659	2737
Apparent retention[7], g d^{-1}	29.9	31.2	0.901	-0.6	4.3
Apparent retention ,% of intake	57.1	57.5	0.821	0.8	3.5
Milk concentration, ppm	648	619	0.68	14	33
Milk secretion, g d^{-1}	3.5	1.7	0.09	-1.4	0.7
Balance[8], g d^{-1}	17.1	20.1	0.720	-1.6	4.3
Balance, % intake	28.5	35.8	0.488	-3.8	5.3

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.12. Mean sulfur balance of cows in early lactation fed diets differing in cation-anion balance.[1]

Sulfur	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Intake, g d^{-1}	31.9	76.7	0.0001	-42.1	2.0
Fecal concentration, %	0.22	0.3	0.003	-0.08	0.020
Fecal excretion, g d^{-1}	10.16	12.88	0.022	-2.77	1.02
Apparent absorption, g d^{-1}	21.8	63.8	0.0001	-39.5	2.3
Apparent absorption, % of intake	67.4	82.7	0.0001	-14.4	1.1
Urinary concentration, ppm	808	3366	0.0001	-2573	295
Urinary excretion, mg d^{-1}	8697	36378	0.0005	-24560	4349
Apparent retention[7], g d^{-1}	13.1	27.4	0.01	-13.8	4.4
Apparent retention ,% of intake	37.8	35.3	0.683	3.5	8.4
Milk concentration, ppm	96.7	110.8	0.43	-15.1	18.4
Milk secretion, g d^{-1}	1.9	2.0	0.62	-0.2	0.4
Balance[8], g d^{-1}	11.2	25.4	0.011	-13.7	4.4
Balance, % intake	31.7	32.5	0.942	0.6	8.6

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine exc.etion
[8] Balance = retention - milk secretion

Table 5.13. Mean plasma mineral concentrations of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Prefeeding					
Ca, mg dL^{-1}	8.7	9.0	0.56	-0.4	0.6
Mg, mg dL^{-1}	2.5	2.4	0.65	0.05	0.1
P, mg dL^{-1}	30.1	32.2	0.52	-6.0	3.4
Na, mmol L^{-1}	125.2	121.6	0.61	3.2	6.1
K , mmol L^{-1}	4.0	4.1	0.74	-0.1	0.2
Cl , mmol L^{-1}	93.1	95.9	0.40	-1.7	2.0
S , mmol L^{-1}	2.2	2.7	0.14	-0.5	0.3
2 h postfeeding					
Ca, mg dL^{-1}	8.8	8.4	0.45	0.3	0.3
Mg, mg dL^{-1}	2.4	2.4	0.50	0.04	0.06
P, mg dL^{-1}	3.7	3.7	0.60	-0.1	0.2
Na, mmol L^{-1}	127.0	129.1	0.83	1.3	5.9
K , mmol L^{-1}	4.0	3.9	0.54	0.1	0.1
Cl , mmol L^{-1}	95.9	95.9	0.58	1.1	2.0
S , mmol L^{-1}	2.6	3.7	0.01	-1.2	0.3
4 h postfeeding					
Ca, mg dL^{-1}	88.5	84.9	0.13	2.9	1.7
Mg, mg dL^{-1}	29.9	24.4	0.05	1.7	0.7
P, mg dL^{-1}	38.1	40.2	0.09	-4.3	2.3
Na, mmol L^{-1}	130.0	128.5	0.27	6.8	5.7
K , mmol L^{-1}	3.9	3.9	0.84	0.03	0.2
Cl , mmol L^{-1}	95.9	95.9	0.84	0.3	2.0
S , mmol L^{-1}	2.7	4.2	0.01	-1.9	0.5

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18

[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimated difference

Blood, urine and milk acid-base parameters: Blood pH and free H^+ ion concentration responded to DCAB at 2 h postfeeding as demonstrated in table 5.14. Feeding a ration with a lower DCAB depressed blood pH. The difference was no longer significant at 4 h postfeeding in terms of pH but the concentration of H^+ still showed a response and was higher for the lower DCAB. Blood concentration of HCO_3^- responded similarly to that of free H^+ and differences between diets were significant at all sampling times. Blood pCO_2 remained relatively constant and similar between diets.

Despite differences in urine volumes between treatments, concentration of H^+ and total excretion were maintained between diets (Table 5.15). However, HCO_3^- fell as DCAB was reduced . This was true for concentration and total daily excretions (Table 5.15).

The trend towards increased concentration of free H^+ and the increase in milk volume as DCAB was increased caused a significant increase in total proton secretion into the milk (Table 5.16).

Blood volume and renal function: Plasma volume, packed cell volume, blood volume, effective renal plasma and blood flow, glomerular filtration rate and the filtration fraction were not affected by dietary treatment (Table 5.17). Table 5.18 shows the fractional excretions of the minerals studied. The fractional excretions of K, Cl and Mg tended to be higher when cows were offered the ration with the lower DCAB but the effects were not significant. Both Ca and S had significantly higher fractional excretions when DCAB was reduced indicating that a larger fraction of the minerals filtered were excreted in the urine. Since GFR was not affected, the increase in fractional excretions of Ca and S can explain some of the increase in urinary excretions observed for these minerals as DCAB was reduced. For S, the higher plasma concentration also contributed to the increased fractional excretion.

Table 5.14. Mean blood pH, free hydrogen ion (H^+) and bicarbonate ion (HCO_3^-) concentrations and carbon dioxide (pCO_2) of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Prefeeding					
pH	7.378	7.370	0.684	-0.608	0.018
H^+, mol L^{-1}	4.22×10^{-5}	4.32×10^{-5}	0.932	0.05×10^{-5}	0.07×10^{-5}
HCO_3^-, mmol L^{-1}	28.47	26.93	0.013	-1.54	0.50
pCO2, mm Hg	48.29	46.12	0.440	1.78	2.23
2 h postfeeding					
pH	7.381	7.345	0.042	0.036	0.015
H^+, mol L^{-1}	4.25×10^{-5}	4.51×10^{-5}	0.026	-0.20×10^{-5}	0.24×10^{-5}
HCO_3^-, mmol L^{-1}	28.00	26.89	0.092	1.11	0.60
pCO2, mm Hg	48.00	48.28	0.279	-2.65	2.32
4 h postfeeding					
pH	7.378	7.374	0.600	0.013	0.025
H^+, mol L^{-1}	4.11×10^{-5}	4.29×10^{-5}	0.016	0.14×10^{-5}	0.19×10^{-5}
HCO_3^-, mmol L^{-1}	28.61	26.44	0.019	2.17	0.78
pCO2, mm Hg	47.16	45.26	0.496	2.04	2.88

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.15. Mean urine volume, free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) excretions of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Volume, L d^{-1}	22.05	18.55	0.024	3.50	1.32
Total free H^+, mol d^{-1}	1.41×10^{-7}	1.32×10^{-7}	0.893	0.10×10^{-7}	0.70×10^{-7}
Free H^+, mol L^{-1}	6.0×10^{-9}	6.9×10^{-9}	0.749	-1.1×10^{-9}	2.0×10^{-9}
Total HCO_3^-, mml d^{-1}	10.35	5.51	0.0001	4.84	0.81
HCO_3^-, mmol L^{-1}	466.1	288.4	0.0001	177.8	29.0
pCO_2, mm Hg	61.7	52.8	0.234	9.0	7.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.16. Mean milk free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) secretion of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Total free H^+, mol d^{-1}	4.9×10^{-6}	4.53×10^{-6}	0.029	3.7×10^{-7}	1.5×10^{-7}
Free H^+, mol L^{-1}	2.56×10^{-7}	2.48×10^{-7}	0.334	1×10^{-8}	1×10^{-8}
Total HCO_3^-, mmol d^{-1}	51.75	49.4	0.303	2.31	2.13
HCO_3^-, mmol L^{-1}	2.70	2.73	0.733	-0.03	0.08
pCO_2, mm Hg	36.53	36.23	0.768	0.30	0.99

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.17. Mean plasma volume, packed cell volume and renal function parameters of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Plasma volume, L	28.46	32.91	0.448	-3.06	3.83
Packed cell volume, %	27.6	27.6	0.53	-0.6	0.9
Blood volume , L	39.29	45.54	0.42	-4.48	5.27
Effective renal plasma flow, L min^{-1}	4.22	5.00	0.904	-0.68	0.54
Effective renal blood flow, L min^{-1}	5.79	6.89	0.846	-0.15	0.74
Glomerular filtration rate, L d^{-1}	692	696	0.686	16	38
Filtration fraction	0.142	0.129	0.961	0.001	0.015

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=17
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.18. Mean fractional excretions (FE) of minerals for cows in early lactation fed diets differing in cation-anion balance.[1]

Fractional excretion	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Na	0.012	0.014	0.677	-0.001	0.002
K	0.082	0.117	0.265	-0.039	0.031
Cl	0.146	0.193	0.257	-0.068	0.055
S	0.220	1.110	0.002	-0.048	0.074
P	0.003	0.005	0.524	-0.002	0.003
Ca	0.008	0.014	0.033	-0.006	0.002
Mg	0.189	0.243	0.865	-0.006	0.031

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=17

[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimated difference

Metabolic parameters: Plasma concentration of ß-hydroxybutyrate, non-esterified fatty acids, NH_3, urea-N, creatinine and glucose remained unaffected by ration prefeeding and at 2 and 4 h postfeeding (Table 5.19). The ration with the lower DCAB increased urinary urea concentration but did not affect its total daily excretion, as was the case for the excretion of NH_3 and creatinine (Table 5.20). The total plasma concentrations of these metabolites (calculated by multiplying plasma volume by concentration of metabolites) were not affected by dietary treatment except for ß-hydroxybutyrate 4 h postfeeding.

Metabolic proton flux: The blood buffering capacity represented by ß in Table 5.21 was not affected by treatment. Considering the time interval of 2 to 4 h postfeeding or 0 to 4 h postfeeding the proton drain from the blood was larger for cows consuming EA *vs* EB but the differences were not significant. Milk yield was included as a covariate in the model but was not found to be significant.

Table 5.19. Mean plasma concentrations of metabolites for cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Prefeeding					
ß-OH-Butyrate, mg dL^{-1}	5.34	5.87	0.83	-0.14	0.63
Urea N[7], mg dL^{-1}	15.57	15.69	0.94	-0.13	1.49
Ammonia, μg mL^{-1}	3.63	3.18	0.28	0.40	0.35
Creatinine, mg dL^{-1}	0.83	0.89	0.18	-0.06	0.04
Glucose, mg dL^{-1}	59.63	55.92	0.23	5.19	4.02
NEFA[8], mEq L^{-1}	0.21	0.24	0.27	-0.03	0.03
2 h postfeeding					
ß-OH-Butyrate, mg dL^{-1}	7.96	9.24	0.25	-1.56	1.26
Urea N[7], mg dL^{-1}	18.06	18.86	0.65	-0.73	1.54
Ammonia, μg mL^{-1}	3.62	3.44	0.61	0.21	0.39
Creatinine, mg dL^{-1}	0.89	0.91	0.97	0.001	0.035
Glucose, mg dL^{-1}	55.95	53.31	0.31	5.04	4.71
NEFA[8], mEq L^{-1}	0.21	0.21	0.78	-0.005	0.019
4 h postfeeding					
ß-OH-Butyrate, mg dL^{-1}	8.24	9.87	0.25	-1.2	0.97
Urea N[7], mg dL^{-1}	19.43	19.65	0.96	-0.09	1.55
Ammonia, μg mL^{-1}	3.26	3.23	0.48	0.15	0.21
Creatinine, mg dL^{-1}	0.86	0.88	0.86	-0.008	0.05
Glucose, mg dL^{-1}	57.79	57.84	0.68	2.21	5.11
NEFA[8], mEq L^{-1}	0.18	0.19	0.21	-0.02	0.02

[1] mEq $(Na^+ + K^+ - Cl^- - S^{--})$ kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen
[8] Non-esterified fatty acids

Table 5.20. Mean urinary excretion of nitrogenous compounds for cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
Urea-N[7], mg dL^{-1}	645.4	794.6	0.017	-146.4	50.0
Urea N, g d^{-1}	77.3	85.5	0.533	-4.9	7.6
Ammonia, μg mL^{-1}	281.7	276.5	0.749	48.3	146.4
Ammonia, g d^{-1}	3.4	2.5	0.734	0.536	1.5
Creatinine, mg dL^{-1}	49.9	59.2	0.126	-10.0	5.9
Creatinine, g d^{-1}	5.8	6.2	0.653	-0.2	0.4

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of . 258.1 mEq kg^{-1} DM
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen

Table 5.21. Mean metabolic proton flux (mmol L^{-1}) of cows in early lactation fed diets differing in cation-anion balance.[1]

	EA[2]	EB[3]	P[4]	EA-EB[5]	SED[6]
β[7]	-12.95	-13.11	0.484	0.414	0.56
Pre to 2h post feeding	0.399	0.262	0.841	-0.160	0.78
2 to 4h post-feeding	-0.813	-0.064	0.735	-0.280	0.81
Pre to 4h post-feeding	-0.336	0.374	0.630	-0.445	0.89
4h post to prefeeding	0.336	-0.374	0.630	0.445	0.89

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +258.1 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +55.5 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Slope of the non-bicarbonate blood buffer line

B. Mid Lactation Experiment

Diet composition: The DM composition of the diets offered to cows in mid lactation is presented in Table 5.22 where MA represents a diet with a high DCAB +372.7 mEq kg^{-1} and MB a ration with a lower DCAB +140.5 mEq kg^{-1}. As in previous experiments K showed a high variation and explains some of the variability observed in DCAB. Although the absolute numbers for DCAB were higher than for the early lactation animals the absolute difference between the diets was maintained. MA contained more K and MB contained more S due to the mineral salts added.

Dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and nitrogen (N) intakes and digestibilities: As was the case with early lactation animals a reduction in DCAB caused a reduction in DMI and BW but no change DM digestibility (Table 5.23). Fitting BW as a covariate in the model to analyze DMI was significant ($P<0.05$) but did not eliminate the effect of ration . Including DMI as a covariate in the model to analyze BW was also significant and eliminated the ration effect. This suggests that DMI mediated the effect of ration on BW and that BW is responsible for only some of the ration effect on DMI.

Table 5.23 presents results obtained for ADF and NDF intakes and digestibilities. Intake and digestibility of ADF were not affected by DCAB. Intake of NDF was significantly increased by a higher DCAB. Digestibility of NDF did not differ between diets. It appears as though the effects on NDF digestion resulted from higher DMI as DCAB increased, in as much as using DMI as a covariate eliminated significant effects of rations observed .

Table 5.22. Mean dry matter composition of diets differeing in dietary cation-anion balance (DCAB)[1] offered to cows in mid-lactation.

Analyte	MA[2]	SD[3]	MB[4]	SD[3]
CP, %	17.2	2.5	17.5	2.5
NDF, %	33.5	4.8	31.1	3.9
ADF, %	24.7	2.3	24.8	1.7
Calcium, %	0.71	0.21	0.71	0.19
Magnesium, %	0.48	0.13	0.43	0.07
Phosphorus, %	0.25	0.05	0.25	0.04
Sodium, %	0.38	0.05	0.41	0.05
Potassium, %	1.50	0.35	1.40	0.30
Chlorine, %	0.21	0.05	0.22	0.05
Sulfur, %	0.18	0.03	0.53	0.12
DCAB[1]	372.7	71.5	140.2	116.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18

[3] Standard deviation

[4] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18

Table 5.23. Mean body weight (BW), dry matter, ADF and NDF intakes and digestibilities for cows in mid-lactation offered rations differing in dietary cation-anion balance (DCAB).[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
BW, kg	523	514	0.020	9	3
DM Intake, kg^{-1} d^{-1}	17.0	15.6	0.001	1.4	0.3
DM Digested, kg d^{-1}	12.1	11.0	0.007	1.0	0.3
DM Digested, % of intake	70.8	70.6	0.980	0.2	1.3
ADF Intake, g d^{-1}	4197	3869	0.110	328	187
ADF Digested, g d^{-1}	2540	2263	0.106	277	156
ADF Digested, % of intake	60.2	58.3	0.337	1.9	1.8
NDF Intake, g d^{-1}	5675	4900	0.010	774	2548
NDF Digested, g d^{-1}	3419	2784	0.031	635	252
NDF Digested, % of intake	59.5	56.0	0.189	3.5	2.5

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Nitrogen intake was significantly increased by a higher DCAB and was probably mediated by the higher BW resulting from MA because the ration effect disappeared when N intake was expressed per kg of BW (Table 5.24). The same observations were made for N absorption and overall balance. In all cases however a trend towards higher values for MA them MB was observed. It is interesting to note that when calculating N retention before considering milk N, cows fed the ration with the higher DCAB retained more N in g d^{-1}.

Water intake and absorption: Cows in mid-lactation responded to a higher DCAB by increasing water intake and absorption (Table 5.25). When offered MA *vs* MB animals consumed 3 L d^{-1} more water.

Milk yield and milk composition: In terms of average milk yield cows in mid-lactation had a positive response to a higher DCAB (Table 5.26) as was the case with early lactation animals. The fat yield was not affected by ration but the percentage of fat in the milk was significantly lower with MA. Protein yield and percentage of protein did not differ between diets. The concentration of protein in the milk was not affected and the total protein yield tended to be higher with MA.

Mineral balances:

Calcium, magnesium and Phosphorous: Intake and absorption of Ca were not affected by dietary treatment as illustrated in Table 5.27. The significantly higher urinary Ca concentration and excretion caused by a reduction in DCAB were not large enough to significantly affect retention and milk content. With both diets Ca balance was positive. Intake of Mg was significantly elevated by MA but the concomitant increase in fecal excretion explains the lack of difference in the amounts absorbed and subsequent retention (Table 5.28). Milk concentration of Mg and total daily secretion were also not affected. Data for intake and retention of P are presented in Table 5.29. Both diets resulted in similar positive P balances. Fecal, urinary and milk concentrations of P were not affected.

Table 5.24. Mean nitrogen balance of for cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
N Intake, g d^{-1}	469.4	434.4	0.044	35.0	15.2
Fecal, N %	2.34	2.37	0.328	-0.04	0.04
Fecal, N g d^{-1}	117.10	107.60	0.258	6.64	5.54
N Apparent absorption					
N Absorbed g d^{-1}	353.5	325.4	0.052	28.1	12.8
N Absorption % of intake	74.9	74.6	0.826	0.2	1.0
Urine N, g L-1	6.43	6.57	0.072	-0.08	0.04
Urine N, g d^{-1}	74.77	70.43	0.490	3.16	4.40
N Apparent retention[8]					
N Retention ,g d^{-1}	279.3	254.3	0.041	24.9	10.6
N Retention, % of intake	59.3	58.4	0.535	0.9	1.4
N Balance[9]					
N Balance, g d^{-1}	187	165.3	0.066	21.7	10.5
N Balance, % of intake	39.2	37.5	0.33	1.9	1.6

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[6] Standard error of the estimated difference
[7] Body weight
[8] Retention = intake - fecal excretion - urine excretion

Table 5.25. Mean milk yield and milk composition of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Yields					
Milk, kg d^{-1}	18.9	18.2	0.031	0.68	0.27
Fat, kg d^{-1}	0.75	0.76	0.807	-0.004	0.01
Protein, kg d^{-1}	0.58	0.56	0.098	0.02	0.01
Lactose, kg d^{-1}	0.97	0.94	0.053	0.04	0.02
Concentrations					
Fat, %	4.00	4.16	0.042	-0.16	0.07
Protein, %	3.04	3.05	0.724	-0.01	0.03
Lactose, %	5.15	5.14	0.898	0.01	0.04

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.26. Mean water intake and apparent absorption of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Free water consumed, L d^{-1}	70.5	61.6	0.009	10.4	3.2
Feed water, L d^{-1}	16.0	14.7	0.520	1.1	1.6
Total water intake, L d^{-1}	86.4	76.3	0.0003	11.49	2.2
Water absorbed L	61.2	53.6	0.0002	9.7	1.7
Water absorbed % intake	70.6	70.1	0.027	1.8	0.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.27. Mean calcium balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Calcium	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	123.0	111.0	0.092	11.3	6.1
Fecal concentration, %	1.45	1.45	0.868	-0.03	0.15
Fecal excretion, g d^{-1}	73.54	66.19	0.577	4.98	8.63
Apparent absorption, g d^{-1}	49.4	44.7	0.385	6.3	7.0
Apparent absorption, % of intake	37.2	40.3	0.913	-0.7	6.1
Urinary concentration, ppm	7.9	25.5	0.009	-13.2	4.1
Urinary excretion, mg d^{-1}	97.1	211.8	0.031	-81.7	32.5
Apparent retention[7], g d^{-1}	49.3	44.5	0.379	6.4	7.0
Apparent retention ,% of intake	37.2	40.1	0.927	-0.6	6.1
Milk concentration, ppm	985	967	0.500	50	72
Milk secretion, g d^{-1}	18.5	17.8	0.320	1.6	1.5
Balance[8], g d^{-1}	30.8	26.7	0.510	4.8	7.0
Balance, % of intake	21.6	23.0	0.994	-0.05	6.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.28. Mean magnesium balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Magnesium	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	82.28	66.10	0.005	16.36	4.50
Fecal concentration, %	1.06	0.97	0.078	0.10	0.05
Fecal excretion, g d^{-1}	53.19	44.29	0.031	8.53	3.41
Apparent absorption, g d^{-1}	29.09	21.82	0.133	7.83	4.78
Apparent absorption, % of intake	34.26	33.39	0.73	1.69	4.75
Urinary concentration, ppm	326.30	357.70	0.288	39.00	34.71
Urinary excretion, mg d^{-1}	3786.30	3480.1	0.571	315.93	539.59
Apparent retention[7], g d^{-1}	25.30	18.34	0.148	7.51	4.79
Apparent retention, % of intake	29.28	28.00	0.681	2.13	5.03
Milk concentration, ppm	106.50	99.10	0.22	11.42	8.74
Milk secretion, g d^{-1}	1.99	1.82	0.14	0.28	0.17
Balance[8], g d^{-1}	23.31	16.51	0.17	7.23	4.89
Balance, % intake	26.72	25.13	0.669	2.30	5.22

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.29. Mean phosphorus balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Phosphorous	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	43.5	38	0.127	4.3	2.6
Fecal concentration, %	0.57	0.6	0.188	-0.034	0.024
Fecal excretion, g d^{-1}	28.4	27.1	0.686	0.6	1.4
Apparent absorption, g d^{-1}	15.1	10.9	0.134	3.8	2.3
Apparent absorption, % of intake	32.9	27.2	0.195	5.9	4.2
Urinary concentration, ppm	7.4	5.1	0.704	0.9	2.4
Urinary excretion, mg d^{-1}	66.0	44.8	0.582	9.8	17.1
Apparent retention[7], g d^{-1}	15.1	10.9	0.134	3.7	2.3
Apparent retention ,% of intake	32.8	27.1	0.194	5.9	4.2
Milk concentration, ppm	502	525	0.950	-3	46
Milk secretion, g d^{-1}	10.5	9.6	0.340	1.1	1.1
Balance[8], g d^{-1}	4.6	1.3	0.370	2.6	2.8
Balance, % intake	7.3	0.8	0.450	5.5	6.9

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Sodium, potassium, chloride and sulfur: As was the case in early lactation, Na intake and balance were not affected by diet in mid lactation (Table 5.30). However, in mid-lactation urinary concentration of Na was increased by a reduction in DCAB. A difference of 362 ppm was observed. This change in urinary concentration of Na did not increase total excretion. The significant dietary effects observed for K metabolism in early lactation became trends in mid-lactation (Table 5.31). This was probably related to the larger standard errors observed because the estimated differences between diets were larger than in early lactation. For example, intake of K differed by 36 g d^{-1} in mid lactation and this difference was not significant whereas a 16.9 g difference was significant in early lactation. The same observation was made for the overall retention which differed by 30 g in mid-lactation *vs* 11 g in early lactation. In mid-lactation cows tended to have higher K balances at higher DCAB . Dietary cation anion balance did not affect Cl intake and retention in mid lactation. The same was observed with cows in early lactation (Table 5.32). Intake, fecal excretion, absorption , urinary excretion and retention of S were all significantly increased by MB *vs* MA (Table 5.33). Milk content of S and the total S secretion via the milk were not altered.

Plasma minerals: Table 5.34 presents the results of plasma mineral analysis of the samples taken at different times in relation to feeding. Large effects of diets were observed for plasma concentrations of S which were increased by the reduced DCAB for all samples taken.

Table 5.30. Mean sodium balance of cows in mid-lactation fed diets differing in cation anion balance.[1]

Sodium	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	65.4	63.2	0.518	1.5	2.3
Fecal concentration, %	0.06	0.06	0.65	-0.01	0.02
Fecal excretion, g d^{-1}	2.82	2.59	0.874	-0.12	0.72
Apparent absorption, g d^{-1}	62.6	60.6	0.542	1.7	2.6
Apparent absorption, % of intake	95.5	95.7	0.815	0.3	1.3
Urinary concentration, ppm	1262	1606	0.032	-344	145
Urinary excretion, mg d^{-1}	14921	15870	0.672	-773	1774
Apparent retention[7], g d^{-1}	47.7	44.7	0.314	2.4	2.3
Apparent retention ,% of intake	72.2	69.5	0.331	2.6	2.6
Milk concentration, ppm	297	326	0.400	-29	33
Milk secretion, g d^{-1}	5.5	5.7	0.640	-0.2	0.5
Balance[8], g d^{-1}	42.2	38.8	0.300	2.8	2.6
Balance, % intake	63.7	59.7	0.225	3.5	2.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.31. Mean potassium balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Potassium	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	256.5	218.2	0.122	36.0	21.3
Fecal concentration, %	0.27	0.28	0.92	-0.01	0.06
Fecal excretion, g d^{-1}	13.56	12.44	0.836	0.64	3.00
Apparent absorption, g d^{-1}	242.9	205.8	0.128	35.4	21.3
Apparent absorption, % of intake	94.8	94.3	0.634	0.7	1.4
Urinary concentration, ppm	1528	1557	0.890	-14	99
Urinary excretion, mg d^{-1}	19354	17011	0.174	3485	3379
Apparent retention[7], g d^{-1}	223.5	188.8	0.154	31.9	20.7
Apparent retention ,% of intake	87.0	86.1	0.800	0.5	1.7
Milk concentration, ppm	1331	1277	0.53	79	123
Milk secretion, g d^{-1}	24.7	23.5	0.43	1.9	2.3
Balance[8], g d^{-1}	198.9	165.3	0.146	30.0	19.0
Balance, % intake	77.0	74.7	0.451	1.3	1.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.32. Mean chloride balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Chloride	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	37.3	35.0	0.466	1.6	2.2
Fecal concentration, %	0.20	0.22	0.210	-0.02	0.01
Fecal excretion, g d^{-1}	10.3	10.0	0.974	0.02	0.6
Apparent absorption, g d^{-1}	27.1	25.0	0.457	1.6	2.1
Apparent absorption, % of intake	72.1	70.8	0.360	1.4	1.5
Urinary concentration, ppm	371	462	0.38	-101	111
Urinary excretion, mg d^{-1}	4785	5129	0.851	-293	1517
Apparent retention[7], g d^{-1}	30.1	19.8	0.139	1.9	1.2
Apparent retention, % of intake	71.3	56.4	0.189	3.3	2.3
Milk concentration, ppm	526	550	0.900	-4	28
Milk secretion, g d^{-1}	4.0	4.1	0.680	-0.5	1.2
Balance[8], g d^{-1}	12.4	9.7	0.216	1.7	1.3
Balance, % intake	32.9	25.3	0.156	4.9	3.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^-$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.33. Mean sulfur balance of cows in mid-lactation fed diets differing in cation-anion balance.[1]

Sulfur	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Intake, g d^{-1}	32.2	82.9	0.0001	-52.4	4.3
Fecal concentration, %	0.23	0.29	0.004	-0.06	0.02
Fecal excretion, g d^{-1}	11.58	13.18	0.043	-2.23	0.96
Apparent absorption, g d^{-1}	20.7	69.7	0.0001	-50.2	4.6
Apparent absorption, % of intake	63.9	81.4	0.0004	-17.6	3.4
Urinary concentration, ppm	717	3133	0.0001	-2490	232
Urinary excretion, mg d^{-1}	7528	32246	0.0001	-25400	1782
Apparent retention[7], g d^{-1}	13.1	37.5	0.0002	-24.8	4.4
Apparent retention, % of intake	39.5	37.2	0.831	2.1	9.5
Milk concentration, ppm	123	117	0.860	2.6	14.3
Milk secretion, g d^{-1}	2.25	2.18	0.920	0.03	0.30
Balance[8], g d^{-1}	10.8	35.3	0.0002	-24.9	4.4
Balance, % intake	32.4	34.2	0.832	-2.2	10.0

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.34. Mean plasma mineral concentrations of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Prefeeding					
Ca, mg dL^{-1}	8.5	8.8	0.26	-0.4	0.4
Mg, mg dL^{-1}	2.5	3.6	0.36	-0.8	0.8
P, mg dL^{-1}	4.4	4.4	0.60	0.08	0.2
Na, mmol L^{-1}	117.0	117.2	0.56	-0.16	0.26
K, mmol L^{-1}	3.8	3.8	0.85	0.03	0.2
Cl, mmol L^{-1}	95.3	93.9	0.41	0.9	1.1
S, mmol L^{-1}	1.6	2.4	0.003	-0.8	0.2
2 h postfeeding					
Ca, mg dL^{-1}	8.8	8.6	0.84	0.1	0.6
Mg, mg dL^{-1}	2.6	2.3	0.08	0.3	0.1
P, mg dL^{-1}	5.1	4.8	0.47	0.2	0.3
Na, mmol L^{-1}	119.0	124.0	0.29	-4.4	3.95
K, mmol L^{-1}	3.8	3.8	0.86	0.04	0.2
Cl, mmol L^{-1}	94.2	93.7	0.54	1.0	1.5
S, mmol L^{-1}	2.4	3.2	0.01	-0.8	0.2
4 h postfeeding					
Ca, mg dL^{-1}	8.6	8.9	0.55	-0.2	0.4
Mg, mg dL^{-1}	2.5	2.4	0.43	0.1	0.1
P, mg dL^{-1}	4.7	4.9	0.46	-0.2	0.2
Na, mmol L^{-1}	116.5	123.2	0.09	-6.7	4.7
K, mmol L^{-1}	3.7	3.7	0.57	-0.09	0.15
Cl, mmol L^{-1}	94.8	94.0	0.83	0.28	1.13
S, mmol L^{-1}	2.0	3.6	0.0001	-1.6	0.18

[1] mEq $(Na^{+} + K^{+} - Cl^{-} - S^{--})$ kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Blood, urine and milk acid-base parameters: All of the effects observed in early lactation in terms of blood pH, free H^+ and HCO_3^- concentrations were eliminated in mid-lactation (Table 5.35). This observation was paralleled by a reduced urinary concentration of free H^+ and total excretion by cows in mid-lactation and there was no difference between diets (Table 5.36). Urine volume was increased by a higher DCAB as were urinary HCO_3^- concentration and pCO_2.

Effect of DCAB on total milk proton secretion was observed in early lactation remained significant in both early and mid-lactation ($P<0.007$) and was greater for the diet with a higher DCAB (Table 5.37). The concentration of free H^+ was also significantly increased. Milk HCO_3^- was not affected by dietary treatment but pCO_2 was increased by MA.

Blood volume and renal function: Neither plasma volume, packed cell volume, blood volume, effective renal plasma and blood flow, glomerular filtration rate nor the filtration fraction were affected by dietary treatment (Table 5.38) in mid-lactation as was the case for early lactation. Again using BW or water consumption as covariates in the statistical model did not affect the results.

Table 5.39 presents the fractional excretions of the studied minerals. Only Ca was affected and its fractional excretion was increased by a higher DCAB contrary to the situation in early lactation. The effects found for S in early lactation were not significant in mid-lactation.

Table 5.35. Mean blood pH, free hydrogen ion (H^+) and bicarbonate ion (HCO_3^-) concentrations and carbon dioxide (pCO_2) of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Prefeeding					
pH	7.373	7.379	0.796	-0.006	0.020
H^+, mol L^{-1}	4.31×10^{-5}	4.19×10^{-5}	0.959	0.10×10^{-5}	0.32×10^{-5}
HCO_3^-, mmol L^{-1}	29.85	29.85	0.990	-0.008	0.51
pCO_2, mm Hg	50.96	50.56	0.790	0.716	2.62
2 h postfeeding					
pH	7.381	7.368	0.647	0.013	0.030
H^+, mol L^{-1}	4.20×10^{-5}	4.34×10^{-5}	0.958	-0.17×10^{-5}	0.40×10^{-5}
HCO_3^-, mmol L^{-1}	30.15	30.11	0.958	0.03	0.62
pCO_2, mm Hg	49.67	52.99	0.595	-1.79	3.26
4 h postfeeding					
pH	7.387	7.374	0.600	0.009	0.032
H^+, mol L^{-1}	4.24×10^{-5}	4.12×10^{-5}	0.532	0.11×10^{-5}	0.21×10^{-5}
HCO_3^-, mmol L^{-1}	31.06	30.14	0.131	0.91	0.55
pCO_2, mm Hg	53.52	50.12	0.250	2.48	2.02

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.36. Mean urine volume free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) excretions of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Volume, L d^{-1}	26.4	22.0	0.051	4.4	1.9
Total free H^+, mol d^{-1}	5.95×10^{-7}	4.57×10^{-7}	0.146	1.0×10^{-7}	1.0×10^{-7}
Free H^+, mol L^{-1}	2.34×10^{-9}	2.26×10^{-9}	0.734	0.07×10^{-9}	1×10^{-9}
Total HCO_3^-, mol d^{-1}	16.87	12.35	0.149	0.50	2.89
HCO_3^-, mmol L^{-1}	686.14	490.9	0.0001	195.2	42.67
pCO_2 mm Hg	57.87	33.01	0.0002	24.86	4.45

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=19
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.37. Mean milk free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) secretion of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Total free H^+, mol d^{-1}	4.62×10^{-6}	4.27×10^{-6}	0.007	3.50×10^{-7}	1.00×10^{-7}
Free H^+, mol L^{-1}	2.45×10^{-7}	2.34×10^{-7}	0.063	0.10×10^{-7}	0.02×10^{-7}
Total HCO_3^-, mmol d^{-1}	59.40	57.90	0.501	1.49	2.14
HCO_3^-, mmol L^{-1}	3.16	3.20	0.694	-0.04	0.11
pCO_2, mm Hg	41.07	40.22	0.068	0.86	0.42

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.38. Mean plasma volume, packed cell volume and renal function parameters of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Plasma volume, L^{-1}	36.3	34.7	0.640	0.8	1.6
Packed cell volume %	29.3	28.0	0.240	1.1	0.9
Blood volume, L^{-1}	51.5	48.4	0.450	1.7	2.2
Effective renal plasma flow, L min^{-1}	3.67	3.74	0.490	-0.17	0.24
Effective renal blood flow, L min^{-1}	5.22	5.20	0.650	-0.15	0.33
Glomerular filtration rate, L d^{-1}	632	636	0.530	-47	72
Filtration fraction	0.124	0.117	0.920	0.001	0.009

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1}, n=18 DM
[3] Ration with a DCAB of +140.2 mEq kg^{-1}, n=18 DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.39. Mean fractional excretions (FE) of minerals by cows in mid-lactation fed diets differing in cation-anion balance.[1]

Fractional excetion	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Na	0.010	0.011	0.97	-0.00004	0.001
K	0.058	0.059	0.70	0.0057	0.014
Cl	0.090	0.095	0.79	0.0066	0.025
S	0.712	0.940	0.44	-0.370	0.457
P	0.002	0.002	0.81	0.0002	0.001
Ca	0.002	0.005	0.05	-0.001	0.001
Mg	0.278	0.250	0.22	0.06	0.046

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1}, n=18 DM
[3] Ration with a DCAB of +140.2 mEq kg^{-1}, n=18 DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Metabolic parameters: Plasma concentrations of ß-hydroxybutyrate, non-esterified fatty acids, urea-N, NH_3, creatinine and glucose are presented in Table 5.40 for the three sampling times. None were significantly affected by dietary treatment. The total plasma content of none of these metabolites was affected by treatment. Urinary urea and NH_3 concentrations and total NH_3 concentration were increased by MB *vs* MA (Table 5.41).

Metabolic proton flux: Blood buffering capacity did not differ treatments (Table 5.42). However, a reduction in DCAB resulted a decrease in blood proton drain between 2 and 4 h postfeeding. Using milk yield as a covariate in the statistical model eliminated the effect of ration.

Table 5.40. Mean plasma concentrations of metabolites in cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Prefeeding					
ß-OH-Butyrate mg dL^{-1}	5.12	4.91	0.77	0.11	0.40
Urea N[7] mg dL^{-1}	13.47	13.65	0.77	-0.34	1.13
Ammonia μg mL^{-1}	3.66	3.28	0.25	0.27	0.22
Creatinine mg dL^{-1}	0.75	0.79	0.89	0.01	0.05
Glucose mg dL^{-1}	51.8	47.22	0.38	5.44	5.94
NEFA[8] mEq L^{-1}	0.15	0.15	0.93	-0.002	0.019
2 h Postfeeding					
ß-OH-Butyrate mg dL^{-1}	7.35	7.13	0.49	0.44	0.62
Urea N mg dL^{-1}	15.19	14.71	0.86	0.34	1.94
Ammonia μg mL^{-1}	3.70	3.97	0.33	0.23	0.22
Creatinine mg dL^{1}	0.72	0.81	0.22	-0.06	0.05
Glucose mg dL^{-1}	49.73	45.41	0.36	4.60	4.80
NEFA mEq L^{-1}	0.14	0.12	0.31	0.02	0.01
4 h Postfeeding					
ß-OH-Butyrate mg dL^{-1}	7.12	8.30	0.21	-0.92	0.69
Urea N mg dL^{-1}	14.65	16.69	0.42	-1.53	1.82
Ammonia μg dL^{-1}	3.69	3.47	0.12	0.30	0.18
Creatinine mg dL^{-1}	0.74	0.79	0.23	-0.04	0.03
Glucose mg dL^{-1}	50.13	47.79	0.56	2.79	4.68
NEFA mEq L^{-1}	0.13	0.12	0.67	0.004	0.01

[1] mEq (Na^{+} + K^{+} - Cl^{-} - S^{--}) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen
[8] Non-esterified fatty acids

Table 5.41. Urinary excretion of nitrogenous compounds in cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
Urea-N[7], mg dL^{-1}	469.6	531.3	0.044	-94.5	41.1
Urea N, g d^{-1}	56.9	57.6	0.61	-2.3	4.4
Ammonia, μg mL^{-1}	299.0	621.2	0.099	-259.8	159.8
Ammonia, g d^{-1}	3.8	4.6	0.031	-2.7	1.3
Creatinine, mg dL^{-1}	42.3	46.3	0.005	-7.2	2.0
Creatinine, g d^{-1}	4.6	4.6	0.255	0.3	0.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen

Table 5.42. Mean metabolic proton flux (mmol L^{-1}) of cows in mid-lactation fed diets differing in cation-anion balance.[1]

	MA[2]	MB[3]	P[4]	MA-MB[5]	SED[6]
β[7]	-13.80	-13.31	0.192	-0.50	0.36
Pre to 2h postfeeding	-0.321	-0.286	0.745	0.26	0.77
2 to 4h postfeeding	-0.977	-0.118	0.037	-1.44	0.59
Pre to 4h postfeeding	-1.298	-0.369	0.174	-1.19	0.80
4h post to prefeeding	1.298	0.369	0.174	1.19	0.80

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +372.7 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +140.2 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Slope of the non-bicarbonate blood buffer line

C. Late Lactation Experiment

Diet composition: The DM composition of the diets offered to the cows in late lactation is shown in Table 5.43. Potassium was variable. The highest DCAB value (LA) was similar to the value obtained for MA. However the DCAB of LB was higher than expected.

Dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and nitrogen (N) intakes and digestibilities: The effect of DCAB on DMI that was noted earlier in lactation was less in late lactation (Table 5.44). The ration with the higher DCAB improved NDF intake (Table 5.44) Nitrogen intake and retention tended to be higher as DCAB increased but differences were not significant statistically as they were in mid-actation (Table 5.45). Fecal and urinary N concentrations and total excretions were not affected by dietary treatment.

Table 5.43. Mean dry matter composition of diets differeing in dietary cation-anion balance (DCAB)[1] offered to cows in late lactation.

Analyte	LA[2]	SD[3]	LB[4]	SD[3]
CP %	17.3	1.9	17.6	2.5
NDF, %	35.1	5.3	34.3	4.8
ADF, %	27.3	3.5	27.3	2.3
Calcium %	0.61	0.19	0.62	0.21
Magnesium %	0.39	0.11	0.38	0.13
Phosphorus %	0.22	0.04	0.21	0.05
Sodium %	0.44	0.06	0.46	0.05
Potassium %	1.64	0.55	1.50	0.35
Chlorine %	0.35	0.07	0.36	0.05
Sulfur %	0.23	0.04	0.50	0.03
DCAB[1]	374.6	107.9	199.8	106.5

[1] mEq $(Na^+ + K^+ - Cl^- - S^{--})$ kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18

[3] Standard deviation

[4] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18

Table 5.44. Mean body weight (BW), dry matter, ADF and NDF intakes and digestibilities for cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
BW,. kg	603	596	0.089	6	3
DM Intake, kg^{-1} d^{-1}	17.8	16.8	0.090	1.0	0.5
DM Digested, kg^{-1} d^{-1}	12.6	11.7	0.085	0.9	0.5
DM Digested, % of intake	70.9	70.0	0.551	1.0	1.6
ADF Intake, g d^{-1}	4851	4565	0.080	285	146
ADF Digested, g d^{-1}	3218	2907	0.090	311	166
ADF Digested, % of intake	65.8	63.5	0.355	2.3	2.3
NDF Intake, g d^{-1}	6194	5793	0.022	400	148
NDF Digested, g d^{-1}	4011	3628	0.074	383	192
NDF Digested, % of intake	63.8	62.5	0.598	1.3	2.4

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.45. Mean nitrogen balance of cows in late lactation fed diets differing cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
N intake, g d^{-1}	496.2	473.6	0.169	22.7	15.7
Fecal N, %	2.57	2.52	0.408	0.06	0.07
Fecal N, g d^{-1}	131.70	128.50	0.645	4.70	9.89
N Apparent absorption					
N Absorbed, g d^{-1}	363.8	345.8	0.170	12.0	12.1
N Absorption, % of intake	72.7	72.9	0.899	0.2	1.7
Urine N, g L^{-1}	5.76	6.67	0.188	-0.06	0.04
Urine N, g d^{-1}	74.3	78.1	0.553	-2.5	4.0
N Apparent retention[8]					
N Retention, g d^{-1}	288.8	268.4	0.192	20.4	14.6
N Retention, % of intake	57.4	56.5	0.652	1.0	2.1
N Balance[9]					
N Balance, g d^{-1}	203.7	186.6	0.227	13.3	17.1
N Balance, %of intake	40.00	39.22	0.730	2.2	0.8

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1}, n=18 DM
[3] Ration with a DCAB of +199.8 mEq kg^{-1}, n=18 DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[6] Standard error of the estimated difference
[7] Body weight
[8] Retention = intake - fecal excretion - urine excretion

Water intake and absorption: Table 5.46 presents results obtained for water consumption and absorption. Total water consumption liters apparently absorbed daily were increased by LA.

Milk yield and milk composition: In late lactation, DCAB had no effect on average milk yield or composition (Table 5.47).

Mineral balances.

Calcium, magnesium and phosphorus: Results of Ca balance are presented in Table 5.48. As in-mid lactation the only parameter affected was the urinary concentration of Ca which was higher as DCAB was reduced ($P<0.10$). Regardless of diet cows were in positive Ca balance. The same observations were made for Mg balance (Table 5.49). Phosphorus balance, was significantly lower when animals were offered LB *vs* LA due to their lower intake and apparent absorption (Table 5.50).

Sodium, potassium, chloride and sulfur: There was no effect of DCAB on Na and K intakes, absorptions, retentions and secretions in the milk (Tables 5.51 and 5.52). Urinary concentration of Cl was elevated by a reduction in DCAB but the difference between diets was small (25 ppm) and did not result in differences in total excretion nor overall balance (Table 5.53). Milk chloride was not affected by treatment Reducing the DCAB resulted in a higher S intake (Table 5.54). Daily fecal S excretion was increased by a reduction in DCAB as was the urinary excretion but these changes were not enough to prevent an increased S retention as DCAB was reduced. As reported for cows in mid and early lactation the milk S content was not significantly affected by ration.

Table 5.47. Mean water intake and apparent absorption of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Free water consumed, L d^{-1}	75.6	72.4	0.156	4.0	2.6
Feed water, L d^{-1}	15.0	14.2	0.098	1.0	0.6
Total water intake, L d^{-1}	90.7	86.7	0.068	5.0	2.4
Water absorbed, L d^{-1}	63.4	59.4	0.063	4.9	2.3
Water absorbed, % of intake	69.7	68.4	0.380	1.5	1.6

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.46. Mean milk yield and composition of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Yields					
Milk, kg d^{-1}	15.3	14.9	0.316	0.4	0.2
Fat, kg d^{-1}	0.67	0.65	0.347	0.02	0.02
Protein, kg d^{-1}	0.53	0.51	0.245	0.02	0.02
Lactose, kg d^{-1}	0.74	0.72	0.271	0.02	0.02
Concentrations					
Fat, %	4.38	4.41	0.592	-0.04	0.06
Protein, %	3.50	3.49	0.706	0.01	0.03
Lactose, %	4.84	4.83	0.837	0.01	0.03

[1] mEq ($Na^+ + K^+ - Cl^- - S^-$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.48. Mean calcium balance of cows in late lactation fed diets differing in cation-anion balance.[1]

Calcium	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	112.6	104.0	0.164	7.1	4.7
Fecal concentration, %	1.26	1.30	0.657	-0.04	0.08
Fecal excretion, g d^{-1}	65.97	60.09	0.722	1.5	4.11
Apparent absorption, g d^{-1}	46.6	39.9	0.412	5.6	6.5
Apparent absorption, % of intake	40.9	39.3	0.930	0.4	4.0
Urinary concentration, ppm	18.42	35.59	0.100	-16.11	8.80
Urinary excretion, mg d^{-1}	259.3	394.3	0.187	-135.6	94.8
Apparent retention[7], g d^{-1}	43.4	39.5	0.682	2.7	6.3
Apparent retention, % of intake	39.2	39.0	0.819	-1.0	4.1
Milk concentration, ppm	166.5	221.7	0.810	-16.7	65.9
Milk secretion, g d^{-1}	3.27	4.28	0.590	0.50	0.89
Balance[8], g d^{-1}	29.2	26.1	0.721	2.4	6.6
Balance, % of intake	24.6	25.1	0.793	-1.2	4.5

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.49 Mean magnesium balance of cows in late lactation fed diets differing in cation-anion balance.[1]

Magnesium	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	70.6	66.2	0.341	4.5	4.5
Fecal concentration, %	0.87	0.93	0.338	-0.059	0.059
Fecal excretion, g d^{-1}	44.38	46.91	0.599	-1.95	4.91
Apparent absorption, g d^{-1}	26.2	19.3	0.107	6.4	3.6
Apparent absorption, % of intake	35.7	28.8	0.227	6.3	4.9
Urinary concentration, ppm	219.9	335.4	0.051	-106.2	47.1
Urinary excretion, mg d^{-1}	2785.1	3864.0	0.029	-1029.5	398.0
Apparent retention[7], g d^{-1}	21.1	15.4	0.113	6.8	3.9
Apparent retention, % of intake	30.3	22.8	0.148	8.3	5.3
Milk concentration, ppm	101.0	163.4	0.330	-50.6	49.2
Milk secretion, g d^{-1}	0.39	1.74	0.430	-0.27	0.34
Balance[8], g d^{-1}	19.9	14.0	0.113	6.8	3.8
Balance, % of intake	27.9	20.5	0.144	8.4	5.3

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.50. Mean phosphorus balance of cows in late lactation fed diets differing in cation-anion balance.[1]

Phosphorus	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	39.4	35.6	0.079	3.7	1.9
Fecal concentration, %	0.50	0.53	0.094	-0.03	0.02
Fecal excretion, g d^{-1}	25.66	26.84	0.650	-0.88	1.88
Apparent absorption, g d^{-1}	13.8	8.8	0.018	4.6	1.6
Apparent absorption, % of intake	31.6	21.5	0.035	10.0	4.1
Urinary concentration, ppm	4.4	4.2	0.956	0.1	2.4
Urinary excretion, mg d^{-1}	59.2	47.1	0.800	9.5	36.4
Apparent retention[7], g d^{-1}	13.6	8.7	0.039	3.8	1.6
Apparent retention, % of intake	30.7	21.4	0.075	8.6	4.3
Milk concentration, ppm	154.3	148.1	0.970	0.8	19.9
Milk secretion, g d^{-1}	2.6	3.3	0.760	0.1	0.4
Balance[8], g d^{-1}	5.1	0.1	0.039	4.0	1.7
Balance, % of intake	7.2	-4.1	0.043	10.6	4.5

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.51. Mean sodium balance of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	77.0	77.2	0.802	-1.1	4.3
Fecal concentration, %	0.08	0.06	0.226	0.01	0.01
Fecal excretion, g d^{-1}	3.98	3.43	0.301	0.53	0.49
Apparent absorption, g d^{-1}	73.0	73.8	0.706	-1.6	4.2
Apparent absorption, % of intake	94.9	95.8	0.218	-0.8	0.6
Urinary concentration, ppm	1842	2051	0.444	213	2662
Urinary excretion, mg d^{-1}	22551	22925	0.770	-784	2608
Apparent retention[7], g d^{-1}	50.9	50.9	0.767	-1.3	4.4
Apparent retention, % of intake	65.1	66.4	0.677	-1.5	3.4
Milk concentration, ppm	120.3	136.8	0.940	3.9	52.8
Milk secretion, g d^{-1}	1.6	2.3	0.760	0.1	0.4
Balance[8], g d^{-1}	45.1	50.9	0.808	-1.0	4.1
Balance, % of intake	57.5	66.4	0.764	-1.0	3.1

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.52. Mean potassium balance cows in late lactation fed diets differing in cation-anion balance.[1]

Potassium	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	302.2	281.7	0.620	21.600	42.0
Fecal concentration, %	0.35	0.30	0.101	0.05	0.03
Fecal excretion, g d^{-1}	17.62	16.32	0.397	1.88	2.12
Apparent absorption, g d^{-1}	284.5	265.3	0.639	19.7	40.7
Apparent absorption, % of intake	9335	93.1	0.657	-0.4	0.9
Urinary concentration, ppm	1553	1537	0.553	39	64
Urinary excretion, mg d^{-1}	20424	19434	0.298	817.	740
Apparent retention[7], g d^{-1}	266	246	0.969	-1.6	39.1
Apparent retention, % of intake	85.7	86.1	0.664	-0.8	1.9
Milk concentration, ppm	205	437	0.370	-93	98
Milk secretion, g d^{-1}	4.7	7.6	0.28	-1.7	1.5
Balance[8], g d^{-1}	248.2	227.0	0.984	0.8	39.1
Balance, % of intake	78.6	78.6	0.919	-0.3	3.2

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.53. Mean chloride balance of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	61.3	59.2	0.517	2.0	3.0
Fecal concentration, %	0.23	0.22	0.940	0.001	0.01
Fecal excretion, g d^{-1}	11.59	11.71	0.920	-0.08	0.78
Apparent absorption, g d^{-1}	49.7	47.5	0.534	2.1	3.2
Apparent absorption, % of intake	80.7	80.4	0.800	0.4	1.6
Urinary concentration, ppm	1175	1182	0.025	-117	44
Urinary excretion, mg d^{-1}	15787	14075	0.272	-763	653
Apparent retention[7], g d^{-1}	34.6	33.5	0.719	1.4	3.7
Apparent retention, % of intake	55.5	55.4	0.528	2.9	4.4
Milk concentration, ppm	224.4	228.5	0.310	-54.5	51.0
Milk secretion, g d^{-1}	3.2	3.4	0.760	0.4	1.4
Balance[8], g d^{-1}	24.2	22.9	0.671	1.8	4.1
Balance, % of intake	38.0	37.7	0.619	2.9	5.7

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Table 5.54.Mean sulfur balance of cows in late lactation fed diets differing in cation-anion balance.[1]

Sulfur	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Intake, g d^{-1}	34.8	87.4	0.0001	-50.5	6.1
Fecal concentration, %	0.23	0.3	0.0004	-0.078	0.015
Fecal excretion, g d^{-1}	11.86	15.70	0.019	-3.80	1.36
Apparent absorption, g d^{-1}	22.9	71.7	0.0001	-50.5	6.1
Apparent absorption, % of intake	64.8	81.9	0.266	-13.6	15.0
Urinary concentration, ppm	470	3257	0.0001	-2841	236
Urinary excretion, mg d^{-1}	5921	37855	0.0001	-32393	2093
Apparent retention[7], g d^{-1}	16.5	33.8	0.033	-16.0	6.3
Apparent retention, % of intake	47.1	37.2	0.340	12.5	14.1
Milk concentration, ppm	128.6	193.6	0.300	-39.7	35.9
Milk secretion, g d^{-1}	1.9	1.3	0.350	0.3	0.3
Balance[8], g d^{-1}	14.6	30.9	0.008	-13.9	6.7
Balance, % of intake	41.6	34.3	0.275	-10.0	13.0

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} , n=18DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Retention = intake - fecal excretion - urine excretion
[8] Balance = retention - milk secretion

Plasma minerals: The only mineral that was affected by manipulating the DCAB of the ration was S (Table 5.55). As the DCAB was reduced the plasma concentration of S increased prefeeding and at 2 and 4 h postfeeding.

Blood, urine and milk acid-base parameters: No differences due to DCAB were found in terms of blood pH and free H^+ concentration (Table 5.56). Blood concentration of HCO_3^- was significantly reduced at 2 and 4 h postfeeding for LB *vs* LA. Urinary indicators of acid-base status were responsive to dietary treatment (Table 5.57). Bicarbonate concentration and total excretion and pCO_2 levels were all higher when cows were offered LA instead of LB. The total secretion of HCO_3^- in the milk was reduced by reducing the DCAB (Table 5.58).

Blood volume and renal function: Plasma volume, packed cell volume, blood volume, renal plasma flow, glomerular filtration rate and the filtration fraction all were unaffected by DCAB (Table 5.59). Using days in calf as a covariate in the model had no significant effect. Body weight was found to be significant ($P<0.0012$) when included as a covariate in the models to analyze plasma and blood volumes and, when included it made the treatment effect significant ($P<0.01$).

Fractional excretions of minerals were not affected by dietary treatment (Table 5.60).

Metabolic parameters: No significant effect of DCAB was found for plasma metabolic indicators (Table 5.61) or for urinary excretions of NH_3, urea or creatinine (Table 5.62). The total plasma contents of these metabolites were not significantly affected by DCAB.

Metabolic proton flux: Postfeeding H^+ drain from the blood compartment was higher for cows fed LA *vs* LB and the difference was significant between 0 and 4 h postfeeding (Table 5.63). The opposite situation was observed between 4 h postfeeding and the prefeeding value.

Table 5.55. Mean plasma mineral concentrations of cows in late lactation fed diets differing cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Prefeeding					
Ca, mg dL^{-1}	8.9	8.6	0.36	0.3	0.3
Mg , mg dL^{-1}	2.6	2.5	0.82	0.02	0.09
P, mg dL^{-1}	3.9	3.8	0.77	0.05	0.2
Na, mmol L^{-1}	119.8	115.5	0.16	8.3	5.3
K, mmol L^{-1}	4.0	3.9	0.18	0.2	0.1
Cl, mmol L^{-1}	99.9	99.0	0.56	0.6	1.1
S, mmol L^{-1}	1.4	2.4	0.01	-1.2	0.4
2 h postfeeding					
Ca, mg dL^{-1}	8.7	8.6	0.57	0.3	0.4
Mg , mg dL^{-1}	2.4	2.5	0.71	-0.04	0.1
P, mg dL^{-1}	4.2	4.2	0.94	0.02	0.3
Na, mmol L^{-1}	122.4	124.4	0.75	-1.4	4.3
K, mmol L^{-1}	3.8	3.8	0.75	0.05	0.2
Cl, mmol L^{-1}	98.7	101.6	0.12	-2.1	1.2
S, mmol L^{-1}	1.9	3.1	001	-1.3	0.3
4 h postfeeding					
Ca, mg dL^{-1}	8.5	8.7	0.13	-0.2	0.3
Mg , mg dL^{-1}	3.7	2.4	0.20	0.5	0.1
P, mg dL^{-1}	4.4	4.2	0.27	0.3	0.2
Na, mmol L^{-1}	119.5	123.6	0.93	-0.5	5.3
K, mmol L^{-1}	3.8	3.9	0.94	0.01	0.2
Cl, mmol L^{-1}	100.1	99.9	0.49	1.4	1.9
S, mmol L^{-1}	2.2	116	0.01	-1.3	0.3

[1] mEq (Na^{+}+ K^{+}- Cl^{-} - S^{--}) kg^{-1} dry matter (DM)

[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18

[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18

[4] Probability associated with the F test

[5] Estimate of the difference between treatment means

[6] Standard error of the estimated difference

Table 5.56. Mean blood pH, free hydrogen ion (H^+) and bicarbonate ion (HCO_3^-) concentrations and carbon dioxide (pCO_2) of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Prefeeding					
pH	7.359	7.355	0.865	0.004	0.023
H^+, mol L^{-1}	4.40×10^{-5}	4.46×10^{-5}	0.860	-0.04×10^{-5}	-0.10×10^{-5}
HCO_3, mmol L^{-1}	28.10	27.59	0.341	0.51	0.51
pCO_2, mm Hg	50.24	48.77	0.792	0.80	2.93
2 h postfeeding					
pH	7.363	7.387	0.406	-0.024	0.027
H^+, mol L^{-1}	4.40×10^{-5}	4.14×10^{-5}	0.647	0.27×10^{-5}	0.32×10^{-5}
HCO_3, mmol L^{-1}	28.81	27.04	0.004	0.77	0.48
pCO_2, mm Hg	50.78	44.51	0.100	5.42	2.99
4 h postfeeding					
pH	7.380	7.38[illegible]	0.970	-0.001	0.027
H^+, mol L^{-1}	4.17×10^{-5}	4.25×10^{-5}	0.934	-0.09×10^{-5}	0.30×10^{-5}
HCO_3, mmol L^{-1}	28.94	26.50	0.002	2.45	0.57
pCO_2, mm Hg	45.58	45.31	0.255	4.01	3.32

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.57. Mean urine volume, free hydrogen ion (H^+) bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) excretions of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Volume, L d^{-1}	25.32	24.56	0.50	0.76	1.08
Total free H^+, mol d^{-1}	$1.0x10^{-7}$	$1.1 x10^{-7}$	0.760	$0.1 x10^{-7}$	$0.3 x10^{-7}$
Free H^+, mol L^{-1}	$3.95 x10^{-9}$	$5.43 x10^{-9}$	0.391	$-0.50 x10^{-9}$	$0.97 x10^{-9}$
Total HCO_3^-, mol d^{-1}	15.0	9.7	0.0003	5.3	0.9
HCO_3^-, mmol L^{-1}	504.9	417.3	0.0001	177.6	24.8
pCO_2, mm Hg	70.52	46.07	0.0002	24.45	4.04

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.58. Mean milk free hydrogen ion (H^+), bicarbonate ion (HCO_3^-) and carbon dioxide (pCO_2) secretion of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Total free H^+, mol d^{-1}	3.3×10^{-6}	3.4×10^{-6}	0.799	-4.0×10^{-9}	1.4×10^{-7}
Free H^+, mol L^{-1}	2.25×10^{-7}	2.32×10^{-7}	0.359	0.09×10^{-7}	0.13×10^{-7}
Total HCO_3^-, mmol d^{-1}	59.81	50.62	0.089	9.19	4.88
HCO_3^-, mmol L^{-1}	3.73	3.39	0.228	0.34	0.27
pCO_2, mm Hg	40.06	38.84	0.006	1.22	0.35

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.59. Mean plasma volume, packed cell volume and renal function parameters of cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Plasma volume, L	39.3	39.8	0.49	-1.8	2.5
Packed cell volume, %	28.3	27.6	0.14	1.65	1.02
Blood volume, L	54.8	55.2	0.62	-1.7	3.3
Effective renal plasma flow, L min^{-1}	4.3	4.2	0.41	0.4	0.4
Effective renal blood flow, L min^{-1}	6.0	5.8	0.24	0.7	0.6
Glomerular filtration rate, L d^{-1}	860.7	837.5	0.52	72.9	110.0
Filtration Fraction	0.146	0.162	0.56	-0.012	0.002

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.60. Mean fractional excretions of minerals in cows in late lactation fed diets differing in cation-anion balance.[1]

Fractional excretion	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Na	0.011	0.012	0.59	-0.001	0.002
K	0.137	0.131	0.98	-0.00004	0.012
Cl	0.219	0.201	0.88	0.003	0.021
S	0.296	1.392	0.15	-1.13	0.700
P	0.002	0.002	0.84	-0.0002	0.001
Ca	0.004	0.008	0.27	-0.003	0.003
Mg	0.161	0.213	0.41	-0.036	0.040

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference

Table 5.61. Mean plasma concentrations of metabolites in cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	I.B[3]	P[4]	LA-LB[5]	SED[6]
Prefeeding					
ß-OH-Butyrate mg dL^{-1}	4.01	4.78	0.06	-0.65	0.3
Urea N[7] mg dL^{-1}	11.05	12.61	0.09	-1.34	0.71
Ammonia μg mL^{-1}	3.82	3.43	0.09	0.49	0.26
Creatinine mg dL^{-1}	0.74	0.78	0.38	-0.08	0.08
Glucose mg dL^{-1}	47.18	49.97	0.49	-2.19	3.07
NEFA[8] mEq L^{-1}	0.12	0.13	0.68	-0.008	0.019
2 h postfeeding					
ß-OH-Butyrate mg dL^{-1}	5.32	5.94	0.25	-0.89	0.73
Urea N mg dL^{-1}	13.66	14.87	0.43	-0.98	1.19
Ammonia μg mL^{-1}	3.80	4.18	0.18	-0.35	0.25
Creatinine mg dL^{-1}	0.77	0.80	0.59	-0.03	0.05
Glucose mg dL^{-1}	48.11	52.97	0.46	-3.60	4.73
NEFA mEq L^{-1}	0.12	0.12	0.91	-0.001	0.009
4 h postfeeding					
ß-OH-Butyrate mg dL^{-1}	6.30	6.46	0.91	-0.17	0.57
Urea N mg dL^{-1}	15.73	16.41	0.69	-0.41	0.49
Ammonia μg mL^{-1}	3.68	3.62	0.44	0.13	0.16
Creatinine mg dL^{-1}	0.77	0.80	0.95	-0.002	0.04
Glucose mg dL^{-1}	53.45	49.84	0.37	4.14	4.43
NEFA mEq L^{-1}	0.12	0.12	0.54	0.007	0.012

[1] mEq $(Na^+ + K^+ - Cl^- - S^{--})$ kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Nitrogen
[8] Non-esterified fatty acids

Table 5.62. Mean urinary excretion of nitrogenous compounds in cows in late lactation fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
Urea-N[7], mg dL^{-1}	458.6	522.7	0.370	-33.3	35.9
Urea N, g d^{-1}	5.9	2.2	0.970	-0.2	4.4
Ammonia, μg mL^{-1}	150.5	197.7	0.302	-70.8	65.1
Ammonia, g d^{-1}	60.3	61.9	0.412	-0.7	0.8
Creatinine, mg dL^{-1}	49.9	54.2	0.732	-1.0	2.9
Creatinine, g d^{-1}	5.9	6.0	0.681	0.2	0.4

1 mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
2 Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
3 Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
4 Probability associated with the F test
5 Estimate of the difference between treatment means
6 Standard error of the estimated difference
7 Nitrogen

Table 5.63. Mean metabolic proton flux in cows in late lactation. fed diets differing in cation-anion balance.[1]

	LA[2]	LB[3]	P[4]	LA-LB[5]	SED[6]
β[7]	-13.45	-13.13	0.12	-0.75	0.44
Pre to 2h postfeeding	-0.379	-0.204	0.12	-0.88	-0.51
2 to 4h postfeeding	-0.393	0.710	0.21	-1.31	-0.97
Pre to 4h postfeeding	-0.772	0.563	0.03	-2.19	0.87
4h post to prefeeding	0.772	-0.563	0.03	2.19	0.87

[1] mEq ($Na^+ + K^+ - Cl^- - S^{--}$) kg^{-1} dry matter (DM)
[2] Ration with a DCAB of +402.6 mEq kg^{-1} DM, n=18
[3] Ration with a DCAB of +199.8 mEq kg^{-1} DM, n=18
[4] Probability associated with the F test
[5] Estimate of the difference between treatment means
[6] Standard error of the estimated difference
[7] Slope of the non-bicarbonate blood buffer line

4. DISCUSSION

A. Early lactation

An increase in the DCAB of the ration resulted in a significant increase in DMI. Similar results had been obtained by West et al. (1991), Tucker et al. (1988a) and Escobosa et al. (1984) who manipulated DCAB by modifying the Na, K and Cl of their rations. Tucker et al. (1991a) compared two diets in which DCAB was modified using S-containing salts and observed a decrease in DMI of 0.7 kg d^{-1} when DCAB was reduced from +150 to 0 mEq kg^{-1} but the difference did not reach significance. In the present experiment the difference in DCAB between treatments was larger and the difference in DMI was statistically significant. The switchback experimental design used could have played a role. In addition, the cows used by Tucker et al. (1991a) were at a more advanced stage of lactation. A positive effect of increasing DCAB in early lactation was also observed in terms of BW. From the data collected it is impossible to say if cows on EA gain more weight or if cows on EB lost more but the difference in BW was significant. Statistical analysis demonstrated that the higher DMI observed with EA was not mediated only by the concomitant increase in BW. A similar beneficial effect of increasing DCAB on BW was observed by Escobosa et al. (1984) comparing a ration high in Cl *vs* a ration high in Na.

The increase in DMI caused by increasing DCAB tended to increase N intake when cows were offered EA *vs* EB. This difference was maintained when N retention was calculated because the higher fecal excretion observed with EA was compensated by a reduced urinary N excretion. The increased protein secretion in the milk observed with EA eliminated the difference between diets resulting in similar N balance. The increased urinary urea excretion could have resulted from a differential use of urea by the kidneys in the countercurrent multiplier system but could also have resulted from a more catabolic metabolism created by the acid stress accompanying EB when compared to EA (May et al. 1987, 1986). Even though increases in HCO_3^- have been demonstrated not to result automatically in increased ureagenesis (Halperin et al., 1986), a reduced availability of HCO_3^- could still limit urea formation. The blood HCO_3^- concentration resulting from the consumption of EB was not low and neither was its urinary concentration demonstrating that HCO_3^- was not limiting urea synthesis. When HCO_3^{-1} is not limiting, ureagenesis appears to

be directly proportional to the availability of NH_3 (Cheema-Dhadli et al., 1987). It can thus be suggested that the higher acid load caused by EB resulted in an increased protein breakdown. The higher rate of protein catabolism could have resulted in a larger quantitiy of NH_3 entering the blood. The combination of higher blood NH_3 and adequate HCO_3^- supply increased rates of ureagenesis, consequently blood NH_3 concentration was maintained constant. Why the catabolic conditions caused by EB did not result in increased nutrient levels in the blood available for milk synthesis cannot be explained from the present results but may be related to increased amounts of energy being required for Na and HCO_3^- reabsorption (Halperin et al., 1985), or, alteration in mammary blood flow or, changes in some transport systems as seen in the kidneys.

Milk production was also improved by a higher DCAB supporting previous results of West et al. (1991a) and Tucker et al. (1991a, 1988a). Discrepancies between these studies and the present one arise when comparing results obtained for the different milk components. West et al. (1991a), Tucker et al. (1991a) and Escobosa et al. (1984) reported a higher fat percentage in milk as DCAB was increased whereas, Tucker et al. (1988a) did not observed any benefit. These different results can be attributed to two major factors namely, the stage of lactation of the animals and the range of DCAB used in the different experiments. The ranges of DCAB used by the authors mentioned above were lower than the one used in the present experiment. In addition, the cows used in these experiments were, in general, in later stages of lactation and less homogenous than the cows used in the present experiment.

Observations of the effects of DCAB on lactose production cannot be compared to those of other authors since they did not report the effect of DCAB on lactose. The observation that the concentration of lactose in the milk was affected by DCAB indicates that lactose is not the only regulator of milk volume as often suggested (Peaker, 1983). However, recent evidence has recognized an inverse relationship between milk lactose concentration and the concentration of ions in milk (Peaker and Taylor, 1975, Peaker, 1983, Polychroniadou and Vafopoulo, 1985). Results of Peaker and Taylor (1975) indicated a positive correlation between K and lactose and a negative correlation between Na and lactose in the milk of rabbits.

Polychroniadou and Vafopoulo (1985) observed the same correlations in the milk of ewes.

Calcium secretion in the milk is normally associated with protein and citrate secretion (Peaker, 1983). Although citrate was not determined it appears that the increase in protein secretion observed with EA was not sufficient to cause an increase in milk Ca in view of the variability observed in the latter measurement.

The protons secreted in milk could arise from the blood but may also have originated from the metabolism of the mammary epithelial cells as suggested by Peaker (1983). The total secretion of free H^+ in the milk of cows with the highest yield (EA) was significantly higher than the total free H^+ secretion in the milk of cows consuming EB. The animals consuming EA had a lower blood H^+ concentration at 2 and 4 h postfeeding and a higher drain of blood H^+ over the same period of time. Although more frequent sampling of blood and milk would be necessary to establish a definite link, it can be hypothesized that an increase DCAB caused a higher metabolic rate in the mammary gland augmenting the quantity of protons secreted or that the milk was used as a mode of excreting some extra protons generated. The total urinary excretion of H^+ was not affected by DCAB nor was the blood pCO_2 content suggesting that the protons drained from the blood over this period were not eliminated via these routes. From these observations it cannot be established if free proton secretion in the milk is required for milk production, if protons are used to maintain electrical neutrality , or if free protons appeared in the milk because a higher DCAB (due to its alkalogenic properties) allowed a higher metabolic rate and increased production of protons. The possibility that DCAB changes the free proton content of milk simply by altering the milk buffering capacity cannot be ruled out since buffering capacity of the milk was not monitored. The three major buffers of milk are: phosphate, citrate and protein (Linzell and Peaker, 1971). Citrate was not measured. A higher DCAB was shown not to affect milk P and to increase milk protein suggesting, unless marked differences in citrate were caused, a higher buffering capacity with EA. This would indicate that the measurement of free H^+ actually underestimated the total quantity of H^+ secreted.

As a general comment, the milk concentrations of minerals reported in the present experiment were within similar ranges reported for ewes by Polychroniadou and Vafopoulou (1985) except for Ca and P which were lower in the present experiment probably because of the lower protein and fat content.

The urine and blood acid-base parameters were indicative of a mild metabolic acid stress when EB was fed as blood concentration of H^+ was increased and blood HCO_3^- was decreased by EB at 2 and 4 h postfeeding. The reduced blood pH caused by EB was paralleled by lower total urinary excretion of HCO_3^- which is a normal renal compensatory mechanism to bring the blood pH back to normal (Vander 1991). Similar effects of DCAB on blood and urine acid-base parameters were observed by West et al. (1991), Tucker et al. (1988a), and Escobosa et al. (1984) even though the cows used in these experiments differed in stage of lactation, in the ranges of DCAB in the salts used to manipulate DCAB and in ration ingredients.

Since EA and EB differed in their content of K and S different intakes were observed for these minerals as was the case for the dry cows studied previously. Mechanisms as to how such dietary differences can cause an acid stress were discussed in chapter III. Significantly higher retention of K was observed for EA whereas S retention was increased by EB. The increased K retained was probably in the intracellular fluids since no increase of K concentration in the blood was observed. Normally K enters the cells in exchange for H^+(Vander, 1991) which would should affect blood pH. Howerver the changes in intracellular K were not large enough to acidify the blood to the same degree as S did because EB resulted in lower blood pH values. As was the case for the dry cows reducing the DCAB resulted in elevated urinary excretion of Ca. The trend towards a higher absorption of Na as DCAB was increased during the dry period was not found in the present experiment and thus cannot be used to explain the higher water intake and water absorption observed for EA. Increased absorption of K and Cl (causing an osmotic gradient), increased milk production and increased DMI can all be suggested as factors having affected water consumption. The correlations between milk yield, DMI and water intake are well established (Murphy, 1991). The estimated difference in water absorption between EA and EB was 6 L d^{-1}. The simultaneous increases in urine volume and milk yield accounted for 3.5 L and 1.2 L (solids were

not subtracted), respectively. The remaining fraction cannot have contributed to an increased plasma volume since EA tended to reduce plasma volume and not to increase it, consequently contributing to the increase in BW observed with EA (assuming equal water evaporative losses). Plasma volume did not change and concentrations of plasma minerals (except for S) remained constant, therefore it can be concluded that the dietary manipulations used were not large enough to overload the osmolarity and volume homeostatic systems.

Renal function was not drastically affected by DCAB. Since EB was associated with a trend towards a higher blood volume it resulted in a slightly higher RBF. However, because GFR is tightly controlled (Vander 1991), the elevation of RBF did not affect GFR. The FE of S was increased by a reduction of DCAB. Since the value obtained for the FE of S was higher then 1 it indicated that tubular secretion undoubtedly contributed to the increased S excretion via the urine when EB was offered. The FE of Ca was also increased by a reduction in DCAB. The increased FE resulted from a decrease in reabsorption by the renal tubules and not from a reduced filtered load since GFR and plasma Ca concentration remained unchanged. Increased urinary excretion of Ca associated with a reduction of DCAB has been reported consistently and attributed to acid stress (Wang and Beede, 1992a, West et al., 1991, Fredeen et al., 1988). The reduced ability of the kidney to reabsorb Ca has also been demonstrated to result from acidosis (Sutton et al., 1979, Stacy and Wilson, 1970), however, the mechanisms involved are still not well understood (Vander, 1991, Beck and Webster, 1976). Although not significantly affected, EB tended to produce higher plasma and blood volumes. Volume expansion has been demonstrated to increase Ca excretion without necessarily increasing the filtered load of the mineral (Suki and Rousse 1991, Blythe et al., 1968).

The trend for the FE of Cl to increase as DCAB was reduced could be related to an increased activity of the Cl^-/HCO_3^- exchanger present in the proximal tubules since lower urinary HCO_3^- concentration was also associated with EB. The lowered HCO_3^- in the urine of cows could also be attributed to a reduced ability of the kidneys to form new HCO_3^-. This is not likely since this mechanism is triggered only when urinary reabsorption of the filtered HCO_3^- is almost complete (Vander, 1991). The trends towards increased FE of Mg and K associated with EB could have resulted from the higher S content in the fluid of the tubules making the potential

gradient unfavorable to cation absorption. The minimal FE of P can be explained by its low plasma value and ultimately by the low dietary concentration. Phosphate reabsorption is markedly increased when dietary intake and thus plasma concentrations are low (Trohler et al., 1976, Brazy et al., 1980).

The FE of Na was low which is normal since most of the Na is normally reabsorbed (Vander, 1991). However, the FE of Na did not differ between diets. From this observation and the knowledge that HCO_3^- is reabsorbed with Na (Alpern et al., 1991) it can be hypothesized that more Na or energy was available for organic solute and Cl^- reabsorptions, both being Na dependent to a large extent (Berry and Rector, 1991) as the need for HCO_3^- reabsorption was reduced (as DCAB was increased). The same situation could have happened in the gastrointestinal tract where most Na is being absorbed with SO_4^{-2} limiting its availability for absorption of amino acids and glucose (Hopfer, 1987). Since no studies have been reported in cattle showing the major location of the Na_2SO_4 cotransporter in the intestine, and since there seems to be some species differences, it cannot be stated that this transporter is located close to the Na-dependent organic molecules transporter. Thus a definite competition cannot be established (Conway et al., 1989, Lucke et al., 1981). The same reasoning could apply to the transport systems of the mammary epithelial cells, however, the identity of the transport systems in these cells still requires further investigation before anything can be inferred as to their relative contribution to nutrient and mineral transport and their interrelationships.

Greater intestinal absorption and renal reabsorption of organic nutrients in addition to an increased DMI could explain some of the observed increases in milk, protein and lactose yields observed at higher DCAB.

The manipulation of DCAB is also likely to have affected the uptake of nutrients by the mammary gland by altering mammary blood flow or altering the transport systems since the blood concentrations of glucose, NEFA and ß-hydroxybutyrate were not altered by DCAB; if anything, due to the trend towards increased total plasma volume observed with EB the total amount present in the blood tended to be increased with a reduced DCAB. The effects of DCAB on mammary blood flow could be of interest in view of the important correlation

existing between blood flow and milk production (Tucker, 1981). It cannot be inferred either that EB caused a diversion of nutrients towards increasing body mass since the cows were found to have a reduced BW when offered EB.

B. Mid lactation

As was the case for the early lactation experiment, manipulation of the DCAB in mid-lactation resulted in a higher K concentration in the diet having the more positive DCAB (MA) and resulted in a higher S content of the diet having the lowest DCAB (MB).

Several of the parameters measured were similarly affected by DCAB in mid-lactation as they were in early lactation. A higher DCAB in mid-lactation also caused higher DMI, water consumption, and urine volume with no real effect on plasma volume or renal GFR or blood flow. All these changes were associated with increases in K intake ($P < 0.12$) and balance ($P < 0.15$), reduced S intake and retentions and lowered urinary Ca excretion as DCAB was increased. As in early lactation, DCAB did not have any significant effect on the concentration of macrominerals in milk.

The difference between the diets in terms of DCAB was similar in mid and early lactation; this explains the similar differences observed in terms of urinary HCO_3^- excretion. These results indicated that the acid stress caused by the diets in mid-lactation was similar in magnitude to the stress caused in early lactation. This suggests that the difference in DCAB is more important in altering urinary acid-base parameters than the actual range of DCAB since the latter was higher in mid-lactation. Further direct comparison will be required to establish the relative importance of these two factors.

Differences between responses to DCAB of mid-lactation and early lactation cows occurred in terms of milk composition, milk acid-base parameters, blood acid-base parameters and FE of the some of the minerals.

Milk lactose yield still tended to be improved by a higher DCAB but the difference observed in terms of protein yield was reduced and a significant difference in percentage of fat was observed in mid-lactation. The fat concentration of milk was reduced by a higher DCAB because the milk volume increased but fat secretion did not. These results indicate that DCAB seems to affect the osmoregulatory ability of the mammary gland more then its ability to synthesize the various milk component in mid lactation. The milk fat percentage was higher in mid lactation than in early lactation for similar milk yields. Possibly fat secretion was maximal in relation to the nutrients supplied to the mammary gland and altering the DCAB at this point could not have altered the fat synthetic rate nor rate of transfer to the milk. The blood concentration of NEFA was not altered by dietary treatment suggesting that tissue mobilization was not rate limiting. Contrary to the observations in early lactation, the plasma of cows in mid lactation consuming MA tended to have higher glucose concentration, the difference being accentuated when the total plasma glucose content was calculated. The higher supply of glucose as DCAB is increased could explain some the increased lactose yield observed in mid lactation (Kuhn et al., 1980) as DCAB is increased. West et al. (1991) demonstrated a positive effect of increasing DCAB on milk fat percentage with cows in mid-lactation. However, the effect was much smaller as the percentage fat reached 4%. Tucker et al. (1988a) did not observe any beneficial effect of increasing DCAB in the ration for cows in mid lactation in terms of milk fat percentage or yield. These results support the idea that in mid lactation DCAB acted more on the fluid portion of the milk than on the actual fat synthetic and/or secreting ability of the mammary gland.

The milk protein yield of cows in mid lactation was affected less but in a similar manner as the protein yield of cows in early lactation. The ration with the highest DCAB caused an improved N balance in part related to higher intakes and to lower urinary excretion of N as ammonia and urea compared with MB. Urinary ammonia was not affected in early lactation and suggests that the protein catabolic effect of an acid stress is of greater magnitude in mid lactation or that the synthetic ability is less causing more N to be excreted as ammonia. Both acidosis and advancing lactation are associated with insulin resistance (DeFronzo and Beckles, 1979, Bauman et al., 1989) favoring protein mobilization over protein synthesis in peripheral tissues. Increasing DCAB could have reduced the effect of these factors explaining the lower NH_3 and urea excretion in the urine but instead of rendering

the extra N consumed available for milk protein synthesis it was diverted more towards peripheral tissue and increases in BW. DCAB probably did not have such effect in early lactation because of the often reduced insulin secretion, thus no insulin resistance associated with early lactation (Vernon, 1988).

Despite similar changes in urinary HCO_3^- concentration and total excretion, the blood and milk acid-base parameters were not affected similarly in mid vs early lactation. Despite similar trends, the effects observed on blood H^+ and HCO_3^- concentrations as DCAB was modified were not statistically significant in mid lactation as they were in early lactation. Interestingly the differences in milk proton secretions were much larger in mid-lactation compared to early lactation. The highest DCAB resulted in a higher concentration and total secretion of protons in the milk with no change in HCO_3^-. This was paralleled not only by a trend towards increased H^+ drain from the blood but also by a significant increase as DCAB was increased. Since urinary proton excretion did not change with dietary manipulation it suggests that the increased proton drain from the blood was in the milk. A respiratory elimination is not likely since as in early lactation, blood pCO_2 remained unaffected at all times. A possible mechanism that could explain the even stronger relation between milk H^+ and blood H^+ would be a higher secretion of glucocorticoids in mid lactation, which could increase the activity of a Na^+/H^+ exchanger as they do in other epithelial cells (Freiberg et al. 1982). The presence of glucocorticoids in the mammary gland has been well documented (Tucker and Scwalm 1977) but the presence of the ion exchanger and its regulation remain to be studied.

C. Late lactation

Despite a slightly smaller difference in DCAB between the late lactation diets than was used in mid and early lactation diets, the treatment difference observed in urine HCO_3^- due to changes in DCAB was of the same magnitude as observed in the earlier stages of lactation. The differences observed in mineral balances were similar in late lactation to those observed previously but sometimes of smaller importance probably due to the smaller differences achieved in intakes of S and K. This later observation can be attributed to the lack of effect of DCAB on DMI in late lactation.

The beneficial effect of a higher DCAB on milk yield and BW also disappeared in late lactation. These results explain why supplementation of $NaHCO_3^-$ in late lactation was found to have no positive effect (Block, 1988b). The effects of DCAB previously observed on water consumption and absorption were still evident in late lactation but were less significant. Differences in urine volume due to DCAB were absent in late lactation.

The concentration of Ca and P in the milk were reduced in late lactation paralleling reductions in protein and fat yields. These correlations have been well established (Jenness, 1985). The concentrations of Na, K and Cl were also markedly reduced in late lactation. Similar changes in terms of K were reported in the guinea pig (Anderson and Sheffield, 1988) but the changes occurring in milk Na and Cl remain unexplained. As lactation progresses, the permeability of the mammary epithelium and thus the relative importance of the paracellular transport of several compounds is believed to increase as lactation progresses (Peaker, 1983). Such a phenomenon would normally mean an increase in Na and Cl content of the milk with advancing lactation which is exactly the opposite of what was observed in this experiment. Results in ewes did not demonstrate an increase in either Na or Cl as lactation progressed (Polychroniadou and Vafopoulou, 1985).

Of interest is also the absence of effect of DCAB on milk proton secretion paralleling the disappearance of an effect on milk yield. However, the increased proton drain out of the blood observed with an increase in DCAB was still significant in late lactation as it was in mid lactation. No increase in urinary proton excretion was observed but blood H^+ concentration tended to be higher for LA *vs* LB. These observations suggest that in early and mid lactation the increased milk production was responsible for the higher proton drain observed or that the cows in early and mid lactation can use the milk as a mode of excreting protons. Since in late lactation, the secretion of most minerals and of H^+ in the milk differed from the earlier stages of lactation, the changes in mammary gland metabolism brought about by advanced stages of lactation and involution appear to affect the ion transport system in addition to the synthetic capacity of the gland (Hurley, 1989).

The changes in the mammary gland mineral metabolism occurring in late lactation could have occurred in conjunction or independent of the general physiological alterations in mineral metabolism brought about by advancing pregnancy. A comparison between indicators of renal function between the different stages of lactation is not possible because different animals with different body weights were used but pregnancy has definitively been established to have marked hemodynamic effects in other species. Both GFR and ERPF are known to increase during pregnancy (Lindheimer and Katz, 1985). Since GFR can affect Na, K and Cl excretions (Vander, 1991) large increases brought about by pregnancy may reduce the possibility of altering the retention of these minerals and thus the retention of H^+ and HCO_3^- by manipulating DCAB; these minerals are major determinants of DCAB. The same could apply to S metabolism but since it has not been as intensively studied a definite affirmation cannot be made. In addition, studies in rats have demonstrated a down regulation of pregnancy on ANP and a reduced responsiveness of angiotensin to changes in blood pressure during pregnancy (Novak and Kaufman, 1991, Potvin and Varma, 1991).

5. CONCLUSION

From these 3 experiments it can be concluded that similar changes in DCAB resulted in similar changes in urinary acid-base parameters at all stages of lactation. However milk and blood H^+ and HCO_3^- concentrations did not respond similarly at all stages of lactation. In early and mid lactation the increased proton drain from the blood compartment measured for the first 4 h postfeeding was paralleled by an increased milk proton secretion as DCAB increased. This association disappeared in later lactation resulting in an accumulation of protons in the blood.

The milk production and DMI of cows in early and mid lactation responded positively to an increase in DCAB. However the milk components were not affected in the same manner which suggests that similar perturbations of the acid-base status will affect different biochemical or transport processes at different stages of lactation.

Parameters of renal function were not affected by DCAB at any stage of lactation but an increase in DCAB consistently resulted in a higher water consumption and a higher water intake. In early and mid lactation urine volume was also increased but this effect was eliminated in late lactation probably due to a reduced sensitivity of the osmoregulatory and volume regulatory systems caused by pregnancy.

More experiments will be necessary to establish which metabolic pathways are affected by DCAB at different stages of lactation and to determine the DCAB which will maximize production without adversely affecting health for the different production stages of the dairy cow.

VI. GENERAL DISCUSSION

Manipulating DCAB by the addition of mineral salts has proven effective in reducing the incidence of milk fever (Dishington, 1975, Block, 1984, Gaynor et al., 1989) by minimizing the hypocalcemia associated with the onset of lactation (Leclerc and Block, 1989). Results obtained in goats, sheep and dry non-pregnant cows (Fredeen et al., 1988a, Takagi and Block, 1991, Wang and Beede, 1992) suggested that DCAB acted on Ca metabolism by altering the acid-base status of the animals. The measurements of total urinary excretion of HCO_3^- and of urinary concentration of HCO_3^- obtained with the present work confirmed that the overall acid-base metabolism of dry pregnant cows was affected by manipulating DCAB, even when comparing 2 diets with positive DCAB. Previous authors had demonstrated that reducing the DCAB during the dry period resulted in increases in urinary Ca excretion (Gaynor et al., 1989, Takagi and Block, 1991a, Fredeen et al., 1988a). These results were not reproduced in the present experiment. Several hypothesis can be formulated to explain this discrepency: 1- the small difference in CAB between the 2 diets compared in the present study, 2- the fact that both diets had positive CAB, 3- the observation that either diet resulted in a real acidosis, 4- no changes in plasma Ca concentration were observed. Interestingly manipulating the DCAB of lactating cows had similar effects on urinary excretion of HCO_3^- at all stages of lactation as it did during the dry period. In addition, trends ($P < 0.20$) towards increases in urinary Ca excretion were observed at all stages of lactation These results indicated that manipulating DCAB of the ration of lactating animals also alters their acid-base status. Even though total urinary HCO_3^- excretion was increased by a higher DCAB and changes were of similar magnitude at all stages of lactation, blood and milk acid-base parameters did respond similarly at all stages of lactation. These observations demonstrated that urinary HCO_3^- is a good indicator of the relative acid stress caused by dietary manipulations but, that it is not necessarily a good indicator of how the blood and milk buffering systems or metabolisms are responding.

Cows in early and mid-lactation responded to an increase in DCAB by increasing DMI and milk production supporting results of West et al. (1991). These effects were absent in late lactation demonstrating that a similar change in urinary

HCO_3- does not affect production parameters similarly at all stages of lactation. The changes noted in DMI and milk production were not large: 1.02 kg and 1.2 kg respectively in early lactation and, 1.4 kg and 0.7 kg respectively in mid-lactation. However, if these observations are expressed in percentage DMI was increased by 6.7% and milk production by 6.5% in early lactation. In mid lactation, the increase in DMI was of 9% and the increase in milk production was of 3.7%. It can be hypothesized that these increases could be of greater importance for higher producing cows or cows of higher parity. The low milk productions observed in the present work can be explained by the tact that cows used in early and mid-lactation were at their first parity. Further research should also determine if milk production could be affected more by larger increases in DCAB.

Because cows in late lactation also showed increases in urinary HCO_3^- but did not increase their milk production or DMI as DCAB was increased, we can conclude that DCAB did affect acid-base parameters which in turn affected production parameters rather than vice-versa. However, from the present results it cannot be determined if the increases in DMI observedin early and mid-lactation were mediated by the increases in milk production or vice versa.

Although most of the effects of DCAB appear to be mediated by alteration of the acid-base status of the animals the effects on water metabolism are difficult to reconcile with changes in acid-base metabolism. At all stages of production cows absorbed a larger volume of water and excreted larger volumes of urine at higher DCAB confirming results obtained by others (Fredeen et al. 1988a, Escobosa et al., 1984). Even though DMI and water consumption have been demonstrated to be highly correlated (Murphy, 1992) the changes observed as DCAB was increased cannot all be attributed to higher DMI since late lactation cows also absorbed more water but did not show higher DMI. Because the total concentration of dieary minerals was maintained between treatments the higher water intake and apparent absorption observed at higher DCAB cannot be attributed to a higher ash concentration in the more positive diet. It may however, have resulted from a higher total sh consumption resulting from higher total DMI. Glomerular filtration rate and renal plasma flow not being affected demonstrated that the increased urine olume observed at higher DCAB resulted from a reduced water reabsorption or increased

water secretion by the kidneys. A reduced secretion of ADH might have resulted from a higher DCAB. However, the exact mechanism involved cannot be identified from the results obtained.

Higher DCAB also increased milk proton secretion in early and mid-lactation. These effects can be attributed to the effects of DCAB on acid-base metabolism. A higher DCAB resulting in a higher drain of proton from the blood into the milk. The relations between milk proton secretion and milk production and, between milk proton secretion and the maintenanace of acid-base balance still remain to be determined.

When the ingredients of the ration offered to cows in early lactation were modified keeping DCAB constant no change in milk production nor DMI were observed. A small reduction in urinary concentration of HCO_3^- was observed when a ration based on alfalfa haylage was offered instead of a ration based on corn silage. However, the total urinary HCO_3^- excretion was affected in the opposite direction due to the higher urinary volume caused by the alfalfa haylage based diet. These results indicated that changing the ingredients of the ration but keeping DCAB constant and minimizing differences in proximate analysis did not impact on acid-base status nor performance of cows in early lactation. The effects of DCAB were of greater magnitude.

Cows consuming the alfalfa haylage-based ration absorbed more water without consuming more DMI than cows offered a corn silage based ration. The increased urine volume observed with the alfalfa haylage-based diet was not enouh to compensate for the increased water apparent absorption consequently, plasma volume was increased. The total water intake was however, similar between the 2 diets. This indicates that care should be taken in studying water metabolism since a higher water consumption does not necessarily result in a higher water absorption or vice versa. The changes in water metabolism observed could be related to the increased intake of K, Cl and S when cows were offered the alfalfa haylage-based diet.

VII. GENERAL CONCLUSION

The results obtained in the present experiments demonstarted that manipulating DCAB by adding K or S-containing salts causes changes in the acid-base status of dairy cows at all stages of production. The acid-base parameters mostly affected were urinary HCO_3^- concentration and daily excretion. When DCAB was maintaned constatnt but ingredients used toformulate the rations were modified no effect on acid-base status were observed despite differences in K, Cl and S concentrations. These results emphasize the importance of DCAB in the determination of the acid-base status of dairy animals. However, the experiments presented did not investigate a possible interaction between the source of forage included in the ration and DCAB (alfalfa haylage vs corn silage) nor the interaction between the absolute concentration of K, Cl, Na and S, and DCAB.

Increasing the DCAB for cows in early and mid-lactation resulted in increased DMI and milk production but did not affect the production of cows in late lactation. Because only 2 DCAB were ompared at each stage of lactation more experiments wil be needed to determine the ideal DCAB for each stage of lactation. More data will also be required to establish minimal and maximal levels of inclusion in the ration of each of the four minerals used in the equation so that deficiencies and toxicities can be avoided and the benefits of DCAB can be maximized.

Becuse the parameters of renal function monitored in these experiments were not affected by DCAB it can be concluded that in that range of DCAB the effects observed in terms of DMI, milk production and mineral metabolism were mediated by changes in acid-base balance and not to impaired or altered renal function.

Modifying DCAB did not affect the metabolism of Ca, Mg or P at any stage of production. The metabolism of these macrominerals was not affected either by changing from an alfalfa haylage-based diet to a corn silage based diet suggesting that their bioavailalbilty from these two forages is equivalent.

Higher DCAB increased water intake and urine volume at all stages of lactation These changes could be attributed to changes in Na intake. The results obtained indicated a need for a better understanding of water metabolism in dairy cows and its possible impacts on the maintenance of acid-base balance and the milk production by these animals. More knowledge as to the modes of secretion of minerals in the milk could also help understand the control of milk osmolarity and pH.

From these results one can suggest that rations offered to cows in early and mid lactation should have a DCAB at least as high as 350 meq kg^{-1} DM and that rations formulated to prevent milk fever should have a slightly negative DCAB if Ca metabolism is to be affected.

STATEMENTS OF ORIGINALITY

To the best of the author's knowledge, the following information contained in this thesis constitute an original contribution to the scientific literature.

1- The evaluation of the effects of modifying dietary cation-anion balance or the ingredients used in the formulation of rations on protons and bicarbonate excretions in the urine and secretions in the milk using total collection of both fluids and correction factors for the changing ionic strength of milk and urine.

2- The evaluation of changing dietary cation-anion balance or the utilization of different ingredients in the ration on metabolic hydrogen production by cows at different stages of production.

3- The evaluation of modifying dietary cation-anion balance or the ingredients of the ration on macromineral balances of dairy cows measured by total collection of feces, urine, and milk with a simultaneous determination of their metabolic profile.

4- The effects modifying dietary cation-anion balance or the ingredients of the ration on water consumption and water absorption.

5- The effects modifying dietary cation-anion balance or the ingredients of the ration of the dietary manipulations on blood volume, glomerular filtration rate and renal plasma flow.

6- The evaluation of the effects of manipulating dietary cation-anion balance of lactating animals on all the above mentioned parameters considering each stage of lactation separately.

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