Manipulation of Dietary Cation-Anion Difference on Nutritionally Related Production Diseases, Productivity, and Metabolic Responses of Dairy Cows[1]

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ABSTRACT

Dietary cation-anion difference has been defined as milliequivalents of (Na + K) − (Cl + S) per kilogram of DM and has a direct impact on blood acid-base metabolism. As this difference decreases, one or more of the following blood parameters change: increased H^+, decreased HCO_3^-, and decreased pH. These changes are accompanied by reduced urinary HCO_3^- excretion and pH as compensatory mechanisms. Although other minerals have an impact on acid-base metabolism, the four minerals used in dietary cation-anion difference have the greatest effect. Manipulation of acid-base balance can be used to manipulate other biological functions to benefit health and productivity of cows. Low cation-anion difference prepartum can mitigate hypocalcemia peripartum via increased urinary Ca, blood-ionized Ca, and responsiveness to Ca homeostatic hormones. These changes reduced the incidence of paresis and increased productivity by reducing the severity and length of hypocalcemia in all cows (periparturient), regardless of the occurrence of paresis. Reduced cation-anion differences prepartum have been related to a reduced severity of udder edema, likely related to increased renal loss of water and unchanged water intake. However, the effects on acid-base balance cannot be ruled out because of effects on biochemical and transport processes. Elevated cation-anion difference in lactation has been shown to increase DMI and production and to mitigate the effects of heat stress. Because production and heat stress are acidogenic, elevated cation-anion difference improves blood-buffering capacity to cope with H^+. In heat stress, elevated water intake with elevated cation-anion difference cannot be ignored. Other diseases related to metabolic acid, such as laminitis and ketoacidosis, may be influenced by elevated cation-anion difference in lactation; however, research in these areas has not been forthcoming.

(**Key words**: cation-anion difference, blood acid-base balance, milk fever, calcium homeostasis)

Abbreviation key: **DCAD** = dietary cation-anion difference; **1,25-$(OH)_2D_3$** = 1,25-dihydroxyvitamin D_3, **pCO_2** = partial pressure of CO_2, **PTH** = parathyroid hormone.

INTRODUCTION

Much attention has been paid to requirements for maintenance and production of the major nutrients: protein, carbohydrate, fat, vitamins, and minerals. Requirements usually are established in feeding trials using graded levels of nutrients and defining optimal performance or blood concentrations by a factorial approach utilizing the knowledge of nutrient costs for maintenance and production or by simulating the animal itself as a computer model. Recently, ruminant nutritionists have been devoting much effort to subdividing the major nutrients into more specific subclasses to define requirements more precisely and to allow better ration formulation from chemical analyses of feeds. Protein, for example, is expressed as rumen-degradable and rumen-undegradable protein and NPN; carbohydrates

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are being divided into structural and nonstructural as well as rumen-degradable and rumen-undegradable components.

For the most part, the subdivisions of the major nutrients have been defined into three or four subclasses, which has allowed researchers to evaluate probable levels for inclusion in rations. Because of the small number of subclasses, the interactions among nutrients have been relatively easy to study. Minerals present a very different picture. This class of nutrients presents a plethora of individual components that each are required by the animal, yet minerals have been subdivided into only two subclasses, macromineral and micromineral. Furthermore, this subdivision is based not on any biological phenomenon or function but instead is based on the quantities at which these minerals are found in the animal body and rations—hardly a basis for ration formulation. Yet minerals are a more integral part of all biological functions in the animal than any other nutrient. The functions include expression and regulation of genes, enzyme systems that regulate cellular function, osmotic balance, detoxification, acid-base balance, and their structural roles (i.e., bone).

The objective of this paper is to focus on the balance of dietary fixed cations Na^+ and K^+, the fixed anion Cl^-, and the acidogenic anion $S^=$ as SO_4 on acid-base balance in the cow and the potential effect of this balance on production and health.

Balancing Anions and Cations in Rations

The concept of balancing rations for anions and cations is not new in animal nutrition and has been used in formulating rations for poultry (42). However, ruminant nutritionists have not utilized ration balancing, probably because of scarce research data and confusion as to how the concept applies to rations for cattle. Most of the confusion lies in ill-defined terminology; terms such as anion gap, alkali-alkalinity, cation-anion difference, and cation-anion balance of rations have been used to denote the same concept. Additionally, there are no guidelines for application of the concept.

In balancing rations, the term "fixed ions" refers to bioavailable ions that are not metabolized, namely, Na^+, K^+, and Cl^-. The fixed ion balance plays a major role in determining acid-base balance in biological fluids (52). Some researchers (2, 10) include S, although S is not a fixed ion, because sulfates directly acidify biological fluids and can alter acid-base balance if included at high dietary concentrations (7, 62). Therefore, for this text, we will only consider dietary cation-anion difference (**DCAD**) as milliequivalents of $(Na^+ + K^+) - (Cl^- + S^=)$ per kilogram of dietary DM.

The anions Cl^- and $S^=$ should be balanced in a ration against the cations Na^+ and K^+ to optimize the physiological functions of the animal. Ultimately, the cells of the body would be presented with these minerals that will have to be utilized in metabolism. Particular minerals (Na^+, K^+, Cl^-, $S^=$) have been chosen to calculate DCAD because their importance in ruminant metabolism lies in their indirect participation in osmotic balance, acid-base balance and integrity, and pumping mechanisms of cell membranes. Ration balancing also necessitates that the minerals in question are not in deficient or toxic levels in the ration, and any deficiency or toxicity must be rectified before any effect can be seen from adjusting the balance of anions and cations.

Following the concepts of Mongin (42) for poultry and Dishington (10) for dairy cows, the DCAD is calculated as milliequievalents of $(Na^+ + K^+) - (Cl^- + S^=)$. The equation itself is easily calculated. However, some of the reasoning behind its use as it appears in the literature is faulty; hence, misconceptions appear. It must be remembered that only some of the total dietary anions and cations are used to calculate DCAD. Indeed, if all feed ions were included, the total anions and cations would have to be in equivalent amounts, for a DCAD of zero, because feeds were once living tissues that must be electrically neutral.

Another concept leading to confusion is that anions form acidic residues (acidogenic) and that cations form alkaline residues (alkalogenic) in the organism. This concept is incorrect. For example, HPO_4^{2-} and NH_4^+ both act as proton donors (alkaline buffer) even though one is an anion and the other is a cation. Similarly, no reactions occur when Na^+ or K^+ form alkalis; however, these ions, as well as Cl^- and SO_4^{2-}, indirectly affect the H^+ concentration in the body via buffer systems, kidney function, and cellular respiration.

Therefore, DCAD, as described, does not

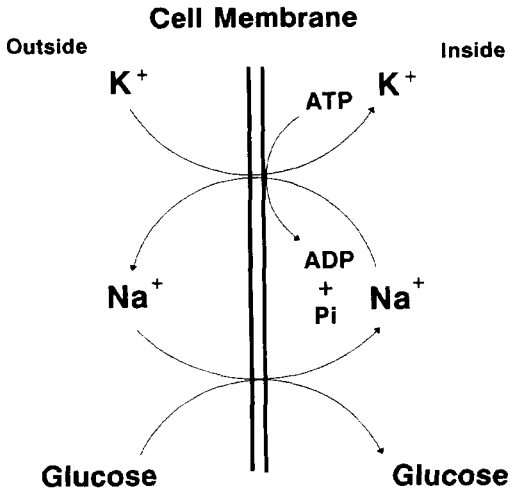

Figure 1. The Na^+-K^+ ATP-dependant pump mechanism in the entry of glucose to cells.

determine the acidogenic or alkalogenic properties of feeds but can affect metabolic processes in the animal by the absorption and metabolism of these ions. Probably the indirect participation of the ions in kidney function, buffer systems, and cellular maintenance is responsible for any effects observed from altering the balance of these ions.

Potential Roles in Metabolic Processes for Balanced Anions and Cations

Some researchers (10, 11, 40) have suggested that cations and anions are alkalogenic or acidogenic. This assertion probably is not true; however, much evidence exists for their role in the acid-base status of the animal. In this role the balance of anions and cations probably has its primary, but not sole, effect on metabolism.

The Na^+-K^+ pumping mechanism of cells often is taken for granted. This mechanism actively maintains high levels of K^+ and low levels of Na^+ intracellularly and requires energy in the form of ATP. In fact, this mechanism has been estimated to require up to 40% of the energy required to maintain cells (41). The Na^+-K^+ pump operates constantly and independently of other metabolic processes; however, Figure 1 illustrates how the pump also operates in conjunction with entry of glucose into a cell. Because glucose is the main source of cellular energy, a slowdown of the Na^+-K^+ pump would not allow cells to operate at full potential, especially for the active mammary gland for which high quantities of glucose are used for lactose synthesis. Obviously, excesses of one cation in relation to the other can cause the pump to slow (causing the cell to use less energy in pumping but obtain less glucose) or to speed up beyond an optimal level (creating a higher energy requirement to maintain the cell). Even though Na^+ and K^+ pumping requires ATP, the entry of a glucose molecule yields a positive ATP balance within the cell. An area of mineral metabolism that has not been investigated is the potential for manipulating dietary Na^+ and K^+ to cause an increase in cellular energy expenditure with a net increase of cellular glucose uptake.

Excretion of Na^+ and K^+ in urine involves a reciprocal relationship by which K^+ is conserved by the body at the expense of Na^+. Excess of one cation versus the other can induce deficiency. For example, if dietary K^+ is high relative to Na^+, the kidney may induce Na^+ deficiency even if dietary Na^+ is in accordance with nutrient requirements and vice versa.

Another process involves intestinal absorption. In the posterior segment of the intestine, Cl^- is absorbed, when it is in excess of Na^+, in exchange for a HCO_3^- to maintain electrical neutrality. If insufficient Na^+ is present to allow the absorption of (neutral) NaCl, an excessive drain of blood HCO_3^- can lead to an acidotic condition. Alternatively, there is potential for intestinal exchange of ingested Na^+ with circulating H^+ in blood when Na^+ is in the intestine in excess of Cl^-, which would lead to a metabolic alkalosis. In fact, this particular mechanism may explain why Schneider et al. (49) found a response in FCM when either $NaHCO_3$ or NaCl were added to a ration for cows. The Na^+ exhibited an alkalogenic effect as suggested by Leach (35). In addition, the alkalogenic effect of Na^+ potentially can be detrimental under certain circumstances (if not balanced with Cl^-) with current practices of supplementing dairy rations with $NaHCO_3$ without altering dietary NaCl to compensation for extra Na^+.

The mobilization of H^+ in the proximal tubules of the kidney, the secretion of H^+, and

the production of ammonia in the distal tubules of the kidney all depend on reabsorption of Na^+ to neutralize electrically the absorption of HCO_3^- from the tubular cell to the blood (Figure 2). If excess Cl^- is present in the glomerular filtrate, Cl^- in the filtrate and HCO_3^- in the cell may exchange, resulting in NaCl reabsorption and a reduction of HCO_3^- absorption. Furthermore, when the animal is under the stress of mild acidosis in extracellular fluids, the kidneys conserve HCO_3^- ions by reabsorption; the reverse is true for alkalosis (19). To maintain electrical neutrality, the Cl^- ion is exchanged with HCO_3^- from tubular fluid because of a preponderance of Cl^- in extracellular fluids. In this manner, optimal Cl^- in relation to other ions is needed to maintain acid-base balance.

The final mechanism to be discussed is the phenomenon called the chloride shift. This phenomenon is illustrated in Figure 3, which depicts an integrated approach of erythrocytes in tissue, plasma, and lung while electrical neutrality is maintained, Figure 3 shows the principal protein buffer in blood, which is the potassium salt of oxyhemoglobin ($KHbO_2$) in erythrocytes. Carbon dioxide produced from tissue metabolism reacts with H_2O to form H_2CO_3 inside the erythrocyte, catalyzed by carbonic anhydrase. Some of the H_2CO_3 enters the plasma, and the rest reacts with $KHbO_2$ to form HCO_3^-, thus liberating oxygen for respiration and K^+ from $KHbO_2$. The HCO_3^- enters the plasma in exchange for Cl^-. Sodium bicarbonate is formed in plasma, and the Cl^- that entered the erythrocyte is neutralized by the K^+ released in the exchange of HCO_3^- with Cl^-. This reaction is reversible in the lung where Cl^- transfers back to the plasma, liberating K^+ to buffer a newly formed $KHbO_2$. The Cl^- that transfers back to plasma neutralizes the Na^+ released when HCO_3^- reenters the erythrocyte for removal of CO_2 in respiration. If these ions are not balanced with each other, even if they are present in adequate and nontoxic quantities, the production of alkalosis or acidosis is possible via insufficient exchange of HCO_3^- and H^+ (19, 22).

Responses of Ruminants to Balancing Dietary Anions and Cations

Very few trials reported in the literature deal with the subject of DCAD for ruminants. In fact, a majority of research trials reported on the subject of any one of the minerals in question disregards the other minerals in that they are not presented in ration composition tables. This result is especially disturbing because, if Cl^- is being studied, it can only be added to a ration as a salt in combination with a cation (e.g., Na^+, K^+, or Mg^{2+}). Thus, the study of one mineral in diets for cows necessitates altering its balance with other minerals.

Based on the previous discussion, the major impact of DCAD obviously will be on acid-base regulation. However, this impact may not be reflected in a measurement of blood pH. Blood pH is the sum total of all reactions in the body, is highly buffered, and is maintained within narrow limits by kidney and respiratory functions. The changes in acid-base status must occur within the cell and exert their effects on cell function by altering the activity of enzyme systems because enzymes, being proteinaceous compounds, require particular pH conditions for optimal activity.

Erdman et al. (14) demonstrated that the indices of acid-base status in cows tend toward alkalinity with increasing time postpartum. Blood pH, HCO_3, and partial pressure of CO_2 (pCO_2) increase with DIM. However, the literature is devoid of any associations between whole animal acid-base status and productivity. Under normal conditions, urinary pH of cows is regulated by HCO_3^- and ammonium ion excretion with an alkaline urinary pH (50, 51), which is unlike other species that use phosphate excretion to control urinary acid excretion (13, 14). Therefore, as discussed, the ions Na^+ and Cl^- are intricately involved in urinary acid excretion.

Erdman (12), in a review of buffer requirements of dairy cows, made a good case for perturbations in blood pH, HCO_3, and pCO_2 caused by environmental temperature. However, changes in these parameters caused by diet have not been investigated. Erdman (12) also pointed out that, although $NaHCO_3$ tends to increase blood pH, HCO_3^-, and pCO_2 when these values are depressed by high environmental temperatures (heat stress), whether the response is due to $NaHCO_3$ or to Na^+ alone is uncertain. Schneider et al. (49) suggested that production and intake responses to $NaHCO_3$ when cows are heat-stressed are due to increased dietary Na^+ and not to a need for

HCO_3^-. In the same review, Erdman (12) cited literature showing that responses of cows appear to be more consistent with added dietary Na^+ in the form of $NaHCO_3$ than as NaCl, leading the reader to assume that the added HCO_3^- from $NaHCO_3$ has some specific role.

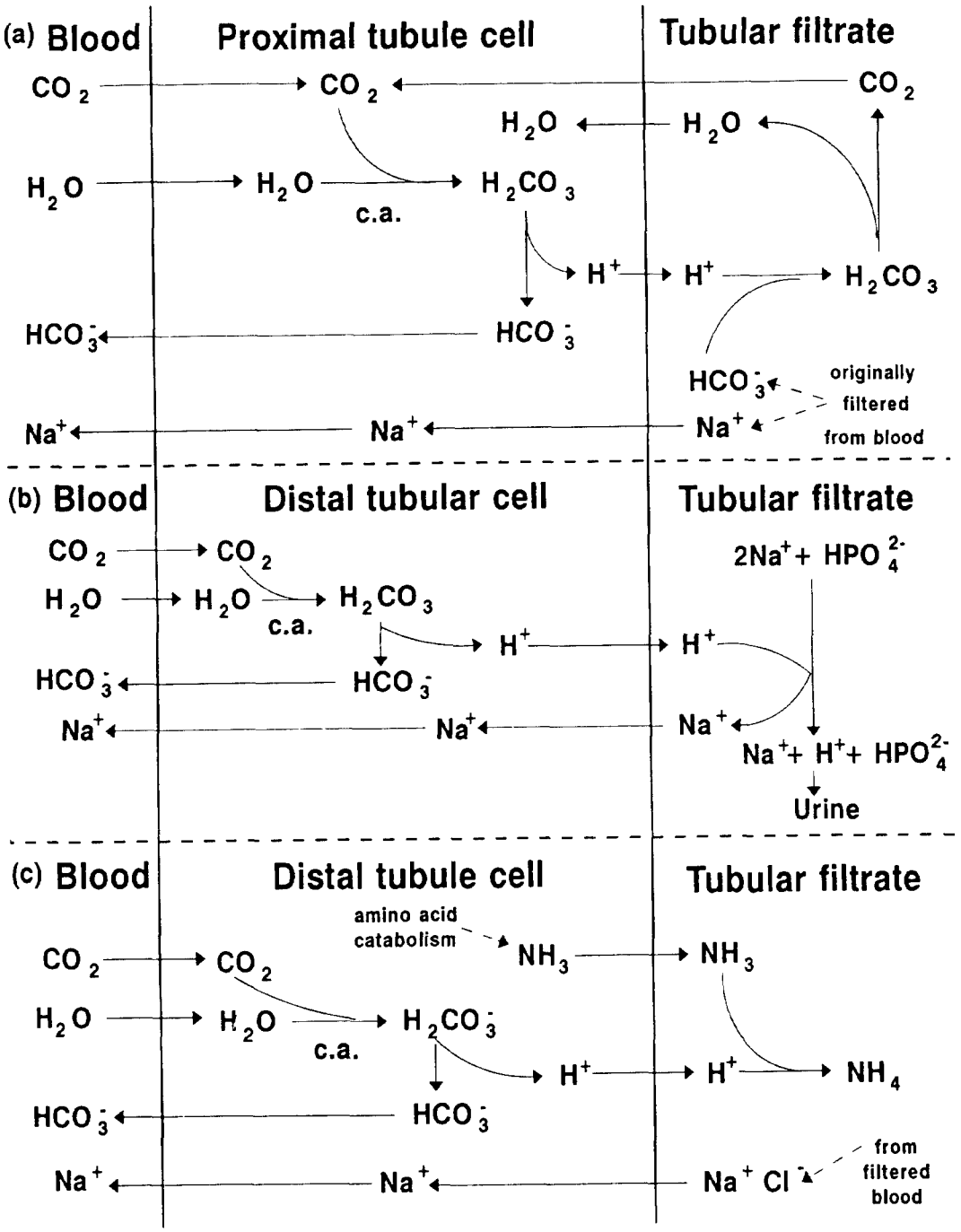

Figure 2. Role of Na^+ in mobilization of H^+ in proximal tubules (a), secretion of H^+ in distal tubules (b), and ammonia production in distal tubules (c). c.a. = Carbonic anhydrase.

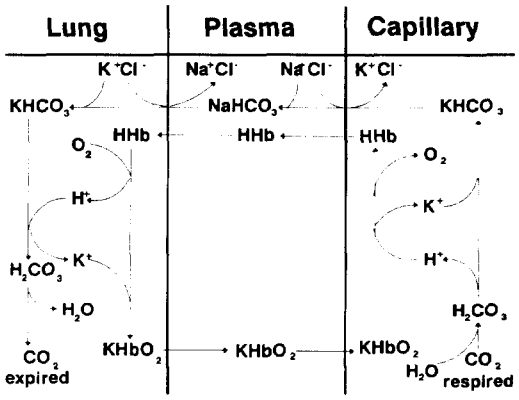

Figure 3. Reaction of erythrocyte in tissue and lung, and plasma changes in respiration, in relation to Na^+, K^+, Cl^-, and the chloride shift.

Using the previous discussion on DCAD, however, a specific role for HCO_3^- cannot be determined, unlike the conclusions by Erdman (12). When DCAD is calculated, added $NaHCO_3$ leads to a more positive number than NaCl because HCO_3^- is not in the equation. Therefore, $NaHCO_3$ would be more alkalogenic than would NaCl even at equivalent inclusion rates of Na^+ from both sources.

This theory is further illustrated from literature data when $NaHCO_3$ was included in diets and DCAD was calculated. It is well documented (43) that $NaHCO_3$, added to rations for dairy cows with low concentrations of fat in their milk, partially corrects milk fat when the low concentration of milk fat is due to low ratios of forage to concentrate in the diet. The response in this case probably was due to a specific buffering effect by $NaHCO_3$ in the rumen and replacement of $NaHCO_3$ that was lost because of low salivary flow rates. Tucker et al. (57) suggests that rumen pH does increase with increasing DCAD. However, this conclusion is equivocal because they increased dietary cations (Na and K) by using HCO_3 salts; the HCO_3 may have influenced rumen pH more than either Na or K. The effects of added $NaHCO_3$ to rations that do not create a depression in milk fat are less clear, and the reason for the lack of clarity may be in the DCAD of the ration. For example, Kilmer et al. (34) added $NaHCO_3$ to rations that did not depress milk fat and found little or no response of cows over the duration of the trial. However, the control and buffered rations contained an equivalent DCAD (220 and 270 meq·kg^{-1} of ration DM, respectively) when calculated as milliequivalents ($Na^+ + K^+ - Cl^-$). The equivalent DCAD were because the Na^+ of $NaHCO_2$ replaced the Na^+ from NaCl in the buffered ration. Conversely, St. Laurent and Block (53) found more responses to $NaHCO_3$ when $NaHCO_3$ was added in addition to the basal level of NaCl in the control ration, thus increasing the DCAD in the buffered ration.

The importance of DCAD in ruminant nutrition was reviewed by Wheeler (61). He reviewed a number of papers and summarized the improvement of weight gain in steers and in milk production of cows occurs when the DCAD [milliequivalents ($Na^+ + K^+ - Cl^-$)] was approximately 100 kg^{-1} of ration DM. Although no specific recommendations can be made on optimal DCAD, a case can be made for the need for further research in this area. For example, Fettman et al. (16) studied the effects of supplemental Cl^- in rations for dairy cows. They found that, as dietary Cl^- was increased from .10 to .45% of the ration DM, feed intake, live weight, and milk production increased. The rations fed differed only in Cl^- (Na^+ and K^+ were held relatively constant). The DCAD [milliequivalents ($Na^+ + K^+ - Cl^-$)] was decreased from 279 to 177 meq·kg^{-1} of ration DM as Cl^- increased.

The problems in extrapolating data from the review by Wheeler (61) or Fettman et al. (16) is that milk production was not sufficiently high to make a specific recommendation for DCAD in lactation. Unfortunately, few other cases are reported for which any recommendations can be made on optimal DCAD for lactating dairy cows because of inadequate data. However, it appears logical to keep the DCAD highly positive (cationic) for lactating cows because these cows have a high metabolic rate and because the cellular environment tends to be acidotic. Keeping the balance highly positive would necessitate higher dietary Na^+ and K^+ relative to Cl^-, thus counteracting the acidotic condition with the alkalogenic effects of Na^+ and K^+. Support for this hypothesis was given by Tucker et al. (57), who used cows from 3 to 8 mo postpartum and demonstrated that cows fed a positive DCAD of +200 meq·kg^{-1} (calculated as milliequivalents of Na + K – Cl) produced

more milk than cows fed a DCAD of −100 meq·kg^{-1}. Additionally, blood pH and HCO_3 and urinary pH increased linearly with DCAD. Similar results were reported by West et al. (60). The ideal DCAD for lactating cows, however, would change as lactation progressed and milk production decreased (i.e., metabolic activity declines). Theoretically, the DCAD should be high at the beginning of lactation and decrease progressively throughout the lactation, which may explain why buffers such as $NaHCO_3$ have little effect on cows that are beyond 100 DIM and receiving a ration that does not depress milk fat (43). Support for this hypothesis was shown by Delaquis and Block (9). Cows in early and midlactation, but not in late lactation, responded to a positive DCAD with higher milk yield. Cows fed the positive DCAD also excreted more HCO_3^- in urine at all stages of lactation.

West et al. (59) demonstrated that increased DCAD increased DMI in heat-stressed cows. They hypothesized that the increase in blood-buffering capacity with higher DCAD was responsible for the increase in DMI. The increases in blood-buffering capacity and DMI have also been reported for cows that were not heat-stressed (8, 60). Although elevated blood buffering may be part of the reason for increased DMI with increased DCAD, water intake also must increase with increases in DMI, milk production, or both. Delaquis (8) showed that there were increases in water intake, water absorption, and urine volume with elevated DCAD but no significant alterations to glomerular filtration rate or effective renal plasma flow.

More work has been generated on the role of DCAD for prepartum cows for the prevention of milk fever. Basically, milk fever occurs at the initiation of lactation when Ca^{2+} is drained from blood for colostrum synthesis and is not replaced rapidly enough from intestinal absorption, bone mobilization (resorption), and reabsorption in the kidney, resulting in paresis tetany of muscles and, if untreated, death of the cow.

Prevention of Milk Fever

Prevention is the most desirable means of reducing the economic losses occurring from milk fever. These losses include loss of milk, veterinary costs, labor costs, and possible loss of the cow (2). Because of the importance of Ca metabolism in the etiology of milk fever, preventive approaches have been focused in this direction. Dietary manipulations and injections of vitamin D_3 and its metabolites have been reported as possible methods for reducing the incidence of the disease.

Oral and intramuscular doses of vitamin D_3 have prevented milk fever successfully (25, 31, 32). However, repeated treatments necessitated by inaccurate prediction of date of parturition may lead to toxicity (39). The metabolites of vitamin D_3 (hormones) are more active in metabolism of Ca and have been used successfully to prevent the disease (20, 47, 48). However, the active metabolite, 1,25-dihydroxyvitamin D_3 (**1,25-$(OH)_2D_3$**), was reported to be higher in the blood of cows with milk fever (27, 29, 33). Therefore, Host and Reinhardt (28) hypothesized that cows with milk fever have a reduced sensitivity (via interference or low receptors for the hormone) to 1,25-$(OH)_2D_3$.

Parathyroid hormone (**PTH**) is involved in Ca homeostasis; however, PTH also is higher in blood of cows with milk fever (27). Therefore, the direct cause of milk fever does not lie in hormone production but, somehow, in the activity of the hormones on their target tissues (bone, intestine, and kidney) to keep Ca constant in the blood at calving. Might the activity of hormones be affected by the acid-base status within the target cells?

Manipulation of DCAD has also been shown to prevent milk fever in dairy cows. Dishington (10) successfully prevented milk fever in 92% of cases when prepartum dairy cows were fed rations with a negative DCAD [calculated as milliequivalents $(Na^+ + K^+) - (Cl^- + S^-)$] and a high Ca content. A better response to the diet occurred when dietary concentration of Ca was high (11), as explained by Lomba et al. (40), who showed that absorption of Ca from the intestine increased as DCAD decreased. Block (2) showed a 47% incidence of milk fever when prepartum cows were fed a ration with a DCAD [calculated as milliequivalents of $(Na^+ + K^+) - (Cl^- + S^-)$] of +330.5 meq·kg^{-1} of DM and a zero incidence when the prepartum ration had a balance of −128.5 meq·kg^{-1} DM. Oetzel et al. (46) also showed a reduction in the incidence of milk

fever, regardless of dietary Ca, when a DCAD of −75 meq·kg^{-1} of DM was fed compared with a DCAD of +189 meq·kg^{-1} of DM in prepartum diets. Sulfur was included by these workers because of the acidifying effect of SO_4 in biological fluids as demonstrated by Whiting and Draper (62).

Block (2) and Goff et al. (23) found that the concentration of plasma Ca was higher in cows fed the negative DCAD during the periparturient period, and Oetzel et al. (46) found that plasma ionized Ca was higher at calving when DCAD was negative. This result was also reported in a kinetic study with sheep (56). Digestibility of Ca was not determined in the trial by Block (2). However, he found that the maintenance of blood Ca in cows fed the negative DCAD was partly a result of an increase in bone mobilization, as indicated by hydroxyproline.

Leclerc and Block (36) fed four different rations to prepartum cows with DCAD [milliequivalents of $(Na^+ + K^+) - (Cl^- + S^=)$] of 400, 200, 100, and 50 meq·kg^{-1} of DM and found that the correlation between DCAD and concentration of plasma Ca was −.51 from d 2 prepartum to d 1 postpartum (Table 1). In other words, as DCAD was reduced, concentration of plasma Ca increased, regardless of paresis. No change was observed in apparent digestibility of Ca, which is in contrast to results of Lomba et al. (40). The reason for the higher blood Ca in the trial by Leclerc and Block (36) was a result of higher bone mobilization, indicated by hydroxyproline, as dietary DCAD was reduced.

In feeding trials with sheep, Takagi and Block (54) showed that, as DCAD was reduced, apparent digestibility of Ca did not change, but retention of Ca was reduced by an increase in urinary excretion. In a subsequent trial, those researchers (55) fed rations to sheep with progressively lower DCAD and infused EDTA to deplete Ca from blood. The results indicated that sheep fed the lower DCAD were more resistant to depletion of blood Ca. Although not measured, we hypothesized that at the lower DCAD the bone was in a state of mobilization, thereby preventing a sharp decline in plasma Ca upon infusion of EDTA.

Gaynor et al. (21) found results similar to those of Block (2). Gaynor et al. (21) fed Jersey cows diets containing high Ca concentrations

TABLE 1. Correlation between the concentration of plasma calcium and dietary anion-cation difference during the periparturient period of dairy cows (n = 20).[1]

Time	Coefficient of correlation	$P > F$
(h)		
Prepartum		
48	−.471	.048
36	−.379	.121
24	−.334	.162
12	−.463	.046
Parturition	−.548	.015
Postpartum		
12	−.590	.013
24	−.287	.248
36	−.455	.058

[1]Leclerc and Block (36).

prepartum (>1.0% Ca) with three different DCAD, calculated as milliequivalents $(Na^+ + K^+) - (Cl^-)$, at 22.0 (anionic), 59.9 (intermediate), and 125.8 (cationic) meq·100 g^{-1} of DM. Interestingly, these DCAD are equivalent to those of Block (2) if SO_4^{2-} were removed from the equation [22.1 and 50 meq·100 g^{-1} of DM for the anionic and cationic diets of Block (2), respectively]. Gaynor et al. (21) found that their anionic diet produced the fewest cases of milk fever and produced higher urinary excretions of Ca and Mg. Those workers (21) also measured 1,25-$(OH)_2D_3$ in blood and found that cows fed the anionic diet had elevated concentrations of the vitamin at 3 d prepartum. As an explanation, Gaynor et al. (21) cited research using rats and dogs showing that tissues are refractory to PTH during metabolic alkalosis (i.e., cationic DCAD), thereby reducing 1,25-$(OH)_2D_3$ production. Goff et al. (23) showed that a reduction in DCAD increased the production of 1,25-$(OH_2)D_3$ per unit of PTH and thus reversed the tissue resistance to PTH that develops at the end of pregnancy and onset of lactation.

Further support for anionic DCAD to prevent milk fever is found in work by Fredeen et al. (17), who compared DCAD calculated without S of 40 to 50 meq·100 g^{-1} of DM to a DCAD of >85 meq·100 g^{-1} of DM. They found that elevated DCAD caused hypocalciuria, diminished Ca and P absorption, and diminished bone turnover. Further, they

showed that a DCAD of <40 meq·100 g^{-1} of DM caused metabolic acidosis, and, when DCAD was >50 meq·100 g^{-1} of DM, a metabolic alkalosis was produced.

Therefore, negative DCAD in rations for prepartum cows prevents a decline in blood Ca at the initiation of lactation by one or more of the following mechanisms: increasing the rate of bone mobilization of Ca directly, increasing the rate of bone mobilization of Ca indirectly via increased excretion (reduced retention) of Ca, or increasing intestinal absorption of Ca. Regardless of how these increases occur, excretion of endogenous Ca must follow because plasma concentration of Ca is maintained within the range of 10 ± 2 mg·dl^{-1} unless a disorder such as milk fever occurs. The question that arises is whether a metabolic basis exists for the described mechanisms to increase the entry of Ca to the blood by altering DCAD.

It is important to emphasize that, for the trials in which negative DCAD aided in prevention of milk fever (2, 21, 23, 46), dietary concentrations of Ca were high (1.5% Ca). As pointed out later, negative DCAD increases urinary excretion of Ca (8, 55, 56, 58). Therefore, if dietary Ca is low with a negative DCAD, hypocalcemia may occur, regardless of and separately from milk fever. Conversely, high dietary Ca with low DCAD may be necessary for this method to be successful. However, the optimal dietary Ca content has not been established.

Metabolic Possibilities for Increasing Entry of Ca to Blood by Altering DCAD

Intestinal Absorption of Ca. There is no readily apparent mechanism by which a decrease in DCAD necessarily would increase intestinal absorption. Lomba et al. (40) summarized data showing that calcium absorption increased because of the acidogenic nature of anions in the intestine. However, Leclerc and Block (36) and Takagi and Block (54) did not find an increase in apparently absorbed Ca. It is true that when intestinal Ca is high the direct acidification of diets increases the rate of passive absorption of Ca from the intestine (18, 24), but no evidence exists that neutral mineral salts resulting in high anionic concentrations can increase intestinal H^+ concentration. In fact, the opposite effect is more likely. When Cl^- is present in excess of Na^+ in the intestine, intestinal Cl^- is exchanged for blood HCO_3^-, creating metabolic acidosis and intestinal buffering with HCO_3^-. When dietary Ca is low in relation to requirements, active absorption of Ca from the intestine occurs, mediated by renal production of 1,25-$(OH)_2D_3$ (26, 28). When diets are acidified directly, this 1,25-$(OH)_2D_3$ fails to increase in response to dietary Ca restriction (5, 6). Furthermore, the active absorption process of Ca is inhibited directly when the H^+ concentration of the intestine increases (pH decreases) (15).

Dairy cows that are near parturition begin to remove Ca from blood for colostrum synthesis (2) and, therefore, should respond by increasing renal production of 1,25-$(OH)_2D_3$. This response, in turn, should increase the active absorption of Ca from the intestine. If it were true that excessive anions in relation to cations were forming acids in the intestine, then active absorption of Ca would be inhibited at the time it is most needed: parturition.

Based on this discussion, true (versus apparent) Ca absorption is unlikely to increase as a result of decreasing DCAD. However, this discussion indicates that both passive and active absorption of intestinal Ca actually may decrease when excessive anions are present. Therefore, even when dietary Ca is high, the excessive anions may cause a reduced absorption of Ca, which would stimulate renal production of 1,25-$(OH)_2D_3$ and release of PTH, causing bone mobilization prior to parturition. This result is difficult to substantiate because few data exist on circulating 1,25-$(OH)_2D_3$ or PTH, or on true absorption of Ca when the DCAD is reduced. The values for apparently absorbed Ca that are in the literature are difficult, at best, to interpret because the intestine is a major excretory route for Ca (22). However, this hypothesis would help explain the increase in bone mobilization observed by Block (2), Leclerc and Block (36), and Goff et al. (23) in cows prior to parturition fed rations with reduced DCAD.

Kidney Function. Good theoretical evidence shows that excessive anions in relation to cations can produce metabolic acidosis, as discussed earlier. Chronic metabolic acidosis increases urinary excretion of Ca (24, 38). If excessive anions produce metabolic acidosis

and increase the excretion of Ca, as shown by Takagi and Block (55, 56) and Oetzel et al. (45), then Ca retention decreases and causes formation of 1,25-$(OH)_2D_3$ and release of PTH to stimulate bone mobilization. This mechanism may function with or without the intestinal mechanism described to maintain adequate circulating Ca in periparturient cows.

Another possible mechanism, albeit farfetched, is that the production of renal 1,25-$(OH)_2D_3$ is an enzyme-dependent process that is pH-sensitive. Any changes in intracellular pH would alter the activity of enzymes. Excessive anions possibly create an intracellular pH that is more favorable for the production of 1,25-$(OH)_2D_3$. This possibility is supported by the work of Gaynor et al. (21).

Bone Mobilization. The discussion on intestinal and renal responses shows how bone mobilization can be stimulated indirectly when DCAD is reduced. However, specific mechanisms also exist whereby bone may be stimulated directly by increasing dietary anions in relation to cations. These mechanisms, again, rely on the premise that metabolic acidosis can be produced when dietary anions exceed cations.

Bone has three types of cells: osteoblasts, osteocytes, and osteoclasts. The osteoclast moves along the surface of bone, actively resorbing bone and leaving behind resorption lacunae (small trenches). The exact mechanisms of bone resorption have not been elucidated; however, certain facts are known (1):

1. Resorption is hormonally mediated by PTH and 1,25-$(OH)_2D_3$.
2. Lysosomal and mitochondrial enzyme activity increases in osteoclasts when resorption occurs.
3. These enzymes are dependent on H^+ for optimal activity (i.e., succinate dehydrogenase and acid phosphatase).
4. Other acids are formed within the cytoplasm and lysosomes of the osteoclasts such as hyaluronic acid and lactic acid.
5. A localized reduction on pH occurs that probably contributes to mineral dissolution.

As pointed out earlier, paretic cows are not deficient in circulating 1,25-$(OH)_2D_3$ or PTH. However, milk fever can be prevented when pharmacological doses of vitamin D_3 or its metabolites are administered prepartum.

The possibility exists that, when prepartum cows are fed rations with highly positive DCAD, the bone cannot respond (is not sensitive) to the hormones mediating resorption because of a drainage of H^+ or excessive HCO_3^- within the bone cells. As discussed, excessive plasma Na^+ can exchange for intracellular H^+, and insufficient Cl^- can prevent HCO_3^- from entering plasma. Drainage of H^+ or excess HCO_3^- can prevent the osteoclasts from resorbing bone because resorption obviously is an acid-dependent process.

When anions are in excess of cations, mild acidosis ensues, which then allows the full expression of hormonally mediated bone resorption. Block (2), Leclerc and Block (36), and Goff et al. (23) showed an increase in bone mobilization beginning a few days prepartum when DCAD was reduced. This increase is approximately coincident with the theoretical increase in 1,25-$(OH)_2D_3$ and PTH in blood in preparation for lactation. Therefore, by reducing DCAD, bone is mobilized in response to hormones normally present.

Further evidence that DCAD affects sensitivity to Ca homeostatic hormones was shown by Goff et al. (23), who showed that cows fed high and low DCAD secreted similar amounts of PTH in the peripartum period; however, the amount of 1,25-$(OH)_2D_3$ produced per unit of PTH secreted was greater in cows fed reduced DCAD accompanied by higher concentrations of blood Ca at parturition and postpartum. These results, along with the lack of any consistent increase in intestinal absorption of Ca with low DCAD, as previously discussed, indicates that the mode of action of reduced DCAD in preventing milk fever lies in the kidney (hormone production and Ca excretion) and bone (osteoclastic resorption activity caused by the mild acidosis produced). Another factor to consider is that the mild acidosis produced with negative DCAD has been shown to cause an increase in ionized Ca in blood (56, 58). This ionized Ca is the form used by cells with the remaining blood Ca being bound to albumins, which must dissociate into ionized Ca prior to use.

TABLE 2. Calculated dietary cation-anion balance (DCAD) of individual feedstuffs.[1]

Feed	Na^+	K^+	Cl^-	$S^=$	DCAD[2]
	(% of DM)				
Alfalfa hay (late vegetative)	.15	2.56	.34	.31	+431.1
Timothy hay (late vegetative)	.09	1.6	.37	.18	+232.0
Corn silage	.01	.96	. . .	.15	+156.4
Corn grain	.03	.37	.05	.12	+18.8
Oats	.08	.44	.11	.23	-26.95
Barley	.03	.47	.18	.17	-23.4
Distillers grain	.10	.18	.08	.46	-219.38
Soybean meal	.03	1.98	.08	.37	+266.37
Fish meal	.85	.91	.55	.84	-75.6

[1]From NRC (44) for Na^+, K^+, Cl^-, and $S^=$.

[2]Calculated as milliequivalents of $(Na^+ + K^+) - (Cl^- + S^=)$ kg^{-1} of DM.

Low Ca and P Versus Anion-Cation Balances in the Prevention of Milk Fever

Rations that contain low Ca and P and fed to prepartum cows prevented milk fever (3, 4, 31). The mode of action is that the rations stimulate 1,25-$(OH)_2D_3$ production and PTH release early in the prepartum period. Therefore, the active absorption mechanism for Ca in the intestine and the bone resorption mechanism are being continually stimulated throughout the prepartum period.

When either equation for calculating DCAD (with or without $SO_4^=$) is applied to rations for prepartum cows, the results usually are a highly positive balance (excess cations), regardless of forage source. Although reduction of dietary Ca and P prepartum reduces the incidence of milk fever, it does not necessarily eliminate it in a herd. A question to consider is whether sufficiently high DCAD and reduced dietary Ca will help to prevent milk fever. Therefore, dietary Ca is not as much the causative factor in milk fever as is the metabolic status of the cow at parturition. Furthermore, no evidence exists that reduced dietary Ca prepartum aids in mitigating the ubiquitous hypocalcemia at parturition, regardless of clinical paresis.

Dietary Cation-Anion Balances of Feeds and Its Implications

As stated, DCAD is usually positive or highly cationic. Table 2 was developed using standard North American values (44). The table demonstrates that all typical forage sources are cationic and that alfalfa hay is the most cationic, which almost precludes the use of alfalfa as a prepartum feed because the cationic nature of the feed predisposes cows to milk fever. The amounts of Cl^- or $SO_4^=$ salts, devoid of Na^+ or K^+, to decrease DCAD of alfalfa becomes impractical because of the low palatability of anionic salts (45). Obviously, timothy forage is a more likely choice for these prepartum cows. The possibility exists, therefore, that grasses fed prepartum will decrease the incidence of milk fever because of their lower DCAD and not because of their lower Ca content compared with that of legumes.

From Table 2, we can conclude for practical purposes that DCAD for typical grains (corn, barley, and oats) is approximately zero (range of -27 to +19 meq·kg^{-1} of DM). The protein sources in Table 2 indicate that DCAD is negative except for soybean meal, primarily because of the S content of these feedstuffs. Interestingly, fish meal has a less negative DCAD than other protein sources in spite of its high concentration of S because of its equally high content of Na.

For high producing cows in early lactation, it is difficult to presume an ideal DCAD. Because most grains are assumed to have a DCAD around 0 meq·kg^{-1} of DM, a ration of 50% alfalfa, 50% grain would have a DCAD of approximately 200 to 300 meq·kg^{-1} of DM (possibly lower, depending on protein supplementation). For lower producing cows in midlactation, the diet probably would contain more grass forage and less legume for a DCAD of 100 to 200 meq·kg^{-1} of DM. Be-

cause some cows fed alfalfa and corn diets that do not depress milk fat show a response to $NaHCO_3$ in early but not late lactation, we must presume that DCAD should be >200 $meq \cdot kg^{-1}$ of DM at high milk production and can be <200 at lower production. Furthermore, during the dry period, DCAD should be negative, and probably a minimum negativity of -75 $meq \cdot kg^{-1}$ is necessary.

Creation of more positive DCAD is no problem with the plethora of Na and K salts in the form of carbonates. Theoretically, Na and K should be equal on an equivalent basis, but this has yet to be proved. Creation of more negative DCAD with Cl or SO_4 salts (without Na or K) has been tested by Oetzel et al. (45). Six anionic salts [$MgCl_2$, $MgSO_4$ $CaCl_2$, $CaSO_4$ NH_4Cl, $(NH_4)_2SO_4$], when included in diets to obtain the same negative DCAD, produced the same effects (compensated metabolic acidosis; decreased blood HCO_3, urinary pH, and urinary base excess; and increased fractional excretion of urinary Ca) (45). Therefore, DCAD seems more important than the specific salt used. Oetzel et al. (45) cautioned that mixtures of salts would be best to avoid toxicities or induced deficiencies of minerals (Mg and NH_4 being of greatest concern).

CONCLUSIONS

Much more research is needed before specific recommendations can be made regarding optimal DCAD in rations for dairy cows. Based on the discussion, if some biological functions can be manipulated by altered ion balance, then certainly others can also follow suit. However, optimal DCAD will not be the same for all productive functions.

Indications exist in the literature for a relationship of cationic DCAD and calf performance (30) and for anionic DCAD and prevention of udder edema (37) and alleviation of heat stress (59). Other production diseases that are associated with acid-base balance or buffering capacity of blood include laminitis and ketoacidosis. Investigations should be directed toward the relationship of these diseases with DCAD.

Some biological functions respond better when the balance is positive, but others do so when the balance is negative. The combined efforts of researchers in basic and applied sciences in more fully describing rations fed to animals in experimental trials with regard to mineral content will increase the knowledge in this obscure area of nutrition.

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