

Anion, Vitamin E, and Se Supplementation of Diets for Close-Up Dairy Cows

David K. Beede and Thomas E. Pilbeam

Department of Animal Science, Michigan State University, East Lansing, MI 48824-1225 U.S.A.

Take Home Messages

Anion Supplementation

- Supplementation of anions (Cl^- and SO_4^{2-}) in diets of close-up dairy cows has become more common in recent years to aid in control of hypocalcemia and related peripartum health disorders. These anions are helpful to counteract the deleterious effects of high dietary K on Ca status in the peripartum period.
- Anions are used to decrease the dietary cation-anion difference (DCAD) which in turn results in mild systemic metabolic acidosis. This is efficacious to increase blood Ca in response to hypocalcemia.
- Recent research suggests that SO_4^{2-} is a less powerful acidifier than Cl^- . This suggests that perhaps we should re-think which is the most appropriate DCAD equation. Suggestions are made for practical formulation and feeding management approaches to improve success with anion supplementation.
- Whatever the equation one chooses, urine pH can be an effective on-farm management tool to determine whether or not anion supplementation is having the desired physiological effects. An approach for this is outlined.

Vitamin E Supplementation

- Vitamin E and Se are both antioxidants which have complementary effects to maintain cell integrity and immune function.
- The vitamin E content of most feedstuffs (except fresh forages) is low and variable; thus common feeds are unreliable sources of these nutrients. Therefore, supplementation is recommended.
- The absolute vitamin E requirement (IU/ d) of close-up cows is unknown.
- Plasma α -tocopherol concentrations of cows decrease as calving approaches and newborn calves are deficient.
- For close-up cows dietary supplementation of at least 1000 IU/ d is recommended. Recently, Ohio workers showed that higher supplementation (>1000 IU/ d) in the peripartum period reduced incidence of clinical mastitis.
- Injected vitamin E has a relatively short half-life in the body. Practically, weekly injections of 3000 IU/ cow are needed to maintain plasma α -tocopherol concentrations of close-up cows.

Se Supplementation

- In the US, 0.3 ppm in the total ration is the legal limit.
- In research to date, improvements in health have not been shown by feeding close-up cow diets with >0.3 ppm of Se.
- Supplementation of vitamin E appears to be the preferred approach to improve cows= antioxidant status and health.

Introduction

This paper focuses on practical considerations for supplementation of anions, vitamin E and Se in diets of close-up dairy cows. Each of these nutrients can have profound effects on peripartum health, and subsequent lactational and reproductive performance.

Anion Supplementation

In a survey of 583 Michigan dairy producers attending the Managing the Dry Cow for More Profit Program in 1996, 33% indicated use of "anionic salts" in diets for close-up dairy cows (21). In a recent characterization of dry cow management programs of six high producing Wisconsin herds, anions were supplemented in close-up diets in each herd (18).

Use of supplemental anions in diets of close-up cows is becoming more common for several reasons.

- First discovered in Norway (7) and followed by considerable research in North America starting with Block (3), the dietary cation-anion difference (DCAD) of close-up dry cow diets is recognized as an important nutritional consideration to control peripartum hypocalcemia and related metabolic disorders.
- Among cows with higher milk yield potential and better prepartum nutrition programs, peripartum colostrum yields have increased; thus, the metabolic challenge to maintain Ca homeostasis in the peripartum period also has increased.
- The K (a dietary cation) concentrations of many farm-grown forages has increased dramatically due to aggressive application of commercial fertilizers and manure; with this has come the concomitant realization that higher dietary K concentrations in forages and diets increases the likelihood that hypocalcemia may occur in the peripartum period (12).
- In recent years, it has become apparent that subclinical hypocalcemia occurs with greater frequency than previously realized; resulting in higher incidences of metabolic disorders, even though the manifestation of clinical hypocalcemia (e.g., clinical milk fever) may be relatively low (2).

The objectives of this section are: 1) to provide a brief background on the etiology of hypocalcemia and related factors, and characterize the occurrence of associated metabolic disorders in peripartum dairy cows; 2) to summarize some recent research findings which may alter somewhat previous recommendations and approaches (1, 4) for practical anion supplementation of close-up diets; and 3) to suggest practical anion supplementation and nutritional management stratagems to improve peripartum health, and subsequent lactational and reproductive performance.

Background

Calcium and Acid-Base Status. Milk fever results from failure of cows to maintain a normal blood Ca pool (e.g., about 2.5 to 3.0g circulating Ca). Each litre of colostrum contains about the same amount of Ca (2.5g) as normally is present in the whole blood volume (17). Around parturition, colostrum formation draws a large amount of Ca (e.g., 20 to 40 g) from the blood in a short period of time.

Some animals are more likely than others to experience clinical (parturient paresis or milk fever) or subclinical hypocalcemia; for example, third and greater parity compared with first and second parity cows, and Jersey compared with Holstein cows (16).

When the amount of Ca in blood drops below normal, parathyroid hormone (PTH) is secreted to stimulate entry of Ca into the blood pool (15). First PTH causes a very rapid increase in renal reabsorption of Ca from the glomerular filtrate. PTH also facilitates the renal enzyme (1- α -hydroxylase) to convert 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃ (the active form). If renal reabsorption of Ca does not completely correct the Ca deficit, PTH in concert with 1,25-dihydroxyvitamin D₃ acts to increase blood Ca. About 24 h of 1,25-dihydroxyvitamin D₃ stimulation is needed before Ca absorption from the intestine is increased significantly; PTH does not participate directly in this process. Osteoclastic bone resorption of Ca, requiring both PTH and 1,25-dihydroxyvitamin D₃, is not significantly activated until about 48 h after PTH stimulation (16). The degree of response to PTH is dependent upon the number and receptivity of vitamin D receptors (VDR) which "recognize" the PTH molecule.

Several important ideas about the causes of hypocalcemia and methods for its correction can be drawn from relatively recent research. 1) When the amount of Ca in blood drops PTH is secreted. There is little difference in the PTH response and concomitant increase in 1,25-dihydroxyvitamin D₃ to the drop in blood Ca between cows that become hypocalcemic and those that do not. However, some cows are less able to respond to PTH stimulation than others, apparently because of lack of recognition of PTH at the VDR of bone and renal tissues (14). 2) Additionally, cows that are in a mild state of systemic alkalosis (e.g., blood pH on the high end of the normal range) are less or non-responsive to secretion of PTH; whereas, cows which are in a state of mild metabolic acidosis (e.g., blood pH near the lower end of the normal range) are more responsive to PTH. Thus, manipulating the cow's acid-base status prior to calving can affect responsiveness to PTH.

Stewart (31) proposed the strong-ion difference theory which greatly enhanced our understanding of how various factors (including dietary factors such as cations and anions) affect acid-base status of simple solutions and extracellular fluids of animals. The basic principle of the strong-ion difference theory is that the electrical charge of solutions and fluids must always be neutral. Positively charged ions (cations) must equal negatively charged ions (anions) to maintain electrical neutrality. If the equivalents of cations in solution exceed that of anions, the pH of the solution will increase; whereas, if anions are in excess of cations the pH of the solution decreases (e.g., more H⁺ ions go into solution to neutralize the negative electrical charge of the anions present).

Blood pH of the cow is ultimately determined by the number of equivalents of cations and anions present. If relatively more anions than cations enter the blood from the diet and digestive tract, blood pH will decrease. This has given rise to the concept of dietary cation-anion difference which is expressed in its fullest theoretical form as $\text{meq}(\text{Na}^+ + \text{K}^+ + \text{Ca}^{+2} + \text{Mg}^{+2}) - (\text{Cl}^- + \text{SO}_4^{-2} + \text{P}^{-3}) / 100\text{g}$ of dietary DM. In this form, each ion is assumed to be completely released in the digestive tract and absorbed into blood (e.g., 100% bioavailable); the equation reflects the net summation of how these ions influence blood pH as a function of their equivalency or charge. Variations on this equation have been evaluated and proposed; pertinent aspects will be addressed subsequently.

If the relative contribution of anionic equivalents in the diet and to the blood is greater than that of cationic equivalents, then blood pH decreases; animals will experience mild metabolic acidosis. It is this change in acid-base status that is believed to be responsible for affecting and improving Ca status of peripartum cows. A currently proposed mode of action is that lowering the cows' systemic pH increases tissue responsiveness to PTH. Additions of anions to diets increases osteoclastic bone resorption and synthesis of 1,25-dihydroxyvitamin D₃. Better responsiveness of VDR to PTH at lower systemic pH seems to occur. There does not appear to be a change in the numbers of VDR (e.g., in the colon mucosa) of cows fed a diet with lower vs. higher DCAD (13). This relationship is not well-defined for VDR in other tissues.

Monitoring the acid-base status of close-up cows is an important part of nutritional management of Ca status. Urine pH, as discussed subsequently, appears to be a reasonably practical way to monitor the close-up cow's acid-base status in response to supplemental anions.

Ca Status and Associations with Other Metabolic Disorders. There is a growing body of evidence from epidemiological mapping studies and other data that clinical and subclinical hypocalcemia certainly is associated with other health problems in the peripartum period. An overview of this is portrayed by the hypocalcemic cascade (Figure 1). Extensive documentation from the literature of these relationships will not be done here; readers are referred to Curtis et al. (5), Wang (34), and Risco (26).

Recent Research Findings

In the last few years, research has provided new information which should help dairy nutritionists and producers use the DCAD concept more effectively.

Different DCAD Equations. As mentioned previously the complete theoretical DCAD equation equals:

$$\text{meq}(\text{Na}^+ + \text{K}^+ + \text{Ca}^{+2} + \text{Mg}^{+2}) - (\text{Cl}^- + \text{SO}_4^{-2} + \text{P}^{-3}) / 100\text{g of dietary DM} \{\text{Equation 1}\}.$$

However, primarily because of differences in bioavailability of each mineral element in the equation, the functional equation most applicable in practical situations differs. Ender and Dishington (6) used the expression: $\text{meq}(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{-2}) / 100\text{g}$ of dietary DM {Equation 2}. In the past decade, this equation has been used widely in practical formulation of close-up diets when anions were supplemented.

Alternatively, based on bioavailability figures for Ca, Mg and P from NRC (22), Goff et al. (12) suggested that the equation: $\text{meq}(\text{Na}^+ + \text{K}^+ + 0.38\text{Ca}^{+2} + 0.30\text{Mg}^{+2}) - (\text{Cl}^- + 0.60\text{SO}_4^{-2} + 0.50\text{P}^{-3}) / 100\text{g}$ of dietary DM {Equation 3}, was more appropriate; Na,

K and Cl were considered 100% bioavailable and the bioavailability of 60% for S was based on work of Tucker et al. (32).

Subsequently, Goff and Horst (9) determined the abilities of 1.0, 1.5, or 2.0 Eq of hydrochloric acid or sulfuric acid added to diets to acidify the urine of nonlactating nonpregnant Jersey cows. Perhaps surprising, sulfuric acid exhibited only about one-third of the acidifying power (e.g., change in urine pH) of hydrochloric acid. Sulfuric acid would be considered the most bioavailable chemical form of the sulfate (SO_4^{-2}) anion compared with other mineral sources of sulfate, such as magnesium sulfate, calcium sulfate, and ammonium sulfate.

A subsequent study compared the acidifying power of commonly used "anionic salts" and hydrochloric acid with a similar animal model (10). Urine samples were taken 4 h after feeding on d 3, 4, and 5 of each experimental period in which a different anion source was fed. Urine pH's of multiparous nonlactating Jersey cows fed hydrochloric acid, calcium chloride, ammonium chloride, calcium sulfate, magnesium sulfate, and elemental S were 6.2 ± 0.21 , 7.1 ± 0.36 , 7.0 ± 0.20 , 7.6 ± 0.15 , 7.9 ± 0.08 , and 8.2 ± 0.04 , respectively. Overall, the Cl-containing salts were more acidogenic than the SO_4^{-2} -containing salts; and, elemental S had no effect on acid-base status as one should expect; although occasionally elemental S is found as a source of anion in mineral supplements for close-up diets (D. K. Beede, personal observation).

Certainly these new data have been reason to reconsider what the most appropriate practical DCAD equation should be and what anion sources are most appropriate for supplementation. Based on results of these two experiments, Goff et al. (11) suggested that a more biologically or functionally correct DCAD equation might be:

$\text{meq}(\text{Na}^+ + \text{K}^+ + 0.15\text{Ca}^{+2} + 0.15\text{Mg}^{+2}) - (\text{Cl}^- + 0.20\text{SO}_4^{-2} + 0.30\text{P}^{-3}) / 100\text{g}$ of dietary DM {Equation 4}.

Recently, Rodriguez et al. (27) found no difference in urine or blood plasma pH when nonlactating nonpregnant Holstein cows were fed diets with either 0.5 or 2.0% Ca (supplemental Ca from CaCO_3) across diets with DCAD set at about $-10 \text{ meq}[(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{-2})] / 100\text{g}$ of dietary DM. Therefore, the influence of Ca on urine and blood plasma pH seems small, although obviously pH response to Ca, if considered in a DCAD equation, may vary depending upon the sources of Ca in the diet (e.g., calcium chloride, calcium sulfate, or calcium carbonate).

Results of these recent experiments have stimulated considerable discussion of DCAD equations and supplementation of anions. Kirk (19) wrote, "If this revised equation {Equation 4} were valid, calcium sulfate and magnesium sulfate would be considered essentially ineffective anion salts. However, we know from our own experience, and that of many other nutritionists, that these ingredients do help reduce hypocalcemia when included in the close-up diet. Nearly all of the research reported to date on the effectiveness of anionic salts for improving Ca status of the

peripartum cow also include substantial contributions from sulfur sources, including much of the work done by Dr. Goff".

Giesy et al. (8) used four nonpregnant dry Holstein cows given a Ca-chelating agent ($\text{Na}_2\text{-EDTA}$) intravenously to simulate hypocalcemia. The objective was to develop the relationship between DCAD, urine pH, and ionized Ca concentrations in response to $\text{Na}_2\text{-EDTA}$ challenge. The DCAD's (assumed to be the four element equation with Na^+ , K^+ , Cl^- , and SO_4^{2-}) of treatments were 27.6, 11.9, -6.4 and -25.3 meq/ 100g of dietary DM. Sources of anions supplemented to lower DCAD were not reported. Urine pH (before infusion of $\text{Na}_2\text{-EDTA}$) was 8.38, 7.65, 6.42, and 6.04 for 27.6, 11.9, -6.4 and -25.3 meq, respectively. After the infusion $\text{Na}_2\text{-EDTA}$, blood ionized Ca concentrations were 2.82, 3.64, 3.62, and 3.99 mg/100 ml. The diet with the most negative DCAD also reduced feed intake. Although it is not possible to determine which anions were supplemented from this initial report, there was a linear decrease in urine pH as DCAD was lowered and some anions were beneficial to increase blood ionized Ca; however, practically, feed intake depression may be a concern with the -25.3 meq treatment.

In another recent experiment, Moore et al. (20) fed diets for 21 d to close-up cows with DCAD of +14, 0, and -5 meq $[(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^{2-})]$ / 100g of dietary DM; supplemental anions were provided from calcium chloride, magnesium sulfate, and magnesium chloride. Total dietary Ca varied (0.44, 0.97, and 1.5% Ca) with the three decreasing DCAD, respectively; supplemental Ca was from increasing calcium chloride and calcium carbonate in the 0 and -5 meq diets. Urine pH of close-up cows immediately before calving was 7.98, 7.0, and 6.21 for +14, 0, and -5 meq, respectively.

Several main points can be made from recent studies.

- Based on the experimental model used, the two experiments of Goff et al. (9, 10) demonstrate the relative ability of hydrochloric and sulfuric acids, and the anion salts to acidify urine of dairy cows. However, in neither abstract is the DCAD of the total diets listed. If the DCAD concept is important and correct, then the equivalents of cations (e.g., K and Na) in the total diet relative to the equivalents of anions (basal plus supplemental) would have major influence on the measured urine pH. Whether or not the urine pH values corresponding to the various anion sources are correct in absolute in these two studies remains a question.
- Clearly the acid or salts containing Cl^- had relatively more acidifying power than those containing sulfate.
- Perhaps magnesium or calcium sulfates, which are believed to be effective to varying degrees in the field, work because they are typically fed for 2 to 3 wk before calving. This may allow time for a pool of released SO_4^{2-} to become established in the digestive tract and some of this anion is absorbed and does affect systemic acid-base status to some degree. This time course may differ from that in the experiment of Goff et al. (10), where urine pH's were averages of measurements made on 3, 4, and 5 d after commencement of feeding. However, it still seems clear from the comparison of sulfuric acid vs. hydrochloric acid that SO_4^{2-} is not as potent as Cl^- to reduce urine pH (9).
- Therefore, because of the combination of: typically higher dietary concentrations of K, Na and Cl in diets, all with about 100% bioavailability, and because Cl^- is a more potent

supplemental anion, the most practical DCAD equation might be $\text{meq}(\text{Na}^+ + \text{K}^+) - (\text{Cl}^-)/100\text{g}$ of dietary DM.

- Finally, Dr. G.R. Oetzel (University of Wisconsin) made a key point during a Roundtable Discussion of anion and Ca supplementation of close-up diets at the American Dairy Science Association annual meeting at the University of Guelph (June, 1997). He pointed out that supplementing anions is not an all-or-none proposition. Some decrease in urine pH (e.g., from 8.0 to 7.0) and improvements in Ca status occur from lowering DCAD of close-up diets, whether it is to the most desired target DCAD and urine pH or not.

Potential Formulation and Feeding Stratagems

Byers (4) suggested a formulation stratagem utilizing the DCAD concept. A somewhat more detailed description and modifications were suggested (1). The primary objective of anion supplementation and changing the DCAD should be to affect acid-base status enough to cause desired changes in entry of Ca into the blood pool in response to hypocalcemia. This is the goal regardless of what particular anion sources are used or what DCAD equation is considered. Below are suggested initial fundamental steps to increase the likelihood of success with anion supplementation in close-up diets. The simplest DCAD equation: $\text{meq}[(\text{Na}^+ + \text{K}^+) - \text{Cl}^-]/100\text{g}$ of dietary DM, will be used in description of a formulation stratagem, although one may wish to include SO_4^{-2} , and apply some discount factor (something <100% bioavailability) in the equation to lower the theoretical contribution of its acidifying power.

1. All dietary ingredients should be analyzed for Na, K, and Cl concentrations before diet formulation begins. Macromineral element contents of various samples of the same types of feeds vary considerably from standard tabular values (22) and from sample to sample. Laboratory analyses must be done using so-called wet chemistry methods; near infrared reflectance spectroscopy (NIRS) is not appropriate for reliable results of macromineral element contents of forages (30).
2. Select feeds, particularly forages, with K contents as low as practically possible. Formulate the diet and calculate the DCAD of the basal diet as: $\text{meq}[(\% \text{Na divided by } 0.023) + (\% \text{K divided by } 0.039)] - (\% \text{Cl divided by } 0.0355)/100\text{g DM}$. The diet should be formulated to meet desired recommendations for energy and other nutrients.
3. At this point, supplementation with the appropriate anion sources should be considered. First inclusion should be of magnesium sulfate as a source of Mg. This is recommended because there is evidence that low blood Mg often occurs with hypocalcemia; low blood Mg can reduce PTH secretion which reduces the ability of the cow to correct the hypocalcemia. By using magnesium sulfate a readily available source of Mg is supplied, as well as an anion (SO_4^{-2}) which may have some

