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

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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 D_3 to 1,25-dihydroxyvitamin D_3 (the active form). If renal reabsorption of Ca does not completely correct the Ca deficit, PTH in concert with 1,25-dihydroxyvitamin D_3 acts to increase blood Ca. About 24 h of 1,25-dihydroxyvitamin D_3 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 D_3, 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 D_3 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 meq($Na^{+} + K^{+} + Ca^{+2} + Mg^{+2}$) - ($Cl^{-} + S0_4^{-2} + P^{-3}$)/ 100g 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 D_3. 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:

meq($Na^{+} + K^{+} + Ca^{+2} + Mg^{+2}$) - ($Cl^{-} + SO_4^{-2} + P^{-3}$)/ 100g of dietary DM {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: meq($Na^{+} + K^{+}$) - ($Cl^{-} + SO_4^{-2}$)/ 100g 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: meq(Na^{+} + K^{+} + 0.38Ca^{+2} + 0.30Mg^{+2}) - (Cl^{-} + 0.60SO_4^{-2} + 0.50P^{-3})/ 100g 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$\pm$0.21, 7.1$\pm$0.36, 7.0$\pm$0.20, 7.6$\pm$0.15, 7.9$\pm$0.08, and 8.2$\pm$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:

meq(Na^{+} + K^{+} + 0.15Ca^{+2} + 0.15Mg^{+2}) - (Cl^{-} + 0.20SO_4^{-2} + 0.30P^{-3})/ 100g 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 meq[(Na^{+} + K^{+}) - (Cl^{-} + SO_4^{-2})]/ 100g 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 (Na_2-EDTA) intravenously to simulate hypocalcemia. The objective was to develop the relationship between DCAD, urine pH, and ionized Ca concentrations in response to Na_2-EDTA challenge. The DCAD's (assumed to be the four element equation with Na^+, K^{+1}, 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 Na_2-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 Na_2-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[$(Na^+ + K^+) - (Cl^- + SO_4^{-2})$]/ 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 meq($Na^+ + K^+$) - (Cl^-)/ 100g 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: meq[($Na^+ + K^+$) - Cl^-]/ 100 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: meq[(%Na divided by 0.023) + (%K divided by 0.039)] - (%Cl divided by 0.0355)]/ 100g 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 ($S0_4^{-2}$) which may have some

influence on acid-base status. Also, research showed that intake of a concentrate mixture containing magnesium sulfate was greater than for a mixture containing calcium chloride, ammonium chloride or ammonium sulfate (23). Magnesium sulfate certainly would be the preferred source of Mg compared with magnesium oxide. Total dietary Mg should be set at 0.35 to 0.4% of dietary DM. Magnesium chloride, available in some parts of the country, would work well also as a source of Mg, plus supplying the Cl^- anion.

4. The concentrations of anions and cations in mineral sources should be verified always. For example, the concentrations (on a dry basis) may vary because of varying free-moisture content, as well as the degree of hydration of some anionic salts. However, one must be careful not to "double-correct" to account for the water of hydration (part of the chemical structure of the anionic salt). Use only the free-moisture content to convert from an as-fed to a 100% DM basis. The waters of hydration are part of the salt and not free water, when converting from as-fed to DM basis. Various calcium sulfates and calcium chlorides may vary in number of waters of hydration; and magnesium sulfate is hydrated also. These differences result in different anion and cation concentrations on a DM basis, even for minerals given the same name (e.g., calcium chloride).

Sodium and potassium chloride salts cannot be used to change DCAD because they are neutral salts, each contributing a cation and an anion in the DCAD calculation. Therefore, net influence of either neutral salt on DCAD is zero. Also, Na-containing dietary buffers are alkalogenic and can increase blood pH and should not be included in close-up diets.

5. Next, supplementation of enough calcium chloride, ammonium chloride, hydrochloric acid or a combination should be used to achieve the desired initial DCAD. With the equation: meq[(Na^+ + K^+) - Cl^-]/ 100 g of dietary DM, a reasonable target may be +15 to +5. One might also want to check SO_4^{-2} content depending upon how much SO_4^{-2} is present in the diet and how much acidifying power that particular SO_4^{-2} source possesses. However, more important will be determining if the cows' urine pH has changed; this is addressed subsequently.

6. When a desired DCAD is achieved, dietary Ca content can be raised to 1.5 to 1.8% of dietary DM without adverse effects (2). Higher than traditionally recommended Ca supplementation in close-up diets is likely beneficial (24, 25, 33). Calcium chloride

can be used to acquire some of the supplemental Ca simultaneously during formulation of the desired DCAD.

7. Supplementation of all other nutrients (e.g., Se and fat soluble vitamins) should be set to recommendations or preferences.

A Practical Feeding Management Approach

The Difficulties. There have been reports in the field of failed attempts to successfully incorporate the DCAD concept. Some of the possible reasons for difficulties include the following.

- The actual DCAD (by whatever equation one chooses to use) is unknown and likely is more positive than presumed from diet formulation, and feedstuff and diet analyses. Therefore, the desired changes in acid-base status do not occur.
- Dietary Ca concentration and intake were not increased to recommended amounts. This problem has been demonstrated experimentally (25,29). However, there appears to be no difficulty, and in fact there is a benefit, to increasing Ca content and intake, if the DCAD is sufficiently low to affect acid-base status. Diets with a DCAD which affects acid-base status can benefit from 180 to 210 g Ca/ cow per d. Intake of P should be set at 45 to 50g/ Holstein cow per d, or 35 to 40 g per Jersey cow/ d. Higher P intake can be refractory to renal synthesis of 1,25 dihydroxyvitamin D_3 (16).
- The unpalatability, inherent with anionic salts, and improper ration mixing can reduce ration intake prepartum. Also, perhaps the diet formulated was not eaten by all cows because of poor ration mixing. Therefore, some cows became hypocalcemic at calving.

  Moreover, allowing cows choices of feeds (e.g., component feeding), such as free-choice feeding of hay, undermines the most accurately calculated DCAD, and increases the likelihood that hypocalcemia will occur. Insufficient feed bunk space can be a source of problems also. In each case, a total mixed ration works best to help alleviate these problems.

- Additionally, a common problem is the practice of using a specific amount of a product (e.g., commercial close-up supplement) which contains the anion sources, micromineral elements, feed additives, and vitamins at a standard feeding rate in the rations in several dairies. Generally when a standard product is fed, the DCAD's vary among farms because of the difference in forage base. Therefore, responses and health of cows differ among farms and the desired effects are not noted in some farms. Varying the amount of product (which includes all supplemented nutrients) to achieve the DCAD which will affect acid-base status generally is not considered a viable option and typically would increase the expense, because it contains other high-priced nutrients (e.g., trace minerals, vitamins) and feed additives.
- Practically, it is very difficult to make changes in dietary ingredients through time and know the DCAD, monitor uncontrollable changes in DCAD (e.g., when forage base of the diet is changed), and acquire needed and accurate nutrient analysis information in a timely fashion. Therefore, use of urine pH can be a practical tool to monitor the cows'

acid-base status, and help determine whether or not the anion supplementation and diet are having the desired effects.

An Alternative Practical Feeding Management Approach is described below.

- If a herd is having metabolic problems early postpartum (e.g., milk fever, retained placenta, etc. [Figure 1]), the DCAD is likely positive and urine pH's are too high. Cows likely are not in the proper physiological state prepartum to optimize Ca status in the early postpartum period. Use of supplemental anions may be a plausible solution.
- Formulate a close-up diet with the recommended DCAD (+15 to +5 meq/ 100g dietary DM; with the three element equation as a starting point); realizing that anion supplementation may take some adjustment over time to optimize effects on acid-base and Ca status.
- This diet will contain a portion of magnesium sulfate, anion sources, and perhaps other minerals (e.g., calcium carbonate to supply Ca) to meet formulation objectives. Using these mineral sources, prepare a separate dietary ingredient, the "anion mix", which contains only the anion sources, supplemental Mg and Ca sources, and about 50% grain carrier (e.g., ground corn). For example, if about 0.45 kg of anion sources, Mg and Ca minerals are called for in the diet, include another 0.45 kg of carrier to make the 0.90 kg "anion mix". The amount of this mix will vary depending upon each specific diet. This mix should be separate from the supplement used to supply other micronutrients (e.g., microminerals, vitamins and feed additives). By making this separate "anion mix", it will be possible to vary the amount fed to achieve the desired acid-base status through time. This approach was demonstrated experimentally (28).
- Feed the diet as formulated to the close-up group for about 7 d. For example, the diet may contain 0.90 kg of the "anion mix". After 7 d, urine pH should be measured for cows in the close-up group. This can be done effectively on-farm, using standard pH paper or a field pH meter. For better reliability, the urine pH of at least five different cows which have been fed the diet for at least 7 d should be measured; the more cows the better. Evaluating the range in urine pH among cows also may be useful to access animal to animal variation. If average urine pH is 7.0 or greater, it is an indication that the close-up diet is not affecting acid-base physiology as much as desired to optimize Ca status at calving. For example, what if the average measured urine pH is 7.5?
- At this point, one should increase the inclusion rate of the "anion mix". In this example, it could be increased to 1.14 kg per cow/ d. This increase can be done without changing the amounts fed of the other dietary ingredients, or it might be desired to reduce the amount of another ingredient by 0.5 lb, to compensate for the increased inclusion of the "anion mix".
- Then the procedure described in Step 4 above would be repeated. After at least 7 d of feeding the adjusted diet, the urine pH should be re-tested. If the average urine pH is 6.0 to 6.5 (Holstein cows), it is likely that the diet is causing the desired effects. A slightly lower pH for Jersey cows (5.5 to 6.0) is recommended (J.P. Goff, personal communication).
- It would be prudent to monitor urine pH of the close-up cows periodically (e.g., every other week) to ensure that the desired physiological effects are maintained. Our recommended target urine pH is 6.0 to 6.5 for Holstein cows. In a recent Michigan study

(20), a DCAD of 0 meq($Na^+ + K^+$) - ($Cl^- + SO_4^{-2}$)/ 100g of dietary DM resulted in an average urine pH of multiparous cows of 7.3 just before calving and plasma ionized Ca of 3.91 mg/ dl within 24 h after calving; a DCAD of -5 meq resulted in a urine pH of 6.2 just before calving and plasma ionized Ca of 4.43 mg/ dl after calving; plasma ionized Ca of less than or equal to 4.0 mg/ dl is considered too low and defined as hypocalcemia. Applying the three element DCAD equation, meq($Na^+ + K^+$) - (Cl^-)/ 100g of dietary DM, to the most anionic diet in the study of Moore et al.(20) the DCAD was +12.8 meq; this diet yielded an average urine pH of 6.2.

This feeding management approach has been used successfully in Michigan dairies. The advantage of this approach is that it allows the producer, veterinarian, or nutritionist to monitor acid-base status of close-up cows in a timely fashion, very conveniently and inexpensively. Diet adjustments can be made without requiring a repeated analyses of the diet or its ingredients to estimate the DCAD. It also aids in circumventing the problem that the actual DCAD may be more positive than that formulated. Practically, the desired physiological status of the cow can be titrated by dairy producers by monitoring urine pH and adjusting the inclusion rate of the "anion mix".

Vitamin E Supplementation

Function of Vitamin E

Vitamin E is essential for integrity and optimal function of reproductive, muscular, circulatory, nervous and immune systems. It functions as an intracellular antioxidant. Vitamin E is part of the body's intracellular defence against adverse effects of reactive oxygen and free radicals that initiate oxidation of unsaturated phospholipids (e.g., in cell membranes and organelles) and critical sulfhydryl groups. This function is related closely to and synergistic with the role of Se.

Dairy cows had marked decreases in blood α-tocopherol concentrations around parturition (39, 41). Newborn calves begin life deficient or nearly deficient of α-tocopherol (37). Therefore, an increase in plasma and tissue α-tocopherol concentrations of cows and calves may be beneficial to health and well-being.

Dietary Recommendations and Concentrations in Feeds

Currently NRC (42) recommends a dietary concentration of 15 IU vitamin E/ kg dry feed for cattle, but vitamin E is not listed in the tables of absolute requirements (e.g., IU/cow per d). The requirement is unknown. Vitamin E is present in the foliage and seed oils of plants, and is present in high concentrations in diets of grazing animals. Animals consuming fresh, growing forage may receive 2500 IU/ d or more of natural vitamin E (38). Total confinement of dairy herds is a common practice and locally grown and stored feedstuffs may be low in vitamin E (41). Vitamin E in stored forages is oxidized quickly. Losses of over 80% in the first 1 to 3 mo of storage were reported for hay and haylages (43). Corn silage and unensiled whole corn plant are poor sources of vitamin E. Grains generally are lower than forages in vitamin E and supply a

very small portion of the vitamin E needs of ruminants. Ensiling of high-moisture grains and artificially drying grains result in extensive destruction of vitamin E (41). Therefore, the possibility is great that without proper supplementation vitamin E is inadequate in many dairy herds. To compensate for losses of vitamin E activity in feedstuffs, diets commonly are fortified using dl-α-tocopherol acetate. The dl-α-tocopherol acetate does not act as an antioxidant in the feed, rather it has antioxidant activity after being hydrolyzed in the intestine and free dl-α-tocopherol is released and absorbed (44).

Bioavailability. The bioavailability of α-tocopherol was studied by Eicher-Pruiett et al. (36). Sixteen Holstein steers were blocked by body weight and fed the following treatments: 1) control, no supplement; 2) supplemented with 442 mg retinyl acetate; 3) supplemented with 1342 mg d-α-tocopherol; and 4) supplemented with 442 mg retinyl acetate and 1342 mg d-α-tocopherol. Supplementing d-α-tocopherol only, increased plasma α-tocopherol concentrations, whereas supplementing retinyl acetate, or retinyl acetate plus d-α-tocopherol decreased plasma α-tocopherol concentrations. Authors concluded that this study demonstrated an interaction of d-α-tocopherol and retinyl acetate when both were delivered at high concentrations. They suggested a limited availability of fat-soluble vitamin carriers for absorption of both vitamins supplied at high dietary concentrations.

An additional study on the bioavailability of vitamin E was reported by Hidiroglou et al. (39). At drying-off, 36 Holstein cows were orally supplemented as follows: 1) control, no vitamin supplementation; 2) 1000 IU/d of d-α-tocopherol acetate; and 3) 1000 IU/d of dl-α-tocopherol acetate. Supplementation with either form of α-tocopherol increased vitamin E concentrations in blood plasma, red blood cells, and neutrophils, however, the d-α form was more effective than was the dl-α form.

Assessment of Vitamin E Status

Vitamin E in blood is a component of serum lipoproteins. Serum lipoprotein concentrations vary over the entire lactation cycle, the lowest occurring near calving and the highest at 10 to 12 wk postpartum. Fluctuations in serum lipoprotein concentrations likely result in fluctuations in serum vitamin E concentrations (38). To compensate for changes in serum lipoprotein concentrations, vitamin E concentrations can be expressed as ratio values, either as vitamin E-to-total lipids or vitamin E-to-cholesterol. Cholesterol is used because it is a major component of lipoproteins and is readily measured in most clinical laboratories. Expressing vitamin E concentrations on a mg of cholesterol basis can markedly change interpretation of treatment effects (45).

Vitamin E concentrations of <2.0 ug/ml are considered deficient (38). Weiss et al. (45) suggested that vitamin E concentrations <3.0 ug/ml were too low on the basis that cows with such concentrations were 9.1 times more likely to have clinical mastitis than were cows with α-tocopherol concentrations >3.0 ug/ml. On a ratio basis, an α-tocopherol concentration of 5.0 ug/mg of cholesterol was equivalent to about 3.0 ug/ml of α-tocopherol.

Recent Supplementation Experiments

Weiss et al. (48) studied the effects of duration of supplementation of vitamin E and Se in 61 pregnant Holstein cows. Prepartum treatments (d 60 to calving) were: 1) long-term unsupplemented (fed 0.1 ppm Se and 45 IU vitamin E /kg, n=39), and 2) long-term supplemented (fed 0.3 ppm Se and 110.6 IU vitamin E/kg plus on d 21 prepartum injected with 50 mg Se and 300 IU vitamin E, n=21). Rations fed during lactation were: 1) long-term unsupplemented (0.11 ppm Se and 33.5 IU vitamin E, d 1 to d 21), and 2) long-term supplemented (0.38 ppm Se and 50.4 IU vitamin E/kg, d 1 to 51). Cows fed rations from d 21 to d 51 in the long-term unsupplemented group were divided into four subgroups: 1) long-term unsupplemented (0.11 ppm Se and 33.5 IU vitamin E/kg, n=11); 2) short-term vitamin E supplemented (0 ppm supplemental Se and 40 IU of vitamin E/kg, n=6); 3) short-term Se supplemented (0.3 ppm supplemental Se and 0 IU vitamin E /kg plus on d 21 injected with 50 mg Se and 300 IU vitamin E, n=6); and 4) short-term Se and vitamin E supplementation (0.3 ppm supplemental Se and 40 IU supplemental vitamin E/kg plus on d 21 injected with 50 mg Se and 300 IU vitamin E, n=6). Short-term supplementation of vitamin E or Se increased plasma α-tocopherol and Se concentrations, as compared with no supplementation long term. Authors concluded that feeding 0.3 ppm Se and 110 IU of vitamin E/ kg for the entire dry period and injecting 50 mg Se and 300 IU vitamin E at d 21 prepartum maintained the enzyme glutathione peroxidase (GSHpx) in whole blood, and Se concentrations in plasma and whole blood; Se is part of the GSHpx molecule. Also, results of this study indicated that Se or vitamin E supplementation had no effect on feed intake or milk production. Furthermore, vitamin E supplementation had no effect on blood Se concentrations.

In a study by Brzezinska-Slebodzinska et al. (35) the antioxidant status of dairy cows supplemented prepartum with vitamin E and Se was reported. Sixty-four pregnant multiparous dairy cows were assigned in 13 blocks of 4 Holsteins and 3 blocks of Jerseys. Treatments, given orally daily by gelatin capsule the last 6 wk prepartum, were: 1) 1000 IU of vitamin E/ d; 2) 3 mg of Se/ d, as sodium selenite; 3) 1000 IU of vitamin E and 3 mg Se/ d; and 4) control, no supplemental vitamin E or Se. All cows were kept in the same lot and fed grass hay plus an average of 4 kg/ d of 16% protein supplement which contained 0.3 mg/ kg of Se and 33 IU of vitamin E/ kg. Blood serum α-tocopherol concentrations in control cows decreased progressively during the following 6 wk period. Se supplementation had no effect on serum α-tocopherol concentrations. Serum α-tocopherol concentrations for cows receiving vitamin E supplementation only (Treatment 1) increased 75% during the first 2 wk of supplementation compared with controls. Cows not receiving vitamin E supplementation were twice as likely to have retained fetal membranes. Cows with retained fetal membranes were lower in several indices of antioxidant status and the antioxidants were increased with vitamin E supplementation.

The effects of supplementing vitamin E to 26 peripartum Holstein cows was reported (47). The basal diet contained 0.3 ppm Se and treatment groups were: 1) unsupplemented control (n=7); 2) feeding 1000 IU of supplemental vitamin E/ d for 60 d prepartum, and placebo injections on d 10 and 5 prior to anticipated calving; 3) feeding 0 IU of supplemental vitamin E/ d and injecting 3000 IU vitamin E on d 10 and 5 pre-calving; and, 4) feeding of 1000 IU of supplemental vitamin E/ d and injecting 3000 IU vitamin E on d 10 and 5 pre-calving. Results indicated that cows fed supplemental vitamin E during the dry period had higher plasma concentrations of α-tocopherol at d 10 prepartum, and d 7 and 14 postpartum. Injections of vitamin E elevated blood

α-tocopherol concentrations at d 5 and d 0. However, the effect was short-lived because concentrations were similar to placebo-injected cows by d 7 postpartum. The authors concluded that injection of 6000 IU vitamin E at 10 and 5 d before calving elevated α-tocopherol concentrations in plasma, red blood cells, and neutrophils. The elevated α-tocopherol concentrations of neutrophils may protect them from destructive action of toxic oxygen molecules and improve their intracellular killing of ingested pathogens.

Neutrophil Function and Intramammary Infection

Neutrophils are considered a primary defence mechanism against bacterial infections. The importance of neutrophils in host defence against bovine intramammary infection (IMI) is well documented (41). The responsiveness of neutrophils affects the incidence and severity of clinical signs associated with IMI. Herd management practices that result in optimum vitamin E and Se status of dairy cows will also optimize neutrophil responses and increase resistance to IMI. Numbers of circulating neutrophils decrease shortly after parturition (49).

Vitamin E inhibits autoxidation of polyunsaturated fatty acids in neutrophil membranes. Vitamin E supplementation increases intracellular kill of *S. aureus* and *E. coli* by blood neutrophils. Supplementing dairy rations with vitamin E will not significantly increase numbers of circulating neutrophils, however, injections of vitamin E will (41).

Hogan et al. (41) reviewed the role of vitamin E and Se in host defence against mastitis. A known consequence of α-tocopherol or Se deficiency is reduced neutrophil activity. Dietary supplementation of early lactation cows with vitamin E resulted in increased bactericidal activity by blood neutrophils. Subcutaneous injections of vitamin E 10 and 5 d prior to calving elevated the α-tocopherol concentrations of neutrophils during the peripartum period, and negated the suppressed intracellular killing of bacteria by neutrophils that is commonly observed at calving. Authors recommended that both dry and lactating cows should consume 1000 IU of vitamin E/ d. These recommendations are based on significant reductions in IMI, clinical mastitis, and milk somatic cell count that were observed when cows were supplemented with 1000 IU vs. 150 or 300 IU vitamin E/d.

Weiss et al. (46) studied the effects of dietary fat and vitamin E on α-tocopherol and β-carotene concentrations in blood of peripartum cows. Starting 14 d prior to calving, 24 Holstein cows were divided into four treatment groups. Prior to the start of the experiment, all cows were fed 500 IU supplemental vitamin E daily. Treatment groups were: 1) control, no supplemental vitamin E or fat; 2) supplementation of 1000 IU/ d vitamin E and 0 g fat; 3) no supplementation of vitamin E and 200g/ d supplemental fat (Ca salts of long-chain fatty acids); and 4) supplementation of 1000 IU/ d vitamin E and 200g/ d fat. Dry matter intake was not affected by treatments. However, when plasma α-tocopherol concentrations were expressed as a ratio with serum cholesterol concentrations, supplemental fat had no influence. In contrast to earlier studies (47), vitamin E supplementation did not increase α-tocopherol concentrations in blood neutrophils and did not increase the intracellular killing of bacteria by neutrophils. This difference was attributed to the fact that in the earlier study vitamin E was administered by feeding and injections, whereas in the current study supplementation was by feeding only. In

sheep, the specific activity of α-tocopherol in plasma from oral vitamin E administration was only about 30% of that when vitamin E was injected (40).

In another recent study by Weiss et al. (45), the effect of vitamin E supplementation in diets with a low concentration of Se on mammary gland health was studied. Fifty-one Holstein and 15 Jersey cows and heifers were fed a basal diet (50% grass silage: 25% grass hay: 25% concentrate and supplement) containing 0.1 ppm Se and dietary treatments were: 1) 100 IU of vitamin E/ d during the 60-d dry period and 100 IU/ d the first 30 d of lactation; 2) 1000 IU/ d vitamin E during the 60-d dry period and 500 IU/ d during the lactation period; and 3) 1000 IU/ d the first 46 d of the dry period, then 4000 IU/ d the last 14 d of the dry period and 2000 IU/ d during the lactation period. The incidence rates of clinical mastitis (expressed as a percentage infected quarters/ all quarters) during the first 7 d of lactation were 25.0, 16.7 and 2.6% for treatments 1, 2 and 3, respectively. Cows having less than 3.0 ug/ ml of plasma α-tocopherol at calving were 9.1 times more likely to have mastitis during the first 7 d of lactation. Dry matter intake was similar for cows during the early dry period and the lactation period, but cows in treatments 2 and 3 had higher DMI in the late dry period than cows in treatment 1. Milk production and fat content were not affected by treatment. Authors concluded that supplementation of greater than 1000 IU vitamin E/ d was necessary in hay- and silage-based diets during the peripartum period to maintain plasma α-tocopherol concentrations.

Se Supplementation

Function and Role of Se

The most important biological role of Se is through the enzyme glutathione peroxidase (GSHpx). In synergism with vitamin E and other antioxidative agents, Se is capable of reducing the destructive effects on living cells of peroxidative reactions. The antioxidant effects of Se and vitamin E are different, but complementary. Whereas vitamin E prevents formation of lipid peroxides by sequestering free radicals before they initiate lipid peroxidation of membranes, Se, as an essential part of GSHpx, reduces already formed hydroperoxides to less reactive alcohols in the cytosol (60).

In addition to the clinical syndrome of white muscle disease, a number of other Se responsive conditions have been reported in ruminants. Selenium seems to be an integral element for normal reproductive function. Many cases of retained fetal membranes in dairy cattle have responded to, or have been prevented by Se and (or) vitamin E supplementation (54, 56). Abortions, early embryonic death, and fertility have been associated with Se deficiency (58).

Currently NRC (59) recommends 0.3 ppm Se supplementation for dairy cattle. This is the legal limit established by Food Drug Administration in the United States.

Sources of Se

Selenium concentrations in crops grown in the Plain States of the US are generally adequate, but concentrations can be highly variable or deficit in regions. At least 50% of the total Se in most plants is found in selenomethionine and plants contain only insignificant amounts of inorganic

Se. The most commonly used dietary Se supplement in animal feeds is sodium selenite. The main reason for the preference for inorganic Se is probably economic. The Se content of many feedstuffs has not been determined. A list of feedstuffs and their Se concentrations is shown in Table 1. However, these should be taken with great caution, because Se concentrations vary widely among common feedstuffs, depending upon the regions of the country and soils in which the plants were grown. Diets must be supplemented with Se.

Table 1.Selenium concentrations of typical feedstuffs (59).

Feedstuff	**Se, mg/ kg**
Alfalfa meal, 20% CP	0.31
Alfalfa hay, early bloom	0.34
Alfalfa silage	0.33
Barley grain	0.22
Blood meal	0.80
Brewers grains	0.76
Corn gluten meal	1.11
Corn grain, cracked	0.08
Corn silage	0.09
Cottonseed meal, pre-pressed, solv. extd., 44% CP	10.00
Fish, Menhaden	1.47
Sorghum silage	0.22
Soybean meal	0.11
Wheat grain	0.30

Bioavailability. Bioavailability of various Se compounds differs. Research results are often contradictory even within the same animal species. One reason for variability in bioavailability is

the means of evaluation used in various studies. Bioavailability should not simply include the absorption of a Se-containing substance from the gastrointestinal tract and its excretion, but also its biopotency. The concept of biopotency is included in the definition of bioavailability proposed by Fox et al. (51): "a quantitative measure of the utilization of a nutrient under specific conditions to support the organism's normal structure and physiological processes." Problems appear when all potential activities of an element are not fully established, as for Se. Differences in metabolic pathways involving Se among animal species may be so significant that extrapolating results from one species to another should not be done.

Supplementation of diets using sodium selenate or sodium selenite as sources of Se was first approved in 1974. It was assumed that the two forms were of equal biopotency. This assumption is valid for rats, but not for ruminants. When sodium selenite was given orally to sheep, most of the Se was excreted in the feces (57). The lack of absorption was caused by microorganisms and the reducing environment within the rumen that promoted conversion of the selenite to less-soluble forms, such as elemental Se or selenides. Podoll et al. (62) studied the effect of dietary supplementation of sodium selenate vs. sodium selenite for cattle, sheep and horses. Their results indicated that on the basis of serum Se concentrations and GSHpx activities, there were no differences between forms for sheep and horses. For Holstein cows, supplementation with sodium selenate significantly increased serum Se concentrations when compared with sodium selenite, however, GHSpx activity was similar for both forms. The authors concluded that either selenate or selenite when supplemented at the rate of 0.3 mg Se/ kg supported normal serum Se concentrations and GSHpx activities.

Other researchers have reported possible sources of interference on Se absorption. Van Saun (64) reported that the rumen provides a very strong reducing environment, which may convert ingested Se into the reduced, unavailable state. High-starch, high-concentrate diets may promote a lower ruminal pH and could reduce the efficiency of Se absorption (52). Harrison and Conrad (54) reported that dietary Ca content influenced Se absorption in a quadratic manner. Maximum Se absorption occurred when Ca was 0.6 to 0.8% of dietary DM, but contents of greater than 0.8 or less than 0.6% reduced Se absorption and blood concentrations. In contrast to this finding, Gerloff (52) reported no effect of Ca on Se absorption when Ca concentrations were between 0.6 to 1.1% of dietary DM.

Organic forms of Se are mainly selenomethionine and selenocysteine. Selenomethionine primarily is incorporated into proteins as an alternative for methionine and not into GSHpx when diets are deficient in protein. For diets containing sufficient protein, selenomethionine will increase GSHpx activity of erythrocytes of Se-deficient cattle (61). The Se from selenocysteine was incorporated in GSHpx as quickly as from selenite, which is logical because most of the Se in GHSpx is in the form of selenocysteine (60). There are few reports comparing the effects of and responses to organic and inorganic Se supplementation. Pehrson (61) reported that yeast Se or selenomethionine were about twice as active as selenite for increasing GHSpx activity in red blood cells of Se-deficient heifers. It is unknown if similar results would be observed in animals which were in adequate Se status.

Assessment of Se Status

Determination of what constitutes adequate Se status has not been firmly established in the scientific literature (52). Several measures of Se adequacy have been used by different diagnostic laboratories. These include serum or plasma Se and GSHpx, whole blood Se, liver Se, and whole blood GSHpx activity. Van Saun (64) reported that serum or plasma Se concentrations more accurately reflect current supplementation levels and are more sensitive to short-term changes. Whole blood Se reflects previous or historical Se supplementation levels, increasing more slowly with Se supplementation and decreasing more slowly without supplementation, than serum or plasma Se. Glutathione peroxidase activities in serum or whole blood reflect a similar temporal relationship to Se supplementation history (e.g., serum concentrations reflect short-term supplementation changes and whole blood concentrations reflect more historical supplementation levels).

Recent Studies on Se Supplementation and Health

Smith et al. (63) reported that 0.3 mg of Se supplementation/ kg dietary DM decreased the prevalence of infected quarters 42.2%, lactation-days infected by 59%, clinical mastitis by 32.1% and lowered somatic cell count in dairy cows. Experimentally induced *Escherichia coli* mastitis was less severe and of shorter duration in Se-supplemented cows (50). However, in a later study with experimentally induced *Staphylococcus aureus* mastitis, Se supplementation of 2 mg of sodium selenite/ cow per d did not affect the severity or duration of mastitis. This lack of response to Se supplementation was attributed to the insidious nature of pathogenesis of *S. aureus* and the adaptation of *S. aureus* that permits survival. In another study, Hogan et al. (55) reported that although phagocytic activity of neutrophils was not altered when dairy cows were supplemented with 0.3 mg Se (as sodium selenite)/ kg of dietary DM, intracellular kill of *S. aureus* was increased and intracellular kill of *E. coli* tended to be increased.

A deficiency of Se is associated with decreased intracellular kill by bovine neutrophils (52). Furthermore, effects of vitamin E and Se supplementation on intracellular kill of bacteria by neutrophils is not additive. Vitamin E supplementation may spare the requirement for GSHpx by oxidizing free radicals at the membrane, thereby preventing leakage of free radicals into the cytosol. Conversely, GSHpx activity in the cytosol may spare the requirement for vitamin E in membranes.

References for Anion Supplementation

1. Beede, D.K. 1995. Macromineral element nutrition for the transition cow: Practical implications and strategies. Proc. Tri-State Nutrition Conf., Ft. Wayne, IN, p. 175.
2. Beede, D.K., C.A. Risco, G.A. Donovan, C. Wang, L.F. Archbald, and W.K. Sanchez. 1992. Nutritional management of the late pregnant dry cow with particular reference to dietary cation-anion difference and calcium supplementation. Proc. 24th Ann. Convention Am. Assoc. Bovine Practitioners, p. 51.
3. Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939.

4. Byers, D. I. 1992. Formulating anionic dry cow rations (Practice Tips). Proc. 24th Ann. Convention Am. Assoc. Bovine Pract. p. 149.
5. Curtis, C.R., H.N. Erb, C.J. Sniffen, and R.D. Smith. 1984. Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. J. Dairy Sci. 67:817.
6. Ender, F., and I.W. Dishington. 1970. Etiology and prevention of parturient paresis puerperalis in dairy cows. Page 71 in Parturient hypocalcemia. J. J. B. Anderson, ed. Academic Press, New York.
7. Ender, F., I.W. Dishington, and A. Helgebostad. 1962. Parturient paresis and related forms of hypocalcemic disorders induced experimentally in diary cows. II. Studies on the etiological importance of feeding prepartal diets high in Ca and low or normal in P in relation to development of milk fever. Effect of high or low alkalinity of diets. Acta. Vet. Scand. 3(Suppl. 1):1.
8. Giesy, J.G., W.K. Sanchez, M.A. McGuire, J.J. Higgins, L.A. Griffel, and M.A. Guy. 1997. Quantifying the relationship of dietary cation-anion difference to blood calcium in cows during hypocalcemia. J. Dairy Sci. 80(Suppl. 1):142.
9. Goff, J.P., and R. L. Horst. 1997. Comparison of sulfuric acid with hydrochloric acid as a source of acidifying anions. J. Anim. Sci. 75(Suppl. 1):98.
10. Goff, J.P., R. Ruiz, and R.L. Horst. 1997. Relative acidogenic activity of commonly used anionic salts - re-thinking the dietary cation-anion difference equations. J. Dairy Sci. 80(Suppl. 1):169.
11. Goff, J.P., R. L. Horst, T.A. Reinhardt, and D.R. Buxton. 1997. Preventing milk fever in dairy cattle. Proc. Tri-State Dairy Nutrition Conf., Ft. Wayne, IN, p. 41.
12. Goff, J.P., and R. L. Horst. 1997. Dietary potassium, but not calcium, induces milk fever in dairy cows. J. Dairy Sci. 80:176.
13. Goff, J.P., T.A. Reinhardt, and R.L. Horst. 1995. Milk fever and dietary cation-anion balance effects on concentration of vitamin D receptor in tissue of peripartum diary cows. J. Dairy Sci. 78:2388.
14. Goff, J.P., T.A. Reinhardt, and R.L. Horst. 1991. Enzymes and factors controlling vitamin D metabolism and action in normal and milk fever cows. J. Dairy Sci. 69:1184.
15. Horst, R.L., J.P. Goff, T.A. Reinhardt, and D.R. Buxton. 1997. Strategies for preventing milk fever in dairy cattle. J. Dairy Sci. 80:1269.
16. Horst, R.L., J.P. Goff, and T.A Reinhardt. 1994. Calcium and vitamin D metabolism in the dairy cow. J. Dairy Sci. 77:1936.
17. Horst. R. L. 1986. Regulation of calcium and phosphorus homeostasis in the dairy cow. J. Dairy Sci. 69:604.
18. Keuning, J., and S. Gunderson. 1997. How six top Wisconsin herds handle their dry and prefresh cows. Hoard's Dairyman (October 10) 142:706.
19. Kirk, D. 1997. In: Frontiers in Nutrition, Dawe's Laboratories, Arlington Heights, IL, No. 327.
20. Moore, S.J., M.J. VandeHaar, B.K. Sharma, T.E. Pilbeam, D.K. Beede, H.F. Bucholtz, J.S. Liesman, R.L. Horst, and J.P. Goff. 1997. Varying dietary cation anion difference (DCAD) for dairy cattle before calving. J. Dairy Sci. 80(Suppl. 1):170.
21. MSU Program Survey. 1996. Managing the Dry Cow for More Profit Notebook. Michigan State University Dairy Programs Group, East Lansing.

22. National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th. rev. ed. Natl. Acad. Sci., Washington, D.C.
23. Oetzel, G.R., and J.A. Barmore. 1992. Palatability of anionic salts fed in a concentrate mix. J. Dairy Sci. 75(Suppl. 1):297.
24. Oetzel, G.R. 1991. Meta-analysis of nutritional risk factors for milk fever in dairy cattle. J. Dairy Sci. 74:3900.
25. Oetzel, G.R., J.D. Olson, C.R. Curtis, M.J. Curtis, and M.J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302.
26. Risco, C.A. 1992. Calving related disorders. Page 192 in Large Herd Dairy Management. H.H. Van Horn and C. J. Wilcox, eds., Management Services ADSA, Champaign, IL.
27. Rodriguez, L.A., T.E. Pilbeam, and D.K. Beede. 1997. Effects of dietary anion source and calcium concentration on dry matter intake and acid-base status of nonlactating nonpregnant Holstein cows. J. Dairy Sci. 80(Suppl. 1): 241.
28. Rodriguez, L.A., T.E. Pilbeam, R.W. Ashley, S.L. Neudeck, R.J. Templeman, J.A. Davidson, and D.K. Beede. 1996. Concurrently lowering dietary cation-anion difference while raising calcium content prepartum reduces urine pH and peripartum subclinical hypocalcemia. J. Dairy Sci. 79:(Suppl. 1):197.
29. Seymour, W.M., J.E. Nocek, J. Siciliano-Jones, and J.E. English. 1992. Effect of feeding an anionic diet prepartum on blood, health, reproductive and productive parameters in Holstein cows. J. Dairy Sci. 75(Suppl. 1):297.
30. Shenk, J.S., and M.O. Westerhasu. 1994. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In: Forage Quality, Evaluation and Utilization. Am. Soc. Agronomy, Crop Sci. Soc. Am., and Soil Soc. Am., Madison, WI pg. 406.
31. Stewart, P. A. 1983. Modern quantitative acid-base chemistry. Can. J. Physiol. Pharmacol. 61:1444.
32. Tucker, W.B., J.F. Hogue, D.F. Waterman, T.S. Swenson, Z. Xin, R.W. Hemken, J.A. Jackson, G.D. Adams, and L.J. Spicer. 1991. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Dairy Sci. 69:1205.
33. Verdaris, J.N., and J.E. Evans. 1974. Early lactation mineral nutrition and milk production as affected by diet calcium and pH. J. Dairy Sci. 57:623 (Abstr.).
34. Wang, C. 1990. Influence of dietary factors on calcium metabolism and incidence of parturient paresis in dairy cows. Ph.D. Diss., Univ. Florida, Gainesville.

References for Vitamin E Supplementation

35. Brzezinska-Slebodzinska, E., J.K. Miller, J.D. Quigley, III, J.R. Moore, and F.C. Madsen. 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J. Dairy Sci. 77:3087.
36. Eicher, S.D., J.L. Morrill, and J. Velazco. 1997. Bioavailability of α-tocopherol fed with retinol and relative bioavailability of d-α-tocopherol or dl-α-tocopherol acetate. J. Dairy Sci. 80:393.
37. Eicher-Pruiett, S.D., J.L. Morrill, F. Blecha, J.J. Higgins, N.V. Anderson, and P.G. Reddy. 1992. Neutrophil and lymphocyte response to supplementation with vitamins C and E in young calves. J. Dairy Sci. 75:1635.

38. Herdt, T.H., and H.D. Stowe. 1991. Fat-soluble vitamin nutrition for dairy cattle. Vet. Clin. North Am. Food Amin. Pract. 7:391.
39. Hidiroglou, M., T.R. Batra, and X. Zhao. 1997. Bioavailability of vitamin E compounds and the effect of supplementation on release of superoxide and hydrogen peroxide by bovine neutrophils. J. Dairy Sci. 80:187.
40. Hidiroglou, M., and K. Karpinski. 1987. Vitamin E kinetics in sheep. Br. J. Nitr. 58:113.
41. Hogan, J.S., W.P. Weiss, and K.L. Smith. 1993. Role of vitamin E and selenium in host defence against mastitis. J. Dairy Sci. 76:2795.
42. National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th. rev. ed. Natl. Acad. Sci., Washington, D.C.
43. Schingoethe, D.J., J.G. Parsons, F.C. Ludens, W.L. Tucker, and H.J. Shave. 1978. Vitamin E status of dairy cows fed stored feeds continuously or pastured during summer. J. Dairy Sci. 61:1582.
44. Vitamin Nutrition for Ruminants. 1991. Hoffmann-LaRoche Inc., Nutley, NJ, 07110.
45. Weiss, W.P., J.S. Hogan, D.A. Todhunter, and K.L. Smith. 1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J. Dairy Sci. 80:1728.
46. Weiss W.P., J.S. Hogan, K.L. Smith, and S.N. Williams. 1994. Effect of dietary fat and vitamin E on α-tocopherol and β-carotene in blood of peripartum cows. J. Dairy Sci. 77:1422.
47. Weiss, W.P., J.S. Hogan, K.L. Smith, D.A. Todhunter, and S.N. Williams. 1992. Effect of supplementing peripartum cows with vitamin E on distribution of α-tocopherol in blood. J. Dairy Sci. 75:3479.
48. Weiss, W.P., D.A. Todhunter, J.S. Hogan, and K.L. Smith. 1990. Effect of duration of supplementation of selenium and vitamin E on peripartum dairy cows. J. Dairy Sci. 73:2187.

References for Selenium Supplementation

49. Cai, T.O., P.G. Weston, L.A. Lund, B. Brodie, D.J. McKeena, and W.C. Wagner. 1994. Associations between neutrophil functions and perparturient disorders in cows. Am. J. Vet. Res. 55:934.
50. Erskine, R.J., R.J. Eberhart, and R.W. Scholz. 1990. Experimentally induced Staphylococcus aureus mastitis in selenium-deficient and selenium-supplemented dairy cows. Am. J. Vet. Res. 51:1107.
51. Fox, M.R.S., R.M. Jacobs, A.O.L. Jones, B.E. Fry, M. Rakowska, R.P. Hamilton, B.F. Harland, C.L. Stone, and S.H. Tao. 1981. Animal models for assessing bioavailability of essential and toxic elements. Cereal Chem. 58:6.
52. Gerloff, B.J. 1992. Effect of selenium supplementation of dairy cattle. J. Anim. Sci. 70:3934.
53. Harrison, J.H., D.D. Hancock, and H.R. Conrad. 1984. Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci. 67:123.
54. Harrison, J.H., and H.R. Conrad. 1984. Effect of dietary calcium on selenium absorption in the nonlactating dairy cow. J. Dairy Sci. 67:1860.

55. Hogan, J.S., K.L. Smith, W.P. Weiss, D.A. Todhunter, and W.L. Schockey. 1990. Relationship among vitamin E, selenium, and bovine blood neutrophils. J. Dairy Sci. 73:2372.
56. Julien, W.E., H.R. Conrad, and A.L. Moxon. 1976. Selenium and vitamin E and incidence of retained placenta in parturient cows. II. Prevention in commercial herds with prepartum treatment. J. Dairy Sci. 59:1960.
57. Lopez, P.L., R.L. Preston, and W.H. Pfander. 1969. Whole-body retention, tissue distribution and excretion of selenium-75 after oral and intravenous administration in lambs fed varying selenium intakes. J. Nutr. 97:123.
58. Maas, J.P. 1983. Diagnosis and management of selenium-responsive diseases in cattle. Compen. Contin. Educ. Pract. Vet. 5:S393.
59. National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.
60. Pehrson, B.G. 1993. Selenium in nutrition with special reference to the biopotency of organic and inorganic compounds. Page 71 in Biotechnology in the Feed Industry. Proc. Alltech's 9th Annual Symp., Nicholasville, KY.
61. Pehrson, B.G., M. Knutsson, and M. Gyllensward. 1989. Glutathione peroxidase activity in heifers fed diets supplemented with organic and inorganic selenium compounds. Swedish J. Agric. Res. 19:53.
62. Podoll, K.L., J.B. Bernard, D.E. Ullrey, S.R. DeBar, P.K. Ku, and W.T. Magee. 1992. Dietary selenate versus selenite for cattle, sheep, and horses. J. Anim. Sci. 70:1965.
63. Smith, K.K., J.S. Hogan, and H.R. Conrad. 1987. Vitamin E, selenium levels may be linked to mastitis in dairy cows. Feedstuffs, June 8:10.
64. Van Saun, R.J. 1990. Rational approach to selenium supplementation essential. Feedstuffs January 15:15.