In: Proc. Southwest Dairy Nutrition and Management Conf., pages 1-11; Feb. 21, 2013, Tempe, AZ.

DCAD revisited: Prepartum use to optimize health and lactational performance

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SUMMARY

- Normal calcium (Ca) status is extremely important through the transition period to launch a healthy and successful lactation.
- From recent evidence, routine monitoring to identify subclinical hypocalcemia (defined by early postpartum blood serum Ca of less than 8.6 mg/dl) would be appropriate in herds with unacceptable periparturient health disorders.
- Based on available analyses the four-element equation for dietary cation-anion difference [DCAD = $(Na^+ + K^+) – (Cl^- + S^{2-})$] is most appropriate to formulate a reduced DCAD of the close-up ration.
- The most effective first step to lower the DCAD is to reduce K^+ and Na^+ as much as possible before supplementing anions.
- DCAD of -10 mEq/100 g is recommended; a somewhat lower DCAD (-15 mEq/100 g) may be advantageous to help accommodate biological variation among animals within the close-up group.
- Close-up diets with 1.0% Ca and 0.35% Mg are sufficient in properly supplemented anionic diets to support normal periparturient Ca status.
- Various vitamin D-related processes, including regulation of periparturient Ca status, might be improved with supplementation of vitamin D throughout the close-up period.
- The body of evidence accumulated over the last 40+ years highlights the direct and indirect importance of periparturient Ca status and how Ca status can be improved by manipulation of close-up DCAD to improve lactational performance.
- Future research should consider: a) timing of vitamin D supplementation prior to calving; b) factors that contribute to vitamin D receptor regulation in the transition period; and, c) performance of heifers fed anionic diets.

INTRODUCTION

The importance of successfully transitioning dairy cows from pregnancy to lactation has been known for decades. Periparturient hypocalcemia can be detrimental to both cows and dairy producers. If preventive measures to manage blood calcium (Ca) around parturition are not taken, a cascade of metabolic problems can lead to secondary health issues, impaired immune function, reduced milk production, and poor reproductive performance. Elliot Block (1984) was first in North America to report that reducing the dietary cation-anion difference (DCAD) before calving could benefit early postpartum Ca status and aid transition into lactation. In the late 1980s and early 1990s the so-called "anionic salts" were supplemented in close-up diets. Success was variable mainly because of lack of understanding about how to deliver this new technology. Often prepartum feed intake was reduced with the salts. Consequently, commercial anion supplements apparently causing fewer feed intake problems became available. Nonetheless, anion supplementation still does not work as well as desired in some cases. This paper takes a brief look back at the development of the DCAD strategy for transition dairy cows, suggests some possible reasons why it is not more universally efficacious, and addresses current approaches to monitor and reduce risk of hypocalcemia as well as new ideas to consider when implementing the prepartum DCAD strategy.

HYPOCALCEMIA

Subclinical Hypocalcemia

Hypocalcemia is a critical concern due to the enormous physiological demands for Ca at calving and the start of lactation (Oetzel et al., 1988; Horst et al., 1997). Calcium demand of a cow at initiation of lactation is about double compared with when she was non-lactating and pregnant. For example, the Ca requirement of a 1600-pound pregnant dry cow increases from about 11 g/d to support the conceptus to about 23 g/d after calving to support milk production (Goff and Horst, 1997a). In order to suffice, a cow must bring at least 30 g of Ca/d into the plasma pool in very short order by intestinal absorption and(or) bone resorption (Horst et al., 1997). This is a formidable physiological task.

Normal blood plasma Ca concentration is tightly regulated and generally kept between 8.5 to 10 mg/dl (2.1 to 2.5 mM; Cahn and Line, 2005). In the periparturient period with enormous physiological demands, blood Ca may drop below this range; though not characteristically considered subclinical until blood plasma Ca drops to the range of 8.0 to 5.5 mg/dl (2.00 to 1.38 mM), but with homeostasis still maintained (Goff, 2008). Recent findings suggest that the subclinical range might be widened to include blood serum Ca concentrations of 8.6 to 5.5 mg/dl to better identify cows at risk for metritis (Martinez et al., 2012). The point at which a

cow is truly hypocalcemic and biological functions become impaired varies among individual cows; their "capacity" to maintain homeostasis and Ca status as influenced by several factors is discussed subsequently. A possible on-farm strategy might be to more routinely monitor and identify subclinical hypocalcemia as serum Ca concentrations of less than 8.6 mg/dl in herds with unacceptable incidences of periparturient health disorders.

Clinical Hypocalcemia

Milk fever (clinical hypocalcemia) defines an extreme decrease in blood plasma Ca below a certain point around time of calving resulting in homeostatic failure. The point at which it is considered clinical was suggested to be below 5.5 mg/dl (Goff, 2008). Milk fever may or may not be accompanied by other signs such as poor appetite, recumbency, and lethargy (Horst et al., 1997). A study conducted by the USDA National Animal Health Monitoring System (2002) estimated a 5% incidence rate of milk fever in the United States (Reinhardt et al., 2011). Failure to maintain muscle tone and contractility of the gastrointestinal tract and the cardiovascular system quickly leads to death in the majority of milk fever cases (Cahn and Line, 2005). Not all cows that have subclinical hypocalcemia develop milk fever, but it is important that diagnostic and preventive measures are taken before the cow's health declines and subsequent problems arise.

Predisposing Factors

Reasons for susceptibility to hypocalcemia vary and may be multi-factorial. One of the most commonly noted differences in susceptibility is parity. Incidence of subclinical hypocalcemia (< 8.0 mg plasma Ca /dl) typically rises with increasing parity, affecting 25% of heifers and almost half of cows of second and greater parity (Reinhardt et al., 2011). It is thought that heifers especially are less susceptible because they have greater bone depletion/repletion activity and are more able to mobilize bone Ca from their Ca reserves than are later parity cows (van Mosel et al., 1993). Additionally, later parity cows produce more colostrum and milk making the demand for Ca greater. A similar relationship with parity was noted with clinical milk fever affecting less than 1% of heifers, but surpassing 6% for cows of third and greater parity (Reinhardt et al., 2011). This greater milk fever risk and incidence is in part due to parity as well as history of previous milk fever. History of milk fever seems to be a large determinant of whether or not a cow develops hypocalcemia and milk fever at subsequent parturitions. This is presumably due to a decreased ability of these particular cows to respond immediately to biological signals and increase vitamin D receptor (VDR) numbers in a timely manner (Goff et al., 1995a). Blood Ca and vitamin D_3 concentrations are obviously important components in Ca mobilization, bone resorption, and intestinal absorption (Jones, 2008). However, deficiencies in dietary Ca, vitamin D, or biologically active 1, 25 dihydroxyvitamin D are not thought to be the casual reasons for hypocalcemia. For instance, low Ca diets were used successfully to reduce incidence of hypocalcemia (Goings et al., 1974; Thilsing-Hansen et al., 2002) and active vitamin D plasma concentrations usually are considered sufficient in afflicted cows (Goff et al., 1989). A possible explanation for the metabolic cause of hypocalcemia is slow VDR up-regulation, and(or) overall lower VDR numbers between breeds (e.g., Jersey vs. Holstein; Goff et al., 1995b). Jerseys have fewer overall VDR compared with Holsteins (Goff et al., 1995b) and Jerseys are more susceptible to milk fever (Lean et al., 2006). Conversely, Holsteins have similar numbers of receptors compared with Brown Swiss (Lisegang et al., 2008) and both breeds typically have less incidence of milk fever than Jerseys. The regulation of VDR could be a very plausible explanation for differences among breeds and whether or not a particular cow of a particular parity or breed develops hypocalcemia and to what severity the disorder manifests.

DIETARY CATION-ANION DIFFERENCE

History and Current Use

The ability to reduce the incidence of periparturient hypocalcemia through addition of mineral acids to the diet was first documented by Ender and Dishington (1970). Shortly thereafter they showed that multiple dietary anionic salts were effective in stimulating increased blood Ca (Ender et al., 1971; Dishington, 1975). Block (1984) introduced the dietary cation-anion balance theory to transition cow research in North America. He demonstrated that providing more dietary fixed anions than cations via anionic salts in the close-up period increased blood Ca in early postpartum cows. Today, this strategy is commonly known as dietary cation-anion difference (DCAD), which more specifically addresses the calculation of DCAD in ration formulation (Sanchez and Beede, 1991).

The DCAD can be calculated as several variants of an equation involving more or less of the seven dietary essential macromineral elements: milliequivalents (mEq) of $[(Na^+ + K^+ + Mg^{2+} + Ca^{2+}) - (Cl^- + S^{2-} + P^{3-})/100g$ of dietary DM]. As part of a ration formulation and management strategy, a low or negative DCAD is supplied in the diet for 2 to 3 wk prepartum (Block, 1984; Oetzel et al., 1988). An effective DCAD reduction changes the acid-base status of the cow within 36 hr (Goff and Horst, 1998). However, because of variation in actual compared with expected calving day (e.g., 8 to10 d, ±S.D.) sufficient time of dietary provision must be used to accommodate the majority of cows in the close-up group. Anion supplementation effectively reduces the incidence of periparturient hypocalcemia and milk fever by increasing the concentration of blood Ca (Block, 1984; Oetzel et al., 1988). Early on, the so-called anionic salts (e.g., chloride and sulfate salts of ammonium, calcium or magnesium) were used. Certain anionic salts were thought to be more palatable than others (e.g., greater DMI when magnesium sulfate compared with calcium chloride was supplemented; Oetzel and Barmore, 1993). In the USDA survey, an estimated 27% of U.S. dairy operations used "anionic salts" (as specifically worded in

the survey) to decrease DCAD in an effort to reduce incidence of periparturient hypocalcemia (USDA, 2007). Use of commercial products to supplement anions rather than anionic salts is much more popular today than in the past due to their ease of use and often the added benefit as a source of protein. The use of palatable carriers and addition of flavoring in commercial sources may help dilute or mask the presumably bitter taste thought to accompany pure anionic salt sources. The first-line, most efficacious strategy for lowering DCAD is to reduce the strong cations (i.e., K^+ and Na^+) before adding anion sources. About 47% of U.S. dairy operations specifically aimed to lower dietary K in efforts to reduce DCAD of prepartum diets (USDA, 2007).

DCAD Equations

There are a variety of ways to calculate DCAD depending on which macromineral elements (fixed ions) are considered important (Ender et al., 1971; Goff, 2004). Most equations include Na, K, Cl, and S, but variations on this come from addition of Ca and Mg, estimated coefficients of absorption for the different mineral elements, and the inclusion or exclusion of S and P. Overall, the most widely used equation in the industry is that originally used by Ender et al. (1971) [DCAD = $(Na^+ + K^+) – (Cl^- + S^{2-})$]. Based solely on the acidifying effect of dietary S in urine and blood, Spanghero (2004) suggested that S had negligible influence on the cow's acid-base balance in the basic four-element equation. Thus, the simpler three-element equation [DCAD = $(Na^+ + K^+) – (Cl^-)$] was suggested. However, increased S in the diet was associated with decreased incidence of milk fever and the source of S (as part of an organic compound or as supplemental sulfates) could be a factor (Lean et al., 2006). Note that supplementation of elemental sulfur (e.g., the flowers of sulfur) is worthless as it does not provide absorbable S nor does it have acidifying properties as part of the DCAD calculation.

The meta-analysis of Lean et al. (2006) assessed the ability of four relevant DCAD equations to both affect acid-base status and to predict milk fever of Holstein, Norwegian and Swedish Red and White, and Jersey cows. It is important to note that using an equation to predict the effects of macromineral elements on milk fever is not necessarily the same as using an equation to predict overall acid-base status. For example, Jerseys have a greater susceptibility to milk fever than Holsteins (Lean et al., 2006), while Mg inclusion in the diet reduced risk of milk fever but made the DCAD value more positive. A criterion for data inclusion in the study of Lean et al. (2006) was that they could calculate DCAD from information given in the original reports; however, lactation number, which is a recognized risk factor for milk fever (Reinhardt et al., 2011) was not in the model. The analysis also did not consider Ca in the DCAD equation noting that diets with both low Ca (0.5 %) and high Ca (> 1.0 %) were effective in preventing milk fever. Additionally, in their analysis there was a time by treatment interaction on the efficacy of Ca inclusion rate and lack of sufficient data with dietary Ca inclusion greater than 1.1%. Results indicate that [DCAD = $(Na^+ + K^+) – (Cl^- + S^{2-})$] was the most effective in describing acid-base status as well as predicting reduced incidence of milk fever. Similarly, a meta-analysis by Charbonneau et al. (2006) concluded that [DCAD = $(Na^+ + K^+) – (Cl^- + 0.6S^{2-})$] correlated with milk fever incidence and acid-base status prediction. The 0.6 coefficient was based on findings of Tucker et al. (1991a) and later supported by Goff et al. (2004) indicating that S had less acidifying effect compared with Cl. Diet composition and desired outcome (i.e., risk prevention or acidifying ability) should be important considerations when determining which equation to use. Based on all of the available information the four-element equation seems most acceptable. Whether or not the 0.6 coefficient for S should be incorporated likely depends upon the sources of supplemental S in a particular diet.

Mechanism of Action

Metabolic acidosis or alkalosis results from the change in electrical charge of biological fluids due to either more anions (i.e., acidosis) or more cations (i.e., alkalosis) entering the blood. The change in electrical charge of the blood results in transfer of H^+ ions and changes in blood pH (i.e., acid-base balance; Goff, 2008). Blood pH is tightly regulated within a narrow normal physiological range, keeping arterial blood pH between 7.35 to 7.45 and venous blood at a slightly lower pH (Nagy et al., 2001). Though blood pH can be reduced through use of negative DCAD, these changes may not be significant (Charbonneau et al., 2006; Grünberg et al., 2011). A meta-analysis indicated a significant negative relationship between decreasing DCAD (from +30 to 0 mEq/100g) and decreasing blood pH; however, the change in blood pH was very small and not statistically significant (Charbonneau et al., 2006). Compared with sulfate salts, chloride anionic salts are thought to have a greater acidifying effect in blood (Goff et al., 2004; Charbonneau et al., 2006).

A more easily accessible proxy for monitoring systemic acid-base status in the field is urine pH. Typically if urine pH is below 7.0 then systemic acidosis has been generated (Jardon, 1995). Charbonneau et al. (2006) suggested that inducing acidosis beyond the point where urine pH drops below 6.8 is not warranted in field application of the DCAD strategy because risk of hypocalcemia was not further reduced. Typical anionic salts fed in close-up rations with negative DCAD include chloride and sulfate salts of calcium, magnesium, and ammonium. Hydrochloric acid was most effective at lowering urine pH compared with the anionic salts (Goff et al., 2004).

The exact mechanism by which a low or negative DCAD aids in the reduction of hypocalcemia during the periparturient period is not completely understood. It apparently has to do with two independent mechanisms. First, the mildly acidic systemic environment created by lowered DCAD stimulates osteoclasts to start mineralizing bone Ca to correct the acidosis. Under hypocalcemic conditions parathyroid hormone (PTH) is secreted. This hormone assists in the subsequent formation of the active 1, 25 dihdroxyvitamin D

hormone (1,25 $[OH]_2$ D). Through both PTH and biologically active vitamin D, bone resorption of Ca is stimulated (Horst and Reinhardt, 1983). Additionally, there is increased renal tubular resorption of Ca and increased efficiency of Ca absorption in the small intestine (Goff and Horst, 2003). Together these mechanisms and newly available Ca sources contribute to the return of Ca homeostasis.

Further connection between anion supplementation and Ca status is thought to be due to the decrease in blood pH by anions (Goff, 2008). When both pH is low and PTH is active, osteoclastic activity is even greater and thus Ca resorption increases significantly (Bushinsky, 2001). It is commonly accepted that the reason for this increased activity is that the creation of an acidic metabolic environment allows increased tissue responsiveness to PTH. When blood pH is more alkaline (>7.35), PTH cannot tightly bind to its receptors on the bone surface and in renal tissue. However, when blood is at its normal physiological pH of 7.35, PTH is tightly bound to its receptors and can therefore better stimulate its target cells (Bushinsky, 2001; Goff, 2008).

"Optimal" DCAD for Close-up Cows

It is not necessary for the prepartum DCAD to be negative in order to stimulate homeostatic acid-base mechanisms and increase blood Ca. Lowering DCAD from +30 to 0 mEq/100g resulted in a decrease of urine pH from a normal of 8.09 to 7.01 (Charbonneau et al., 2006). Furthermore, this mild systemic acidosis resulted in significant increase in total blood Ca concentration immediately postpartum and reduced incidence of milk fever from 16.4 to 3.2%. In another study, cows fed close-up diets with a negative DCAD (-10 to -12 mEq/100 g) with supplemental calcium chloride and calcium sulfate (personal communication with authors) or a commercial supplement did not have increased blood Ca after calving compared with cows fed a control ration (+22 mEq/100g) (DeGroot et al., 2012).

It was suggested that systemic acidic conditions decrease the ability of insulin to bind to its receptor (Whittaker et al., 1982). This causes reduced insulin sensitivity resulting in decreased glucose uptake and utilization (Bigner et al., 1996). Because of the shift in energy demand at onset of lactation, loss of utilizable glucose can be detrimental to both milk production and cow health. Grünberg et al. (2011) did not observe an effect on insulin response or sensitivity when mild systemic acidosis was induced through use of anion supplementation (-9 mEq/100 g). Similarly, Ramos-Nieves et al. (2009) and DeGroot et al. (2010) found no difference in plasma glucose concentrations during the periparturient period in relation to DCAD prepartum (-15 mEq/100 g and -12 mEq/100 g, respectively).

The influence of anion supplementation on DMI varies among studies. Both Block (1984) and Oetzel et al. (1988) found no changes in prepartum DMI when anion sources were provided (-13 mEq/100 g and -7.6 mEq/100 g, respectively). DeGroot et al. (2010) also observed changed acid-base status without compromising prepartum DMI in multiparous cows, but observed increased postpartum DMI (2.1 kg/cow per d) for cows receiving anion supplementation prepartum. These findings differ from a meta-analysis that indicated a decrease in prepartum DMI of 1.3 kg/cow per d when DCAD was reduced from +30 to 0 mEq/100g (Charbonneau et al., 2006). Ramos-Nieves et al. (2009) also reported a significant decrease in DMI (15.6 vs. 14.4 kg/cow per d) when prepartum anion supplementation was provided (+10 vs. -15 mEq/100, respectively). This discrepancy could be due to the amount and(or) source of anions supplemented to reach a desired DCAD. Sodium and K are recognized as strong cation contributors with greater dietary concentrations being linked to increased incidence of milk fever (Goff and Horst, 1997b; Lean et al., 2006). For most effective DCAD formulation, dietary Na and K should be reduced as much as practically possible before adding supplemental anions. In order to avoid possible adverse effects of supplemental anions on DMI while still achieving mild systemic acidosis, a DCAD of -10 mEq/100 g is suggested. Using a somewhat lower DCAD (e.g., -15 mEq/100 g) may be advantageous to help accommodate biological variation among individual cows (i.e., fluctuations in daily DMI and differences in biological ability to maintain acid-base balance).

Management of Heifers (Primiparous Cows)

Although primiparous cows do not typically experience as many or the severity of Ca-related metabolic problems during transition as multiparous cows, both pregnant heifers and multiparous cows are often in the same group and fed the same close-up ration. The USDA (2007) survey suggested that about 20% of dairy farms use anion supplementation in close-up rations for heifers. A possible adverse effect of providing a negative DCAD to heifers is reduced DMI (Tucker et al., 1991b; Moore et al., 2000); but, other negative effects are largely not reported. DeGroot et al. (2010) demonstrated that using various anion sources (commercial supplements, or calcium sulfate and calcium chloride) to create a negative DCAD during the close-up period did not result in any obvious negative side effects in pregnant heifers. Acid-base balance was effectively altered with heifers having lower prepartum urine pH compared with multiparous cows (6.24 and 6.80 respectively). Similar to the multiparous cows, the prepartum DMI of heifers was not impacted and early postpartum DMI increased (1.4 kg/cow per d). Overall, there is insufficient evidence to say whether or not heifers/primiparous cows will be impacted negatively in major ways if provided a negative DCAD diet in late gestation. The lowered prepartum urine pH (i.e., affected acid-base status) of heifers could be an unnecessary consequence considering there was no significant change in postpartum plasma Ca concentrations. Overall management of heifers must still be considered when deciding on

anion supplementation. Improper monitoring of mixed parity close-up groups may lead to competition among animals and reduced DMI of typically smaller heifers.

DCAD and Milk Yield Response

Prepartum anion supplementation improved lactation performance in some studies. Block (1984) reported a 6.8% increase in milk yield of cows given anionic salts (DCAD: -13 mEq/100 g) in the close-up period with no reported change in postpartum DMI. Likewise, high producing multiparous cows provided diets supplemented with anions (-10 to – 12 mEq/100 g) prepartum increased milk yield by 18% (6.5 kg/cow per d) compared with cows fed a control diet (+22 mEq/100 g; DeGroot et al., 2010). This increase was likely due to greater postpartum DMI of cows fed anions prepartum. Differences in milk yield are not always found. No changes in milk yield, milk composition, or postpartum DMI were found when comparing prepartum anionic vs. cationic diets (-15 vs. +15 mEq/100g by Moore et al., 2000; or, -15 vs. + 10 mEq/100g by Ramos-Nieves et al., 2009). Similarly, milk yield and postpartum DMI did not differ among cows in a pasture system provided a prepartum diet with a DCAD of +50 vs. +7 mEq/100g (Roche et al., 2003). The improvement in some studies from feeding a low or negative DCAD prepartum on postpartum milk yield is most likely due to improved periparturient Ca status and health, and improved postpartum DMI.

MACROMINERALS

Cations

Whether or not feeding less or more dietary Ca than the cows' requirement in close-up diets is still questioned. To truly restrict Ca intake below requirement to affect Ca homeostasis, less than 20 g/cow per d is required (Thilsing-Hansen et al., 2002) or possibly less than 15 g/cow per d (Goings et al., 1974). The current NRC (2001) recommendation for absorbed Ca for a pregnant Holstein dry cow (1600 lb BW) is approximately 11 g/d (or about 20 - 30 g of total dietary Ca/cow per d). By markedly restricting dietary Ca prepartum, hypocalcemic-like conditions are induced prior to calving. This results in increased fractional absorption of Ca from the small intestine, renal reabsorption, and bone resorption (Goff and Horst, 2003). Because these mechanisms are stimulated before calving, increased Ca in blood at calving can result (Goings et al., 1974; Thilsing-Hansen et al., 2002).

The relatively high Ca content of some forages and byproduct feeds can make dietary restriction of Ca difficult. Furthermore, the concentration of Ca in the diet is not as important a factor in occurrence of milk fever as other macromineral cations such as K, Na and Mg (Goff and Horst, 1997b; Lean et al., 2006). Whether or not there is an interaction between DCAD and Ca has been questioned (Goff and Horst, 1997b; Oba et al., 2011; Goff and Horst, 2012). In a classic study, Goff and Horst (1997b) were the first to directly assess the effects of increasing dietary Ca and K (that is increasing DCAD) on hypocalcemia. They found no change in incidence of milk fever of multiparous Jersey cows with 0.5% vs. 1.5% Ca in the close-up diet. However, hypocalcemia increased when greater concentrations of Na and K were incorporated into the diet regardless of Ca concentration. This response demonstrated clearly that the overall effect of DCAD, independent of dietary Ca, greatly influenced occurrence of hypocalcemia. The more influential effect of Na and K compared with Ca on incidence of milk fever was later confirmed by meta-analysis (Lean et al., 2006). They also observed increased milk fever when close-up diets had greater Ca (0.5 vs. 1.0%). An even more significant increase in milk fever was observed when cows were fed close-up diets with >1.5% Ca. In their study, length of time of feeding high Ca diets and few data with high Ca close-up diets may be contributing factors explaining why dietary Ca did not affect incidence of milk fever. Higher dietary Ca might be beneficial if fed longer than 20 d (Lean et al., 2006), though a 14-d period was a long enough to show a beneficial effect of high Ca in another study (Oba et al., 2011). From these studies it appears that a Ca inclusion rate of 1.0% in close-up diets is sufficient to maintain periparturient blood Ca provided that an anionic diet is fed.

Calcium and DCAD

The aforementioned difference in response to DCAD rather than dietary Ca could be explained in a couple of ways. Limiting Ca in a positive DCAD diet causes some degree of hypocalcemia and stimulates PTH to increase blood Ca before calving (Thilsing-Hansen et al., 2002). On the other hand, the mild metabolic acidosis that results from a negative DCAD increases hypercalciurea (Vagnoni and Oetzel, 1998). Thus, feeding greater Ca with anion supplementation could aid in maintaining blood Ca concentrations around calving. Oba et al. (2011) examined both of these concepts by supplementing diets with moderate or low Ca (0.9% vs. 0.3%) with DCAD of -6.4 or +9.1 mEq/100 g and challenging cows with intravenous EDTA (a blood Ca-chelator) infusion. The time for cows to recover to 90% of their pre-challenge blood ionized-Ca concentration was shorter for those fed +9.1 mEq/100 g DCAD with 0.3% dietary Ca and for those fed -6.4 mEq/100 g DCAD with 0.9% Ca; whereas, the time for cows to reach their pre-challenged ionized-Ca status was longer for those fed +9.1 mEq/100 g DCAD with 0.9% Ca as well as those fed the -6.4 mEq/100 g DCAD with 0.3% Ca. A significant interaction of dietary Ca% by DCAD on time to recover to 90% of pre-challenge ionized blood Ca also was detected. Conversely, Goff and Horst (2012) found no advantage in Holstein cows' ability to increase blood Ca when fed 1.5% Ca compared with 0.5% Ca while on negative DCAD diets (-6.1 and -6.8 mEq/100 g). Also, Chan et al. (2006) noted no change in blood Ca with anion supplementation (-6 mEq/100 g) and either moderate (0.99%) or high (1.5%) dietary Ca. These findings support the idea that the DCAD in the close-up diet is much more influential on development of hypocalcemia than dietary Ca concentration *per se*.

Perhaps the differences in recovery time of blood ionized-Ca in response to DCAD with varying dietary Ca found by Oba et al. (2011) was actually due to differences in DCAD. Theoretically, the treatment group (+9.1 mEq/100 g with 0.9% Ca) that had the greatest recovery time also would have the greatest DCAD value due to increased Ca inclusion.

Calcium Status and Milk Yield

Besides troublesome health effects associated with periparturient hypocalcemia (Curtis et al., 1983), lactation performance can be impacted by Ca status in early lactation. Kimya et al. (2005) reported increased milk production of both primiparous and multiparous cows (1.7 and 3.9 kg/cow per d, respectively) when blood Ca status was maintained on a low Ca diet (0.46%). Additionally, milk Ca, P, and Mg were not affected by changes in close-up Ca concentration (0.46% vs. 0.86%) for any parity. In another study where normal blood Ca was maintained around parturition and moderate or high Ca was fed prepartum (0.99% vs. 1.5%) there was no difference in milk yield or composition (Chan et al., 2006). Interestingly, Jawor et al. (2012) reported increased milk yield of hypocalcemic cows during the first few weeks of lactation. The biggest difference was in third lactation cows where milk yield remained greater for hypocalcemic compared with normocalcemic cows through 280 DIM before declining. However, other researchers observed no differences in the milk yield of hypocalcemic compared with normocalcemic cows (Martinez et al., 2012). In another study, milk yield was reduced by hypocalcemia (-3.2 kg of milk/cow per d; Chapinal et al., 2012). These differences in lactational performance among various studies demonstrate the importance of proactive approaches for monitoring and treating hypocalcemia.

Magnesium and Calcium

Magnesium is extremely important in homeostatic mechanisms to correct hypocalcemia. Magnesium deficiency impairs PTH secretion and PTH receptor response resulting in blunted Ca mobilization and hypocalcemia (Johannsson and Raisz, 1983); thus, exacerbating and predisposing hypocalcemic conditions around parturition. Cows that do not maintain normocalcemia around parturition had reduced blood Mg concentrations as well (Martinez et al., 2012). Range of normal plasma Mg concentration is 1.8 to 2.4 mg/dl (0.75 mM to 1.0 mM; Goff, 2008). Whereas, Mg did not have a strong influence on predicting acid-base response to varying DCAD (Charbonneau et al., 2006; Lean et al., 2006) dietary Mg concentration is thought to influence occurrence of milk fever. An increase in dietary Mg concentration (0.3 to 0.4%) greatly reduced milk fever incidence (Lean et al., 2006). The recommended concentration of dietary Mg is 0.35 to 0.40% (Goff, 2008).

An interaction between high dietary Ca and Mg absorption may exist. Close-up cows fed 1.36% Ca and 0.18% Mg had lower apparent Mg digestibility and urinary Mg excretion compared with cows provided 0.45 or 0.90% Ca (Kronqvist et al., 2011). It should be noted that prepartum DCAD was positive (~20 mEq/100g); based on urinary Mg excretion all cows in this study were subclinically hypomagnesmic. In another study, blood Mg was not changed when 55 to 91 g of Ca/cow per d (0.46 to 0.86% Ca) were provided with 0.18% dietary Mg (Kamiya et al., 2005). In that study the DCAD was not reported and there was no increase in blood PTH or bone resorption markers.

VITAMIN D

Vitamin D Activation and Receptor Regulation

Vitamin D metabolites such as 1,25 $(OH)_2$ D_3 have a variety of important physiological roles such as cellular differentiation, Ca signaling (Jones, 2008), and immunological functions (Kimura et al., 2006; Nelson et al., 2010). Failure of these biological functions due to vitamin D deficiency can exacerbate and complicate the effects of hypocalcemia. As a result, risk of secondary health problems such as mastitis, displaced abomasum (Goff, 2008), and poor reproductive performance (Ward et al., 1970) are increased greatly.

Vitamin D is needed when plasma Ca drops below its homeostatic range. Through Ca and PTH signaling the vitamin (hormone) is released from its stores and transported to the liver via binding proteins (Jones, 2008). In the liver vitamin D is hydroxylated, forming 25 hydroxyvitamin D (25OHD). This newly formed vitamin D metabolite is then transported through blood to the kidney where it is fully activated by another hydroxylase and converted to the active form 1,25 $(OH)_2$ D (Jones, 2008).

The role of active vitamin D in intestinal Ca absorption is crucial (Goff and Horst, 2003; Jones, 2008). There are a couple of theories as to how vitamin D status might influence hypocalcemia. Questioned initially was whether or not hypocalcemia could be caused by deficient vitamin D activation to form the active 1,25 $(OH)_2$ D hormone. This was rare with hypocalcemia incidence rate with low 1,25 $(OH)_2$ D occurring in less than 10% of cows (Goff et al., 1989). However, cows afflicted with milk fever in successive parturitions apparently do have delayed 1,25 $(OH)_2$ D production and response. These cows also have greater plasma PTH in the days surrounding parturition compared with cows that have not had a prior incidence of milk fever (Goff et al., 1989). Interestingly, there may be a compounding negative influence of PTH on vitamin D receptors (VDR; Reinhardt and Horst, 1990). Goff et al. (1995a) also observed a slight decrease in VDR at time of calving when PTH activity would be elevated. How exactly PTH and vitamin D

metabolites influence each other and their receptors is not clearly understood. Perhaps by providing enough vitamin D in the prepartum period to stimulate 1,25 $(OH)_2$ D production and VDR up-regulation, the negative effects of PTH at calving would not be so detrimental to VDR numbers and 1,25 $(OH)_2$ D stimulation.

Further action of vitamin D is thought to be indirectly involved in the occurrence of hypocalcemia through VDR regulation around time of parturition (Goff et al., 1995a; Goff et al., 1995b). For vitamin D to control Ca absorption efficiently it is important that VDRs are present in adequate numbers and are effectively stimulated. It was thought that dairy cows with clinical milk may have inadequate numbers of prepartum receptors for 1,25 $(OH)_2$ D, the active hormone needed for Ca uptake. Goff et al. (1995a) showed a significant increase in colon VDR numbers in pregnant compared with non-pregnant Jersey cows, suggesting preparation for lactational demands. However, there was no significant change in receptor numbers in the time immediately around parturition. A slight decrease in receptor numbers at time of calving was observed; although the reason for this decrease was unclear as complications with hypocalcemia were an issue. Also observed was a delayed increase in VDR numbers of cows with a previous history of milk fever; but, eventually typical numbers were reached. Liesegang et al. (2008) explored the idea of decreased VDR function in third and greater lactation Holstein and Brown Swiss cows compared with first and second lactation cows. There was no difference in intestinal VDR numbers between breeds or as lactation number increased. Importantly, none of the cows experienced periparturient hypocalcemia and all cows were in mid to late lactation at time of sampling. The entire impact of VDRs around parturition is still unclear. There is an obvious link between VDR existence and hypocalcemia (Beckman and DeLuca, 2002), though how they are regulated during the cow's transition period is not entirely clear. Other factors affecting VDRs are thought to include DCAD (Goff et al., 1995a), VDR gene alterations (Deiner et al., 2012), VDR anatomical locale (Beckman and DeLuca, 2002), and glucocorticoids (Hidalgo et al., 2010). Altogether, definitive evidence of the role of VDRs in hypocalcemia of dairy cows has not been fully elucidated.

Controlling Hypocalcemia and Milk Fever

The ability to decrease incidence of hypocalcemia in dairy cows with greater susceptibility by supplementing vitamin D during the close-up period is well documented. Julien et al. (1976) demonstrated that cows of third and greater lactation with a prior history of milk fever were less likely to develop milk fever during their subsequent lactation if injected with 10 million IU of vitamin D_3 1 wk prepartum. Furthermore, Hibbs and Conrad (1960) were able to increase protection against milk fever in the more susceptible third and greater lactation Jersey cows by as much as 38% by supplementing vitamin D (15 and 30 million IU/cow per d) as part of the TMR or in capsule form for a week before calving. However, this protective effect of vitamin D was not always observed (Taylor et al., 2008) and supplemental vitamin D benefits to cows thought to be less susceptible is not well understood. These differences in response may be due to a variety of reasons such as history of milk fever (Hibbs et al., 1976), parity, level of milk production, or length of period of Ca stress (Muir et al., 1968). A low or negative DCAD during the close-up period may very well play an important part in the effectiveness of vitamin D as well (Wilkens et al., 2012).

Supplementation

Whereas it is relatively unlikely for vitamin D to be deficient in most modern dairy farms, there is reason to believe that supplemental vitamin D_3 may be beneficial. Vitamin D_2 and D_3 can be supplied orally or by intramuscular injection. Injection was thought to be more effective as the vitamin would not be degraded in the rumen if given orally (Sommerfeldt et al., 1979). However, Hymøller and Jensen (2010) reported no ruminal degradation of orally supplemented vitamin D_2 or D_3. Drawbacks to using injectable vitamin D are optimal timing relative to actual parturition, labor, and increased possibility of toxicosis. Because vitamin D is a fat-soluble vitamin, it can accumulate in body tissues with frequent or large dose injections and result in vitamin D toxicosis (Littledike and Horst, 1980). Tolerance of supplementary vitamin D by injection can be 100 times less than to oral delivery (Littledike and Horst, 1980).

Vitamin D can be supplemented in its various plant and animal-derived forms (Sommerfeldt et al., 1983; Taylor et al., 2008; Wilkens et al., 2012). It was suggested that vitamin D_3 supplementation is more effective than vitamin D_2 because vitamin D_3 more efficiently increased vitamin D blood metabolites (Sommerfeldt et al., 1983; Hymøller and Jensen, 2010). Vitamin D_3 is thought to be more effective at increasing vitamin D blood metabolites due to differences in absorption mechanisms for D_2 and D_3 in the intestine (Sommerfeldt et al., 1983; Hymøller and Jensen, 2010). Furthermore, Hymøller and Jensen (2011) suggested vitamin D_2 supplementation might have a direct negative influence on D_3 utilization in the body. Thus, supplementing with vitamin D_2 could be misleading when looking at the overall effects of vitamin D supplementation on vitamin D plasma metabolite concentrations. Because vitamin D_3 is the naturally occurring form in animals and has the most biological activity, vitamin D_3 supplementation is considered best.

The most efficacious amount of vitamin D_3 needed by close-up and lactating dairy cows is not clear. In general, 30 IU/kg of BW per d is recommended for cows in late pregnancy and early lactation. For a 1600 lb cow, this is roughly equal to 20,000 IU/cow per d (NRC, 2001). Due to the greater Ca demand of high producing cows, 30,000 to 40,000 IU/cow per d may improve Ca absorption and milk production (NRC, 2001).

Appropriate timing of administration of vitamin D supplementation relative to actual calving date can be difficult. Additionally, length of time of supplementation prior to calving to help prevent hypocalcemia is unclear. Research referenced previously found positive results by D_3 injection for about 1 wk before calving (Julien et al., 1976; Hibbs and Conrad, 1960). Taylor et al. (2008) were able to increase blood 25 hydroxyvitamin D (25-OHD) after calving by orally supplementing 600,000 IU of 25-OHD/cow per d in gel capsules within 6 d of calving, but there was no increase in blood Ca around parturition. It is likely that for vitamin D supplementation to work optimally all dietary and physiological conditions (i.e., acid-base status, dietary Ca, VDRs) must be in alignment. Perhaps dietary supplementation of vitamin D at recommended doses for a longer period of time (e.g., 20,000 IU/d for entire close-up period) would effectively stimulate and up-regulate VDRs thus allowing for more efficient Ca absorption. This could allow adequate time for vitamin D activation and subsequent stimulation of important biological processes.

RELATIONSHIPS WITH HEALTH AND REPRODUCTION

Periparturient Metabolic Disorders

Calcium is a major cellular signal needed for a range of biological functions (Jones, 2008). A classic path analysis by Curtis et al. (1983) showed significant associations between poor Ca status and muscle contraction-associated problems (i.e., dystocia and displaced abomasum) and ketosis. Cows experiencing hypocalcemia spend 10% more time standing but less time at feed and water in the days just before calving (Jawor et al., 2012). With decreased intake and insufficient Ca for proper gut motility, risk of displaced abomasum postpartum increased (Curtis et al., 1983). Furthermore, hypocalcemia predisposes cows to increased concentrations of metabolites associated with ketosis. Both primiparous and multiparous cows fed a close-up diet with a negative DCAD had decreased prepartum and postpartum blood non-esterified fatty acid (NEFA) concentrations (DeGroot et al., 2010). The deleterious side effects of greater blood NEFA concentrations and increased liver triglyceride accumulation might be reduced as a result of the negative DCAD prepartum and suggests a possible reason to provide a negative DCAD diet to close-up heifers (DeGroot et al., 2010). Others did not observe changes in peripartum NEFA concentrations of cows provided an anion DCAD; perhaps changes in NEFA at this time are more likely due to reduced DMI rather than the anionic diet *per se* (Ramos-Nieves et al., 2009). Cows that develop hypocalcemia have greater NEFA concentrations than those that maintain normal blood Ca; and, as a result those hypocalcemic cows are at greater risk of developing ketosis (Reinhardt et al., 2011). Lower postpartum β-hydroxybutyrate (BHBA) concentrations were observed for cows that maintained normocalcemia (DeGroot et al., 2010; Martinez et al., 2012).

Reproduction

In the USDA (2007) survey, approximately 79% of cows in the U. S. were culled for poor reproductive performance. Through better control of periparturient Ca status, subsequent reproductive management and success might be improved. Evidence to support this possibility was first reported by Ward et al. (1970) who observed a decrease in number of days to first ovulation postpartum by feeding 200 g Ca/d prepartum compared with 100 g/d. Additionally, vitamin D supplementation decreased both days to first estrus and days to conception (Ward et al., 1970). Calcium status is not thought to alter estrous cyclicity directly (Ward et al., 1970; Martinez et al., 2012); however, cows that experience difficulty at parturition (i.e., dystocia, stillbirth, and retained placenta) have reduced normal cyclicity (Martinez et al., 2012). Furthermore, cows that maintain normal Ca status around parturition are more likely to have increased pregnancy rate and reduced days open (Martinez et al., 2012). Also, Chapinal et al. (2012) reported a link between incidence of periparturient disease and pregnancy rate. Cows identified as being at greater risk for clinical diseases (i.e., increased blood NEFA and BHBA, and(or) hypocalcemia) postpartum and dystocia had lower odds of pregnancy at first artificial insemination (AI). Cows that maintained normal Ca status around parturition were 1.5 times more likely to conceive at first AI.

Immunity

Relationships between blood Ca concentrations and the cow's ability to combat infections have been established (Kimura et al., 2006; Nelson et al., 2010; Martinez et al., 2012). Calcium is an important second messenger in the immune response. In an attempt to adjust the depleted Ca pool during times of hypocalcemia, peripheral mononuclear cells release Ca from their stores. In a short time these Ca stores are depleted and this limits the immune cells' ability to send Ca as a signal when triggered as an immune response (Kimura et al., 2006). Martinez et al. (2012) linked greater risk of metritis with drops in serum Ca concentrations. Four times as many cows developed metritis if they displayed both birthing problems (e.g., dystocia, twinning, stillbirths, or retained placenta) and subclinical hypocalcemia compared with cows that did not have birthing difficulties and maintained normal Ca status.

Inadequate vitamin D in the transition period compounds problems associated with hypocalcemia by limiting the cow's ability to increase Ca absorption and resorption (Goff and Horst, 2003); thus, prolonging hypocalcemia and subsequent health problems. It has been understood for a while that active vitamin D has a large role in immune function as a signal hormone for monocytes (Reinhardt and Hustmyer, 1987). More recently, vitamin D was shown to be a crucial component of cow monocyte function (Nelson et al., 2010). Previously this relationship between cow monocytes and vitamin D was not known. It is now understood that monocytes not only have the ability to respond to active vitamin D, but also actually produce the hormone (Nelson et al., 2010). The amount of vitamin D needed for appropriate immune responses is unknown; it is simply assumed that the NRC (2001) recommended daily intake

is sufficient for all known biological needs. Given these recent findings, further investigation into supplementation of vitamin D and increased dietary Ca in rations of sick and(or) cows predisposed to hypocalcemia could prove beneficial.

CONCLUSIONS

Subclinical hypocalcemia and milk fever doubtless are detrimental disorders that affect mineral element status, periparturient health, and lactational and reproductive performance of too many dairy cows. Calcium status around parturition can be affected by a variety of factors surveyed in this paper. Utilizing a low or negative DCAD in the close-up ration is a tried and often effective way to increase blood Ca in the periparturient period. However, more proactive approaches (i.e., reconsideration of "normal" blood Ca range, vitamin D supplementation, more enhancement of immune function by modulating Ca status, diets tailored specifically to late pregnant heifers, targeted approaches for predisposed cows) may prove efficacious and should be considered. Together these findings highlight both the direct and indirect importance of Ca status around parturition and how subsequent milk production and future performance and profitability can be affected.

REFERENCES

Beckman, M.J. and H.F. DeLuca. 2002. Regulation of renal vitamin D receptor is important determinant of 1-alpha, 25-dihydroxyvitamin D(3) levels in vivo. Arch. Biochem. Biophys. 401:44-52.

Bigner, D.R., J.P. Goff, M.A. Faust, J.L. Burton, H.D. Tyler, and R.L. Horst. 1996. Acidosis effects on insulin response during glucose tolerance tests in Jersey cows. J. Dairy Sci. 79:2182-2188.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939-2948.

Bushinsky, D.A. 2001. Acid-base imbalance and the skeleton. Eur. J. Nutr. 40:238-244.

Cahn C.M., and S. Line. 2005. The Merck Veterinary Manual. 9th ed. Whitehouse Station, NJ.

Chan, P.S., J.W. West, and J.K. Bernard. 2006. Effect of prepartum dietary calcium on intake and serum and urinary mineral concentrations of cows. J. Dairy Sci. 89:704-713.

Chapinal, N., S.J. LeBlanc, M.E. Carson, K.E. Leslie, S. Godden, M. Capel, J.E.P. Santos, M.W. Overton, and T.F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Charbonneau, E., D. Pellerin, and G.R. Oetzel. 2006. Impact of lowering dietary cation-anion difference in non-lactating dairy cows: A meta-analysis. J. Dairy Sci. 89:537-548.

Curtis, C.R., H.N. Erb, C.J. Sniffen, R.D. Smith, P.A. Powers, M.C. Smith, M.E. White, R.B. Hillman, and E.J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J. Amer. Vet. Med. Assoc. 183:559–561.

DeGroot, M.A., E. Block, and P.D. French. 2010. Effect of prepartum anionic supplementation on periparturient feed intake, health, and milk production. J. Dairy Sci. 93:5268-5279.

Deiner, C., M. Reiche, D. Lassner, D. Grienitz, S. Twardzoik, A. Moesch, P. Wenning, and H. Martens. 2012. Alleic variations in coding regions of the vitamin D receptor gene in dairy cows and potential susceptibility to periparturient hypocalcaemia. J Dairy Res. 79:423-428.

Dishington, I.W. 1975. Prevention of milk fever (hypocalcemic paresis puerparalis) by dietary salt supplementation. Acta. Vet. Scand. 16:503.

Ender, F., and I.W. Dishington. 1970. Etiology and prevention of paresis puerperalis in dairy cows. Page 71 *in* Parturient hypocalcemia. J.J.B. Anderson, ed. Academic Press, New York, NY.

Ender, F., I.W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. Z. Tierphysio. Tierernähr., Futtermittelkd. 28:233.

Goff, J.P., T.A. Reinhardt, R.L. Horst. 1989. Recurring hypocalcemia of bovine parturient paresis is associated with failure to produce 1,25 dihydroxyvitamin D. J. Endocrinology 125:49-53.

Goff J.P., T.A. Reinhardt, and R.L. Horst. 1995a. Milk fever and dietary cation-anion balance effects on concentration of vitamin D receptor in tissue of periparturient dairy cows. J. Dairy Sci. 78:2388-2394.

Goff, J.P., T.A. Reinhardt, D.B. Beitz, and R.L. Horst. 1995b. Breed affects tissue vitamin D receptor concentration in periparturient dairy cows: a milk fever risk factor? J. Dairy Sci. 78(Suppl. 1):184. (Abstr).

Goff, J.P. and R.L. Horst. 1997a. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Goff, J.P and R.L. Horst. 1997b. Effects of the addition of potassium or sodium, but not calcium, to prepartum rations on milk fever in Dairy Cows. J. Dairy Sci. 80:176-186.

Goff, J.P., and R.L. Horst. 1998. Use of hydrochloric acid as a source of anions for prevention of milk fever. J. Dairy Sci. 81:2874-2880.

Goff, J.P. and R.L. Horst. 2003. Role of acid-base physiology on the pathogenesis of parturient hypocalcemia (milk fever) – the DCAD Theory in Principal and Practice. Acta. Vet. Scand. Suppl. 97:51-56.

Goff, J.P., R. Ruiz, and R.L. Horst. 2004. Relative acidifying activity of anionic salts commonly used to prevent milk fever. J. Dairy Sci. 87:1245–1255.

Goff, J.P. 2008. The monitoring, prevention and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176:50-57
Goff, J.P., and R.L. Horst. 2012. Comparison of low versus high calcium "anionic" diets for prevention of hypocalcemia and milk fever. J. Dairy Sci. 95(Suppl. 2):444.
Goings, R.L., N.L. Jacobson, D.C. Beitz, E.T. Littledike , and K.D. Wiggers. 1974. Prevention of parturient paresis by a prepartum calcium deficient diet. J. Dairy Sci. 57:1184-1188.
Grünberg, W., S.S. Donkin, and P.D. Constable. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J. Dairy Sci. 94:727-745.
Hibbs, J.W. and H. R. Conrad. 1960. Studies of milk fever in dairy cows. VI. Effect of three prepartal dosage levels of vitamin D on milk fever incidence. J. Dairy Sci. 43:1124-1128.
Hibbs, J.W. and H.R. Conrad. 1976. Milk Fever in Dairy Cows. VII. Effect of continuous vitamin D feeding on incidence of milk fever[1]. J. Dairy Sci. 76:1944-1946.
Hidalgo, A.A., D.L. Trump, and C.S. Johnson. 2010. Glucocorticoid regulation of the vitamin D receptor. J Steroid Biochem. Mol. Biol. 121:372-375.
Horst, R.L. and T.A. Reinhardt.1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. J. Dairy Sci. 66:661-678.
Horst, R.L., J.P. Goff, and T.A. Reinhardt. 1997. Strategies for preventing milk fever in dairy cattle. J. Dairy Sci. 80:1269-1280.
Hymøller, L. and S.K. Jensen. (2010). Stability in the rumen and effect on plasma status of single oral doses of vitamin D and vitamin E in high-yielding dairy cows. J. Dairy Sci. 93:548-5757.
Hymøller L. and S.K. Jensen. 2011. Vitamin D_2 impairs utilization of vitamin D_3 in high-yielding dairy cows in a cross-over supplementation regimen. J. Dairy Sci. 94:3362-3366.
Jardon, P.W. 1995. Using urine pH to monitor anionic salt programs. Compend. Contin. Educ. Pract. Vet. 17:860–866.
Jawor, P.E., J.M. Huzzey, S.J. LeBlanc, and M.A.G. von Keyserlingk. 2012. Associations of subclinical hypocalcemia at calving with milk yield, and feeding drinking and standing behaviors around parturition in Holstein cows. J. Dairy Sci. 95:1240-1248.
Jones, G. 2008. Pharmocokinetics of Vitamin D toxicity. Am J Clin Nutr. 88(Suppl). 582S-586S.
Johannsson, A.J., and L.G. Raisz. 1983. Effects of low medium magnesium concentration on bone resorption in response to parathyroid hormone and 1,25-dihydroxyvitamin D in organ culture. Endocrinology 113:2294-2298.
Julien, W.E., H.R. Conrad, J.W. Hibbs, and W.L. Crist. 1976. Milk Fever in Dairy Cows. VIII. Effect of injected vitamin D_3 and calcium and phosphorus intake on incidence[1]. J. Dairy Sci. 76:431-436.
Kamiya, Y., M. Kamiya, M. Tanaka, and S. Shioya. 2005. Effects of calcium intake and parity on plasma minerals and bone turnover around parturition. Anim. Sci. Journal. 76:325-330.
Kimura, K., T.A. Reinhardt, and J.P. Goff. 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J. Dairy Sci. 89:2588:2595.
Kronqvist, C., R. Emanuelson, R. Spörndly, and K. Holtenius. 2011. Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J. Dairy Sci. 94:1365-1373.
Lean, I.J., P.J. DeGaris, D.M. McNell, and E. Block. 2006. Hypocalcemia in dairy cows: meta-analysis and dietary cation-anion difference theory revisited. J. Dairy Sci. 89:669-684.
Liesegang, A., K. Singer, and A. Boos. 2008. Vitamin D receptor amounts across different segments of the gastrointestinal tract in Brown Swiss and Holstein Friesian cows of different age. J. Anim. Physiol. Anim. Nutr. 92:316-323.
Littledike, E.T., and R.L.Horst.1980.Problems with vitamin D injections for prevention of milk fever: Toxicity of large doses and increased incidence of small doses. J. Dairy Sci. 63(Suppl.1):89.
Martinez. N., C.A. Risco, F.S. Lima, R.S. Bisinotto, L.F. Greco, E.S. Ribeiro, F. Maunsell, K.N. Galvão, J.E.P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158-7152.
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. 2000. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. J. Dairy Sci. 83:2095-2104.
Muir, L.A., J.W. Hibbs, and H.R. Conrad. 1968. Effect of vitamin D on the ability of cows to mobilize blood calcium. J. Dairy Sci. 67:1046-1050.
Nagy, O., G. Kovác, H. Seidel, T. Weissová. 2001**.** The effect of arterial blood sampling sites on blood gases and acid-base balance parameters in calves. Acta. Vet. Hung. 49:331-340.
National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Washington, D.C.
Nelson, C.D., T.A. Reinhardt, T.C. Thacker, D.C. Beitz, and J.D. Lippolis. 2010. Modulation of the bovine innate immune response by production of 1α,25-dihydroxyvitamin D_3 in bovine monocytes. J. Dairy Sci. 93:1041-1049.
Oba, M., A.E. Oakley, and G.F. Tramblay. 2011. Dietary Ca concentration to minimize the risk of hypocalcemia in dairy cows is affected by the dietary cation-anion difference. Anim. Feed Sci.Tech. 164:147-153.
Oetzel, G.R., J.D. Olson, C.R. Curtis, and M.J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302-3309.
Oetzel G.R., and J.A. Barmore. 1993. Intake of a concentrate mixture containing various anionic salts fed to pregnant, nonlacting, dairy cows[1]. J. Dairy Sci. 76:1617-1623.

Ramos-Nieves J.M., B.J. Thering, M.R. Waldron, P.W. Jardon, and T.R. Overton. 2009. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. J. Dairy Sci. 92:5677-5691.
Reinhardt, T.A., and F.G. Hustmyer. 1987. Role of vitamin D in the immune system. J. Dairy Sci. 70:952-962.
Reinhardt, T.A., and R.L. Horst, 1990. Parathyroid hormone down regulates 1,25 dihydroxyvitamin D receptors (VDR) and VDR mRNA in vitro and blocks homologous up-regulation in vivo. Endocrinology 127:942-948.
Reinhardt, T.A., J.D. Lippolis, B.J. McCluskey, J.P. Goff, and R.L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122-124.
Roche, J.R., D. Dalley, P. Moate, C. Grainger, M. Rath, and F. O'Mara. 2003. A low dietary cation-anion difference precalving and calcium supplementation post calving increase plasma calcium but not milk production in a pasture-based system. J. Dairy Sci. 88:2658-2666.
Sanchez, W.K., and D.K. Beede. 1991. Interrelationships of dietary Na, K and Cl and cation-anion difference in lactation rations. In Proc. Florida Rum. Nutr. Conf. University of Florida, Gainesville. p.31.
Sommerfeldt, J.L., R.L. Horst, E.T. Littledike, and D. C. Beitz. 1979. In vitro degradation of cholecalciferol in rumen fluid. J.Dairy Sci. 62(Suppl. 1):192–193.
Sommerfeldt, J.L., J.L. Napoli, E.T. Littledike, D.C. Beitz, and R.L. Horst. 1983. Metabolism of orally administered [3H] ergocalciferol and [3H] cholecalciferol by dairy calves. J.Nutr.113:2595– 2600.
Spanghero, M. 2004. Prediction of urinary and blood pH in non-lactating dairy cows fed anionic diets. Anim. Feed Sci. Tech. 116:83-92.
Taylor, M.S., K.V. Knolton, M.L. McGilliard, W.M. Seymour, and J.H. Herbein. 2008. Blood mineral, hormone and osteocalcin responses of multiparous Jersey cows to an oral dose of 25-hydroxyvitamin D_3 or vitamin D_3 before parturition. J. Dairy Sci. 91:2408-2416.
Thilsing-Hansen, T., R.J. Jørgensen, and S. Østergaard. 2002. Milk fever control principles: a review. Acta. Vet. Scand. 43:1-19.
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. 1991a. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Dairy Sci. 69:1205-1213
Tucker, W., Z. Xin, and R. W. Hemkin. 1991b. Influence of calcium chloride on systemic acid-base status and calcium metabolism in dairy heifers. J. Dairy Sci. 74:1401-1407.
USDA. 2007. Animal and Plant Health Inspection Service (APHIS). Veterinary Services. National Animal Health Monitoring System (NAHMS), Part 1: Reference of dairy cattle health and management practices in the United States.
Vagoni, D.B., and G.R. Oetzel. 1998. Effects of dietary cation-anion difference on the acid-base status of dry dairy cows. J. Dairy Sci. 81:1643-1652.
van Mosel M., A.T. Van't Klooster, F. van Mosel, and J.V.D. Kuilen. 1993. Effects of reducing dietary $[(Na^+ + K^+) - (Cl^- + SO_4)]$ on the rate of calcium mobilization by dairy cows at parturition. Res. Vet. Sci. 54:1-9.
Ward, G., G.B. Marion, C.W. Campbell, and J.R. Dunham. 1970. Influences of calcium intake and vitamin D supplementation on reproductive performance of dairy cows. J. Dairy Sci. 51:204-206.
Wilkens, M.R., I. Oberheide, B. Schröder, E. Azem, W. Steinberg, and G. Breves. 2012. Influence of the combination of 25-hydroxyvitamin D_3 and a diet negative in cation-anion difference on peripartal calcium homeostasis of dairy cows. J. Dairy Sci. 95:151-164.
Whittaker, J., C. Cuthbert, V.A. Hammond, and K.G. Alberti. 1982. The effects of metabolic acidosis in vivo on insulin binding to isolated rat adipocytes. Metabolism. 31:553-557.