EFFECTS OF FEEDING MODERATE-ENERGY HIGH-FORAGE DIETS WITH REDUCED DCAD FOR TWENTY-ONE OR FORTY-TWO DAYS PREPARTUM ON MINERAL HOMEOSTASIS AND POSTPARTUM PERFORMANCE

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Dedication

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Effects of feeding moderate-energy high-forage diets with reduced DCAD for twenty-one or forty-two days prepartum on mineral homeostasis and postpartum performance

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Abstract

In spite of decades of research, feeding strategies that prevent milk fever and hypocalcemia in the transition cow are not completely understood. Extended negative dietary cation-anion difference (DCAD) feeding in the prepartum diet would allow producers to utilize a one group dry cow pen; reducing social stress on cows while providing protection against hypocalcemia. High-fiber prepartum diets including wheat straw are inherently low in potassium and have been shown to reduce risk of hypocalcemia. Few studies examine effects of inclusion of DCAD lowering feed additives in low-potassium dry cow diets. The combined effects of extended low DCAD in a low-potassium diet remaining unexplored. To test objectives, Holstein and Holstein-cross dairy cows (n = 49) blocked by breed, parity, body weight, body condition score and previous milk production were randomly assigned to one of three treatments 42 d prior to expected calving to evaluate effects of feeding negative DCAD for 21 or 42 d during the dry period on postpartum production and mineral homeostasis. Treatments included: 1) CON, DCAD = +12 mEq/100 g DM, 2) 21-ND, DCAD = +12/-16 mEq/100 g DM, 3) 42-ND, DCAD = -16 mEq/100 g DM. Prepartum diets were similar in nutrient composition, averaging 17.0% CP, 42.0% NDF and 1.5 Mcal/kg DM. Control and anionic diets were achieved using two isonitrogenous protein mixes: 1) 97.5% soybean

meal and 2) 52.8% Bio-Chlor® (Church and Dwight, Franklin Lakes, NJ), 45.8% soybean meal. CON was fed high DCAD prepartum for 42 d. 21-ND received high DCAD for the first 21 d of the dry period, and the anionic diet from d 22 until calving. 42-ND received anionic diet for the entire dry period. Supplementing anions induced a metabolic acidosis reducing urine pH for 21-ND and 42-ND compared to CON. Prepartum DMI was not affected by prepartum anionic supplementation. Postpartum DMI tended to be higher for anionic diets than the control diet (20.1 vs. 18.1 kg/d). Prepartum anionic supplementation significantly affected milk production, with CON, 21-ND and 42-ND averaging 39.1, 45.7 and 43.8 kg/d, respectively. Also, overall postpartum total blood calcium increased with extended feeding of the anionic diet. Blood magnesium through calving was highest for 42-ND. Diets had no effect on postpartum energy-related metabolites or liver composition, however overall means for liver total lipid, liver triglyceride and blood ketone concentrations were highest for CON. These data suggest low DCAD in high fiber diets for 21 or 42 d during the dry period can have positive effects on postpartum mineral homeostasis and production.

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Introduction and Objectives

Calcium (Ca) homeostasis is vital for normal function of the cow, with hypocalcemia (blood Ca < 8 mg/dL) having detrimental effects on skeletal muscle function, smooth muscle function, including gastrointestinal tract motility, and immune function (Daniel, 1983, Hurwitz, 1986, Kimura et al., 2006). The dramatic increase in Ca needs for production of colostrum and the ensuing lactation rapidly depletes the cow's blood Ca pool, leaving the cow susceptible to hypocalcemia and milk fever (blood Ca < 5 mg/dL). Hypocalcemia most recently has been quantified to clinically affect upwards of 10% of multiparous dairy cows and 50% sub-clinically, depending on lactation number (Reinhardt et al. 2011). Feeding reduced dietary cation-anion difference (DCAD) through supplementation of anionic feeds or selection of low-potassium forages remain widely used strategies to prevent hypocalcemia, with 26.7% and 46.9% of producers using these methods, respectively (USDA, 2007). Lowered DCAD (more anions than cations) of the prepartum diet induces a mild metabolic acidosis of the cow with remodeling of bone responsible for providing elements to negate the systemic pH imbalance (Bushinsky, 2000). This action beneficially frees calcium complexed in the bone matrix, making more Ca available to the cow at calving while promoting bone Ca resorption mechanisms that will be further utilized during lactation. Others have reported increased Ca around calving when supplying anions to reducing DCAD during the close-up period (~ 3 wk prepartum) beyond that achieved by feeding low-K ingredients, although additional effects on production were not observed (Moore et al., 2000, Ramos-Nieves et al., 2009).

Accompanying hypocalcemia, maintaining dry matter intake around calving bears significant importance in determining the outcome of transitioning. Of the many things that can influence dry matter intake (DMI), we have identified three factors: pen or diet changes, anion source, and prepartum diet type as management practices that the producer can focus on to improve DMI status in the transitioning dairy cow.

Reduction in DMI frequently occurs leading into calving leaving the cow in a state of negative energy balance as lactation begins. Subsequently, energy that is necessary for milk production is attained through mobilization of adipose tissue in the form of non-esterified fatty acids (NEFA). During periods of elevated NEFA, synthesis of triglycerides (TG) in the liver exceeds the rate at which they can be exported, leading to hepatic TG accumulation (Grummer, 1993). Hepatic steatosis or fatty liver ensues if the animal too heavily relies on her adipose stores. Reduced ability for hepatic gluconeogenesis results as TG accumulates in the liver (Grummer, 1993), with the reduction in glucose output decreasing insulin secretion, leading to greater lipid mobilization and hepatic uptake for production of energy. Greater catabolism of fat to ketone-bodies follows during this time of high energy demand and ketosis can occur (Squires, 2011). Effects of ketosis include loss of appetite, decreased milk yield, and further development of fatty liver. Effects are only exacerbated by hypocalcemia as low Ca inhibits normal function of the gastrointestinal tract, decreasing strength of ruminal contractions, slowing rate of passage and eventually depressing DMI. With the detrimental effects of depressed DMI and fatty liver noted, it is critical to choose management practices that maintain DMI through calving so the cow relies less on her adipose stores and retains normal liver function.

Administration of anionic supplements usually occurs in the close-up period, however, incurring diet changes or moving cows to a close-up pen 3 wks prior to expected calving has potential to generate additional feed adaptation time and social stress on cows with the addition and removal of cows (Nordlund, 2006, 2009). This data shows as cows reestablish a feeding hierarchy after pen changes, displacements from the feed bunk are increased and DMI can be reduced in an already delicate period for the cow. Grouping cohorts of cows with similar calving dates together in one pen, following the "all-in, all-out" strategy for dry cow grouping, ensures that herd dynamics will be more stable at calving and drops in DMI due to social stress will be reduced. When feeding anionic diets, it is also important to consider a palatable source of anions. Anionic salts can be unpalatable at doses needed to induce bone Ca resorption and can reduce prepartum DMI (Oetzel and Barmore, 1993). Modern acidified fermentation products contain an anion source (chlorine) in a protein-rich by-product making them more palatable than their bitter counter-parts, allowing producers a method to feed negative DCAD with less concern of reducing prepartum DMI.

Cows that are overweight during the dry period impose an additional dilemma for producers as they are more predisposed to fatty liver and hypocalcemia (Squires, 2011). Evidence shows that overconditioned cows at calving have greater than twofold more liver lipid accumulation within 1 wk postpartum compared to cows fed to normal body condition (Reid et al., 1986, Grummer, 1993). Addition of wheat straw, or other low-energy forages, in the prepartum diet allows for formulation of moderate-energy high-fiber (MEHF) prepartum diets permitting cows to ad libitum intake while not excessively overfeeding energy. Dann et al., 2006, showed that reducing prepartum energy intake

through addition of wheat straw to the diet or restricting feed intake to more closely meet NRC energy requirements can have positive effects on energy status around calving. Cows receiving restricted energy during the transition period had lower (P <0.05) body condition as calving approached which resulted in less ketone production (P <0.05) and NEFA mobilization (P > 0.05) immediately prepartum with similar results seen postpartum. Postpartum DMI was greater (P > 0.05) for cows with lowered prepartum energy intake which correlated to numerically higher milk yield in the first 10 DIM. However, moderate-energy high fiber (MEHF) diets frequently result in more gradual starts in milk compared to high-energy, lower fiber prepartum diets. Utilizing MEHF diets through inclusion of wheat straw in the prepartum diet can also work to reduce the cationic load on the cow and reduces the amount of anions needed to properly manage DCAD.

Information exists on extended feeding (> 21 d) of reduced DCAD during the dry period (Block, 1984) and negative DCAD in MEHF diets (Siciliano-Jones, 2008); however, none have assessed effects of, or the need for, extended reduced DCAD utilizing modern acidified fermentation products in MEHF diets. Furthermore, this strategy has the ability to allow producers to minimize dry cow pen moves and diet changes, incorporating the “all-in, all-out” management practice to reduce stress received by the cows. Accordingly, objectives for this experiment were to 1) confirm positive effects on mineral homeostasis seen by reducing DCAD in low-cation prepartum diets, as well as, to 2) determine if feeding anionic fermentation products to reduce DCAD in low-cation MEHF diets for the entire dry period would affect mineral homeostasis, energy metabolism and performance of multiparous cows during the transition period in

comparison to traditional 21 d anionic feeding periods. Questions would also be answered related to the cow's ability to compensate and overcome the metabolic acidosis when supplementation of anions extends for > 21 d.

Chapter 1

Review of Literature

A well-managed dry cow feeding strategy provides producers a method to reduce risk of postpartum health complications and lower treatment costs associated with the transition period. Smooth transitioning into lactation is critical and provides the foundation needed for cows to maintain maximum health and reach production potential.

Milk Fever – A Background

Milk fever, synonymous with clinical hypocalcemia, has plagued dairy producers for centuries, with the first occurrence reported in Germany, 1793, although it remained largely a non-concern until the early 19th century as selection for higher milk yielding dairy cows became popular (Dryerre and Greig,1925, Hibbs, 1950). Milk fever is caused by circulating total blood calcium falling below ~5 mg/dL and is due to increased demand for Ca in colostrum and milk synthesis. Susceptibility to milk fever rises as the animal ages, with the risk of developing milk fever increasing by approximately 9% per lactation (DeGaris and Lean, 2008) due to decreased ability to mobilize Ca from bone (van Mosel et al., 1993) and possibly decreased 1,25 dihydroxy-cholecalciferol receptors in the small intestine (Horst et al., 1990, Goff et al., 1995). Developing hypocalcemia puts cows at increased risk of dystocia, retained placenta, ketosis, displaced abomasum, mastitis and even death if timely treatment is not administered (Curtis et al., 1983, Curtis et al., 1985). Nadirs in Ca homeostasis observed by others (Block, 1984, LeClerc and Block, 1989, Goff et al., 1995, Goff, 1998) usually occur within 12 to 24 h after calving; however, sub-clinical hypocalcemia can persist for greater than 1 wk postpartum. The

lag in normocalcemia (~10 mg/dL) is a result of systemic measures to increase Ca resorption from bone taking up to 14 d to adapt to the onset of lactation (Squires, 2003). Quantification of sub-clinical hypocalcemia remains more elusive to grasp as cows don't display obvious visual indicators of the condition as seen in clinical cases, and is often overlooked in many dairy operations. Sub-clinical hypocalcemia is more costly than clinical milk fever cases because it affects a much higher percentage of cows (Oetzel, 2011), and can decrease milk production up to 7% compared to healthy herd mates (Oetzel, 2012). Estimates by Guard, 1996 suggests that approximately 8% of cows that experience sub-clinical hypocalcemia will die due to development of secondary conditions, and that an additional 12% of sub-clinically hypocalcemic cows will be culled.

Hibbs, 1950 offers an intriguing review of research on milk fever from its earliest depictions through the first successful hypothesis on the cause of the perplexing disease. In retrospect, remedies for milk fever prior to Dryerre and Greig's successful determination of the cause of milk fever in 1925 are bizarre and were ultimately dismissed; however they drew much attention in their era. Crude counteractive measures ranged from application of blankets and cayenne pepper as diaphoretics, to bloodletting. Modern science has since allowed researchers to pinpoint factors leading to hypocalcemia through determination of blood Ca and hormone levels around calving, ultimately leading to development of prepartum feeding strategies that aid in maintaining blood mineral homeostasis around calving, as well as many treatments for cows that succumb to milk fever.

Through decades of research, the cause of milk fever is now thoroughly understood (Goff, 2008, Degaris and Lean, 2008), although complete prevention of the disease is still not achievable. More current information reported by producers reiterates that milk fever remains a chronic issue in transitioning dairy cattle, affecting roughly 5% of the herd population (NAHMS, 2007).

Milk Fever – Etiology

Colostrogenesis

Maintaining Ca homeostasis around calving has been identified as an essential aspect of maximizing postpartum health and production. During the dry period cows require ~15 g Ca per day for supplementation of fetal growth and maintenance, although needs upwards of 50 g Ca per day are necessary to counteract blood Ca loss due to colostrogenesis and the onset of lactation (DeGaris and Lean, 2008). Colostrum is the first milk produced by the cow and contains twice as much dry matter as normal milk; supplementing the calf with vitamins and minerals, as well as immunoglobulins, to provide protection against disease until its own immune system becomes fully functional (Davis and Drackley, 1998). In the process of colostrogenesis, approximately 2.5 g Ca are removed from blood to produce each kilogram of the nutrient rich fluid (Horst, 1986); however demand for Ca does not diminish, as milk produced after colostrum remains at a Ca content around 1.5 g/kg milk (Foley and Otterby, 1978). Collectively, the demands of colostrogenesis and milk production induce a lactational osteoporosis which places a significant strain on the cow's skeleton as the main source of replenishing its blood Ca

pool, which results in a skeletal loss of 9-13% in the first month of lactation (Ellenberger et al., 1932).

Parathyroid Hormone (PTH) and Vitamin D

PTH is the main hormone contributing to restoration of blood Ca in hypocalcemic cows. Blood Ca declining from prepartum levels (~10 mg/dL) as colostrogenesis and lactation ensue stimulates secretion of PTH from the chief cells of the parathyroid glands which monitor circulating Ca levels through Ca^{2+} sensing G-protein-coupled receptors (Norman and Litwack, 1997). PTH contributes four biological functions to increase blood Ca: 1) stimulates movement of available Ca in bone fluid to extracellular fluid providing a more rapid mechanism to maintain Ca homeostasis; 2) increases rate of conversion of 25(OH) D_3 to 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$) in the kidney; 3) increases osteoclastic activity and proliferation; 4) increases urinary reabsorption of Ca and urinary excretion of phosphate (Norman and Litwack, 1997, Squires, 2011). Within minutes of PTH secretion blood Ca levels can rise, quickly alleviating the deficiency; although more drastic mechanisms are needed to displace Ca needs for milk production. Binding of PTH to G-protein-coupled receptors in the kidneys stimulates production of active vitamin D. Vitamin D is not active in the ingested plant form (vitamin D_2 or ergocalciferol) or vitamin D_3 (cholecalciferol) form derived from the interaction of sunlight and cholesterol. Hydroxylation in the liver through 25α-hydroxylase forms calcidiol (25-OH-D_3) which then becomes hydroxylated for the second time in the kidneys by 1α-hydroxylase to form the active secosteroid form of the vitamin (calcitriol or 1,25 $(OH)_2D_3$) when stimulated by PTH. Active vitamin D acts principally on the proximal tubule resulting in excretion of phosphate and reabsorption of Ca from the

glomerular filtrate (Norman and Litwack, 1997, Goff, 2008). The excretion of phosphate that follows ensures that less Ca will be lost due to reformation of bone constituents if Ca and phosphate interact. Furthermore, being that blood phosphate and Ca levels are interrelated, the increase in phosphate excretion provides means to protect against an antagonist feedback mechanism that would decrease the PTH-1,25 $(OH)_2D_3$ response for Ca if high blood phosphate levels were present (Norman and Litwack, 1997). When Ca or phosphate are properly maintained in circulation and PTH is low, regulation of 24α-hydroxylase becomes more active in the kidneys forming 24,25 $(OH)_2D_3$, an inactive form of the vitamin.

Calcitriol acts in steroid hormone fashion by passing into the target cell and binding to specific receptors vitamin D receptors (nVDR) mainly in bone, kidneys and intestine (Norman and Litwack, 1997). These complexes are capable of binding a DNA response-elements, allowing up-regulation of calbindin-D and transient receptor potential vanilloid type-6, both of which increase intestinal Ca absorption. Calcitriol works much the same way in the kidneys to up-regulate gene expression for 1α-hydroxylase and reabsorption of Ca.

Lastly, PTH and active vitamin D work in combination to stimulate osteoclast activity and proliferation; however no binding sites for these hormones exist on this type of cell. It is believed that PTH and calcitriol activation of osteoclasts works through interactions with osteoblasts. Binding of the two hormones to osteoblasts activates RANK-Ligand and monocyte colony-stimulating factors which work in unison to increase monocyte differentiation into osteoclasts (Hollinger et al., 2005). Unknown

binding effects of PTH also cause osteoblasts to vacate the bone surface, allowing osteoclasts clean surfaces to bind and resorb Ca (Hollinger et al., 2005).

As the animal ages, the ability to resorb Ca from bone decreases due to reduced number of osteoblasts (Squires, 2011). Production of active vitamin D lessens as the animal becomes older as well. The lower amount of active vitamin D translates to a reduction in intestinal Ca absorption, kidney Ca reabsorbtion and phosphate excretion. Lowered synthesis of active vitamin D in older animals is attributed to an increase in 24α-hydroxylase which inactivates and ceases production of 1,25 hydroxyvitamin D (Squires, 2011).

Metabolic Alkalosis

Feeding excess cations, especially potassium (K) and sodium (Na) induces a metabolic alkalosis of the prepartum dairy cow which can significantly increase the risk for developing milk fever (Ender et al., 1971, Goff and Horst, 1997). Cow's blood is inherently alkaline due to high amounts of cations in forages that are regularly fed in their diets (NRC, 2001). Ca, K, Na and magnesium (Mg) are cations that are all associated with higher risks of milk fever. Metabolic alkalosis blunts the response of the cow to PTH during hypocalcemia (Gaynor et al., 1989, Leclerc and Block, 1989, Goff et al., 1991, Goff, 2008). Decreased responsiveness to PTH is contributed to alteration of the PTH tissue receptor during metabolic alkalosis, resulting in decreases tissue sensitivity to PTH (Goff, 2008). Failure of bone and kidneys to respond to PTH prevents activation of osteoclastic bone resorption and reduction in renal reabsorption of Ca. Conversion of the non-active 25-hydroxyvitamin D to the active 1,25 dihydroxyvitamin D_3 is also impaired

under metabolic alkalosis, and the failure to convert to the active form of vitamin D no longer allows for increased intestinal Ca absorption.

Hypomagnesemia

The Mg-ATP complex is utilized for every ATP-requiring reaction including enzymatic reactions needed to restore normocalcemia (Fontenont et al., 1989). Subsequently, lowered circulating Mg due to high lactational needs (~12 mg/100 ml) can negatively affect Ca homeostasis. Upon binding to bone or kidney tissue receptors, PTH initiates activation of enzymes to produce secondary messengers that lead to pathways stimulating Ca homeostasis (Fontenont et al., 1989, Rude, 1998). Two main enzymes in this pathway, adenylate cyclase and phospholipase C, require binding of Mg for full enzymatic activity (Rude, 1998). Active transport of Mg across the rumen wall is the main route of Mg absorption in ruminants. High-K diets have been shown to increase susceptibility to hypocalcemia by inducing a metabolic alkalosis; however, high ruminal K concentrations also hinder ruminal Mg absorption leading to secondary mechanisms affecting Ca homeostasis. Martens and Schweigel, 2000 report that high ruminal K concentrations work to depolarize the luminal membrane, impairing Mg uptake, although dietary Mg fed to correct levels ensures that Mg uptake will not be limited due to passive transport across intestinal membrane occurring with proper supplementation. To reduce possibility of affecting Mg and Ca homeostasis around calving, selection of forages lower in K for the prepartum diet could effectively reduce risk of the aforementioned issues.

Breed

Certain breeds of cows are recognized to have higher risk of developing milk fever, although the reason for increased susceptibility is not well understood. Breeds

including Jerseys, Norwegian Red breeds and the Swedish Red and White have all been linked with higher prevalence of milk fever (NRC, 2001). Increased amounts of Ca in colostrum from Jersey cows compared to Holsteins has been suggested as the predominant factor predisposing this breed to milk fever (NRC, 2001). Jersey cows have also been reported to have significantly fewer intestinal receptors for 1, 25-dihidroxyvitamin D_3 compared to Holstein cows (Goff et al., 1995). On pasture based operations, Jerseys have been quantified to be 4.96 times more likely to develop milk fever than Holsteins, whereas Holstein Jersey crossbreds were 2.44 times more likely to get milk fever (Roche and Berry, 2006). No research has been conducted to confirm speculations that other European breeds are more susceptible to hypocalcemia; we can only assume mechanisms for increased milk fever in Norwegian Red breeds and the Swedish Red and White are similar to those described for Jerseys.

Milk Fever – Additional Symptoms and Effects

Hypocalcemia negatively affects cows in a variety of aspects, with clinical cases reducing productive life of the dairy cow by an estimated 3 to 4 yrs (Curtis et al., 1983), while costing the dairy $334 per case (Guard, 1996). Cows that develop clinical milk fever are readily diagnosable as they most notably lose nerve and skeletal muscle function, resulting in lateral recumbency or the "downer cow" syndrome of milk fever. Ca is required for normal function of all types (cardiac, skeletal, smooth) of muscle, and if the condition is allowed to worsen, clinical hypocalcemia has been reported to result in death due to inability of the cow to eructate, with the ensuing bloat leading to asphyxiation (Rebhun, 1995). Timely treatment results in death essentially being

avoided; however impairments due to sub-clinical and clinical hypocalcemia are extremely broad and extend much further than loss of skeletal muscle function. Additional symptoms of clinical milk fever can be characterized by loss of appetite, rumeno-intestinal stasis, somnolence leading to coma, heavy respiration and lowered surface temperature, especially of the extremities (Ender et al., 1971). The majority of hypocalcemia occurrences may offer few visible symptoms, if any, and most cases are undiagnosable for producers. These factors make the condition frustrating as it undoubtedly affects profitability of the cow through reduction in DMI and milk yield (Block, 1984, Oetzel, 2012). It is to no surprise that studies on feeding strategies to promote Ca homeostasis through calving still remain an inherent aspect of research.

Milk Fever - Prevention

Dry cow feeding strategies to prevent hypocalcemia have been utilized for many decades. In 1925, Dryerre and Greig were the earliest to correctly hypothesize that milk fever is caused through depletion of Ca stores upon calving. Their ideas were founded on the facts that milk fever was prevalent in strains of cows known for deep milking and the disease more commonly affected dairy cows, as opposed to beef cows. Their conclusion also took into consideration that cows were most susceptible to the disease immediately post-calving when Ca content in milk and milk yield were highest. From these early speculations spawned what have become highly developed strategies to maintain normocalcemia, calculating exact dietary amounts of cations and anions to provoke bone Ca resorption during the dry period. The main goal of feeding a dietary cation-anion difference is to elevate circulating Ca at calving and increased bone resorptive

capabilities in hopes to prevent deleterious effects of clinical and sub-clinical hypocalcemia postpartum.

Although numerous treatments were developed shortly after the causes of milk fever were detected, utilizing feeding strategies to promote Ca homeostasis promotes Ca homeostasis and results in less treatment of clinically ill animals. Utilizing feeding strategies that protect against sub-clinical hypocalcemia, saving producers much needed time and money, became the most widely used methods to fight this transition disorder.

Vitamin D

In early research on milk fever, the mechanisms of action for vitamin D were not known; however, the correlation between vitamin D and increased ability for Ca homeostasis had been linked. Five years after his discovery of the cause of milk fever, Greig's research focused on supplementation of vitamin D during the prepartum period as a feeding strategy to prevent milk fever. By feeding 50,000 I.U. of vitamin D per day for 3 wk prepartum, Greig was able to increases serum Ca for a period of 9 d however, after 9 d of supplementation serum Ca levels returned to normal and eventually fell below homeostatic levels after administration of the vitamin was stopped (Greig, 1930, Hibbs, 1950). Continuing on this idea, Hibbs et al., 1946 increased level of vitamin D to 1-5 million I.U. per day for up to 4 wk prepartum in hopes of seeing a larger response in Ca homeostasis at calving. An increase in serum Ca was evident with greater administration of the vitamin, but this feeding strategy was not enough to maintain Ca homeostasis in freshening cows. Unsatisfied with these results still, Hibbs was determined to find an optimal dosage of vitamin D that resulted in milk fever prevention. In 1960, 164 Jersey

cows that had previously experienced milk fever were recruited to determine the effects of 15 to 30 million I.U. of vitamin D per day (Hibbs and Conrad, 1960). Vitamin D supplemented at 20 million I.U. per day for 6 d prepartum resulted in greatest protection against milk fever (82%), and became a recommended feeding strategy to combat milk fever in transitioning dairy cows.

In spite of arriving at an optimal dosage of vitamin D supplementation for prevention of milk fever, toxic and detrimental effects of the excessive administration of vitamin D quickly became evident (Capen et al., 1966), and prevention of milk fever by copious oral or parenteral administration of vitamin D could no longer be recommended (Littledike and Horst, 1982, Goff, 2008). Reasoning for this decision focuses on the fact that the margin between harmful doses and doses needed to prevent milk fever is too narrow. Toxic effects of excessive vitamin D can be noted as early as 10 d after administration and included depressed feed intake, gastrointestinal stasis, calcification of soft tissue, and possibly death (Capen et al., 1966). Current knowledge of this vitamin also suggests that high levels of active vitamin D suppresses PTH secretion, which correlates to results seen by Greig who observed declining serum Ca with extended feeding of excessive vitamin D. These systemic responses to vitamin D supplementation are not favorable for cows approaching calving, and improved feeding strategies exists to promote Ca resorption and absorption at calving. Modern dietary vitamin D recommendations suggest supplying smaller doses of vitamin D (20-30,000 I.U./d) in the prepartum diet in accordance with other strategies to prevent milk fever (Goff, 2008).

Dietary Calcium and Phosphorus

Dietary Ca and P concentrations have long been linked with parathyroid activity and prevention of milk fever. Interestingly, emerging feeding strategies that provided the greatest protection against milk fever included *low* dietary Ca and higher P.

Collip, 1925 was one of the first to positively link parathyroid hormone to increased circulating Ca, while Marine, 1914 had previously described parathyroid gland hypertrophy in fowl receiving low Ca diets consisting mainly of maize. While working with rats, Stoerk and Carnes, 1945 reported increased parathyroid gland size with decreasing dietary Ca: P ratios. The increase in gland size was attributed to lower circulating Ca due to low Ca concentrations of the diets. Continuing research following these researchers' conclusions, Boda and Cole, 1954 designed a trial to test the hypothesis that a low Ca: P ratio of the ruminant prepartum diet could be useful in the prevention of milk fever. If effects of low-Ca, low Ca: P diets followed those of other species, their treatments would result in greater parathyroid size and activity achieving greater Ca homeostatic capabilities and reduced incidence of milk fever upon calving. Over a period of 2 yrs, transitioning cows were fed prepartum diets that varied in Ca: P ratios ranging from 1: 3.3 to 6: 1. The diet that resulted in greatest blood Ca at d 1 and 4 postpartum and no cases of clinical milk fever consisted of the lowest Ca: P ratio, while cows on high Ca: P ratios of 5.9: 1 and 6: 1 had the lowest blood Ca after calving and a milk fever occurrence rate of 30%. Fifteen percent of cows on fed a Ca: P ratio of 1: 1 experienced milk fever. The cause of increased prevention of milk fever was attributed

to hypertrophy of the parathyroid gland induced by feeding low-Ca and high-P diets which increased serum Ca concentrations.

A failure to achieve significant differences in blood Ca at calving in cows fed prepartum diets containing 1: 1 or 2.3: 1 Ca: P ratios led Beitz et al., 1973 to question the efficacy of using Ca: P ratio of the prepartum diet as a method to reduce milk fever. However, their work highlights that researchers (Boda and Cole, 1954) feeding dietary Ca *amounts* significantly below the required dietary levels observed a successful elimination of the disease. Later research suggests that feeding specific amounts of Ca in the prepartum diet rather than P concentration, or ratios of Ca: P is the key to milk fever prevention.

Goings et al., 1974 observed significantly (P <0.05) increased circulating Ca around calving in cows fed prepartum diets deficient in Ca compared to cows fed a control diet with greater amounts of Ca. No occurrences of milk fever were observed in the treatment group, however, five of ten cows in the control group experienced clinical milk fever. Cows fed a Ca deficient diet had higher circulating PTH during the prepartum period compared to the control group. Evidence suggests increased PTH due to low blood Ca caused increased mobilization of skeletal Ca to meet new Ca demands brought on by feeding a Ca-deficient diet (Goings et al., 1974). Feeding a Ca-deficient prepartum diet that stimulates PTH secretion, bone Ca resorption, and intestinal absorption can significantly reduce the lag time needed to adapt to lactational Ca demands upon calving, lessening the risk of milk fever (Goings et al., 1974).

A meta-analysis by Lean et al., 2006 reviewed data from 84 trials to develop predictive models for milk fever based on Ca content of the prepartum diet. The data

revealed a parabolic risk effect for milk fever, with the greatest risk present at a prepartum dietary Ca inclusion rate of 1.35% DM. Risks diminish quickly as dietary Ca approaches 0.2 or 2.5% DM. An increase in dietary calcium from 0.5% DM to 1.0 % DM results in a 327% increase in risk for milk fever (Lean et al., 2006). The reason for decreased risk at high dietary Ca concentrations is unclear; although it is likely attributed to greater blood Ca due to passive absorption that occurs with increased dietary Ca concentration (Lean et al., 2006). When feeding high Ca in the prepartum diet, concerns with calcitonin secretion arise, and are the primary argument for increased milk fever risks with high Ca diets (Goings et al., 1974). Requirements for Ca are low in the prepartum cow, and feeding high Ca diets will undoubtedly increase calcitonin secretion to lower blood Ca levels. Calcitonin works by inhibiting bone Ca resoption, and increasing kidney excretion of Ca (Norman and Litwack, 1997). Inhibiting bone Ca resorptive capabilities by feeding high dietary Ca during the prepartum period excludes a main preventative measure that could aid in the cow avoiding hypocalcemia at the onset of lactation. With this being known, optimal Ca concentration of the prepartum diet remains a controversial topic with many opinions on the best diet concentration.

DCAD Diets

Aside from dietary Ca and P amounts, lowering the dietary cation anion difference of the prepartum diet has been proven to minimize risk of milk fever and hypocalcemia (Block, 1984). Ender et al., 1971 recognized extreme alkalinity of the prepartum diet as a main cause of milk fever and were one of the first to attempt to reduce it by supplementing anions to the prepartum diet. To test their hypothesis,

researchers administrated mineral acids (HCl and H_2SO_4) during the 3 wk period before calving, minimizing alkalinity of the prepartum diet, and were able to reduce milk fever drastically. Twenty-four of twenty-six cows avoided milk fever when fed the prepartum diet including mineral acids. Further testing of this diet allowed the researchers to prevent milk fever in six of seven multiparous cows that had been suffering from milk fever in previous lactations (Ender et al., 1971). To calculate alkalinity of the prepartum diet, researchers subtracted the amount of anions (S and Cl) from the amount of cations (K and Na), represented by the following equation: DCAD (mEq/100g /DM) = (Na + K) – (Cl + S). Interestingly, this equation remains the optimal equation to calculate DCAD that results in the best prevention of milk fever without negatively affecting cows (Charbonneau et al., 2006). A new equation (mEq/100g /DM) = (Na + K) – (Cl + 0.6 S) was recently proposed which was more highly correlated with milk fever prevention, however, this equation also lead to unpalatable diets and large decreases in DMI prior to calving (Charbonneau et al., 2006). When calculating DCAD, optimal levels that result in greatest prevention of milk fever are between -10 and -15 mEq/ 100g /DM (Charbonneau et al., 2006). Feeding DCAD at this level requires supplementation of anions, which results in a mild metabolic acidosis. Blood pH is highly regulated; however, excretion of H^+ ions in the urine to compensate for the metabolic acidosis gives producers a method to accurately access effectiveness of feeding negative DCAD feeding. The urine pH predicted to give the greatest protection against milk fever has been estimated to be 6.2 to 6.8 (Goff, 2008). The mild metabolic acidosis that occurs when feeding negative DCAD diets beneficially causes an increase in circulating Ca. To compensate for the acidosis the bone complex dissociates to accept H^+ ions, freeing

mainly Ca, P and carbonate, while bone Na and K also exchange for H^+ ions (Bushinsky, 2000). Manipulation of prepartum DCAD has been proven to reliably increase circulating Ca prior to calving and increases osteoclastic activity, lessening the lag time needed to meet lactational Ca demands and reducing the risk of milk fever upon calving.

Lowering DCAD

Many strategies exist to lower the DCAD value of the prepartum diet, with the least invasive method being selection of low-cation forages. As previously mentioned, prepartum diets high in K can be detrimental to maintaining Ca homeostasis around calving, and choosing feeds to lower the K and cation load of the prepartum diet is a preferred method to reduce DCAD. By using this method, producers do not need to supply an anion source in the prepartum diet which have the potential to reduce feed intake and increase feed costs. Utilizing this method singularly is an acceptable method for producers experiencing relatively minimal effects of hypocalcemia in their herd; however, for herds with more serious hypocalcemia issues, anions must be supplemented to achieve DCAD levels that correlate to greatest protection against hypocalcemia.

The use of acids to dilute the alkalinity of the prepartum diet was the first method to achieve lower DCAD values (Ender et al., 1971). Block, 1984, continued research on methods to achieve negative DCAD diets after highlighting concerns with previous milk fever prevention strategies, including unavailability of low Ca forages, timing of vitamin D treatment and toxicity, and harmful effects of acid to machinery and user. Block speculated that use of acidogenic minerals ($CaCl_2$ and $MgSO_4$), presently known as anionic salts, could successfully increase blood Ca during the prepartum period via increased bone Ca resorption and intestinal Ca absorption. Cows were offered an anionic

diet containing acidogenic minerals or a cationic diet with no mineral adjustment in a 2 yr switchover design. Cows receiving the cationic diet had an overall milk fever occurrence rate of 47.4% and two deaths attributed to milk fever. No milk fever was seen in cows fed the anionic diet. Cows in the anionic supplemented group maintained significantly ($P < 0.05$) greater blood Ca through calving which led to a 14% greater ($P < 0.05$) milk production over an entire lactation compared to paretic cows. Block's prepartum diets were inherently low in Ca (0.65% DM), however, a great percentage of cows still experienced milk fever in the cation group, proving additional actions must be utilized to prevent milk fever in high producing dairy cattle.

More recent work (Goff and Horst, 1998) agrees that supplemental HCl to lower prepartum DCAD should not be implemented until a less dangerous and corrosive form is available, albeit, anionic salts have become an accepted method to achieve DCAD goals. Shortly after Block, 1984 successfully prevented milk fever by administration of $CaCl_2$ and $MgSO_4$, Oetzel et al., 1988 conducted an experiment to determine usefulness of ammonium based anionic salts, NH_4Cl and $(NH_4)_2SO_4$, as additional options to those previously tested. Cows receiving ammonium salts had higher blood Ca at calving, which reduced milk fever incidence to 4% in comparison to 17% occurrence in the control group. Furthermore, Oetzel, 1991, evaluated effects of six different negative DCAD diets containing an individual anionic salt to determine differences in anion source compared to a control diet. No differences were reported in DMI or blood Ca levels among treatments containing $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, NH_4Cl, or $(NH_4)_2SO_4$. Urine pH was lowest for NH_4Cl and highest for $MgSO_4$. To better understand specific acidifying activity of commonly used anionic salts, Goff et al., 2004

supplied equal doses of NH_4Cl, $CaCl_2$, $CaSO_4$, and $MgSO_4$ for 5 d before analyzing urine pH. Chloride based anionic salts were most potent, lowering urine pH more so than sulfate based anionic salts. These findings allowed producers and nutritionists to fine tune diets to more accurately meet mineral and DCAD needs.

Palatability has always been of particular concern when feeding anionic salts in the prepartum period due to their bitter tastes and ammonium chloride conversion to ammonia at high dietary pH levels, which is irritating when inhaled by cows (Goff, 2008). Reduced prepartum DMI has often been reported when feeding anionic salts (Gaynor et al., 1989, Leclerc and Block, 1989, Oetzel and Barmore, 1993, Joyce et al., 1997, Vagnoni and Oetzel, 1998, Moore et al., 2000). A meta-analysis by Charbonneau et al., 2006 reported a 1.3 kg/d loss in dry matter intake when decreasing DCAD by 30 mEq/ 100g DM, the amount needed to obtain optimal negative DCAD on most farms. Newer anionic fermentation products derived from a fermentation processes making the food additive monosodium glutamate (MSG) give producers a more palatable option to deliver anions. The fermentation effluent is high in Cl and is applied to a substrate consisting mainly of wheat middlings to create the anionic feed product. Products such as Bio-Chlor® and Soy-Chlor® deliver anions at a rate of -3 mEq/ 100g as-fed and are incorporated into a protein source making the product more palatable to cows. Recent studies have shown that inclusion of acidified fermentation products at rates to prevent milk fever are less detrimental on DMI and can improve Ca homeostasis around calving and postpartum production (Siciliano-Jones et al., 2010, DeGroot et al., 2010).

In the decades of research pertaining to milk fever, minimal studies have followed ideas regarding effects or the need of feeding negative DCAD in high-fiber low-K diets,

such as those containing wheat straw. Ramos-Nieves et al., 2009 recently identified this issue as wheat straw inclusion in dry cow diets to reduce the energy density has become more popular. They observed detrimental effects of anionic supplementation, although, anionic salts were used, in partial, to reduce DCAD. Only Siciliano-Jones et al., 2008, assessed effects of feeding negative DCAD MEHF diets solely relying on acidified fermentation products to achieve optimal DCAD. Results are favorable showing increased blood Ca and increased production in multiparous cows receiving the anionic diet.

Research has been done to identify optimal dietary Ca levels when utilizing negative DCAD strategies. Chan et al., 2006, fed Ca at 0.99% or 1.50% DM in coordination with negative DCAD, however no effect of dietary Ca on DMI, blood Ca or milk production was observed. Meta-analyses on negative DCAD studies suggest feeding lower dietary Ca (< 0.9% DM) in order to decrease risk of milk fever (Goff, 2008, DeGaris and Lean, 2008). Most recently, Goff, 2012 presented unpublished work done in his lab showing no effect of dietary Ca in negative DCAD prepartum diets on postpartum mineral homeostasis or performance of dairy cows. Goff suggested feeding Ca during the prepartum period at 0.8 % DM to stimulate bone Ca resorption mechanisms.

Length of Administration

Length of administration has always been in question when supplementing anions. Block, 1984 reported increased Ca and increased postpartum production from cows receiving anionic salts for 45 d prepartum, however, increased blood Ca around calving and reduction of milk fever in cows receiving anionic supplementation for only

21 d prepartum (Oetzel et al., 1989) led to feeding strategies that incorporated anionic diets usually beginning in the 3 wk period prior to calving. This practice has stuck due to concern that cows receiving anionic supplementation for greater that 21 d prepartum will systemically compensate for the metabolic acidosis, negating beneficial effects of the DCAD program (Lean et al., 2006). There is also speculations that greater the 21 d exposure to negative DCAD diets will gradually deplete bone Ca stores, leaving the cow more susceptible to milk fever (Lean et al., 2006). With this being said, few studies assess the risk of increased exposure to negative DCAD diets. DeGaris et al., 2008 used predictive models to estimate milk production from cows as exposure to prepartum diets with negative DCAD was extended. Results suggest that milk production would be negatively affected as days exposure to the prepartum diet increased. Results from a connected study suggest that extended feeding of negative DCAD prepartum diets would have no effect of blood Ca (DeGaris et al., 2010). Intriguingly, a majority of studies that report increased production when feeding prepartum negative DCAD diets do so for greater than 21 d prior to calving (Block, 1984, 45 d, DeGroot et al.,2010, 28 d). In addition to showing promising results on Ca homeostasis at calving and increased postpartum production, feeding a negative DCAD diet for the entire dry period would require less diet changes or pen changes as are needed if 21 d feeding programs are utilized.

Primiparous vs. Multiparous Cows

First lactation heifers are at less risk of developing milk fever due to lower colostrum production and milk production, and may not benefit from prepartum anionic supplementation. Rates of clinical hypocalcemia and sub-clinical hypocalcemia in

primiparous cows have been reported to be 1% and 25%, respectively (Reinhardt et al., 2011). On most farms, primiparous cows will be placed in a common pre-calving pen comingled with multiparous cows. Data exist showing reduced postpartum DMI and milk production from primiparous cows supplemented with anions prepartum (Moore et al., 2000, Siciliano-Jones et al., 2008). Interestingly, increased blood Ca was observed around calving in these cows (Moore et al., 2000), suggesting factors related to Ca homeostasis might be responsible for depressions in DMI and milk production. DeGroot et al., 2010 report contrasting data, observing no effects of anionic supplementation on Ca homeostasis, DMI or production. Chan et al., 2006 reported DMI values similar to DeGroot et al., 2010 in Holstein heifers fed negative DCAD prepartum diets. Feeding of anionic salts and acidified fermentation products have both resulted in adverse effects on primiparous postpartum performance (Moore et al., 2000, Siciliano-Jones et al., 2008). At this point there is not enough evidence to confidently state that negative DCAD prepartum diets can deleteriously affect heifer performance. Detrimental effects on postpartum performance of heifers receiving prepartum negative DCAD diets seen by these researchers might be in part to variation among on farm management practices or feed composition of the prepartum diet.

Dry Cow Management Strategies

Pen Moves

In modern dairies, cows move between pens depending on management practices, stage of lactation and special handling requirements (Nordlund et al., 2006). Grouping situations that appear to be most detrimental to cow health are those when cows are faced

extended stays with frequent arrival of new cows (Nordlund et al., 2006). Upon arrival of new cows, reestablishment of the pecking order disrupts feeding behavior and decreases feeding time (Hasegawa et al., 1997, von Keyserlingk et al., 2008). This behavior can be particularly harmful in vulnerable time periods for the cow, such as the transition period and this disruption in DMI is seen to be a major contributor to postpartum metabolic diseases, ketosis and fatty liver (Nordlund et al., 2006). It is suggested that new cows entering into a pen on a daily basing is the least desirable grouping strategy that results in the greatest turmoil (Nordlund, 2009). New entries should be at a minimum of a week apart, with the optimal strategy grouping cohorts of cows with similar calving dates in a dry cow pen, utilizing the "all-in, all-out" management practice, so there are no new cows entering the pen through calving (Nordlund, 2009). Suggested methods advocate setting up dry cow pens to hold 15-20% of the herd, taking no longer than one week to fill each pen, leaving these groups intact until calving (Nordlund, 2009).

This strategy is complicated for producers feeding a traditional negative DCAD diet during the last 3 wk before calving, as it requires more pen space and diets for far-off dry cows and close-up dry cows. If extending feeding of the negative DCAD diet through the entire dry period proves successful, producers could feed one diet to dry cows, while utilizing optimal dry cow grouping strategies, promoting healthy herd dynamics and maintaining DMI as calving approaches.

Conclusion

A lack of knowledge on effects of extended feeding of negative DCAD diets on the prevention of milk fever is evident. Moreover, to maintain proper BCS at calving and avoid complications associated with obese transition cows, producers have started diluting the energy density of the prepartum diet with high-fiber feeds. Often times these feeds, such as wheat straw, are inherently low in cations, lowering the DCAD of the diet. The effects of feeding negative DCAD in low-cation MEHF diets is minimally explored, and research is warranted to evaluate the combined effects of these diets coupled with extended feeding. If results are positive, producers could eliminate far-off dry cow groupings and more easily utilize grouping strategies that promote healthy herd dynamics while providing protection against hypocalcemia.

Materials and Methods

Animal Housing and Management

The experimental protocol was reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC# 1008A87152). Sixty multiparous Holstein and Cross-bred (Holstein x Montbeliard x Swedish Red) dairy cows housed at the University of Minnesota Dairy Research and Teaching Facility (St. Paul, MN) were assigned to 3 treatments in an optimized design. Cows were housed in a tie-stall barn on rubber mattresses bedded with sawdust daily. Cows were fed once daily prepartum at approximately 1100 h. After calving all cows received a common lactation diet (CLD, Table 2, 3), and were fed twice daily at 0600 h and 1200 h. Feed was offered approximately 10 and 90% of daily allocation for the morning and afternoon feeding, respectively. Cows were milked twice daily at 0200 h and 1400 h.

Assignment to Treatments

Immediately prior to the start of the trial individual cow measurements were collected and used to balance treatments by previous 305 mature equivalent, body condition score and body weight at dry-off, parity and breed (Table 1). On d -42 prior to expected calving date, cows were placed on one of three prepartum treatments until parturition.

Treatments

Positive and negative DCAD diets (Table 2, 3) were utilized to create three prepartum treatments. Altering the ingredient composition of the prepartum protein

mixes (Table 2) was used to obtain the desired dietary DCAD (Table 3). CPM Dairy (Version 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) was used to formulate diets that supplied adequate NE_L and MP for 650 kg dry cows 280 days in gestation. DCAD (DM basis) of the prepartum diets was determined through weekly collection of individual ingredients which were composited by month and analyzed for mineral content (AOAC, #985.01) in addition to nutrient analysis. Nutrient and mineral profiles of each ingredient were input into CPM Dairy to calculate DCAD for each treatment on a monthly basis. Treatments included: 1) 42 d control (CON, DCAD +123 mEq/kg DM), 2) 21 d Bio-Chlor® (21-ND, DCAD +123/-158 mEq/kg DM) and 3) 42 d Bio-Chlor® (42-ND, DCAD -158 mEq/kg DM). The control group received the positive DCAD diet from d -42 until parturition, with cows on 21-ND receiving the positive DCAD diet from d -42 through d -22 and the negative DCAD diet from d -21 to parturition. The 42-ND treatment group received the negative DCAD diet for the entire dry period.

Sample Collection and Preparation

Feed Collection and Analysis.

Individual feed ingredients comprising the dry cow diets and lactation diet (Table 2) were collected weekly, composited monthly and analyzed in a commercial lab using wet chemistry procedures (Dairyland Labs Inc., St. Cloud, MN). Ingredient DM percentages were determined weekly to maintain desired diet dry matter content throughout the trial.

Dry Matter Intake, Nutrient Intake, Milk Production and Milk Processing.

DMI was calculated from -42 d relative to expected calving through 56 d postpartum. Individual daily as-fed feeding weights and refusal weights were collected from a Data Ranger® feed cart. Daily amounts of feed offered and refused were recorded and multiplied by weekly DM percentage of each cow's respective diet to determine DMI. Daily feed refusals were collected at approximately 0900 h. Energy balance (EB) was calculated (NRC, 2001) for each cow on a weekly basis. All equations used units of megacalories per kilogram. Net energy intake (NE_I) was determined by multiplying DMI by the calculated mean NE_L density of the diet. The NE_L value of each individual feed (Dairyland Laboratory, St Cloud, MN) was used to calculate the mean NE_L content of the diet. Net energy required for maintenance (NE_M) was calculated as $BW^{0.75} \times 0.08$. Net energy requirements for pregnancy (NE_P) were calculated as [(0.00318 × day of gestation – 0.0352) × (calf birth weight/45)]/0.218. Milk net energy requirements (NE_{LAC}) were calculated as (0.0929 × fat % = 0.0563 × protein % + 0.0395 × lactose %) × milk. The following equation was used to calculate prepartum energy balance (EB_{PRE}) was $EB_{PRE} = NE_I – (NE_M + NE_P)$. The equation used to calculate postpartum energy balance (EB_{POST}) was $EB_{POST} = NE_I – (NE_M + NE_{LAC})$ Milk weights were recorded individually through 56 DIM. Milk samples were collected from individual cows weekly on wk 0 through 8 from consecutive a.m. and p.m. milkings and were preserved (800 Broad Spectrum Microtabs II; D and F Control Systems, Inc. San Ramon, CA) until analysis using mid-infrared procedures (AOAC, 1995) at DHIA (Zumbrota, MN) for fat, protein, lactose, somatic cell count (SCC) and milk urea nitrogen (MUN).

Body Weight and Body Condition Score.

Body weight and body condition score (BCS) were recorded once weekly for wk -6 through 4 relative to calving. BCS was evaluated using a 1-5 scale with 0.25-unit increments (Wildman et al., 1982; Ferguson et al., 1994). Three trained scorers assigned BCS values which were then averaged.

Blood Collection, Processing and Analysis.

Blood was collected from the coccygeal vein or artery into evacuated serum or plasma tubes (SST or trace element; Beckton Dickinson, Franklin Lakes, NJ) before feeding at approximately 0900 h. Serum was collected -28, -21, -14, -7, -3, -1, 1, 3, 7, 14 and 21 d relative to calving. Blood collection tubes were centrifuged at 1,300 x *g* for 20 min, and the supernatant was removed by disposable pipette and immediately frozen at -20 °C until analysis for non-esterified fatty acids (NEFA; 2Hr NEFA kit; Wako Chemicals USA, Inc., Richmond, VA), *beta*-hydroxybutyrate (BHBA; Precision Xtra Meter; Abbott Laboratories, Abbott Park, IL) and ionized calcium (Michigan State Diagnostic Laboratory, East Lansing, MI). Plasma was collected -72, -24, 12, 24 and 72 h relative to calving and processed similar to serum. Plasma was analyzed by inductively coupled plasma atomic emission spectroscopy (ICP, AOAC, 1995) for total minerals: Ca, K, Mg, P, Na, Zn, Cu and Fe (University of Minnesota Soils Laboratory, St. Paul, MN).

Liver Biopsy, Total Lipids Assay, Glycogen Assay and Triglyceride Assay.

Liver samples were collected -14, 7 and 14 d relative to calving at approximately 0700 h via puncture biopsy (Hughes, 1962; Veenhuizen et al., 1991). Before biopsy, local anesthesia was infiltrated in the intercostal space posterior to the tenth rib followed by aseptic preparation of the surgical site with Bedadine®. Liver cores were immediately

flash frozen in liquid nitrogen, transferred to a -80°C storage freezer and awaited analysis for percent total lipids (Hara and Radin, 1978), percent triacylglycerol (Fletcher, 1968; Foster and Dunn, 1973) and percent glycogen (Lo et al., 1970).

Urine Sampling and Analysis.

Urine samples were collected individually by manual stimulation of the vulva on wk -5, -3, -2 and -1 before calving. Urine was collected at approximately 1100 h and analysis was completed within 1 h of sample collection. A portable pH meter (Corning, Model 345) was calibrated weekly and used to determine sample pH.

Health Events.

Cows were observed daily from d -42 prior to calving through 60 DIM. Rectal body temperature was monitored for 7 d following calving and for 3 d following each liver biopsy. Adverse health events were recorded, with unhealthy cows treated by the barn staff or attending veterinarian accordingly to barn SOP.

Calf and Colostrum Data.

When observed, calving ease scores and complications were recorded. Fresh cows were milked last at the next milking session after calving. Colostrum weights were recorded as the first a.m. or p.m. milking weight after calving. Calves were weighed within 24 h after birth.

Statistical Analysis

Statistical analysis was completed using SAS® version 9.2 (SAS Institute Inc., Cary, NC). Data were analyzed as a completely randomized design. Data with multiple measurements over time were processed using the REPEATED statement in the MIXED procedure and analyzed using preplanned contrasts (21-ND and 42-ND vs. CON, and 21-

ND vs. 42-ND) to identify significant differences between treatments on a time interval basis. The model included treatment, time, treatment by time, and breed. Calving date was not included as a block in the model as it did not have significant effects on analysis. Data were analyzed using compound symmetry and auto-regressive order 1 for covariate structures. The covariate structure that resulted in the akaike information criterion closest to zero was used (Littell et al., 1996). Data not analyzed over time were subjected to ANOVA by using the MIXED procedure of SAS (Littell et al., 1996). Least squares means for treatment, week and treatment × week interactions were separated using the PDIFF statement. Prepartum and postpartum data sets were analyzed separately. Significant data were declared at $P<0.05$. Trends are discussed when $P < 0.10$.

Results and Discussion

Final Treatment Distribution

One cow from CON and 21-ND were removed due to unknown health complications 1 wk postpartum. A premature calving (>3 wks early) resulted in one cow being removed from 21-ND. Twinning and resulting complications led to a loss of one cow from CON. Lastly, one cow from 21-ND was removed due to a stomach ulcer. Data from five Jersey sired Holstein-cross cows resulted in mild outliers for milk yield and component yield. Data from this breed was removed from trial due to these affects and because they were unevenly distributed among treatments. Finalized treatment totals after cow removal can be found in Table 1.

Treatment Assignments

Prior to the start of the trial, treatment groups were balanced (Table 1) for previous 305ME (mean ± standard deviation = 11,558.8 ± 1621.5 kg), body weight at dry-off (651.5 ± 96.4 kg), body condition score at dry-off (3.3 ± 0.3 points) and parity (2.0 ± 1.2 lactations). Days dry were similar ($P = 0.93$) among treatments (44.9 ± 7.9 days). Holstein-cross cows had higher ($P = 0.02$) body condition score prior to trial (3.15 vs. 3.36) compared to Holstein cows. Parity, previous milk yield and body weight at dry-off were not different among treatments.

Diet Composition

Diet ingredient composition and nutrient profiles are described in Table 2 and Table 3, respectively. Crude protein concentrations of the dry cow diets were higher than

anticipated. Additional amounts of anions were necessary to maintain a urine pH in the optimal range identified for optimal Ca homeostasis. Ultimately, this led to an increase in dry cow diet crude protein amounts as the DCAD lowering feed additive was blended with the protein source. CP levels of the control diet were adjusted accordingly to keep prepartum diets isonitrogenous.

Urine pH

Urine pH must be assessed when feeding negative DCAD diets. Analyzing urine pH is used as an appropriate measure of blood pH and offers a cheap and accurate method to determine acid-base status (Goff, 2008). When supplementing anions to induce a metabolic acidosis, urine excretion of H^+ increases in attempt to alleviate the imbalance, lowering urine pH. Optimal pH ranges that correlate to greatest prevention of milk fever and hypocalcemia are reported to be between 6.2 and 6.8 (Goff and Horst, 2003, Goff, 2008). As designed, prepartum urine pH of 21-ND and 42-ND were significantly lower ($P < 0.01$) compared to CON (Table 4) while being fed the anionic diet. Urine pH for 42-ND averaged 6.4 with CON averaging 8.2 during the dry period. 21-ND had an average urine pH of 6.9 during the dry period with urine pH being 8.2 during wk -5 and 6.5 while receiving the negative DCAD diet for wk -3 through -1 (Figure 1). Feeding the negative DCAD diet in replacement of the control diet starting at wk -3 relative to parturition helps explain differences observed in average pH when comparing 21-ND and 42-ND as well as the significant trt × wk (trt = treatment) interaction.

Dry Matter Intake and Energy Balance

Prepartum DMI was not different ($P = 0.12$) between cows fed anionic diets for 21 or 42 d and were similar to CON ($P = 0.94$) (Table 4). A significant effect of wk can be explained by an approximately 2 kg drop in DMI in the wk preceding calving (Figure 2). Others feeding acidified fermentation products in MEHF diets containing wheat straw during the dry period have experienced mixed effects on prepartum DMI (Oetzel and Barmore, 1993, Vagnoni and Oetzel, 1998, Siciliano-Jones et al., 2008, Ramos-Nieves et al., 2009, Rezac et al., 2010). Similar to Siciliano-Jones et al., 2008, feeding a negative DCAD high-fiber diet utilizing wheat straw resulted in no differences in prepartum DMI compared to a positive DCAD control diet. Anionic salts supplemented in addition to acidified fermentation products to reduce a high fiber diet DCAD to -15 mEq/ 100 g DM have caused reductions in prepartum DMI (14.4 vs. 15.6 kg/ d) compared to a positive DCAD control diet (Ramos-Nieves et al., 2009). Reduction in prepartum DMI reported by these researchers might be explained by supplementation of anionic salts in addition to acidified fermentation products, which can be unpalatable when fed in amounts needed to prevent hypocalcemia (Oetzel and Barmore, 1993). Albeit, reduction of prepartum DMI has occurred through sole administering acidified fermentation products (Vagnoni and Oetzel, 1998, Rezac et al., 2010). When depressed prepartum DMI was reported by inclusion of acidified fermentation products, an inclusion rate of >6% (DM basis) of the diet was supplied by acidified fermentation products (Vagnoni and Oetzel, 1998, Ramos-Nieves et al., 2009). Acidified fermentation products were supplied at 9.3% (DM basis) in this trial with no detrimental effects on prepartum DMI. These data suggest perhaps other nutritional or management factors led

to decreases prepartum DMI reported by others supplying acidified fermentation products to reduce diet DCAD. In the current study, anions were supplemented at 472 mEq/ kg DM in the negative DCAD diet, well above that (300 mEq/ kg DM) reported by Charbonneau et al., 2006 to have potential to reduce prepartum DMI. This theory can be confirmed by Joyce et al., 1997 who supplemented anionic salts to deliver anions at 471 mEq/ kg DM and reported decreased prepartum DMI. An explanation to why no depression in prepartum DMI was observed in this study could be the addition of acidified fermentation products to reduce dietary DCAD, which are generally accepted as more palatable than anionic salts (Block, 2011, Bowman et al., 2003). Furthermore, cows remained on one diet through the prepartum period and did not experience social stress due to pen movements, possibly contributing to maintained prepartum DMI. 21-ND tended to have higher ($P = 0.06$) DMI on wk -6 in comparison to 42-ND, although reasoning for this increase can be attributed to differences in DMI at dry-off. Postpartum DMI increased (wk = $P < 0.01$) after calving and tended ($P = 0.09$) to be higher for 21-ND and 42-ND compared to CON (Figure 2). 21-ND tended ($P < 0.10$) to have greater postpartum DMI on wk 2, 3, and 4 compared to CON. All treatments followed similar increases in postpartum DMI from wk 1 through wk 4, yielding no trt × wk affect ($P = 0.87$). Prepartum and postpartum EB were not different between 21-ND and 42-ND and were similar to CON (Table 4). Due to the drop in DMI as calving approached, prepartum EB drop from 5.7 to 3.5 Mcal/d (wk = $P <0.001$) 1 wk prepartum. Postpartum EB was most negative the wk following parturition (-10.5 Mcal/d), increasing to -6.7 Mcal/d by wk 4 postpartum. Analyzing data from one wk prepartum through one wk postpartum showed no differences in DMI among treatments, however, numerically

greater postpartum DMI for anionic diets resulted in a tendency ($P = 0.06$) for 21-ND and 42-ND to be in a state of less negative energy balance compared to CON (Table 7).

Body Weight, Body Weight Loss, and Body Condition Score

Prepartum and postpartum means for body weight were similar ($P = 0.88$ and $P = 0.95$) among prepartum treatments (Table 5, Figure 10). Cows from all treatments lost weight at equal rates after calving (trt × wk = $P = 0.63$). Postpartum weight loss through 28 DIM was not different among treatments. Postpartum weight loss was greatest in 42-ND, resulting in 13% and 3% greater losses than CON and 21-ND, respectively. Prepartum BCS was not different comparing anionic diets to CON, but 21-ND tended to have lower prepartum BCS compared to 42-ND (3.1 vs. 3.3, $P = 0.08$). Postpartum BCS was similar between anionic diets and CON, however cows on 42-ND tended ($P = 0.10$) to have greater BCS in contrast to 21-ND.

Circulating Energy-Related Metabolites

Prepartum serum NEFA concentrations were not different among treatments and averaged 228 µEq/ L (Table 9). A significant effect of d is described by prepartum NEFA concentrations for all treatments increasing as calving approached, increasing from 107 µEq/L at d -28 to 425 µEq/ L -1 d prior to calving (trt × d = $P = 0.56$). NEFA levels are reported to be inversely correlated to DMI (Lean et al., 1992), which relates to the reduction in DMI observed as calving approached (Figure 2). Circulating NEFA concentrations during the postpartum period were similar among prepartum treatments. All treatment's serum NEFA concentrations peaked 7 d (671 µEq/L) after calving and returned to pre-calving concentrations by d 21(405 µEq/L). No trt × d interaction was

observed for postpartum NEFA as all treatments followed similar trends. Analysis of postpartum BHBA yielded no significant interactions between anionic diets, however, lower (P = 0.12) concentrations of BHBA were observed in 21-ND and 42-ND compared to CON. Anionic diets had decreasing circulating BHBA from d 1 postpartum through d 14, but circulating BHBA levels in CON increased from d 1 postpartum through d 14 (trt × d = P = 0.56), signifying greater liver oxidative capacity in anionic fed cows, or less reliance on adipose stores for energy due to greater postpartum DMI. Energy-related metabolite results are consistent with others (Moore et al.,2000, Ramos-Nieves et al., 2009) who reported a lack of effect of prepartum anionic supplementation on NEFA and BHBA status during postpartum period. Effects of negative DCAD on energy-related metabolites are likely confined to situations where effects on DMI are observed (Ramos-Nieves et al., 2009).

Liver Composition

Liver composition during the prepartum period was not affected by prepartum treatment; however, anionic treatments resulted in lower postpartum lipid (7.6% vs. 9.8%) and triglyceride (3.8% vs. 5.5%) accumulation in the liver compared to CON, although these interactions were not different (P = 0.12)(Table 9). Prepartum liver triglyceride percent averaged 0.5% during the prepartum period, increasing to 5.5, 3.2, and 4.4% for CON, 21-ND, and 42-ND, respectively, by d 7 after calving. Liver triglyceride percent remained constant for each treatment through d 14 postpartum, resulting in no d or trt × d effects (d = P = 0.21, trt × d = P = 0.88). Prepartum liver glycogen percent averaged 5.6% for treatments. Analysis of postpartum glycogen levels showed a significant (P = 0.04) trt × d effect resulting from higher liver glycogen on d 14

for 42-ND compared to CON and 21-ND. Increasing liver TG results in decreased gluconeogenic capabilities (Grummer, 1993). Greater DMI from anionic diets may have resulted in reduced liver lipid and TG accumulation leading to higher hepatic glucose production and glycogen synthesis.

Milk Production and Components

Milk production and components are shown in Table 6 and Figure 3. In our study, 21-ND and 42-ND produced more milk ($P = 0.01$) for the first eight wks of lactation, on average, than cows receiving the control diet (39.1 kg/d vs. 44.8 kg/d). 21-ND tended to have greater milk for wk 1 postpartum, achieving significantly greater production for the remainder of trial. 42-ND was similar to CON for wks 1 through 3 and tended ($P < 0.10$) to have greater milk than CON wks 3 through 5. 42-ND produced significantly greater milk for wks 6 through 8 compared to CON. No differences in milk production ($P = 0.50$) were observed between cows fed anionic diets for 21 or 42 d, indicating that extended negative DCAD feeding did not have detrimental effects on milk yield. When analyzing data from 1 wk postpartum only, anionic diets had faster starts ($P = 0.03$) in milk, yielding 31.6 kg/d and 29.8 kg/d for 21-ND and 42-ND in comparison to 26.5 kg/d for CON. DMI and milk yield from wk 1 postpartum were positively correlated ($P = 0.07$, $r = 0.27$), stressing the importance of maintaining DMI through the transition period. Block, (1984) was one of the earliest to report increased milk production with extended prepartum anionic supplementation for 45 d prior to expected calving. More recent research provides data to support positive effects of anionic diet supplementation on the subsequent lactation (Siciliano-Jones et al, 2008, DeGroot et al,

2010). Similarities exist among the majority of studies that have reported increased milk in that cows 1) received anionic diets for >21 d prior to expected calving (Block, 1984, DeGroot et al., 2010), and/or 2) DCAD lowering feed additives used were acidified fermentation products (DeGroot et al., 2010, Siciliano-Jones et al., 2010). In 2010, DeGroot et al. reported greater milk yield from cows fed negative DCAD diets prepartum containing acidified fermentation products compared to a positive DCAD control. Cows receiving the anionic diet containing acidified fermentation products also had greater milk production than diets containing anionic salts. Furthermore, Siciliano-Jones et al., 2008 observed increased milk yield; however, offering of the prepartum anionic diet started only 21 d prior to expected calving. Comparably, their diet also included wheat straw as a main ingredient, significantly lowering the energy content of the prepartum diet. Numerically higher blood Ca values were observed for all studies reporting increased milk, which, in partial, can explain milk responses seen, although variables related to rumen efficiency, which have been shown to be positively affected by acidified fermentation products (Lean et al., 2005), were not pursued. Collectively, recent data suggests supplementation of anions through the use of acidified fermentation products to MEHF diets can increase postpartum performance, although increased postpartum blood Ca or improved rumen function, possibly both, cannot be confirmed for causing the positive results. Yield of 3.5% FCM was similar between CON and anionic diets (P = 0.37) and averaged 41.0, 44.1 and 43.2 kg/d for CON, 21-ND and 42-ND, respectively (Table 6). Milk fat yield (kg/d) was similar among all treatments (P = 0.87), although a tendency (P = 0.10) for 21-ND and 42-ND to have higher protein yield was observed. Milk protein yield for treatments followed a similar trend to milk production, increasing

for the first 4 wks postpartum (wk = P = <0.01, trt × wk = P = 0.29). Cows fed anionic diets for 21 or 42 d prepartum produced significantly more milk lactose than cows on the control diet (P = 0.01), and followed similar postpartum increases to those seen in milk production and milk protein yield. Milk lactose yields were 1.7, 2.0 and 1.9 ± 0.1 kg/d for CON, 21-ND and 42-ND, respectively. Milk concentrations of urea nitrogen were highest in anionic diets (P = 0.02), with CON, 21-ND and 42-ND averaging 12.3, 13.3 and 14.1 ± 0.5 mg/dL. A significant effect of wk was caused by increasing MUN concentrations from 12.7 mg/dL at wk 1 postpartum to 13.4 mg/dL at wk 4 after calving. No trt × wk interaction was observed. Others analyzing MUN concentrations when supplementing acidified fermentation products prepartum reported no effect of treatment (Ramos-Nieves et al., 2009). Dairy efficiency was not affected by prepartum anionic diets (P = 0.25), and averaged 2.5, 2.3, and 2.4 for CON, 21-ND, and 42-ND, respectively. Somatic cell score (SCS) was lowered (P = 0.05) by the addition of acidified fermentation products during the dry period, however, SCC was not significantly affected by negative DCAD diets (P = 0.11). SCC was highest at wk 1 postpartum (217,000 cells/mL) declining to 109,000 cells/mL by wk 4 (wk = P = <0.001). Depletion of calcium from peripheral blood mononuclear cells has been shown to suppress immune function in the transitioning cow (Kimura et al., 2006). We speculate that the tendency for increased total serum Ca in 21-ND and 42-ND contributed to the positive effects of anionic diets on SCS through the improvement of postpartum immune function.

Blood Minerals

Diets had no effect on blood mineral concentrations during the prepartum period. Correlations between milk yield, DMI, and blood mineral concentration using data from wk 1 postpartum can be seen in Table 12. Analysis of serum ionized calcium (iCa) and plasma total mineral concentrations can be found in Table 8. Ionized Ca represents the fraction of circulating Ca that is considered free or unbound, and is generally referred to as available Ca. Postpartum iCa was not different (P = 0.28) for 21-ND and 42-ND compared to CON. All treatments increased at similar rates postpartum, increasing from 4.4 mg/dL at 1 d postpartum to 4.9 mg/dL at d 7 (d = P = <0.001). Differences in iCa may have not been observed due to infrequent and late sampling relative to calving (d 1 and 7). More frequent blood sampling within 18 h of calving may have revealed differences in iCa profiles among treatments and is recommended for future transition cow work. More frequent blood sampling within 24 h of calving for total Ca may explain why differences among treatments were detected. Total Ca represents free and bound or complexed Ca in blood. A tendency for anionic diets to have higher (P= 0.07) postpartum total Ca (tCa) than CON was present. Additionally, cows receiving anionic supplementation for 42 d had higher (P = 0.10) tCa than 21-ND (Figure 4). Total Ca levels remained low through 24 h postpartum, however, increases in tCa were observed by 72 h postpartum, resulting in a significant time effect (h = P = 0.001). These data contradict statements from Lean et al., 2006, who speculated the hypercalciuric effect of low DCAD diets would be even more exacerbated if exposure to a negative DCAD prepartum diet was extended beyond 21 d prepartum, leaving lower amounts of available Ca available for mobilization after parturition. All reviewed studies utilizing acidified

fermentation to lower dietary DCAD reported numerically ($P > 0.05$) higher tCa at calving, although only Siciliano-Jones et al., 2008 observed significantly higher postpartum tCa. Nadirs in tCa and occurrence of hypocalcemia by treatment can be seen in Figure 5. All treatments had 90% or greater prevalence of hypocalcemia although 42-ND maintained significantly greater tCa through calving compared to CON and 21-ND. Higher ($P = 0.07$) plasma magnesium (Mg) was observed from 42-ND compared to 21-ND, with CON, 21-ND and 42-ND averaging 1.1, 1.0 and 1.3 ± 0.1 mg/dL, respectively. Figure 6 displays Mg status through calving. After calving, Mg increased through 24 h postpartum and decreased thereafter (h = $P = <0.001$) possibly due to greater milk production as Mg inclusion rates in milk are large (.12 g/ kg) in proportion to their available Mg pool. A large portion (59%) of the body's Mg is stored in bone (Aikawa, 1981) and higher amounts of blood Mg have been reported due to increased postpartum bone resorption when feeding reduced DCAD (Leclerc and Block, 1989). A correlation analysis between postpartum tCa and Mg confirms findings by Leclerc and Block, 1989, as we can report a moderate correlation between circulating levels of these minerals ($P < 0.01$, r = 0.61). Mg contributes to calcium homeostasis as a critical component in the release of parathyroid hormone and synthesis of 1,25-dihydroxycholecalciferol (Lean et al., 2006). Higher circulating amounts of phosphorus were observed prepartum ($P = 0.29$) and postpartum ($P = 0.11$)(Figure 7) in anionic treatments also indicating increased bone remodeling. Calcium and phosphorus are bound together in bone forming hydroxyapatite. When remodeling occurs in cows fed negative DCAD diets, increases in blood concentrations of both minerals has been observed (Block, 1984). Postpartum blood P concentrations followed similar trends to postpartum blood Ca, increasing by 72

h after calving (h = P = 0.01) and were highly correlated with tCa (P < 0.01, r = 0.75). Tendencies for increased K and Na in anionic diets most likely are due to increases in postpartum DMI as K and Na concentrations in bone are very low (Logan, 1935). Postpartum K levels for all treatments was highest 12 h after parturition (75 mg/dL) and decreased to 68 mg/dL by 72 h postpartum (Figure 8). A significant correlation between postpartum K levels and wk 1 milk production was observed (P = 0.02, r = 0.37). Knowing its functions in muscle stimulation, the postpartum increase in K observed from anionic treatments could have contributed to increased muscle function and DMI seen in these treatments, explaining the link between increased milk and greater postpartum K.

Cow Health and Calving Data

Cow numbers were not sufficient to determine differences in prevalence of health disorders. Adverse health events can be seen in Table 10. Treatment means for colostrum yield, calf weight and calving ease score were not affected by prepartum treatment (Table 11).

Conclusions and Implications

In conclusion, our results indicate that feeding negative DCAD (-15 mEq/100 g) for 21 or 42 d prepartum utilizing acidified fermentation products positively affected mineral homeostasis, postpartum DMI, and milk production in multiparous cows with no effect on prepartum DMI. The prepartum anionic diet significantly reduced prepartum urine pH to optimal values correlated with greatest protection against hypocalcemia and resulted in a tendency for increased tCa in 21-ND and 42-ND. We speculate that this increase in tCa contributed to improved smooth and skeletal muscle function, as well as increased immune function, which correlated to greater postpartum DMI, lowered somatic cells and reduced adverse health events in anionic treatments. Higher postpartum DMI in anionic treatments resulted in improved EB and cows relying less on adipose stores for energy, contributing to the lowered BHBA and liver lipid accumulation seen in 21-ND and 42-ND. Lowered liver lipid accumulation allows for greater hepatic gluconeogenic capacity as the liver can focus more on converting glycogen and propionate to glucose and less on catabolism of TG to meet energy needs. Lactose is a major osmoregulator of milk production, and production of lactose relies on the availability of glucose. The speculative increase in glucose from 21-ND and 42-ND may have contributed to the increase in lactose yield, and therefore milk yield, from these treatments. In addition to data from Block, 1984, our results suggest that extending feeding of negative DCAD diets may be needed to consistently achieve greater mineral homeostasis and postpartum performance in multiparous dairy cows.

Furthermore, feeding negative DCAD for the entire dry period allows producers to utilize single pen dry cow grouping strategies, however, research to test the efficacy of

extended exposure to negative DCAD in primiparous cows is warranted, as Siciliano-Jones et al., 2008 observed a tendency for decreased milk in heifers fed a similar negative DCAD diet containing acidified fermentation products for 21 d prior to expected calving. To further confirm results from this research, 42-ND should be evaluated against a negative control, such as extended anionic salts feeding, to determine a truer efficacy of this feeding program. Extended feeding of acidified fermentation products as an option to regulate DCAD in high-potassium dry cow diets still needs to be explored to better understand the effects of length of exposure in different varieties of dry cow diets. Added, future research to evaluate lactation responses seen when administering acidified fermentation products is proposed to separate effects of increased postpartum calcium and enhanced rumen efficiency.

Table 1. Least squares means for finalized treatment assignments for multiparous cows 7 d prior to assignment to prepartum treatment with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value
Variable	CON	21-ND	42-ND	Treatment
Cows, #	19	12	18	---
Holstein	7	5	10	---
Crossbred[2]	12	7	8	---
Previous 305ME[3], kg	11664.7 ± 383.6	12056.4 ± 472.1	11244.3 ± 380.9	0.41
BCS[4]	3.3 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	0.44
Body weight, kg	659.7 ± 23.8	633.1 ± 30.5	659.0 ± 24.0	0.75
Parity[5]	2.0 ± 0.3	1.9 ± 0.4	2.0 ± 0.3	0.94
Treatment length[6], d	44.8 ± 2.0	44.2 ± 2.4	45.4 ± 2.0	0.93

[1]Cows assigned to CON received a prepartum diet with DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]Holstein x Montbeliard x Swedish Red.
[3]305 d mature equivalent milk yield.
[4]BCS = body condition score (1-5 scale, 0.25 unit increments).
[5]Number of previous lactations.
[6]Days dry.

Table 2. Ingredient composition for prepartum and postpartum diets fed to multiparous dairy cows from 42 d prepartum through 56 d postpartum.

	Dry Cow Diets		Lactation Diet
	Negative DCAD	Positive DCAD	CLD[1]
Ingredient	% of Diet (DM basis)		
Corn silage, processed	38.6	38.6	32.7
Wheat straw, chopped	22.8	22.8	0.0
Bio-Chlor® protein mix[5]	17.6	0.0	0.0
Control protein mix[4]	0.0	17.6	0.0
Alfalfa hay, chopped	12.1	12.1	16.1
Molasses-based LF[2]	6.0	6.0	2.6
Dry corn, ground	2.9	2.9	19.8
Lactation protein mix[3]	0.0	0.0	21.3
Cottonseed, whole fuzzy	0.0	0.0	7.5

[1]CLD = common lactation diet
[2]Molasses-based liquid feed, Quality Liquid Feeds, Dodgeville, WI; 63.0% DM, 39.7% sugar, 26.3% ash, 5.9% Ca, 3.5% K.
[3]34.1% CP, 14.1% ash, 8.0% sugar, 3.8% fat.
[4]97.5% soybean meal (48% CP), 1.1% urea (281% CP), 1.4% MgO, 53.9% CP, 14.8% sugar, 7.5% ash, 0.4% Ca, 2.4% K, 0.1% Cl, 0.4% S.
[5]52.8% Bio-Chlor, Arm & Hammer Animal Nutrition, Princeton, NJ, 45.8% soybean meal (48% CP), 1.4% MgO, 49.9% CP, 9.9% sugar, 8.7% ash, 0.3% Ca, 1.9% K, 4.9% Cl, 1.1% S.

Table 3. Nutrient composition for prepartum and postpartum diets (DM basis) fed to multiparous dairy cows from 42 d prepartum through 56 d postpartum (CPM Dairy 3.0.08). Feed stuffs comprising diets were collected weekly, composited by month, and analyzed by wet chemistry to determine actual diet nutrient compositions.

	Dry Cow Diets		Lactation Diets
	Negative DCAD	Positive DCAD	CLD[1]
DM, %	61.13	61.95	63.96
Forage, %	74.09	74.09	50.83
CP, %	16.65	17.33	16.60
RUP, % CP	21.38	19.70	38.89
RDP, % CP	78.62	80.30	61.11
RDP, %	13.06	13.91	10.14
Sol prot, %CP	55.57	47.83	30.94
NE_L, Mcal/kg	1.47	1.47	1.66
ADF, %	28.32	28.10	22.24
NDF, %	42.54	41.35	31.56
peNDF, %	37.25	37.72	23.95
Lignin, %	4.51	4.36	4.48
NFC, %	31.30	32.00	42.84
Sugar, %	6.11	6.82	5.57
Starch, %	12.25	11.03	26.12
Sol fiber, %	10.13	11.34	8.68
EE total, %	2.00	1.83	3.97
Ash, %	9.01	8.80	7.42
Calcium, %	0.80	0.82	0.80
Phosphorus, %	0.41	0.40	0.47
Magnesium, %	0.39	0.39	0.32
Potassium, %	1.42	1.51	1.43
Sulfur, %	0.40	0.29	0.27
Sodium, %	0.18	0.07	0.46
Chlorine, %	1.23	0.42	0.49
Vitamin D, IU/kg	2118.64	2118.64	849.42
Vitamin E, IU/kg	105.98	105.98	42.48
DCAD1[2], mEq/100g	-15.79	12.34	25.68
DCAD2[3], mEq/100g	-6.94	18.85	28.50

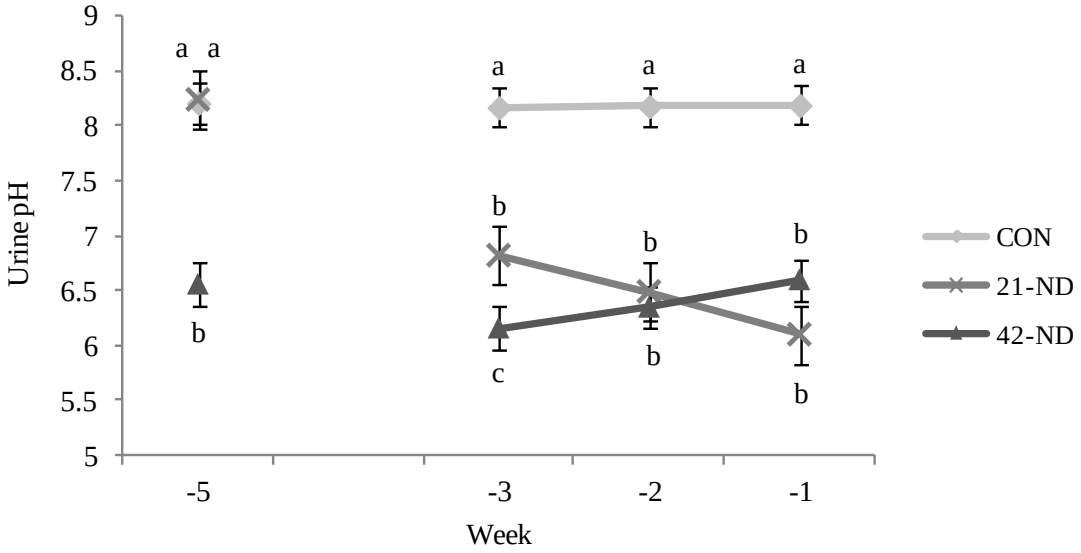
[1]CLD = common lactation diet.
[2]DCAD1 = (mEq/100g) = (Na + K) – (Cl + S).
[3]DCAD2 = (mEq/100g) = (Na + K + 0.38 Ca + 0.30 Mg) – (Cl + 0.6 S + 0.5 P).

Table 4. Least squares means of pre- and postpartum dry matter intake, energy balance and prepartum urine pH from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
	Prepartum				
DMI[3], kg/d	13.7 ± 0.7	14.7 ± 0.9	12.9 ± 0.7	0.94	0.12
DMI[3], %BW	2.0 ± 0.1	2.0 ± 0.2	1.8 ± 0.1	0.72	0.21
EB[4,9], Mcal/d	5.7 ± 1.2	6.0 ± 1.5	3.7 ± 1.2	0.56	0.12
EB[4,10], %Req.	138.5 ± 8.4	139.8 ± 10.6	126.2 ± 8.6	0.61	0.32
Urine pH[5,6]	8.2 ± 0.1	6.9 ± 0.2	6.4 ± 0.1	<0.01	0.01
	Postpartum				
DMI[7], kg/d	18.1 ± 0.9	20.8 ± 1.1	19.4 ± 0.9	0.09	0.36
DMI[7], %BW	2.6 ± 0.2	3.0 ± 0.2	2.8 ± 0.2	0.14	0.33
EB[8,9], Mcal/d	-8.8 ± 1.9	-6.8 ± 2.4	-7.4 ± 1.9	0.48	0.84
EB[8,10], %Req.	76.6 ± 4.0	86.4 ± 5.0	81.6 ± 4.0	0.15	0.46

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]DMI = dry matter intake collected from d -42 through d 0 relative to calving.
[4]EB = energy balance calculated from d -42 through d 0 relative to calving.
[5]Sampled at weeks -5, -3, -2 and -1 relative to calving.
[6]Mean value for 21-ND for weeks -3, -2 and -1.
[7]DMI = dry matter intake collected from d 0 through d 28 relative to calving.
[8]EB = energy balance calculated from d 0 through d 28 relative to calving.
[9]EB = energy intake – energy requirements.
[10]EB = energy intake / energy requirements*100.

Figure 1. Urine pH collected prior to calving from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.



[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
Different superscripts within week differ by $P<0.05$.

Prepartum Standard Errors of the Mean:
SEM = 0.2
Prepartum P-values:
Trt: P = <0.001
Week: P = <0.001
Trt × Week: P = 0.001
21-ND and 42-ND vs. CON: P = <0.001
21-ND vs. 42-ND: P = 0.02

Table 5. Least squares means of pre- and postpartum body weight, body condition score, postpartum body condition loss and postpartum body weight loss from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
	Prepartum				
Body weight[3], kg	708.4 ± 22.2	694.2 ± 28.9	712.3 ± 23.3	0.86	0.63
BCS[3,5]	3.3 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	0.29	0.08
	Postpartum				
Body weight[4], kg	629.5 ± 18.5	622.7 ± 23.2	632.1 ± 18.8	0.93	0.76
BCS[4,5]	2.9 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	0.38	0.10
Weight loss[4], kg	43.8 ± 8.4	44.2 ± 11.0	52.0 ± 8.5	0.70	0.58
BCS[5] loss, points	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.55	0.51

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]Measured d -42 through d 0 relative to calving.
[4]Wk 1 weight – wk 4 weight, measured d 0 though d 28 relative to calving.
[5]BCS = body condition score (1-5 scale, 0.25 unit increments).

Table 6. Least squares means of milk yield, 3.5% fat corrected milk (FCM) yield, milk component concentration, component yield, fat: protein, dairy efficiency, SCC and MUN d 0 through d 28 relative to calving from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
Milk[3], kg/d	39.1 ± 1.7	45.7 ± 2.1	43.8 ± 1.7	0.01	0.50
3.5% FCM[4], kg/d	41.0 ± 2.2	44.1 ± 2.8	43.2 ± 2.3	0.37	0.82
Milk fat, kg/d	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.65	0.84
Milk lactose, kg/d	1.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	0.01	0.51
Milk protein, kg/d	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.10	0.97
MUN[5], mg/dL	12.3 ± 0.5	13.3 ± 0.6	14.1 ± 0.5	0.02	0.35
Fat : protein	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.26	0.61
Dairy efficiency[6]	2.5 ± 0.1	2.2 ± 0.2	2.3 ± 0.1	0.25	0.77
SCS[7]	3.1 ± 0.4	2.1 ± 0.4	2.3 ± 0.4	0.05	0.80
SCC[8]	185.5 ± 37.9	103.3 ± 47.4	109.3 ± 38.4	0.11	0.92

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]Milk through 56 d postpartum.
[4]3.5% FCM = 0.4324 × (kg milk) + 16.2162 × (kg fat), through 28 d postpartum.
[5]Milk urea nitrogen.
[6]3.5% FCM divided by DMI.
[7]Somatic cell score (1-9 scale, 1 = lowest, 9 = highest).
[8]Somatic cell count (1000's of cells).

Table 7. Least squares means of DMI, EB and milk yield from 1 week prepartum through 1 week postpartum from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
			Week -1		
DMI[3], kg/d	13.2 ± 0.9	13.2 ± 1.1	11.2 ± 0.9	0.41	0.18
DMI[3], %BW	1.9 ± 0.1	1.9 ± 0.2	1.6 ± 0.1	0.40	0.21
EB[3,5], Mcal/d	5.1 ± 1.4	4.1 ± 1.8	1.4 ± 1.5	0.22	0.27
			Week 1		
DMI[4], kg/d	14.3 ± 1.0	16.3 ± 1.2	15.3 ± 1.0	0.23	0.52
DMI[4], %BW	2.1 ± 0.2	2.5 ± 0.2	2.2 ± 0.2	0.38	0.38
EB[4,5], Mcal/d	-13.2 ± 1.7	-8.4 ± 1.9	-10.0 ± 1.5	0.06	0.54
Milk[4], kg/d	26.5 ± 1.5	31.6 ± 1.9	29.8 ± 1.5	0.03	0.46

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]Dry matter intake and energy balance from days -7 through -1 relative to calving.
[4]Dry matter intake, energy balance and milk yield from days 1 through 7 relative to calving.
[5]EB = energy intake – energy requirements.

Table 8. Least squares means of serum ionized calcium, total plasma calcium and other blood minerals from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
	Prepartum				
Ca, Ionized[3], mg/dL	4.78 ± 0.1	4.74 ± 0.1	4.81 ± 0.1	0.95	0.49
Ca, Total, mg/dL	7.53 ± 0.4	7.79 ± 0.5	8.17 ± 0.4	0.41	0.58
Cu, mg/dL	0.03 ± <0.1	0.03 ± <0.1	0.03 ± <0.1	0.66	0.88
Fe, mg/dL	0.07 ± <0.1	0.12 ± <0.1	0.09 ± <0.1	0.25	0.26
K, mg/dL	71.58 ± 5.4	82.48 ± 7.0	72.25 ± 5.4	0.41	0.26
Mg, mg/dL	0.98 ± 0.1	1.04 ± 0.2	1.22 ± 0.1	0.26	0.31
Na, mg/dL	255.01 ± 13.6	275.10 ± 17.4	262.03 ± 13.6	0.45	0.56
P, mg/dL	7.60 ± 0.5	8.31 ± 0.6	8.28 ± 0.5	0.28	0.97
Zn, mg/dL	0.03 ± <0.1	0.03 ± <0.1	0.03 ± <0.1	0.86	0.60
	Postpartum				
Ca, Ionized[3], mg/dL	4.60 ± 0.1	4.68 ± 0.1	4.69 ± 0.1	0.28	0.91
Ca, Total, mg/dL	6.52 ± 0.3	6.80 ± 0.3	7.53 ± 0.3	0.07	0.10
Ca, Nadir, mg/dL	5.62[a] ± 0.3	5.68[a] ± 0.4	6.65[b] ± 0.3	0.16	0.05
Cu, mg/dL	0.04 ± <0.1	0.04 ± <0.1	0.04 ± <0.1	0.61	0.78
Fe, mg/dL	0.07 ± <0.1	0.10 ± <0.1	0.09 ± <0.1	0.17	0.83
K, mg/dL	65.97 ± 4.2	76.35 ± 5.1	75.66 ± 4.2	0.07	0.92
Mg, mg/dL	1.06 ± 0.1	1.02 ± 0.1	1.27 ± 0.1	0.48	0.07
Na, mg/dL	243.76 ± 9.3	259.66 ± 11.6	274.65 ± 9.4	0.06	0.32
P, mg/dL	6.66 ± 0.4	7.06 ± 0.5	7.75 ± 0.4	0.11	0.24
Zn, mg/dL	0.03 ± <0.1	0.04 ± <0.1	0.03 ± <0.1	0.75	0.20

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]Ionized Ca was measured at d -7, -3, -1, 1 and 7 relative to calving; total minerals were analyzed at h -72, -24, 12, 24 and 72 relative to calving.

Table 9. Least squares means of energy related metabolites and liver lipid and carbohydrates from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
	Prepartum				
Liver tissue					
Glycogen[3], %	5.4 ± 0.5	5.4 ± 0.7	5.9 ± 0.5	0.69	0.63
Total lipid[3], %	4.3 ± 0.2	4.9 ± 0.3	4.6 ± 0.2	0.19	0.40
Triglyceride[5], %	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.31	0.59
Total lipid: triglyceride	0.1 ± <0.1	0.1 ± <0.1	0.1 ± <0.1	0.17	0.60
Serum					
NEFA[4], µEq/L	233.4 ± 27.5	220.6 ± 34.4	231.2 ± 28.2	0.83	0.82
	Postpartum				
Liver tissue					
Glycogen[7], %	2.2 ± 0.3	2.5 ± 0.3	2.6 ± 0.3	0.38	0.63
Total lipid[7], %	9.8 ± 1.2	6.8 ± 1.5	8.3 ± 1.3	0.12	0.48
Triglyceride[9], %	5.5 ± 0.8	3.2 ± 1.0	4.4 ± 0.9	0.12	0.34
Total lipid: triglyceride	2.8 ± 0.6	2.1 ± 0.7	2.6 ± 0.6	0.48	0.62
Serum					
BHBA[6], mg/dL	13.9 ± 2.0	9.1 ± 2.5	10.7 ± 2.0	0.12	0.72
NEFA[8], µEq/L	584.2 ± 61.5	469.0 ± 76.4	606.3 ± 62.0	0.56	0.17

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]Percent of wet weight from liver samples from day -14 relative to calving.
[4]Non-esterified fatty acid analysis of serum from days -28, -21, -14, -7, -3 and -1 relative to calving.
[5]Percent of TL from day -14 relative to calving.
[6]Beta-hydroxybutyrate analysis of serum from days 1, 7, 14 and 21 relative to calving.
[7]Percent of TL from day 7 and 14 relative to calving.
[8]Non-esterified fatty acid analysis of serum from days 1, 7, 14 and 21 relative to calving.
[9]Percent of wet weight from liver samples from days 7 and 14 relative to calving.

Table 10. Health events recorded d -42 prepartum through d 56 postpartum from multiparous cows fed prepartum diets with positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]		
Variable	CON	21-ND	42-ND
Displaced abomasum	1	0	0
Dystocia[2]	0	2	2
Ketosis	1	0	0
Mastitis	1	0	0
Metritis	2	1	0
Milk fever[3]	0	0	1*
Retained placenta[4]	3	1	1
Twins	1	2	1
Udder edema[5]	7	4	7

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2]1-5 scale (1 = easy, 5 = mechanically assisted pull); score of 4 or higher recorded.
[3]Diagnosed by recumbency.
[4]Placenta retained more than 24 h.
[5]No severity scale used.
*Experienced milk fever in previous lactation.

Table 11. Least squares means of colostrum yield, calf weight and calving ease data from multiparous cows fed prepartum diets with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

	Treatments[1]			P-value	
Variable	CON	21-ND	42-ND	C1[2]	C2[2]
Colostrum[3], kg	6.5 ± 0.8	8.0 ± 1.2	7.3 ± 0.9	0.28	0.64
Calf weight, kg	45.0 ± 1.9	42.8 ± 2.4	44.9 ± 1.8	0.65	0.49
Calving ease[4]	1.7 ± 0.3	1.7 ± 0.4	1.8 ± 0.3	0.87	0.83

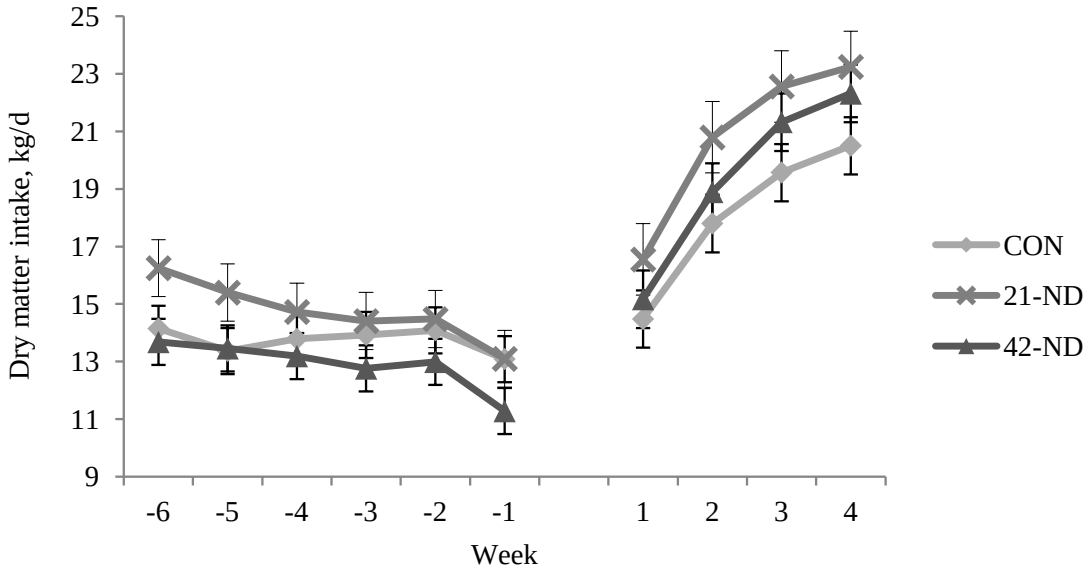
[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
[2] C1: Control vs. 21-ND and 42-ND, C2: 21-ND vs. 42-ND.
[3]First milk weight.
[4]1-5 scale (1 = easy, 5 = mechanically assisted pull).

Table 12. Correlations between DMI, milk yield, and circulating total minerals for wk 1 postpartum from multiparous cows fed prepartum diets with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

Variable	DMI, kg/d	Milk, kg/d	P, mg/dL	K, mg/dL	Mg, mg/dL
DMI, kg/d	~				
	~				
Milk, kg/d	0.27	~			
	0.07	~			
P, mg/dL	0.04	0.15	~		
	0.82	0.36	~		
K, mg/dL	0.09	0.37	0.43	~	
	0.57	0.02	0.01	~	
Mg, mg/dL	-0.09	0.09	0.6	0.46	~
	0.56	0.58	<0.01	<0.01	~
Ca, mg/dL	-0.07	0.12	0.75	0.44	0.61
	0.68	0.46	<0.01	0.01	<0.01

[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.
Top box = pearson's correlation (r).
Lower box = p – value.

Figure 2. Dry matter intake from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

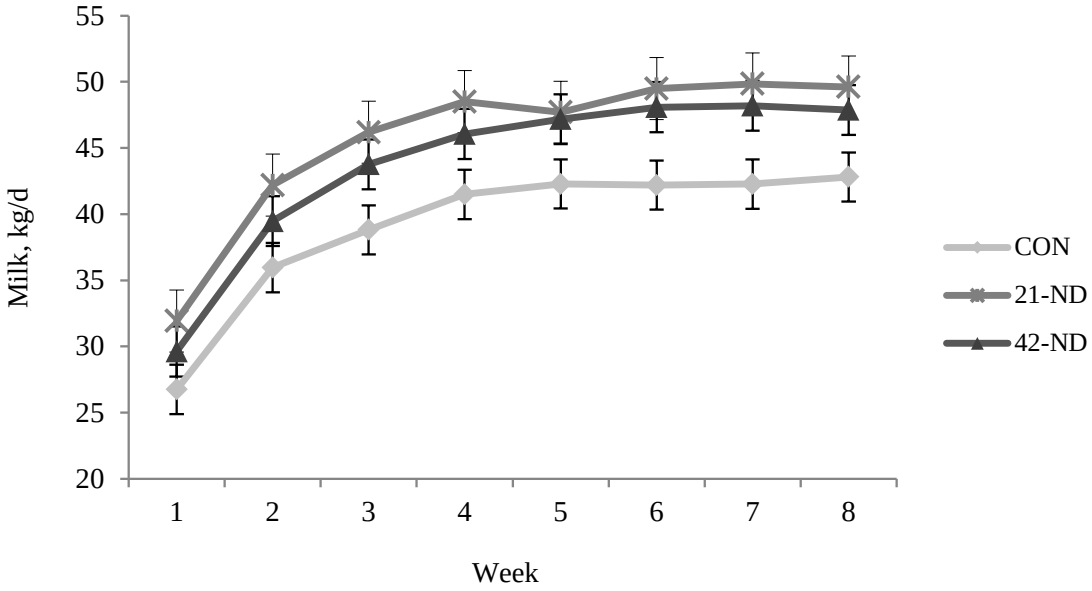


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.90
Prepartum P-values:
Trt: P = 0.30
Week: P = <0.001
Trt × Week: P = 0.53
21-ND and 42-ND vs. CON: P = 0.94
21-ND vs. 42-ND: P = 0.12

Postpartum Standard Errors of the Mean:
SEM = 1.1
Postpartum P-values:
Trt: P = 0.18
Week: P = <0.001
Trt × Week: P = 0.87
21-ND and 42-ND vs. CON: P = 0.09
21-ND vs. 42-ND: P = 0.36

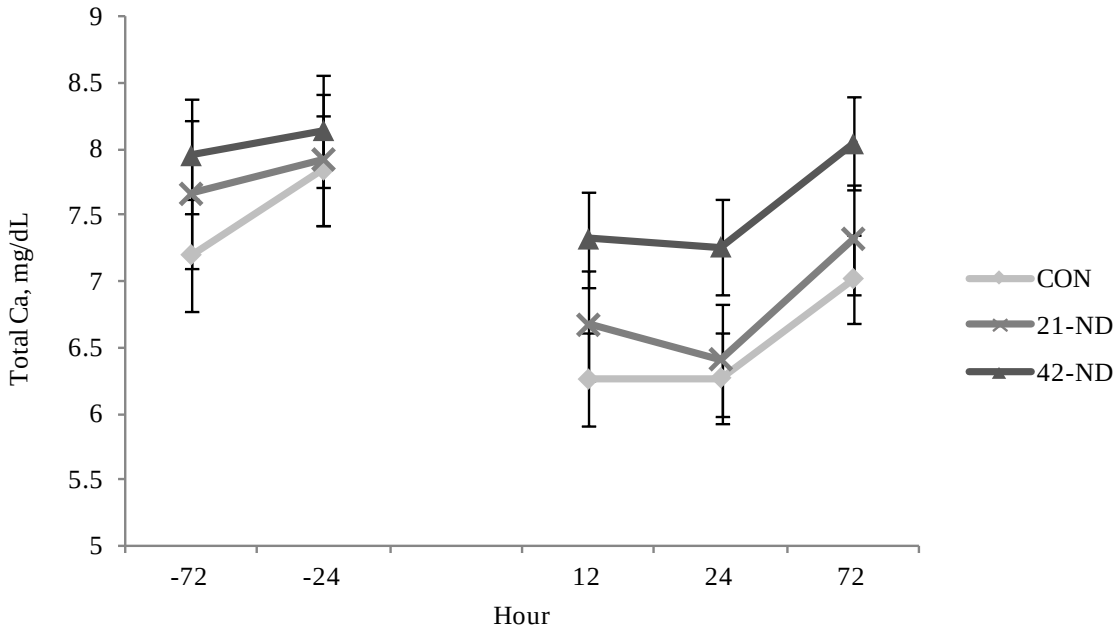
Figure 3. Milk production through 56 d postpartum from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.



[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Postpartum Standard Errors of the Mean:
SEM = 2.1
Postpartum P-values:
Trt: $P = 0.04$
Week: $P = <0.001$
Trt × Week: $P = 0.75$
21-ND and 42-ND vs. CON: $P = 0.01$
21-ND vs. 42-ND: $P = 0.50$

Figure 4. Total plasma calcium from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

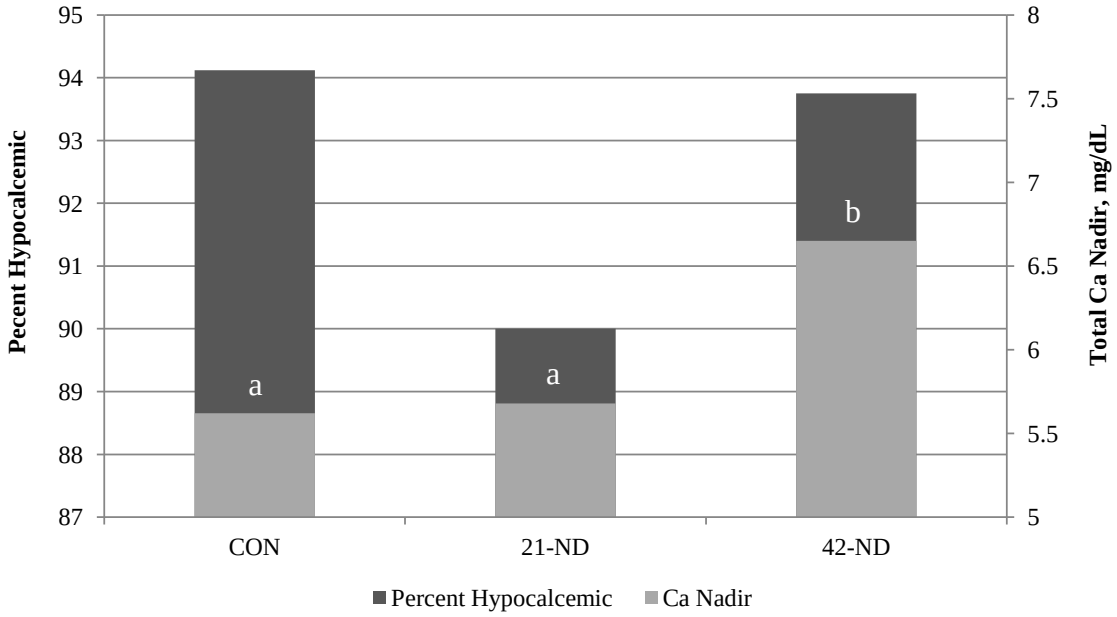


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.5
Prepartum P-values:
Trt: $P = 0.64$
Hour: $P = 0.13$
Trt × Hour: $P = 0.64$
21-ND and 42-ND vs. CON: $P = 0.41$
21-ND vs. 42-ND: $P = 0.58$

Postpartum Standard Errors of the Mean:
SEM = 0.4
Postpartum P-values:
Trt: $P = 0.04$
Hour: $P = 0.001$
Trt × Hour: $P = 0.87$
21-ND and 42-ND vs. CON: $P = 0.07$
21-ND vs. 42-ND: $P = 0.11$

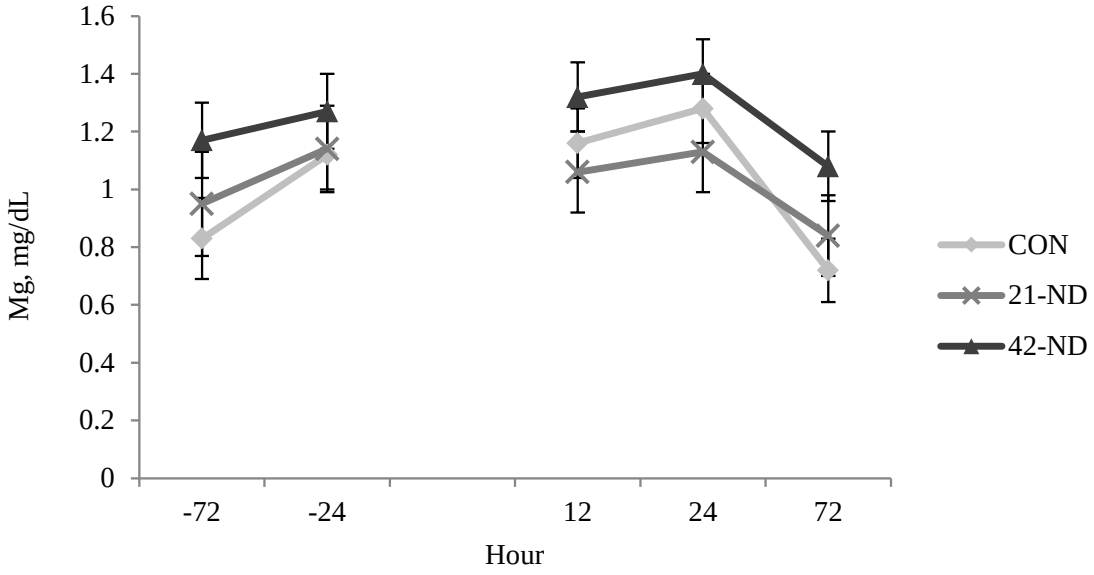
Figure 5. Total plasma calcium nadir and percent hypocalcemic from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.



[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Postpartum Standard Errors of the Mean (Ca Nadir):
SEM = 0.4
Postpartum P-values:
Trt: $P = 0.04$
21-ND and 42-ND vs. CON: $P = 0.16$
21-ND vs. 42-ND: $P = 0.05$

Figure 6. Total plasma magnesium from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

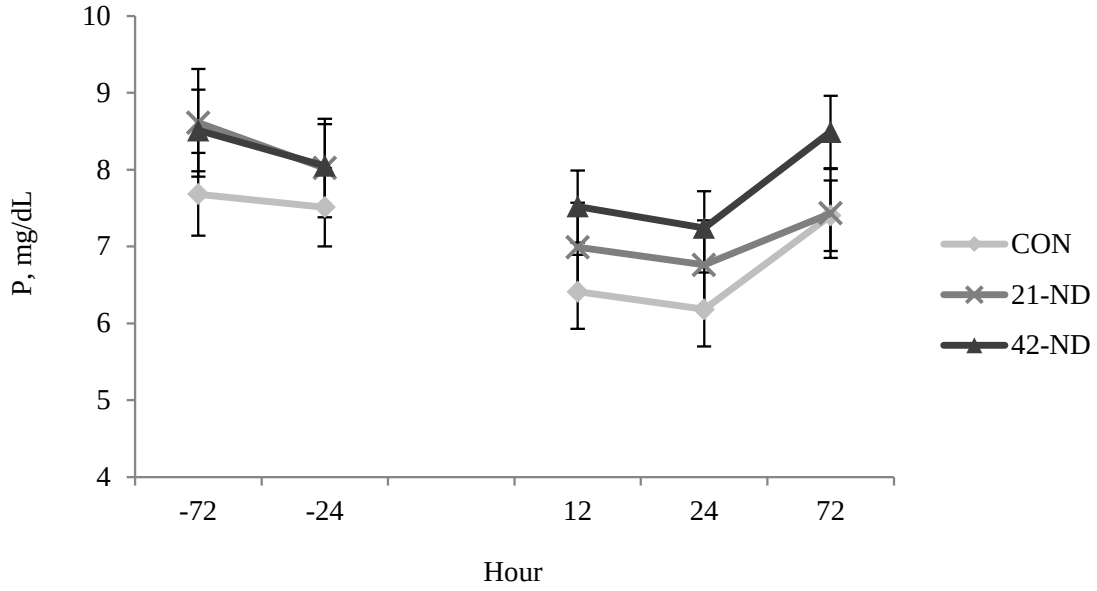


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.1
Prepartum P-values:
Trt: $P = 0.26$
Hour: $P = 0.07$
Trt × Hour: $P = 0.69$
21-ND and 42-ND vs. CON: $P = 0.27$
21-ND vs. 42-ND: $P = 0.31$

Postpartum Standard Errors of the Mean:
SEM = 0.1
Postpartum P-values:
Trt: $P = 0.12$
Hour: $P = <0.001$
Trt × Hour: $P = 0.58$
21-ND and 42-ND vs. CON: $P = 0.48$
21-ND vs. 42-ND: $P = 0.07$

Figure 7. Total plasma phosphorus from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

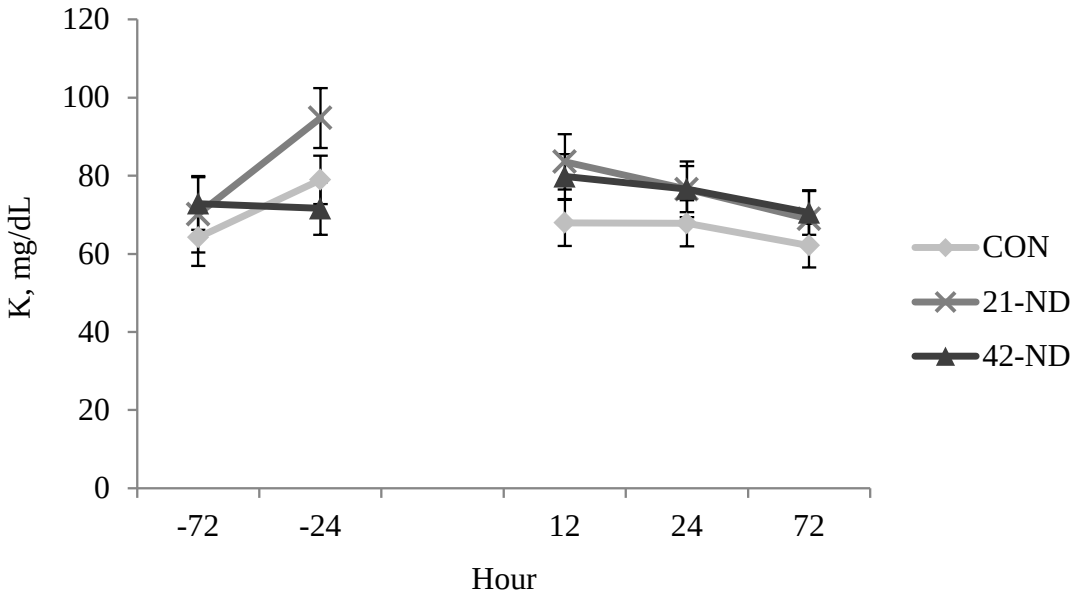


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.6
Prepartum P-values:
Trt: P = 0.55
Hour: P = 0.11
Trt × Hour: P = 0.78
21-ND and 42-ND vs. CON: P = 0.28
21-ND vs. 42-ND: P = 0.97

Postpartum Standard Errors of the Mean:
SEM = 0.5
Postpartum P-values:
Trt: P = 0.11
Hour: P = <0.01
Trt × Hour: P = 0.95
21-ND and 42-ND vs. CON: P = 0.11
21-ND vs. 42-ND: P = 0.24

Figure 8. Total plasma potassium from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

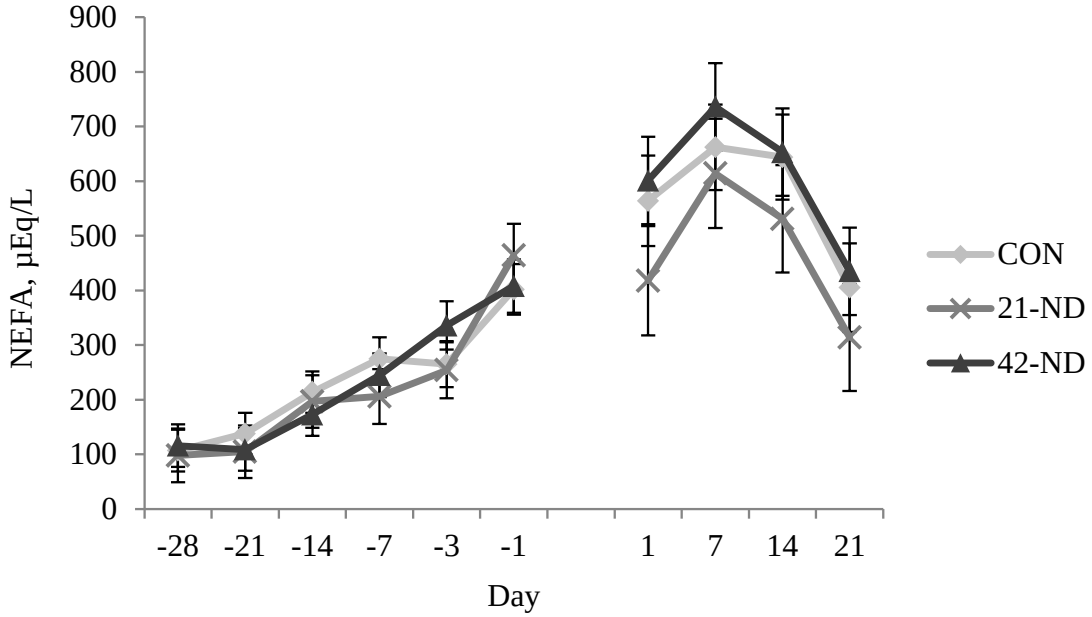


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 7.0
Prepartum P-values:
Trt: $P = 0.42$
Hour: $P = 0.03$
Trt × Hour: $P = 0.16$
21-ND and 42-ND vs. CON: $P = 0.41$
21-ND vs. 42-ND: $P = 0.26$

Postpartum Standard Errors of the Mean:
SEM = 5.1
Postpartum P-values:
Trt: $P = 0.18$
Hour: $P = 0.07$
Trt × Hour: $P = 0.95$
21-ND and 42-ND vs. CON: $P = 0.07$
21-ND vs. 42-ND: $P = 0.92$

Figure 9. Non-esterified fatty acids (NEFA) from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

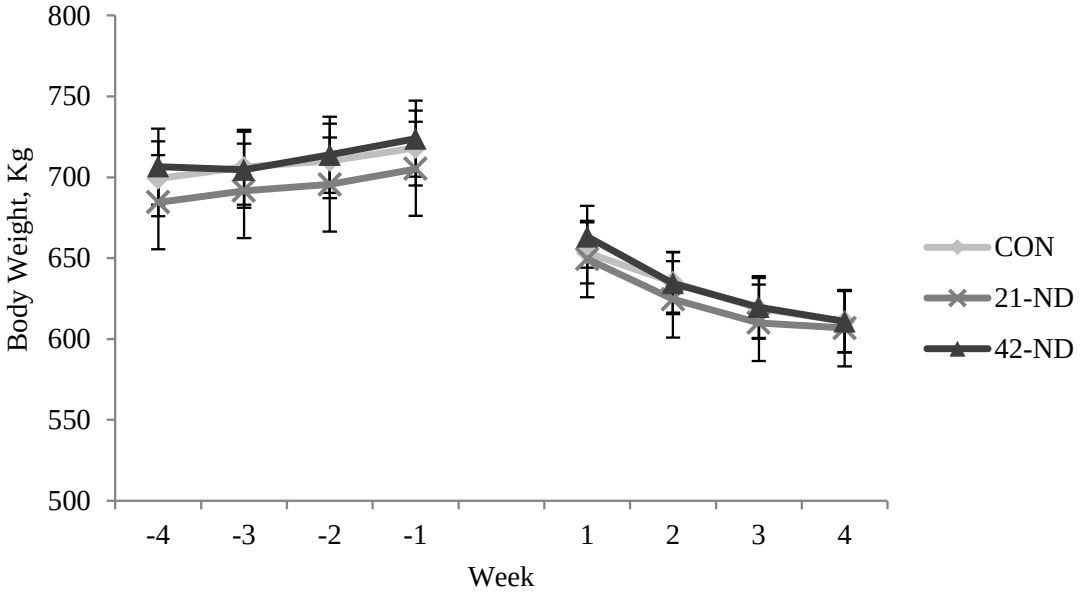


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.03
Prepartum P-values:
Trt: *P* = 0.96
Day: *P* = <0.001
Trt × Day: *P* = 0.56
21-ND and 42-ND vs. CON: *P* = 0.83
21-ND vs. 42-ND: *P* = 0.82

Postpartum Standard Errors of the Mean:
SEM = 0.08
Postpartum P-values:
Trt: *P* = 0.35
Day: *P* = <0.001
Trt × Day: *P* = 0.97
21-ND and 42-ND vs. CON: *P* = 0.56
21-ND vs. 42-ND: *P* = 0.17

Figure 10. Prepartum and postpartum body weight from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.

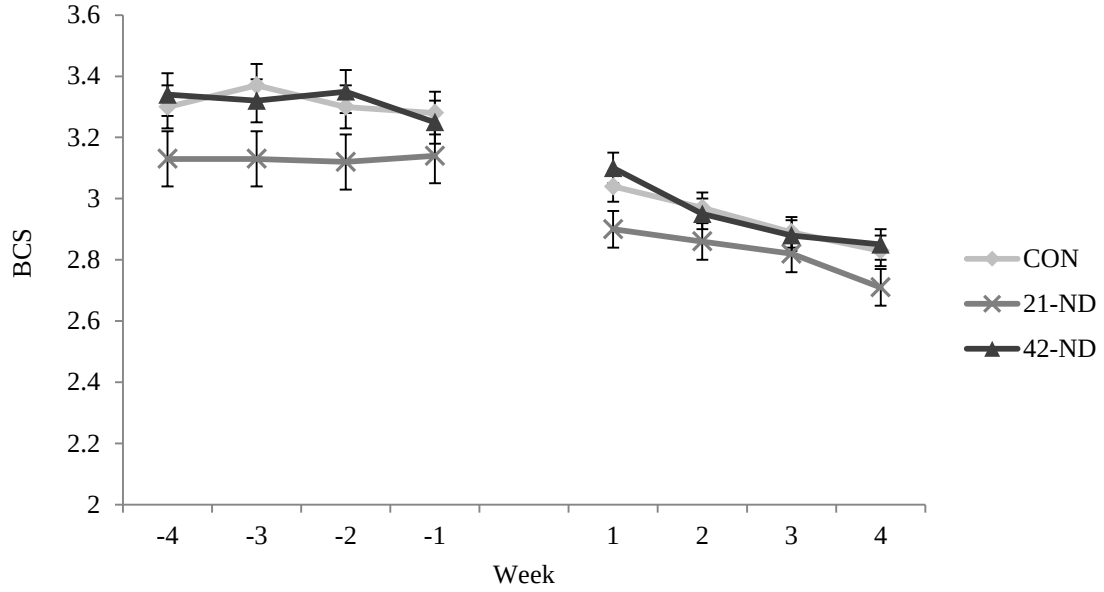


[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 28.9
Prepartum P-values:
Trt: P = 0.88
Week: P = <0.001
Trt × Week: P = 0.63
21-ND and 42-ND vs. CON: P = 0.86
21-ND vs. 42-ND: P = 0.63

Postpartum Standard Errors of the Mean:
SEM = 23.2
Postpartum P-values:
Trt: P = 0.95
Week: P = <0.001
Trt × Week: P = 0.70
21-ND and 42-ND vs. CON: P = 0.93
21-ND vs. 42-ND: P = 0.76

Figure 11. Prepartum and postpartum body condition score from multiparous cows fed prepartum diets[1] with a positive DCAD (CON) for 42 d prepartum or negative DCAD (21-ND or 42-ND) for 21 or 42 d prepartum.



[1]Cows assigned to CON received a prepartum diet with a DCAD value of +12 mEq/ 100 g of DM, 21-ND received a prepartum diet with a DCAD value of -16 mEq/ 100 g of DM for 21 d prepartum and those assigned to 42-ND received a prepartum diet with a DCAD value of -16 mEq/ 100g for 42 d prepartum.

Prepartum Standard Errors of the Mean:
SEM = 0.08
Prepartum P-values:
Trt: $P = 0.14$
Week: $P = 0.25$
Trt × Week: $P = 0.24$
21-ND and 42-ND vs. CON: $P = 0.29$
21-ND vs. 42-ND: $P = 0.08$

Postpartum Standard Errors of the Mean:
SEM = 0.06
Postpartum P-values:
Trt: $P = 0.20$
Week: $P = <0.001$
Trt × Week: $P = 0.34$
21-ND and 42-ND vs. CON: $P = 0.38$
21-ND vs. 42-ND: $P = 0.10$

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