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thesis entitled

VARYING CATION-ANION DIFFERENCE
IN DIETS OF PREPARTUM DAIRY COWS

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

Major professor

Date 8-8-97

VARYING CATION-ANION DIFFERENCE IN DIETS OF PREPARTUM DAIRY COWS

By

Stanley Joseph Moore

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1997

ABSTRACT

VARING CATION-ANION DIFFERENCE
IN DIETS OF PREPARTUM DAIRY COWS

By

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Holstein cows (n=27) and heifers (n=35) were fed a close-up dry cow diet with a varying dietary cation-anion difference (DCAD) of +14.4 (control), 0, or -15 meq/100g dietary DM. Dietary Ca concentration increased (with $CaCO_3$ supplementation) with decreasing DCAD. Cows were fed experimental diets for 24 d prior to expected calving date. Urine pH decreased with decreasing DCAD. Plasma HCO_3 was lower with the -15 DCAD treatment. Prepartum DMI was depressed with the -15 DCAD treatment compared with 0 DCAD treatment. BW also was reduced at 1 wk prepartum in the -15 DCAD treatment. Energy status in the 2 wk prepartum period was lower for cows fed the -15 DCAD diet. Plasma NEFA, IGF-I, and hydroxyproline concentrations were not affected by treatment. Decreasing DCAD increased plasma iCa concentrations both prepartum and at the time of calving in cows but not heifers. Control cows had higher PTH and calcitriol concentrations than cows on the 0 and -15 meq/100 g DM. In conclusion, feeding anionic salts plus $CaCO_3$ to reduce DCAD to -15 and increase Ca in prepartum diets prevented hypocalcemia at calving in cows, but also decreased prepartum feed intake and energy balance. Heifers did not become hypocalcemic and should not be fed anionic salts.

ACKNOWLEDGEMENTS

This project involved the work of many dedicated people. I thank Dr. Herb Bucholtz for serving as my major professor and for his direction throughout my M. S. program. For serving as my guidance committee, I thank Dr. Mike Allen, Dr. Tom Herdt, Dr. Dave Beede, and Dr. Mike VandeHaar. I also thank Dr. Roy Emery for his assistance at the MSU farm. I thank Tom Pilbeam for all his advice and assistance throughout the project. I also thank Jim Liesman for his help with statistical analysis and Dr. Bal Sharma for laboratory expertise and for helping with farm labor supervision.

I wish to thank Michigan State University for the financial support of this experiment through Michigan Animal Industry Initiative Research and Extension Funding.

I wish to thank my parents who started me on my educational journey and supported me throughout. Finally, thank-you Gayle for all your encouragement, support, and dedication in helping me see through this project.

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INTRODUCTION

Milk fever costs the U.S. dairy industry over $120 million/yr in direct costs of treatment and secondary problems (Goff and Horst, 1990). The dynamics of feeding and managing high producing cows are most critical for the period 30 d prepartum through 30 d postpartum.

Besides tremendous changes in energy and protein flux around calving, peripartum cows also experience large changes in mineral element dynamics. Daily body turnover of Ca changes from about 10 g in dry cows to about 35 g in lactating cows (Moodie, 1960). In a recent study, almost 70% of multiparous cows in three commercial farms in Florida, Colorado and Wisconsin suffered from clinical or subclinical hypocalcemia at calving, although only 8% exhibited clinical hypocalcemia (i.e., milk fever) (Wang, 1992). Milk fever, also called parturient paresis, occurs when the output of Ca from the blood exceeds the input of Ca absorbed from the gut and resorbed from bone (Wang et al., 1994; Ward et al., 1953). It has been suggested that because Ca has a role in smooth muscle function, hypocalcemia is either the root cause or a predisposing factor for several other problems in the peripartum cow. Parturient paresis is a risk factor for dystocia (7.5 to12.6 odds ratio), prolapsed uterus (13.1 to 34.6 odds ratio), retained placenta (2.0 to 2.8 odds ratio), and early metritis (1.2 to 1.8 odds ratio) (Grohn et al., 1990) (where odds ratio indicates the increased likelihood that a cow with parturient

paresis would develop one of these metabolic disorders or diseases, compared with cows without parturient paresis).

Decreasing the dietary cation-anion difference (DCAD; meq [(Na + K) - (Cl + S)/100g DM]) during the last 3 to 4 wk before calving can have beneficial effects on systemic acid-base status, Ca metabolism, peripartum health, and postpartum productive and reproductive performance (Beede, 1995; Beede, 1992; Horst et al., 1994; Oetzel et al., 1988; Tucker et al. 1991).

The strategy of feeding anions (Cl or S) to lower the DCAD of prepartum diets is often successful. These anions are often supplied by the so-called "anionic salts" ($MgSO_4 \bullet 7H_2O$, NH_4Cl, $(NH_4)_2SO_4$, $CaCl_2 \bullet 2H_2O$, $MgCl_2 \bullet 6H_2O$, and $CaSO_4 \bullet 2H_2O$). However, the optimum DCAD, and thus the amount and kinds of anionic salts to feed are not well established.

The common recommendation is to add anionic salts until the DCAD value is -10 to -15 meq/100g DM. However, Michigan forages typically are high in K, thus requiring large amounts of anionic salts to achieve a diet with a negative DCAD of -10 to -15 meq/100g DM. Based on experience in an earlier dry cow study at Michigan State University (VandeHaar et al., 1995), protection against hypocalcemia may be achieved with only moderate additions of anionic salts to dry cow diets. Most studies to date have examined DCAD of -10 to -15 to prove that the DCAD concept was valid. However, anionic salts are unpalatable and a moderate inclusion rate (to achieve DCAD = 0 meq/100g DM) may have less effect on feed intake than higher inclusion rate, and thus be desirable to optimize energy, protein, and mineral nutrition simultaneously. If moderate

inclusion rates could be effective, prepartum diets could be formulated to help control hypocalcemia yet maintain high DMI, thus reducing the incidences of ketosis and fatty liver.

The hypothesis tested in this study was that 1) feeding a close-up dry cow diet with a DCAD of 0 meq/100g DM would not prevent hypocalcemia in the peripartum dairy cow to the same extent that a diet with a DCAD of -15 meq/100g DM would, and 2) that feeding a diet with 0 meq/100g DM DCAD diet would not result in a higher DMI compared with a diet of -15 meq/100g DM, and thus would not result in less lipid mobilization before calving.

The alternative hypothesis was that the 0 meq/100 g DM DCAD would prevent hypocalcemia and depression of DMI compared with the -15 meq/100g DM DCAD diet.

The objectives of the research were to examine the effects of a prepartum diet containing no (Control, DCAD = +14.4 meq/100g DM), moderate (DCAD = 0 meq/100g DM) or high (DCAD = -15 meq/100g DM) amounts of anionic salts on the prevention of parturient hypocalcemia, prepartum feed intake, and health and milk production postpartum. By experimental design, the dietary Ca concentration varied among the three treatments. The dietary Ca concentration increased (with supplemental $CaCO_3$) with increased inclusion of the anionic salts (decreasing DCAD).

LITERATURE REVIEW

Dietary Ca effects on Ca status of the peripartum cow

Past research on preventing parturient paresis and field recommendations have focused on changing the Ca/P ratio or reducing the amount of Ca in the prepartum diet (Boda and Cole, 1954; Verdaris and Evans, 1974). A later review of literature on Ca/P ratio showed that Ca/P ratio had no significant effect on the Ca status of the cow (Beitz et al., 1973; Jonsson, 1978).

Reducing the amount of dietary Ca to prevent parturient paresis is considered to be impractical for most dairy farms (Goff et al., 1988). Very low Ca diets (<20 g/d) result in low blood Ca and thus increase parathyroid hormone (PTH) and calcitriol (1,25-$(OH)_2$-D_3) before calving (Bushinsky et al., 1982; Goff, 1992a; Jonsson et al., 1980; Kumar, 1980).

Jonsson (1978) showed that 37 g/d of dietary Ca was not low enough to prevent milk fever. Similarly, Jonsson et al. (1980), showed no effect of dietary Ca concentration on milk fever at the inclusion rate of 37 to 150 g/d. Oetzel (1991) in a meta-analysis of data from the literature showed that incidence of milk fever increased with increasing dietary Ca up to about 1.2% of the diet, and beyond 1.2% milk fever incidence actually declined. Verdaris and Evans (1976) showed that with very high amounts of dietary Ca in the prepartum period (2.1% of DM), the amounts of Ca absorbed and retained were

increased. This increased absorption can be very important in older cows because reduced Ca digestibility and absorption cause older cows to be at increased risk for milk fever (Hansard et al., 1954; Moodie, 1960). Moodie (1960) suggested that decreased gut motility caused the decrease in Ca absorption. The major routes for Ca homeostatic control are absorption, bone tissue deposition and bone resorption, urinary loss, and resorption from kidney tubules (Miller, 1974). Parathyroid hormone, calcitriol, and acid-base status are some of the major controlling factors of Ca absorption, and bone tissue deposition and resorption in the cow.

PTH effects on Ca status of the peripartum cow

Parathyroid hormone (PTH) is secreted by the parathyroid gland in response to low blood Ca (Boda and Cole, 1954; Kumar, 1980; Ramberg et al., 1967) and PTH concentration is inversely proportional to the blood Ca concentration. PTH increases distal tubular reabsorption of Ca in the kidneys (Ganong, 1985), increases bone Ca resorption (Block, 1992; Boda and Cole, 1954), and mediates an increase of calcitriol in blood (Kumar, 1980; Wang et al., 1994).

PTH affects bone by causing a increase in Ca permeability in osteoblasts, osteocytes, and osteoclasts. The osteoclasts erode and resorb previously formed bone Ca (Block, 1992; Ganong, 1985). Bone is composed of both labile (available) and stable (unavailable) forms of Ca (Moodie, 1960). PTH first acts on available bone Ca stores, but will eventually affect even the stable portion of bone Ca (Goff, 1992b). PTH effects on bone can be measured by looking at hydroxyproline concentrations in blood (Leclerc and Block, 1989; Wang et al., 1994). Increased blood hydroxyproline indicates increased

resorption of bone. The Ca that is released from bone due to the action of PTH, is released as Ca phosphate (Barzel and Jowsey, 1969) and dissociates in the blood to form Ca and PO_4^{-3}.

PTH affects calcitriol concentrations in the blood by activating renal mitochondrial 1-alpha-hydroxylase which converts 25-OH-D_3 to calcitriol (Goff, 1992b; Kumar, 1980; Wang et al., 1994). Ikeda et al. (1987) showed that in rats changes in renal 1-alpha-hydroxylase activity generally paralleled those of circulating calcitriol.

PTH is regulated through several mechanisms. As mentioned earlier, PTH is correlated negatively with blood Ca concentration (Goff, 1992a). Ramberg et al. (1967) reported that PTH secretion was inversely proportional to Ca concentration in plasma. D'Amour et al. (1986) showed that in calves, dogs and humans, hypercalcemia caused a decrease in PTH concentration to 40% of basal concentrations. Hypocalcemia increased PTH concentrations 300 to 450% above basal concentrations. Hughes and Haussler (1978) showed that the parathyroid gland has receptors for calcitriol. This may serve as feedback regulation to shut down PTH secretion after calcitriol is produced and low blood Ca is corrected.

Lastly, the effect of PTH on bone tissue and renal production of calcitriol is dependent in part on the acid-base balance of the cow (Goff, 1992b). Metabolic acidosis causes bone to be more sensitive to PTH stimulation, whereas metabolic alkalosis causes bone and renal tissues to be refractory to PTH stimulation (Goff, 1992a). Block (1992) showed that hypocalcemic cows had higher plasma PTH concentrations. This would suggest that low blood Ca is not due to a lack of PTH secretion, but instead a lack of bone and renal tissue sensitivity to the effects of PTH.

Calcitriol effects on Ca status of the peripartum cow

Calcitriol increases blood Ca status by increasing uptake from the intestine (Block, 1992; Evans, 1977; Gaynor et al., 1989; Goff, 1992a). This uptake is by active transport (transcellular) across the intestinal epithelium (Goff, 1992b). Calcitriol also enhances mobilization of Ca from bone (Kumar, 1980; Wang et al., 1994). These influences of calcitriol on Ca utilization have led to experimental use of calcitriol in the prevention of milk fever. Given the correct timing and dose, calcitriol reduces incidence of milk fever when given intramuscularly, intravenously, or orally (Boda, 1954; Hibbs and Pounden, 1956; Littledike and Horst, 1982; Wang et al., 1994). However, administration of calcitriol is not used widely for the prevention of milk fever due to the need for exact timing of administration with respect to actual and potential toxicity. Hibbs and Pounden (1956) showed that feeding 30 million units of calcitriol for 3 to 8 d prepartum would prevent milk fever. When calcitriol was fed longer than 2 to 4 wk, the positive results disappeared, presumably because the increased concentration of blood Ca for too long caused the parathyroid gland to reduce secretion of PTH. Hove and Kristiansen (1982) also found positive results with oral calcitriol. In their experiment, the dose of calcitriol was 500 ug given 1 to 3 d prepartum. In 1980, Reinhardt and Conrad found that giving calcitriol intravenously for 7 d prepartum initially raised circulating concentrations of calcitriol in blood, but caused decreased circulating calcitriol concentrations just prior to calving. These authors suggested that feedback inhibition was overridden initially, but that the inhibition still occurred before parturition. Toxicity generally occurs when calcitriol is given for an extended period of time. This toxicity

may cause calcification of internal organs (Wang et al., 1994) and eventually lead to death. Littledike and Horst (1982) showed that a parenteral dose of calcitriol given 32 d prepartum at 15 to 17.5 X 10^6 IU prevented milk fever, but it also caused vitamin D toxicity and 10 of 17 cows died. Another concern with using calcitriol in prevention of milk fever is cows becoming dependent on calcitriol administration. Calcitriol stimulates Ca absorption from the intestine, making cows more dependent on Ca absorption to maintain plasma concentrations and homeostasis as opposed to activating bone resorption (Wang et al., 1994). Wang et al. (1994) also suggested that because vitamin D metabolites inhibit 1-alpha-hydroxylase in the kidney, there is an increasing dependency on exogenous vitamin D sources.

Production of calcitriol from 25-(OH)-D_3 by the kidneys is affected negatively by dietary Ca intake (Bushinsky et al., 1982; Goff, 1992a; Ikeda et al., 1987; Kaetzel and Soares, 1985; Kumar, 1980), and positively by increasing PTH blood concentrations (Block, 1992; Jonsson, 1978). Calcitriol production is affected negatively by plasma Ca concentrations (Block, 1992; Bushinsky et al., 1982; Goff, 1992a; Ikeda et al., 1987; Jonsson, 1978), negatively by plasma P concentrations (Goff, 1992a; Goff, 1992b; Kumar, 1980), and negatively by feedback inhibition of calcitriol (Gordeladze et al., 1986; Jonsson, 1978; Reinhardt and Conrad, 1980; Wang et al., 1994). Lastly, calcitriol production by the kidneys is affected negatively by a positive DCAD (Block, 1992; Gaynor, 1989; Goff, 1992a).

Acid-base status effects on Ca status of the peripartum cow

Buffer systems are important in the cow as in other animals to maintain body intracellular and extracellular pH. Two of the important buffer systems are: 1) carbon dioxide/bicarbonate system in blood (Freeden, 1993), and 2) bone buffer system (Bushinsky and Lechleider, 1987; Freeden, 1993). Maintaining a narrow range in pH in extracellular and intracellular fluids is critical for enzyme systems which are pH-dependent (Block, 1992).

The carbon dioxide/bicarbonate system is the main system for maintaining acid/base status and is regulated by the kidneys and lungs. The concentration of bicarbonate is determined by the difference in the concentrations of all cations and all anions (excluding HCO_3^-) in plasma (Fredeen, 1993). When cations exceed anions in the blood, the kidney increases the excretion of bicarbonate into urine thus maintaining pH (Block, 1992). When anions exceed cations in blood, the kidney will conserve HCO_3^- in the plasma to maintain pH. Respiratory rate controls the plasma $pC0_2$ concentrations and thus the concentration of carbonic acid (H_2CO_3) in the blood (Fredeen, 1993). When metabolic acidosis occurs carbonic acid increases in the blood as bicarbonate is converted to carbonic acid. The carbonic acid is picked up by the red blood cells and transported to the lungs for respiratory removal of CO_2 (Fredeen, 1993). This metabolic acidosis may or may not include a decrease in plasma pH. If an increase in respiration rate does not occur, a decrease in plasma pH will occur causing metabolic acidemia.

Bone contains a vast reserve of base (carbonate) (Fredeen, 1993). During acidosis the solubility of Ca phosphate in bone (as hydroxyapatite) is increased (Barzel and Jowsey, 1969; Block, 1992), thus increasing the entrance rate of Ca from bone into blood.

Ca phosphate dissociates in blood to form Ca and PO_4^{-3}. Clearance rate of Ca also increases with acidosis as excess Ca in blood is excreted in the urine. Metabolic acidosis thus causes calciuria (increased Ca in the urine) (Fredeen, 1993; Block, 1992; Fredeen et al., 1988). Although metabolic acidosis increases the flux through the exchangeable Ca pool, it does not affect the size of the exchangeable Ca pool (Fredeen et al., 1988).

Because of these buffer systems, the Ca status of the cow can be affected by manipulating the acid-base status of the cow. Any manipulation causing acidosis will cause bone and renal tissues to be more sensitive to PTH stimulation (Goff, 1992a), and cause the bone buffer system to release Ca phosphate (Barzel and Jowsey, 1969; Block, 1992). Manipulation of the acid-base status of the cow can be achieved through changing the dietary cation-anion difference (Beede and Sanchez, 1989).

Dietary cation-anion difference (DCAD) effects on Ca status of the peripartum cow

Decreasing the DCAD of the diet during the last 3 to 4 wk prepartum affects systemic acid-base status and has beneficial effects on Ca metabolism, peripartum health, and postpartum productive and reproductive performance (Beede, 1995; Beede, 1992; Horst et al., 1994; Oetzel et al., 1988; Tucker et al., 1991). DCAD is calculated for the diet by subtracting the milliequivalents of anions from the milliequivalents of cations in the ration. Rather than consider all cations and anions, the commonly used formula is meq [(K + Na) - (Cl + S)]/100g dietary DM (Beede, 1995). Of these minerals elements, K, Na and Cl are considered fixed ions because they are not metabolized. Sulfur, although not a fixed ion, is included in the equation because S directly acidifies biological fluids and can alter acid-base balance if included at high dietary concentrations (Block,

1992; Lemann and Relman, 1959; Tucker et al., 1991). The anionic salts used in the present study ($MgSO_4 \bullet 7H_2O$, $CaCl_2 \bullet 2H_2O$, $CaSO_4 \bullet 2H_2O$) change acid-base status because Cl and S have higher rates of absorption compared to Ca and Mg. The anions, Cl and S, then draw H^+ ions out of base creating metabolic acidosis (Beede and Sanchez, 1989).

Decreasing DCAD in the prepartum period leads to increased plasma Ca concentrations at parturition (Beede, 1995; Gaynor et al., 1989; Leclerc and Block, 1989; Oetzel et al., 1988). This relationship between DCAD and plasma Ca concentration was first reported by researchers in Norway (Dishington, 1975; Ender et al., 1962). The mode of action of negative DCAD to increase plasma Ca is not well understood. Block (1992) suggested that increased excretion of Ca in the urine leads to an initial drop in plasma Ca concentrations. This drop in Ca concentration would increase the release of PTH and increase the formation of calcitriol by the kidneys. Both of these responses to the initial decrease in blood Ca would lead to an eventual increase in blood plasma Ca concentrations.

This suggestion of an initial drop in blood Ca concentrations is not supported by others. Fredeen et al. (1988) suggested instead that the pool size of Ca is not affected by acidosis, but the flux of Ca is increased. An increase in dietary Ca concentration is recommended with decreased DCAD diets because of this increased flux (Beede, 1995; Fredeen, 1993; Wang, 1994). The increase in Ca entering the pool is apparently mostly from increased bone mobilization as evidenced by increases in hydroxyproline concentrations in blood and decreased bone density (Leclerc and Block, 1989; Petito and

Evans, 1984). The increase in Ca flux from bone is due to the bone buffering system trying to compensate for the metabolic acidosis initiated through a negative DCAD diet. There is also evidence that bone may become more sensitive to PTH when animals are kept in a mild state of metabolic acidosis as compared with an alkalinic state (Goff, 1992a; Goff, 1992b; Ochs et al., 1964).

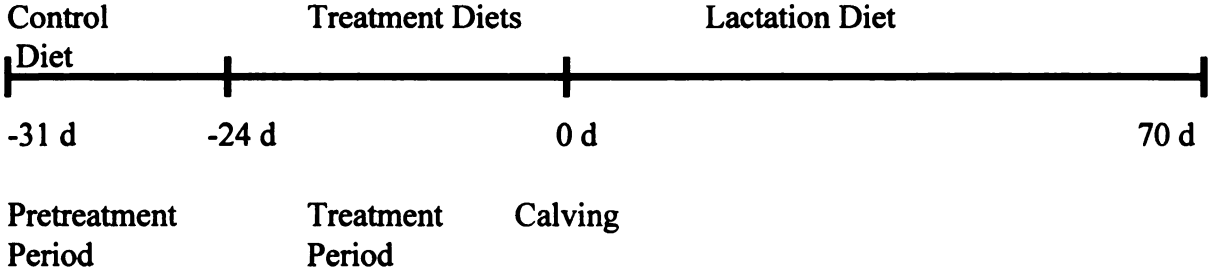
A negative DCAD also may cause an increase in intestinal absorption of Ca (Leclerc and Block, 1989; Wang and Beede, 1992). This increase in absorption may be through increases in plasma calcitriol (Block, 1992; Gaynor et al., 1989; Goff, 1992b), and (or) an increase in tissue sensitivity to calcitriol in the acidotic state created by feeding a negative DCAD diet (Block, 1992; Goff, 1992b).

MATERIALS AND METHODS

Cows

Sixty-two Holstein cows (27 multiparous and 35 primiparous) from the Michigan State University herd in East Lansing were selected for the experiment. Cows were assigned randomly to one of three treatments and were assigned in blocks of three cows each based on parity and date of expected calving. Only two cows were available for the last block and they were assigned randomly to one of three treatments. Cows were housed in individual tie-stalls to enable individual feeding and feed intake measurements. Cows were brought into the experiment approximately 35 d prior to expected date of calving on Monday each week and remained on the Control diet (DCAD = +14.4) for at least 1 wk (see timeline) before being fed a treatment diet.

Timeline of experiment



Treatment diets

Twenty-four days prepartum, cows were assigned to one of three treatment diets. The diets were 1) Control diet (DCAD= +14.4), 2) diet formulated for 0 DCAD, and 3) diet formulated for -15 DCAD (Table 1). After calving all cows were placed on the same lactation diet (Table 2). All diets were fed once daily with orts from the previous feeding removed just prior to the next feeding. Amounts fed and orts removed were recorded to calculate daily feed intakes. All forages were measured twice per week for DM% using a Koster Tester (Koster Crop Tester, Inc., Strongsville, OH) by the farm crew.

Feed samples were collected once per week and analyzed via wet chemistry by North East DHIA Forage Laboratory (NEDHIA) (Ithaca, NY) for mineral element content and DM percent. Full analysis (NE_L, CP, NDF, DM and mineral element content) was done twice per month via wet chemistry through the same laboratory.

Table 1. Ingredient and chemical composition of prepartum diets.

Item	Control	0 DCAD	-15 DCAD
Dietary ingredients, % of dietary DM			
Corn silage	34.9	34.9	34.9
Haylage	20.1	20.1	20.1
Corn, ground	26.0	24.0	22.0
Soybean meal	8.9	8.9	8.9
Pelleted cottonseed hulls	4.2	2.1	0.0
Trace mineral and vitamin premix	5.9	5.9	5.9
Anionic pack	0.0	3.9	7.9
Composition of anionic pack, % of dietary DM			
Magnesium sulfate	0.0	0.24	0.50
Calcium sulfate	0.0	0.44	0.90
Calcium chloride	0.0	0.53	1.09
Limestone ($CaCO_3$)	0.0	0.68	1.40
Dicalcium phosphate	0.0	0.15	0.30
Corn, ground	0.0	1.83	3.74
Calculated nutrient content, % of dietary DM			
NE_L (Mcal/kg)	1.66	1.65	1.63
CP	16.5	16.5	16.3
NDF	31.6	30.0	28.0
K	1.35	1.35	1.35
Na	0.12	0.12	0.12
Ca	0.44	0.97	1.50
P	0.34	0.37	0.39
Mg	0.20	0.24	0.28
Cl	0.44	0.70	0.96
S	0.21	0.32	0.44
DCAD, meq/100g DM	+14.4	0	-15

Table 2. Ingredient and chemical composition of lactation diet.

Dietary ingredients	% of DM
Corn Silage	17.3
Haylage	20.3
Corn, high moisture	17.6
Corn, ground	10.8
Soybean meal	15.6
Cottonseeds	7.8
Distillers grain	4.9
Blood meal	0.5
Trace minerals and vitamins premix	5.2
Nutrient content	
NE_l (Mcal/kg)	1.70
CP	18.4
NDF	26.0
K	1.3
Na	0.36
Ca	1.07
P	0.50
Mg	0.3
DCAD, meq/100g of dietary DM	+30.1

Housing and management of cows

Cows were milked three times per day at 0550, 1400, and 2200 h. Milk yield was measured electronically (Boumatec, Madison, WI) and recorded daily using Dairy Comp 305 (Valley Agricultural Software, Tulare, CA). Milk samples were collected once a week (from the three individual milkings for that day) and analyzed for fat, protein, lactose, and SCC by Michigan DHIA (East Lansing, MI). Milk composition of fat, protein, lactose, and SCC was calculated as the weighted average of all three milkings.

Body weights of all cows were measured weekly (Thursday and Friday) at 0900 h. Body condition (1 to 5 scale) was assessed at the times of body weight measurement by three individuals (Wildman, et al., 1982).

Calf birth weights were measured within 12 h of birth and recorded.

Blood and urine sampling

Blood samples were obtained from the tail vein twice per week (Monday and Friday) at 1600 h, starting 1 wk after cows entered the experiment. Two weeks prior to expected date of calving, blood was sampled three times per week (Monday, Wednesday, and Friday). Beginning 1 wk prior to expected date of calving, cows were bled daily until calving. Cows also were bled after calving within 4 h, and at 12 h, 1 d, 2 d, 3 d, 1 wk, and 2 wk. Lithium heparin vacutainer tubes were used for blood collection. Samples were centrifuged and plasma collected for analysis. Samples were measured for concentrations of iCa, K, Na, Cl, pH, pCO_2, HCO_3, calcitriol and PTH as indicators of the Ca regulatory system, and non-esterfied fatty acids (NEFA) and insulin-like growth factor-I (IGF-I) as indicators of energy status.

Urine pH was measured each Monday, Wednesday, and Friday before calving to assess acid-base status. Hand-held urine pH meters (Hach, Loveland, CO) were calibrated prior to each sampling using a pH 7.0 buffer solution as the standard.

Blood mineral elements and pH analyses

Blood mineral elements and pH analyses were done on plasma samples within 3 d of sampling. Samples were stored at 4^0C prior to analysis. Analyses for Na, K, Cl, iCa, pH, pCO_2, and HCO_3 were done. Analysis was accomplished using a Nova Biomedical Stat Profile 4, blood gas analyzer (Waltham, MA).

NEFA analysis

Samples were analyzed for NEFA using a NEFA-C kit (Wako Pure Chemicals Inc., Richmond, VA) with modifications by Dyk (1995). Any plasma sample with a coefficient of variation greater than 15% for two replications was reanalyzed.

IGF-I analysis

Plasma samples were analyzed for IGF-I using a radioimmunoassay procedure (see Appendix for procedure and all buffer and reagent mixtures) (Sharma et al., 1994).

Energy balance

Energy balance was calculated for each cow by week (-2, -1 wk prepartum and 2 through 9 wk postpartum) using the following equations:

Energy balance (Mcal/day)=(NE_{intake} - $NE_{maint.}$ - $NE_{lactation}$)/efficiency factor.

NE_{intake} = DMI(kg)*NE_l

$NE_{maint.}$ prepartum = $BW(kg)^{.75}$*0.104 $Mcal/kg^{.75}$

$NE_{maint.}$ postpartum = $BW(kg)^{.75}$*0.08 $Mcal/kg^{.75}$

$NE_{lactation}$ = milk(kg)*(41.63*fat+24.13*prot+21.60*lact-11.72)/1000

If energy balance was negative postpartum, the efficiency factor was 0.82 to account for the efficiency of milk production from body tissue. An efficiency factor of 1 was used to calculate energy status when status was positive postpartum and for prepartum energy status.

Calcitriol, hydroxyproline, and PTH analyses

Calcitriol, hydroxyproline and PTH were analyzed by the USDA National Animal Disease Center in Ames, Iowa under the direction of Dr. Jesse Goff. Plasma samples were shipped frozen, on ice. Calcitriol was analyzed using a microassay procedure developed by Reinhardt et al. (1984).

Dabev and Struck (1971) developed the hydroxyproline assay procedure. The assay was modified for use in microtiter plates (Goff, personal communication).

PTH was analyzed with a kit from Nichols Diagnostics (San Juan Capistrano, CA). The test used for PTH was an intact PTH immunoassay, which was a two-site

immunoradiometric assay for the measurement of the biologically intact 84 amino acid chain of PTH. This human PTH kit has been validated for bovine (Goff et al., 1989).

Statistics

The GLM procedure was used in the analysis of all data using a repeat measure design (SAS, 1995). All results were reported as least squares means. The model for ANOVA included parity, block(parity), treatment, parity by treatment, treatment by block(parity), time, treatment by time, parity by time, and parity by treatment by time for: milk yield and components, DMI, body condition score, body weight, energy status, urine pH, plasma mineral elements and pH, plasma pCO_2, plasma HCO_3, plasma IGF-I, and plasma NEFA concentrations. "Time" in the model refers to week, or day depending on the analysis. DMI also was analyzed with the model including only parity, block(parity), treatment, and parity by treatment due to inestimable contrasts when time was included. Calf weights were analyzed with a model that included parity, block(parity), treatment, and parity by treatment. Plasma hydroxyproline, PTH and calcitriol were analyzed with a model that included parity, treatment, parity by treatment, cow(parity by treatment), time, parity by time, treatment by day, and parity by treatment by day.

Due to heterogeneous variance in the PTH and calcitriol data, the log base 10 of the data was used in the analysis.

For statistical analyses of blood plasma variables, pretreatment means of data for up to 14 d on experimental diets were used as covariates.

Milk yield and component data were analyzed for 2 through 9 wk. Data from wk 1 and 10 were not included in the analysis due to missing data and varying time of sampling with respect to calving.

Categorical data (e.g. as metabolic disorder incidences) were analyzed in SAS using Fisher's Exact Test (2-Tail). Data from cows having twins were not included in the analysis for metabolic disorder incidences, but were included in all other analyses.

Treatment means were compared by orthogonal contrasts: Control vs. 0 and -15 meq/100g dietary DM DCAD and 0 vs. -15 meq/100g dietary DM DCAD.

RESULTS AND DISCUSSION

Plasma mineral elements

Table 3 shows the plasma mineral element concentrations for the three treatments. For blood samples taken during the first 2 wks on dietary treatments, iCa concentrations were lower for Control than 0 and –15 DCAD treatments, but did not differ for 0 vs. –15 DCAD ($P < 0.005$, and $P = 0.30$ respectively). Concentration of K was lower for Control than 0 and -15 DCAD ($P < 0.001$), but not different between 0 vs. -15 DCAD ($P = 0.49$). Blood Na concentrations were not different due to treatment (Table 3). Concentration of Cl in plasma was higher for primiparous than multiparous (385 vs. 379 mg/dl respectively $P < 0.05$). Pooled across parities plasma Cl was lower for Control than 0 and -15 DCAD, and lower for 0 than -15 DCAD ($P < 0.05$) (Table 3).

Table 3. Plasma mineral element concentrations (mg/dl) for treatments (first 2 wk on treatments).

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
iCa	4.65	4.77	4.82	0.038	P < .005	P = .30
K	16.90	17.38	17.49	0.11	P < .001	P = .49
Na	333	334	333	0.89	P = .32	P = .51
Cl	379	381	387	1.5	P < .05	P < .05

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

Plasma mineral element concentrations (means of samples taken 0, 12 h, and 1 d after calving), are shown in Table 4. There was an effect of treatment ($P < 0.001$), parity ($P < 0.001$), and parity by treatment ($P < 0.05$) on plasma iCa concentrations (Table 4, Figure 1). Cows on the Control diet had lower plasma iCa than those on 0 and -15 DCAD ($P < 0.01$), and plasma iCa was lower for 0 DCAD than for -15 DCAD ($P < 0.05$) (iCa = 4.1, 4.2, and 4.5 mg/dl for Control, 0 and -15 DCAD, respectively). Figure 1 shows multiparous cows had lower plasma iCa concentrations than primiparous cows (overall means across d = 4.0 and 4.5 mg/dl, respectively). This parity effect on Ca concentration in blood is well documented (Barzel, 1969; Hansard et al., 1954; Moodie, 1960). Plasma iCa concentrations of primiparous cows were similar for all treatments and indicated that primiparous cows were not hypocalcemic at parturition (i.e. iCa > 4 mg/dl) (Figure 1). However, plasma iCa concentration of multiparous cows increased from 3.7 to 3.9 and 4.4 mg/dl for Control, 0, and -15 DCAD, respectively 12 h after calving. Thus, multiparous cows fed the Control and 0 DCAD were hypocalcemic (iCa $\leq$ 4 mg/dl), whereas cows fed the -15 DCAD had mean plasma iCa concentrations well above 4 mg/dl at all sampling times.

Table 4. Effects of treatments, irrespective of parity, on plasma mineral element concentrations immediately after calving (0h, 12h, and 1d)

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
iCa	4.1	4.2	4.5	0.08	$P < 0.01$	$P < 0.05$
K	18.0	18.5	19.0	0.49	$P = 0.57$	$P = 0.49$
Na	337	338	338	1.3	$P = 0.64$	$P = 0.81$
Cl	380	384	383	2.5	$P = 0.22$	$P = 0.68$

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

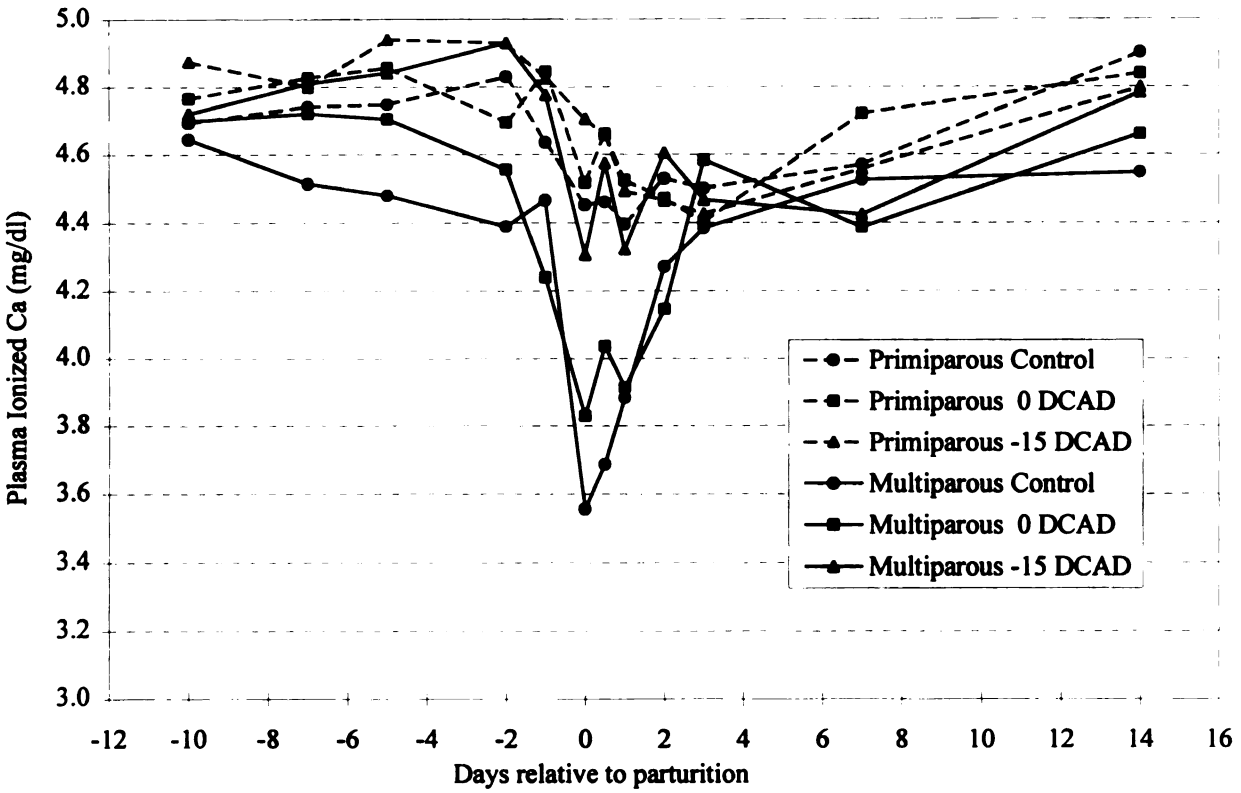


Figure 1. Parity by treatment effect on peripartum plasma iCa concentrations (mg/dl) ($P < 0.05$) SEM = 0.11.

Plasma hydroxyproline, PTH, and calcitriol

Plasma samples were analyzed at -14, -7, -2, -1 d pre-calving, and 0, 12 h, 1, 2, 3 d post-calving for concentrations of hydroxyproline. Hydroxyproline was affected by day, parity, and parity by day ($P < 0.001$, Figure 2), but not by treatment (Table 5). Hydroxyproline is an indicator of bone resorption (Leclerc and Block, 1989; Wang et al., 1994). The lack of a treatment effect on hydroxyproline was unexpected, and suggests a lack of increased bone mobilization with the inclusion of anionic salts. If so, the increased dietary Ca was the source of increased plasma iCa seen with the 0 DCAD and -

15 DCAD diets. Whether the acidifying effects of anionic salts helped potentiate the effects of dietary Ca on plasma iCa cannot be determined in this study because dietary Ca varied among treatments as well as DCAD. Oetzel et al. (1988) showed in a 2 X 2 factorial experiment that dietary Ca intake (53 g/cow per d vs. 105 g/cow per d) alone did not affect serum concentration of iCa, but that higher dietary Ca concentration with a negative DCAD did lower the incidence of hypocalcemia. Leclerc and Block (1989) showed an increase in plasma hydroxyproline with reduced DCAD when using a constant dietary Ca concentration of 1.3 %. In the present study, the potential positive effect of a reduced DCAD on bone resorption may have been masked because of the confounding of DCAD with different dietary Ca concentration (Control = 0.44 %, 0 DCAD = 0.97 %, and -15 DCAD = 1.52 %). The results of Oetzel et al. (1988) indicate that increased plasma iCa concentrations in cows fed the anionic salts was due to both the DCAD and the dietary Ca concentration.

Hydroxyproline concentrations were higher for primiparous cows than multiparous cows, suggesting greater mobilization of bone ($P < 0.001$ SEM = 3.45) (Figure 2). Older cows may not be able to respond as well as primiparous cows to Ca challenges around the time of calving because of a decreased ability to mobilize bone.

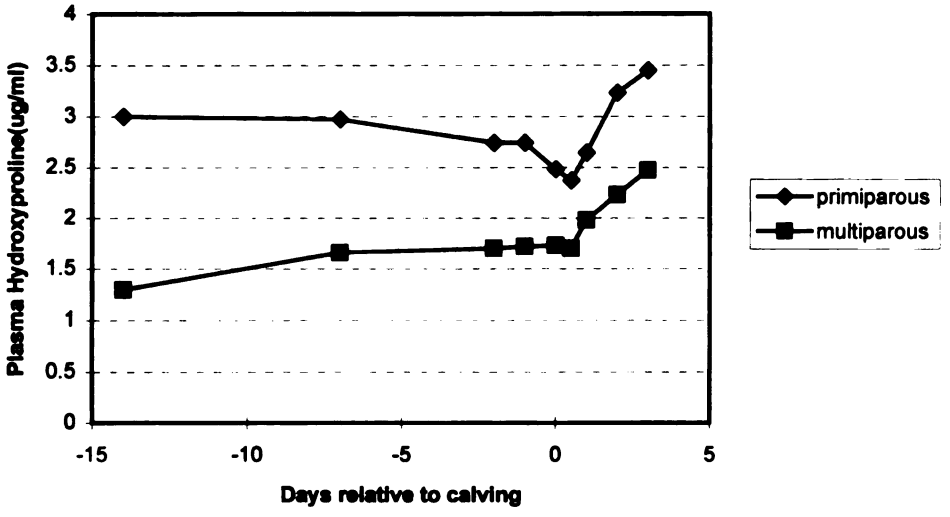


Figure 2. Parity by day effects on plasma hydroxyproline concentrations (ug/ml) ($P < 0.001$) SEM = 3.45.

Table 5. Treatment effects on plasma hydroxyproline[1] (ug/ml), PTH[2] (pg/ml), and calcitriol[3] (pg/ml) concentrations.

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Hydroxyproline	2.37	2.39	2.30	0.15	P = 0.87	P = 0.61
PTH	46.7	52.0	23.5	...	...	...
log PTH	3.30	3.12	2.70	0.16	P < 0.05	P = 0.06
Calcitriol	78	57	41	...	...	...
log Calcitriol	4.13	3.74	3.55	0.09	P < 0.001	P = 0.12

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

[1] Means of samples taken at 14, 7, 2, 1 d prepartum and 0, 12 h, 1, 2, 3 d postpartum.

[2] Means of samples taken at 2, 1 d prepartum and 0 h, 1, 2 d postpartum.

[3] Means of samples taken at 7, 1 d prepartum and 0 h, 1 d postpartum.

Plasma samples were analyzed at -2, -1 d pre-calving, and 0 h, 1, 2 d post-calving for concentrations of PTH. Plasma PTH concentrations were transformed to log base 10 for analysis because of heterogeneous variances. PTH concentrations were affected by treatment ($P < 0.05$), treatment by day ($P < 0.05$), day and parity by day ($P < 0.001$) (Table 5, Figure 3 and 4). Cows fed Control had higher PTH concentrations than the

average of cows fed the 0 and -15 DCAD diets ($P < 0.05$), and there was a trend for higher PTH concentrations with the 0 DCAD diet than in the -15 DCAD diet ($P < 0.1$). Decreased plasma PTH concentrations with 0 and –15 DCAD diets around the time of calving (-2 to +2 d) was consistent with the plasma iCa concentrations, the reason for the elevated PTH with 0 and –15 DCAD compared with Control cannot be explained. The higher PTH concentration of cows on the 0 DCAD (Table 5 and Figure 3) is not consistent with the relative ranking of iCa concentrations among treatments and may be due to high variability or bias (heterogeneity of variances) in PTH data. D'Amour et al. (1986) showed that in calves, dogs and man, plasma PTH concentrations were in close inverse relation with plasma iCa concentrations. This close relationship between plasma concentrations of PTH and iCa is supported by the results of others (Jonsson et al., 1980; Kumar 1980; Ramberg et al., 1967).

The decreased plasma PTH of cows fed the 0 and -15 DCAD diets is one more indicator of the better Ca status of these cows compared with the Control cows.

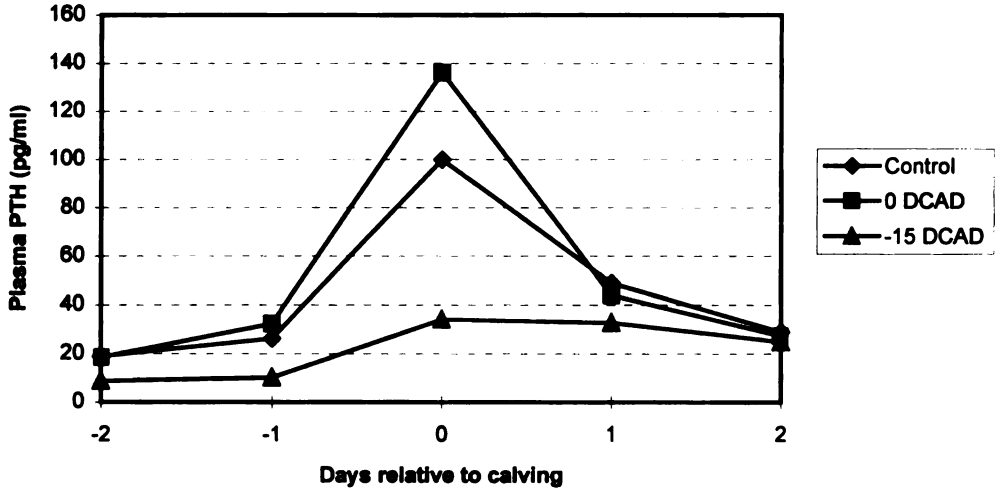


Figure 3. Treatment by day interaction on plasma PTH concentrations (pg/ml).

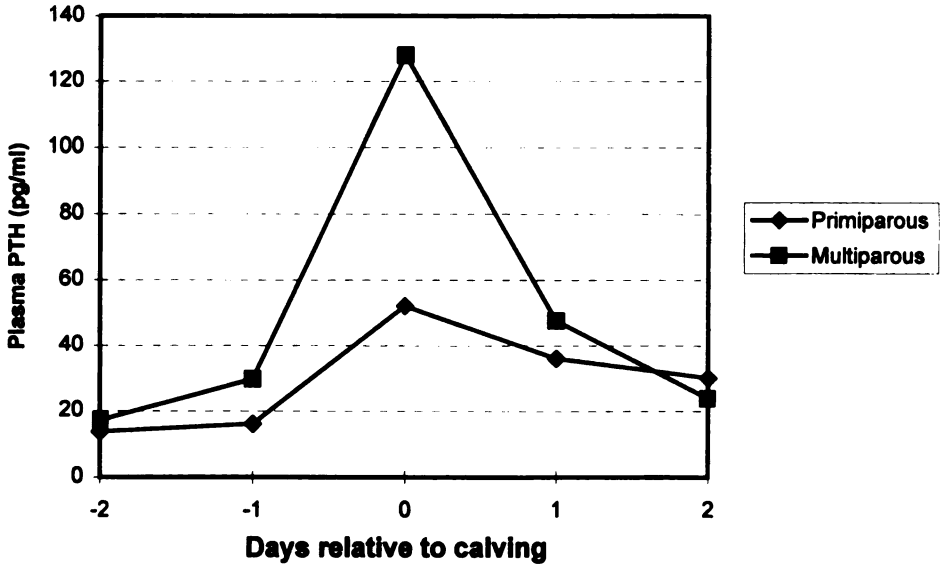


Figure 4. Parity by day interaction on plasma PTH concentrations (pg/ml).

Plasma samples were analyzed at -7, -1 d pre-calving, and 0 h, 1 d post-calving for concentrations of calcitriol. Mean plasma calcitriol concentrations for these sample

points were transformed to log base 10 for analysis because of heterogeneous variances. Calcitriol was affected by treatment, parity, and day ($P < 0.001$). The interactions of parity by treatment, treatment by day, and parity by day were also significant ($P < 0.05$) (Figures 5 and 6). Table 5 gives the actual calcitriol concentrations for the Control and treatment diets. Cows fed the Control diet had higher calcitriol plasma concentrations than cows fed the 0 and -15 DCAD diets ($P < 0.001$), but plasma calcitriol concentrations of cows fed 0 DCAD did not differ from the -15 DCAD ($P > 0.1$). The lower plasma calcitriol concentrations with increased plasma iCa concentration is consistent with the results of others (Bushinsky et al., 1982; Goff, 1992a; Jonsson, 1978; Kumar, 1980). Gaynor et al. (1989) however, reported that anion diets increase plasma calcitriol before calving. However, the increase in calcitriol that they saw was most likely due to an increase in Ca flux and the fact that the cows fed the anion diet actually had lower plasma Ca concentration prior to calving than their cation group (the same time period that calcitriol was analyzed for). The higher plasma PTH and calcitriol concentrations of Control cows (which were also hypocalcemic) is also consistent with the thought that bone and renal tissues of hypocalcemic cows may have reduced sensitivity to the effects of PTH and calcitriol (Block, 1992; Goff, 1992a). The higher calcitriol concentrations of Control cows are also consistent with the positive effects of plasma PTH concentration on calcitriol concentration (Block, 1992; Jonsson, 1978).

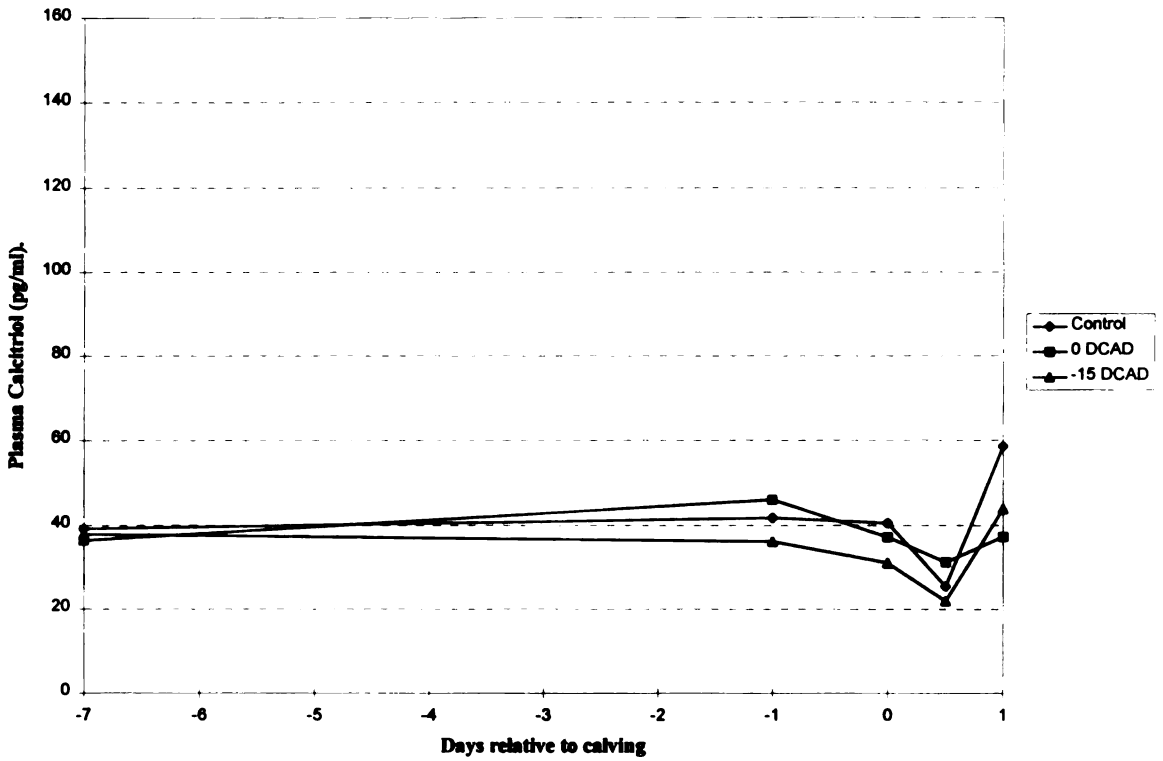


Figure 5. Treatment by day effects on plasma calcitriol concentrations (pg/ml) of primiparous cows in the peripartum period.

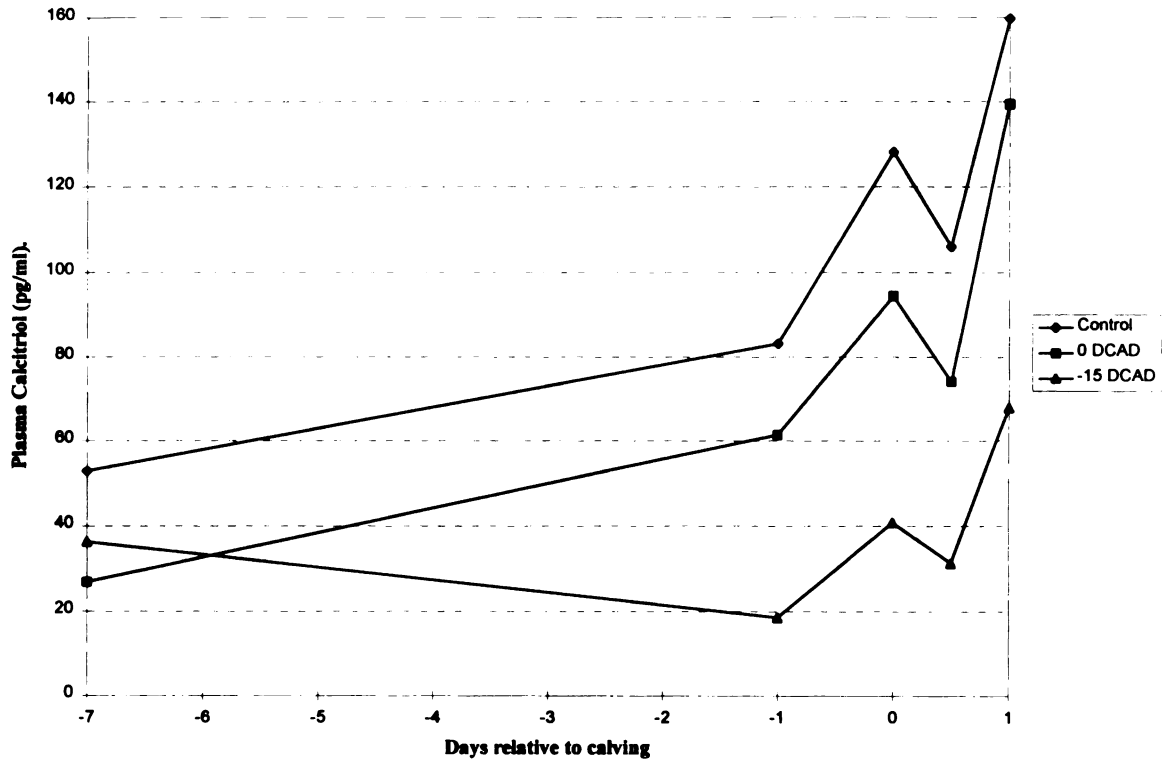


Figure 6. Treatment by day effects on plasma calcitriol concentrations (pg/ml) of multiparous cows in the peripartum period.

Acid-base balance: urine pH, plasma pH, plasma pCO_2, and plasma HCO_3

Cows fed the Control diet had higher urine pH than cows fed the 0 and -15 DCAD diets during the prepartum period ($P < 0.005$) (Table 6). Cows fed the 0 DCAD diet had higher urine pH than cows fed the -15 DCAD diet ($P < 0.005$). The decreased urine pH indicates that the acid-base status of the cows was altered by the addition of anionic salts to their diets (Goff, 1992a; Tucker et al., 1988).

Plasma pH and pCO_2 were not affected by treatment in the prepartum period ($P = 0.18$ and $P = 0.15$ respectively) (Table 6), although numerically they were both lower for cows fed the -15 DCAD diet. Plasma HCO_3 was lower in cows fed the -15 DCAD diet

than cows fed the 0 DCAD diet ($P < 0.001$), and Control cows had higher plasma HCO_3 concentrations than did 0 DCAD and –15 DCAD ($P < 0.001$) (Table 6).

According to the Henderson Hasselbach equation; blood pH = 6.1 + $\log_{10}[HCO_3/(.03 \text{ X } pCO_2)]$. Changing either plasma HCO_3 or pCO_2, will thus have an immediate affect on plasma pH if there is not a concurrent change in the other. In this study as in others (Tucker et al., 1991; Tucker et al., 1988), plasma HCO_3 was decreased with the inclusion of anionic salts but pCO_2 was not. In the present study, HCO_3 was not reduced until urine pH was acidic and DCAD was negative. The necessity for urine pH to be acidic and DCAD to be negative before plasma HCO_3 is affected is not supported by Tucker et al. (1991).

Table 6. Effects of treatment on acid-base status of cows up to 1d prepartum.

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Urine pH	7.98	7.30	6.21	0.082	$P < 0.001$	$P < 0.001$
Plasma pH	7.58	7.58	7.56	0.011	$P = 0.29$	$P = 0.12$
Plasma pCO_2, mm Hg	25.8	25.3	24.0	0.67	$P = 0.16$	$P = 0.16$
Plasma HCO_3, mM	24.1	23.5	21.1	0.38	$P < 0.001$	$P < 0.001$

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

At the time immediately after calving (0 h, 12 h, 1 d), plasma pH was affected by treatment ($P<.05$) with Control and 0 DCAD having higher pH than -15 DCAD (Table 7). The difference in pH soon after calving may have been due to a lag effect of higher anions being present in –15 DCAD cows as compared to Control and 0 DCAD cows. Plasma pCO_2 and HCO_3 were not affected by treatments (Table 7).

Table 7. Effects of treatment on acid-base status of cows immediately after calving (0h, 12h, and 1d).

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Plasma pH	7.6	7.6	7.5	.01	$P < 0.05$	$P < 0.05$
Plasma pCO_2, mm Hg	26.1	26.0	27.5	.98	$P = 0.59$	$P = 0.28$
Plasma HCO_3, mM	25.3	24.4	23.5	.60	$P = 0.08$	$P = 0.30$

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

Dry matter intake

Prepartum DMI data were analyzed as sets for 3 d before to 2 d after cows were offered the experimental diets, as repeat measures during the 3 wk prepartum period, and as average DMI by each cow across the 3 wk prepartum period.

Analyzing the data for -3 to +2 d on treatment diet, showed a significant effect of parity ($P < 0.001$), day ($P < 0.001$), and treatment by day ($P < 0.001$) on DMI (Table 8, Figure 7, respectively). There was also a trend for a parity by day ($P = 0.07$) and treatment ($P = 0.07$) effect on prepartum DMI. DMI was not affected (Figure 7). This is likely due to the 3 d before dietary treatments were administered being included in the analysis. A treatment by day interaction existed for DMI, showing that –15 DCAD decreased DMI over time, whereas DMI was not affected by 0 DCAD or Control (Figure 7).

Table 8. Effect of parity on DMI (kg/d) from -3d to +2d on treatments.

Item	Primiparous	Multiparous	SEM	P
DMI	10.8	16.7	0.55	0.001

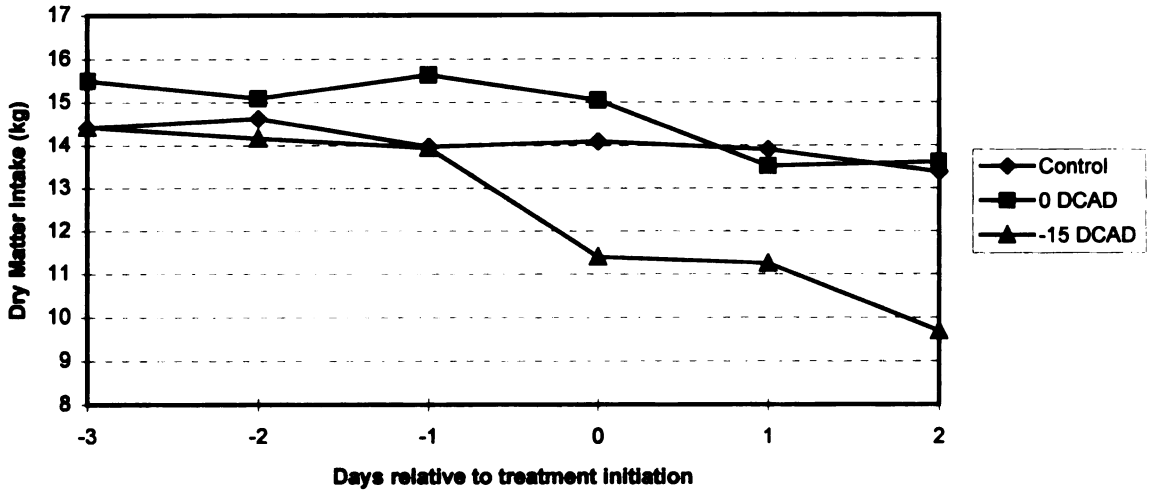


Figure 7. Effect of treatment by day interaction on DMI ($P < 0.001$) SEM = 0.69.

When analyzing the DMI data for 3 wk prepartum to calving, week ($P < 0.001$), parity by week ($P < 0.001$), parity ($P < 0.001$), and treatment ($P < 0.005$) were all significant (Table 9, Figure 8). Over the 3 wk prepartum period, primiparous cows had mean DMI of 9.6 kg/d compared with 14.1 kg/d DMI for multiparous cows (SEM = 0.56).

Table 9. Treatment effects on prepartum[1] DMI (kg/d), BW (kg), and BCS.

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Prepartum						
DMI	12.9	12.5	10.2	0.55	$P < 0.05$	$P < 0.01$
BW	733	729	701	5.2	$P < 0.01$	$P < 0.001$
BCS	3.88	3.77	3.63	0.084	$P = 0.11$	$P = 0.20$

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

[1] Prepartum period equals -3 wk to calving for DMI. Prepartum BW and BCS are at -1 wk adjusted for pretreatment covariate.

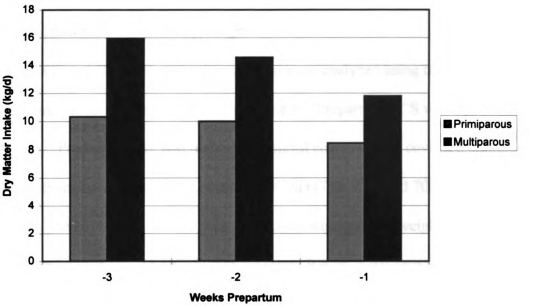


Figure 8. Parity by week interaction on DMI ($P < 0.001$) SEM = 0.56.

Cows fed Control diet had higher DMI than did cows fed the 0 and -15 DCAD diets ($P < 0.05$) during the 3 wk prepartum period (Table 9). Cows fed the 0 DCAD diet had higher DMI than did cows fed the -15 DCAD diet ($P < 0.01$). This DMI depression in the - 15 DCAD diet is consistent with the experimental hypothesis. These results are also consistent with the literature in that anionic salts are not palatable and dietary formulation must account for this reduction in DMI (Beede, 1995; Goff, 1992a). The reduced DMI seen in the prepartum period might be expected to affect body weight and BCS.

Postpartum DMI was analyzed using the same model as prepartum DMI with 10 wk of data per cow. Treatments had no effect on postpartum DMI ($P > 0.42$). There was a trend for a treatment by parity interaction ($P = 0.08$) with the 0 DCAD tending to have greater DMI than the -15 DCAD diet in multiparous cows.

Body condition score and body weight

BCS and BW (kg) at -1 wk prepartum were analyzed using the respective pretreatment mean values as covariates (Table 9). Prepartum BCS was not affected by treatment. Prepartum BW was higher for Control cows than for cows fed anionic salts and higher for cows fed 0 DCAD than -15 DCAD (733, 729, and 701 respectively, $P < 0.001$, SEM = 5.23). Thus although all cows were gaining weight prior to calving, DMI depression in the -15 DCAD diet did lower BW at -1 wk prepartum compared with the 0 DCAD diet.

Postpartum, BCS was only affected by parity and week. Body weight was affected by week, parity by week, and parity ($P < 0.005$). There was a trend for a treatment by parity interaction ($P = 0.1$).

Energy balance

Prepartum (-2 and -1 wk) energy balance was lower for cows fed the -15 DCAD diet than for cows fed the 0 DCAD diet (EB = 0.8 and 5.5 Mcal/day, $P < 0.01$, SEM = 1.14) (Table 10). Reduced energy balance prepartum, resulted from the depression in BW and DMI seen in the prepartum period with cows fed the -15 DCAD diet compared with cows fed the 0 DCAD diet, but also indicated all treatments maintained a positive energy balance in the prepartum period.

Postpartum (2 - 9 wk) energy balance was not affected by treatment (Table 10).

Table 10. Energy balance (Mcal/d) as affected by treatment, prepartum and postpartum.

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Prepartum energy balance	5.1	5.5	0.8	1.14	P = 0.17	P < 0.01
Postpartum energy balance	-2.1	-.74	-1.2	1.2	P = 0.45	P = 0.80

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

NEFA concentrations in plasma

Treatment had no effect on NEFA concentrations (P > 0.9) (Table 11). NEFA concentrations were affected by day, pre and postpartum (Figure 9). NEFA concentrations increased as parturition approached, with peak NEFA concentrations occuring 1 d postpartum. NEFA concentrations gradually declined after parturition.

Diets were formulated to meet the energy and nutrient requirements of a 612 kg cow consuming 10 kg of DMI. These results emphasize the need for the formulation of a nutrient dense diet for the prepartum cow when feeding anionic salts.

Table 11. Plasma NEFA (uM) and IGF-I (ng/ml) concentrations by treatment.

Item	Control	0 DCAD	-15 DCAD	SEM	P
NEFA	550	510	530	53.7	0.9
Prepartum IGF-I	216	195	193	13.4	0.441
Postpartum IGF-I	128	126	112	16.3	0.738

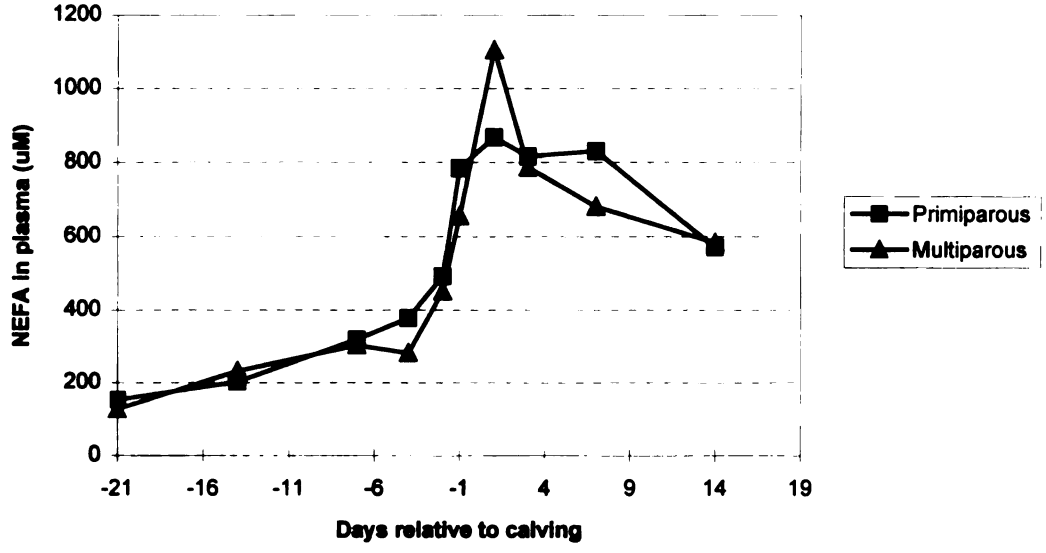


Figure 9. Plasma NEFA concentrations (uM) before and after parturition ($P < 0.001$) SEM = 74.2.

Plasma IGF-I

IGF-I concentrations for prepartum and postpartum samples were analyzed separately. Treatment had no effect on plasma IGF-I concentrations prepartum or postpartum ($P > 0.4$) (Table 11). Prepartum IGF-I concentrations were affected by day and parity ($P < .005$), with IGF-I concentrations decreasing as parturition approached and IGF-I concentrations being lower in multiparous cows than in primiparous cows (165 vs. 237 uM respectively, $P < 0.005$, SEM = 15). Postpartum IGF-I concentrations were only affected by parity ($P < 0.005$), with multiparous cows having lower plasma concentrations than primiparous cows (89 vs. 155 uM respectively, $P < 0.005$, SEM = 13).

Plasma IGF-I concentrations have been positively correlated with energy balance in heifers and cows (Sharma et al., 1994; VandeHaar et al., 1995; Zurek et al., 1995).

Plasma IGF-I concentrations decreased as parturition approached, which is consistent with the decrease in DMI as calving approached.

Calf weight

Calf weight was higher for multiparous than primiparous cows ($P < 0.05$). There was a trend for cows fed the 0 DCAD diet to have larger calves than cows fed the -15 DCAD diet ($P = 0.08$) (Table 12).

As mentioned previously, DMI and body weight gain were both suppressed by the -15 DCAD diet. Whether this contributed to the reduced calf weights in the -15 DCAD diet cannot be determined.

Table 12. Treatment effects on calf weight (kg).

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Calf Weight	42	46	42	1.32	P = 0.27	P = 0.08

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

Milk yield and components

There were no effects of treatments on milk yield or any milk components when analyzed for 2 through 9 wk postpartum (Table 13). There was a treatment by week effect for 4% FCM yield ($P < 0.05$, Figure 10) with cows fed the -15 DCAD diet appearing to start out at a lower level of production. Figure 10 shows that the mean FCM for the Control group dropped sharply after wk 6 postpartum. One cow in the Control group dropped significantly in production during this time period due to mastitis.

Removal of this cow did not change the statistical outcome or make any meaningful change to the graphs of the results, therefore that cow is included in the data analysis.

Table 13. Treatment effects on daily milk yield and components.

Item	Control	0 DCAD	-15 DCAD	SEM	C – 1	C – 2
Milk (kg)	37	37	34	1.3	P = 0.45	P = 0.15
FCM (kg)	36	36	34	1.5	P = 0.70	P = 0.23
Fat %	3.8	3.8	3.8	0.12	P = 0.75	P = 0.98
Fat (kg)	1.4	1.4	1.3	0.07	P = 0.87	P = 0.34
Protein %	2.8	2.9	2.9	0.05	P = 0.56	P = 0.80
Protein (kg)	1.0	1.1	1.0	0.04	P = 0.64	P = 0.14
Lactose %	4.2	4.3	4.3	0.07	P = 0.09	P = 0.65
Lactose (kg)	1.5	1.6	1.5	0.06	P = 0.92	P = 0.13
SCC	392	308	444	114	P = 0.91	P = 0.41

C - 1: +14 vs. 0 and -15 DCAD, C - 2: 0 vs. -15 DCAD

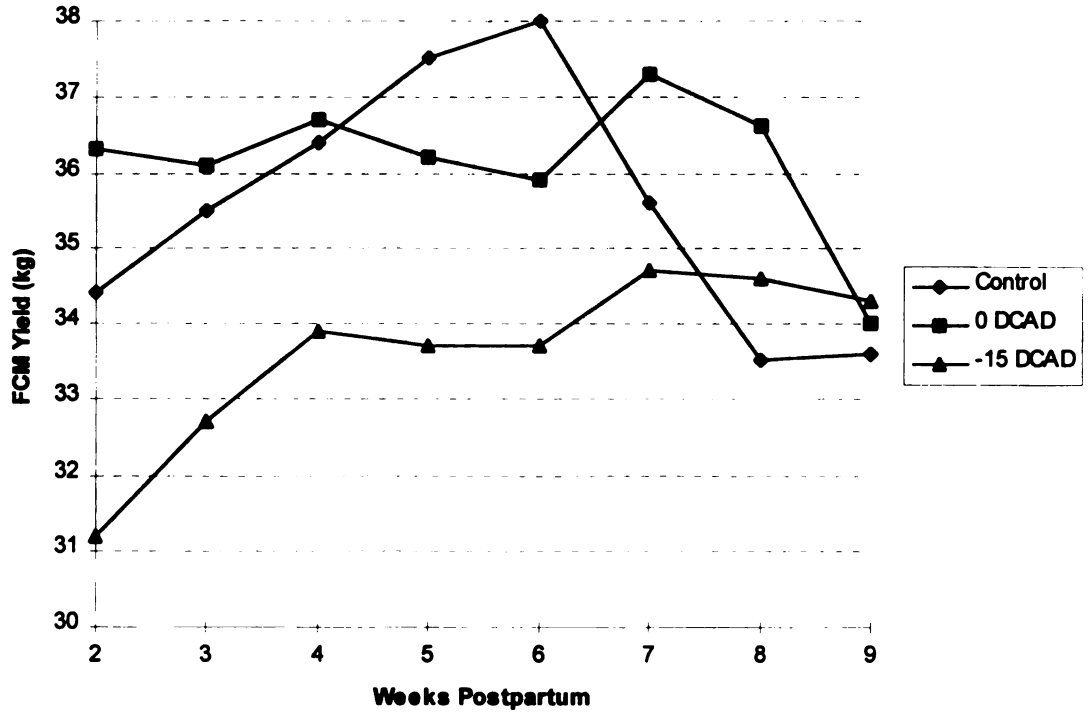


Figure 10. Treatment by week interaction on 4% FCM (kg), (P < 0.05) SEM = 0.938.

Any prepartum dietary effects on milk yield and components would most likely occur shortly after parturition. Milk yield and component data were thus also analyzed for treatment effects from 2 to 5wk postpartum. Milk yield tended to be lower for cows fed the -15 DCAD diet than for cows fed the 0 DCAD diet (milk yield = 32.6 and 36.6 kg/d respectively, P = 0.07, SEM = 1.47). FCM yield did not differ (P = 0.13).

Milk yield and FCM yield responses were not consistent with the literature (Beede, 1995; Block, 1984). Block showed that cows fed an anionic diet prepartum had higher (7% increase) 305-d milk yields than cows fed an cationic diet. Verdaris and Evans (1974) showed that when looking at Ca concentrations in the diet, cows with high Ca (2.48%) during an 86-d dry period and 84-d milk period produced the most milk compared with cows with low-low (prepartum-postpartum, 0.23%), low-high, or high-low dietary Ca concentrations. Increasing inclusion of anionic salts in the present experiment caused a lower DCAD as well as greater dietary Ca concentration. These studies suggest that both effects could increase milk production for the -15 DCAD treatment group. However, in the present experiment cows on –15 DCAD prepartum appeared to have lower FCM production at wk 2 postpartum (Figure 10). Lower milk yield at 2 wk postpartum may have been due to the suppressed DMI during the late dry period with the increased anionic salts (-15 DCAD).

Incidence of Metabolic Disorders

Table 14 shows the incidences of the various metabolic disorders that were recorded during the experiment. Analysis with Fisher's Exact Test (2-Tail) showed that there were no differences in the incidences of any of the metabolic disorders as affected

by treatment. Cows having twins were not included in this analysis. There was a trend (P = 0.09) for a difference in milk fever incidence, with all incidences of milk fever occurring in cows (three of nine multiparous cows) fed the 0 DCAD diet. Number of cows per treatment group was too small to reliably detect differences in metabolic disorder.

Table 14. Incidence of metabolic disorders.

Item	Parity	Control	0 DCAD	-15 DCAD
Milk Fever	Primiparous	0	0	0
	Multiparous	0	3	0
Ketosis	Primiparous	5	5	4
	Multiparous	2	0	2
Displaced Abomasum	Primiparous	2	1	3
	Multiparous	0	0	1
Retained Placenta	Primiparous	2	1	0
	Multiparous	3	1	1
Metritis	Primiparous	2	2	0
	Multiparous	2	0	1

SUMMARY AND CONCLUSIONS

Milk fever (hypocalcemia) has been defined as plasma iCa concentrations ≤ 4 mg/dl (Beede et al., 1992; Oetzel et al., 1988). Low plasma iCa concentrations cost the U.S. dairy industry over $120 million/year in the cost of health disorder treatments and secondary problems (Goff and Horst, 1990). The feeding of anionic salts to the prepartum cow is becoming a popular method to control hypocalcemia. The focus of this experiment was to determine if a lower inclusion of salts (0 DCAD) would have the same positive effects on Ca status in the dairy cow as that currently recommended (-10 to -15 meq/100 g DM DCAD), while improving DMI. In order to accomplish this change in DCAD, an anionic salts pack was developed for the -15 DCAD diet and included at a lower rate for the 0 DCAD diet. This method of adjusting the DCAD would be most consistent with how a dairy producer would adjust the DCAD on the farm. The one confounding factor with this type of approach was that the dietary Ca concentrations increased as more anionic salt pack was fed.

The results of this study are not completely consistent with the hypothesis or alternative hypothesis. Prepartum DMI was depressed in the -15 DCAD treatment compared with 0 DCAD treatment. Cows fed the -15 DCAD diet had lower BW 1 wk prepartum compared with cows on the 0 DCAD diet. Prepartum energy status was

decreased for the cows fed the -15 DCAD diet, but was still positive. Plasma NEFA, IGF-I, and hydroxyproline concentrations were not affected by treatment. Decreasing DCAD increased plasma iCa concentrations both prepartum and in samples shortly after calving (0 h, 12 h, 1 d). There was a parity by treatment effect on plasma iCa concentrations with primiparous cows being unaffected by treatment, whereas iCa increased as DCAD decreased in multiparous cows. Acid-base status of the cows was affected by treatment, with cows fed the -15 DCAD diet having lower plasma HCO_3 concentrations than cows fed the 0 DCAD diet. Urine pH also decreased with decreasing DCAD during the treatment period. Control cows had higher PTH and Calcitriol concentrations than cows on the 0 and -15 meq/100 g DM. There was a treatment by week effect on FCM yield (2 through 9 wk) with cows fed -15 DCAD starting at a lower level of milk production.

The results of this experiment indicate that anionic salts should be fed to prepartum cows at a level to prevent hypocalcemia and maintain DMI. This will require careful diet formulation and careful monitoring of urine pH by dairy producers to evaluate the effectiveness of anionic salts. The lack of hypocalcemia, along with the negative effects of anionic salts on DMI, suggests that dairy producers should not feed anionic salts to primiparous cows.

The present study indicated that urine pH can be used as an indicator of the acid-base status of the cow. The urine pH values found in this experiment will give dairy producers a good tool for estimating the success they are having in reducing the DCAD for their close-up cows. One strategy for producers, would be to formulate diets at 0 DCAD and then titrate urine pH to 7.0 by including more or less anionic salts in the diet.

This may insure a mild metabolic acidosis that would result in less DMI depression than the -15 DCAD diet did in this experiment.

Future research will need to look into separating out the effects of DCAD and dietary calcium concentration on plasma iCa concentrations. Research will also need to look at ways to better determine how anionic salts increase plasma iCa levels. Specifically how much of the increased plasma iCa is from intestinal absorption or bone resorption, and does one source decrease parturient hypocalcemia to a greater extent.

APPENDIX

APPENDIX

Procedure for IGF-I analysis

Iodination was carried out using 2 ug recombinant pure human IGF-I (Bachem Inc., Torrance, CA) dissolved in 10 ul 0.1 N HCl . Thirty-five ul 0.5M phosphate buffer solution (PBS) was added and mixed. One mCi ^{125}I radionucleotide in 0.1 M NaOH (DuPont , Boston, MA) was added and mixed. Chloramine T (25 ul; 1 ng/ml) was added using a Hamilton syringe and mixed. Fifteen seconds later, 50 ul sodium metabisulfite (5 mg/ml) were added and mixed. Twenty seconds later, 100 ul of transfer solution were added and mixed. This reaction mix (about 100 to 200 ul total volume) was then carefully transferred to a column bed (see Appendix for description). Twenty-five 0.5-ml fractions were then collected from the column using elution buffer. The column was used to separate bound (i.e., incorporated) ^{125}I from free ^{125}I. Ten ul from each fraction were counted for 0.1 min. Fractions comprising the first peak (bound ^{125}I) were saved for use in the IGF-1 assay. Free ^{125}I was discarded. Fractions containing the first peak were tested for TCA precipitable counts to determine if the ^{125}I had indeed labeled IGF-I. A test for total binding and specific binding was run on the fractions to determine the level of specific binding in each fraction.

Serum samples for IGF-1 assay were extracted by an ethanol acid procedure (Bruce et al., 1991) with modifications by Sharma et al. (1994) to remove IGF-I binding proteins. To each 50 ul of serum sample, 12.5 ul of formic acid and 250 ul absolute ethanol were added. Samples were mixed, by vortex, and allowed to stand at room temperature for 30 min. Samples were then centrifuged for 30 min at 4°C at 600 x g. An aliquot of supernatant (50 ul) was removed and diluted to 1.05 ml total volume using neutralizing buffer. The total dilution was 1:131.25. Of this extract, 50 ul in triplicate were used for the IGF-I assay.

Standard curve consisted of 0, 10, 20, 30, 40, 60, 80, 100, 150, 200, 300 and 400 pg/tube of diluted standard A. Fifty ul of serum extract were pipetted in triplicate assay tubes. Tubes labeled NSB contained no first antibody. Tubes labeled TC were kept empty. The final volume of all assay and standard tubes were brought to 200 ul with assay buffer.

To these assay tubes, 250 ul of first antibody (diluted 1:40,000) was added (except to TC and NSB samples), tubes were mixed, by vortex, and all samples were incubated at 4°C overnight. To NSB tubes, 250 ul of assay buffer was added. On d 2, 18,000 to 20,000 cpm iodinated IGF-I was added to each tube including TC tubes. Tubes were stored at 4°C for another 48 h. On d 4, 200 ul/tube of Staph A (dissolved in 39 ml assay buffer) were added to all tubes except TC tubes. Samples were mixed, by vortex, and stored at room temperature for 4 h. Two ml of assay buffer were added to each tube and all tubes were centrifuged for 30 min at 4°C and 3000 rpm. The supernatant was discarded properly. All tubes were dried with pellet intact and then counted for 1 min.

Plasma IGF-I content was calculated using the standard curve developed from the standards.

Buffers and reagents for RIA of IGF-I

Recombinant human IGF-I:

rhIGF-1 from (Bachem, Inc., product #DGR012), 50 ug is dissolved in 1 ml of 10 mM acetic acid solution and made aliquots of 40 ul = 2 ug into labeled Eppendorf tubes, dried in Savant and stored at -20C until used.

International reference standard of IGF-I from World Health Organization (WHO):

Obtained 3.1 ug IGF-I containing ampoule from National Inst. of Biological Standards and Control (NIBSC). Dissolved the total content in 1 ml of 0.05M HCl and transferred to already tared 50 ml orange top tube and make final weight of solution to 15.5 g using 0.03 M phosphate buffer pH 7.5. Make 100 ul aliquot and store at -20 C.

8% Bovine Serum Albumin (BSA):

0.8 g BSA Qs to 10 ml total volume with water, pH at 7.5

Protein A positive Staph. aureus cells:

Dissolve 1g of Staph A (BM 100 061) into 10 ml distilled water. And make into 1 ml aliquot. Store at -20 C.

Assay buffer:

It is 0.03 M sodium phosphate, .01 M EDTA, 0.02% protamine sulfate, 0.02% sodium azide and 0.05% Tween-20 with final pH 7.5.

Elution buffer:

0.03 M phosphate buffer with 0.25% BSA.

Neutralizing buffer pH 7.5:

Na_2HPO_4 0.11 moles/liter; NaCl 0.154 moles/liter; Na_2EDTA 0.01 mol / liter; Sodium azide 0.015 moles/liter; Solution Tween-20 .5%w/v. [weigh 15.62 g Na_2HPO_4, 9.0g NaCl, 3.7 g EDTA, 0.975g Na-azide, and add 0.5 ml of 10% solution of Tween-20.

Transfer solution:

Weigh 100 milligram of potassium iodide (KI), and weigh 1.6 g sucrose. Dissolve them in double distilled water to make total volume=10 ml. Make 1 ml aliquot and store at -20C.

PBS solution:

It is .5 M sodium phosphate buffer pH 7.5.

Standard curve for IGF-I assay:

20 nanograms of rhIGF-I (in 100 ul PBS) was made to a total volume of 200 ul with assay buffer. 150 ul of this solution (15 nanograms) was removed and made to a total volume of 3 ml with assay buffer. This solution was called A (5 picograms/ul). Out of the 3 ml of solution A, 1 ml was removed and taken to total volume of 5 ml with assay buffer. This second solution was called B (1 picogram/ul).

Preparation of Column:

1) Prepare a 10 ml column using 2 parts of coarse and 1 part of medium sized Sephadex G-50 swollen in .03 M PBS. Use yellow pipet tip and glass wool to regulate flow. Run plenty of degassed elution buffer through the column. Regulate the flow at about 1 ml/5 min.

2) Run 1 ml of 8% BSA to coat the column material with protein.

3) Prepare a fresh solution of sodium metabisulfite 5 mg/ml.

4) Prepare fresh solution of Chloramine T, 1 mg/ml. Make in dark, wrapped with foil. Weigh 18 mg and dissolve in 18 ml .03 M sodium phosphate buffer with no BSA.

Determining TCA Precipitable Count:

1) 50 ul from each fraction comprising the peaks was placed into a series of duplicate 12x75 tubes, keeping track of cpm that was added to tubes from each fraction.

2) 800 ul of ice cold 8% BSA was added.

3) 200 ul of ice cold 100% TCA was added.

4) All samples were chilled on ice for 30 minutes.

5) 1-2 ml of assay buffer was added.

6) Samples were spun at 300 rpm for 30 minutes.

7) Supernatant was transferred to a new set of tubes using a 1 ml pipet

8) Both sets of tubes were corked and counted in the gamma counter.

9) Total cpm, cpm in supernatant, and % cpm in pellet were calculated.

10) The free iodine peak was determined.

11) Fractions comprising free iodine peaks were discarded as FREE I-waste.

Test for Total Binding and Specific Binding

For each fraction of bound IGF-I, an assay was set up to test the actual specific binding. The table below shows an example of the specific binding assay.

Fraction No.	7	8	9	10	11	12	13
TC (about 15000cpm)	TC	TC	TC	TC	TC	TC	TC
NSB (No 1st AB)	NSB	NSB	NSB	NSB	NSB	NSB	NSB
Zero, 200 ul buffer	0	0	0	0	0	0	0
50 ul=50 pg IGF-I in 200 ul elution buffer	50	50	50	50	50	50	50
100 ul=100 pg IGF-I in 200 ul elution buffer	100	100	100	100	100	100	100

Note: Tubes were labeled as:
NSB got no first AD but everything else.
TC got nothing but iodinated IGF-I from its respective fraction.
Zero got 200 ul buffer and then everything else.

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