THE EFFECTS OF SUPPLEMENTAL ANIONIC SALTS & YEAST CULTURE ON THE PRODUCTION OF DAIRY CATTLE DURING THE PERIPARTURIENT PERIOD

A Thesis presented to the Faculty of the Graduate School
University of Missouri-Columbia

In Partial Fulfillment
Of the Requirements for the Degree

Master of Science

By

REAGAN JANEEN VOGEL BLUEL

Dr. James N. Spain, Thesis Supervisor

DECEMBER 2006

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

THE EFFECTS OF SUPPLEMENTAL ANIONIC SALTS AND YEAST CULTURE ON THE PRODUCTION OF DAIRY CATTLE DURING THE PERIPARTURIENT PERIOD

presented by Reagan J. Bluel,

a candidate for the degree of Masters of Science

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Professor Jim Spain, advisor

_____

Professor Ron Belyea

_____

Professor Barry Steevens

_____

Professor H. Allen Garverick

_____

Professor Mark Ellersieck

ACKNOWLEDGEMENTS

I would like to acknowledge those who helped make this thesis possible. First and foremost, I would like to thank my husband Neal I. Bluel for his dedication and support. Thank you for helping me start each morning off right with words of encouragement and a hot cup of coffee. I would like thank my father, Ray E. Vogel, for his assistance both emotionally and financially through my college years – I would not have been able to do it without you! Dr. Jim Spain deserves a big thanks for his guidance over the past seven years. Also, thank you Dr. Rob Kallenbach for reigniting my invigoration for research.

My graduate committee, Drs. Ron Belyea, Barry Steevens, Allen Garverick, and Mark Ellersieck who provided insight throughout my Masters. Thank you Dr. Belyea for teaching me the intricacies of ruminant nutrition and for being such a great friend. Thanks are extended to Foremost dairy farm's crew. More specifically, I would like to thank farm manager John Denbigh - for his knowledge, assistance and mostly for his patience. Thank you Dawe's and Diamond V Laboratories for their financial contributions in this research endeavor. More specifically, I would like to thank Drs. Dave Kirk and Ilkyu Yoon for their expertise.

I would also like to thank all the undergraduate workers that aided in the completion of the largest transition dairy trial completed at the University of Missouri-Columbia to date: Jesse Cheever, Katie Voelker, Marin Summers, Lindsay Parsons, Fallon Brice. Zach Brockman deserves an especially big thank

TABLE OF CONTENTS

Chapter

    1.   LITERATURE REVIEW

LIST OF FIGURES

LIST OF TABLES

LIST OF ADDENDUMS

THE EFFECTS OF SUPPLEMENTAL ANIONIC SALTS & YEAST CULTURE ON

THE PRODUCTION DAIRY CATTLE DURING THE PERIPARTURIENT

PERIOD

Reagan Janeen Vogel Bluel

Dr. James N. Spain, Thesis Supervisor

To determine nutritional strategies with the strategic use of feed additives during the periparturient period to reduce the negative energy balance of the transitioning dairy cow. Two research trials were conducted to evaluate the effect of a sulfur-based anionic salt fed during late gestation and yeast culture fed during the periparturient period on mineral and energy metabolism, intake, health, and production of Holsteins.

To evaluate the success of anionic salts in mineral and energy metabolism twenty-six mature cows were pair by expected calving date, lactation number, milk production potential, and body weight. Cows within pair were then randomly assigned to one of two diets. The dietary treatments were control (C) and supplemental anionic salt (A). Cows were fed the experimental diets as TMR via electronic feeding gates. Control diet was formulated to achieve a Dietary Cation-Anion Difference (DCAD) of +20 mEq/100 g dry matter. Control diet was predicted to provide 70g of calcium per cow per day. The treatment group was fed 454g per cow per day of a commercially formulated anionic salt supplement which lowered the DCAD level to -10 mEq/100 g dry matter. Treatment diets were formulated to provide a daily intake of 150g of calcium per cow per day.

Diets were fed 30 days prior to expected day of calving. At calving, cows were fed standard lactation TMR for the first 6 weeks of lactation. Feed intake was measured daily. Urine pH was monitored twice each week using an electronic pH meter. Blood samples were collected weekly prepartum as well as on day -3 and day of calving. Postpartum blood samples were collected on day 1, 3, 7, 10 and 14 of lactation and then weekly until day 42. Blood samples were analyzed for Ca and NEFA. Daily milk yields and weekly milk component data were also collected. These data were analyzed for significance using SAS proc mix method.

Sulfur based anionic salts when fed during late gestation results in improved calcium metabolism. This is a result of mild metabolic acidosis that was observed through a significant decline in urine pH, increase in blood chloride, and decreased blood CO_2. Body weight and body condition score was not affected by treatment, however serum nonesterfied fatty acids were lowered for cows fed anionic salt. This suggests the cows fed anionic salts were in a less negative energy balance through the transition period. This concept was further supported with the elevated dry matter intake observed during the first three weeks of lactation. Milk production, four percent fat corrected milk, milk fat, and milk protein were not affected by the addition of anionic salts during gestation. However milk urea nitrogen and the more sensitive measurement of postpartum blood urea nitrogen did differ between treatments with anionic salt cows producing elevated urea nitrogen concentrations. The improvement during the transition from gestation to lactation seen as the result of feeding anionic salts

prepartum is likely a result of improved liver function. This concept was supported with control cows exhibiting elevated alkaline phosphatase and bilirubin concentrations during early lactation.

To evaluate the success of yeast culture energy metabolism ninety-five pregnant Holstein cows were fed one of three treatments from thirty days prepartum through day 77 post-partum. Dietary treatments consisted of: 1) no supplemental yeast culture (Control, C), 2) 56g of yeast culture (YC), or 3) 14g of concentrated yeast culture (CYC). Individual feed intake and milk production were measured daily. Body weight and body condition score were recorded weekly. Metabolic status was measured by analysis of blood samples collected sequentially throughout the study.

Yeast culture treatment did not affect DMI prepartum. Body weight and change in body weight were similar among treatments during late gestation and early lactation. Cows fed CYC had a decrease in body condition score after calving that differed ($P = 0.03$) from cows fed the control diet. Yeast culture resulted in a significant quadratic response ($P < 0.002$) in DMI as a percent of body weight after calving, with a concurrent quadratic increase in 4% FCM compared to control. These results suggest that cows supplemented with yeast culture experienced improved rumen function during transition leading to increased feed intake, milk fat percentage and FCM yield during early lactation.

CHAPTER 1

LITERATURE REVIEW

THE TRANSITION DAIRY COW

The periparturient dairy cow experiences a significant metabolic and physiological transition from pregnant, non-lactating through calving to high levels of milk production in early lactation. The periparturient period as defined by Grummer (1995) is three weeks prepartum to three weeks postpartum. The changes associated with transition include: exponential growth of the fetus (Bell et al., 1995), changing endocrine profiles (Grummer, 1995), compromised immune response (Mallard et al., 1998), increased incidence of metabolic diseases (Goff and Horst, 1997), rapid change in dry matter intake (Hayirli et al., 1998), and mobilization of adipose tissue (Hayirli and Grummer, 2004). In addition to the endocrine and physiological changes, dietary composition and therefore the status of the reticulorumen also experience a significant shift (NRC, 2001). Proper nutrition is paramount to a successful transition (Ingvartsen, 2006). Goff and Horst (1997) identified the nutritional objective of the transition period to involve maintaining normal energy metabolism and normal mineral metabolism. The majority of metabolic diseases have been reported to occur within the first two weeks of lactation (Goff and Horst, 1997). More recently, Godden et al. (2003) supplied supporting evidence when they found approximately 25% of all cows culled in Minnesota between 1996 and 2001 were removed within the first

sixty days of lactation. Therefore, caring for and feeding the prepartum dairy cow is important for optimizing animal production during the subsequent lactation. Much research has been focused on the periparturient dairy cow, as this transition has the most profound effect on the success or failure of the subsequent lactation. The purpose of this review, is to associate the importance of energy and mineral metabolism during the periparturient period with the use of yeast culture and anionic salts as promoters of metabolic energy to assist transitioning dairy cows through this period of stress.

Energy balance

A negative energy balance is associated with the transition dairy cow. As a result, non-esterfied fatty acids (NEFA) are mobilized from body fat. Ingvartsen and Anderson (2000) speculated a down regulation of feed intake is a result of elevated NEFA concentrations in the transition dairy cow. During the periparturient period, DMI and NEFA concentrations are usually inversely related (Overton and Waldron, 2004).

Non-esterfied fatty acids begin to rise 2 to 3 weeks prior to calving resulting in peak concentrations at calving or the first week of lactation (Ingvartsen and Anderson, 2000). Bell et al. (1995) described the parturition in dairy cows as a dramatic shift in adipose tissue metabolism. Many agree the decline in insulin prior to calving results in the initiation of the switch from lipogenesis to lipolysis (Grummer, 1993; Bell et al., 1995; Ingvartsen and Anderson, 2000).

Elevated NEFA concentrations are associated with increased risk of fatty liver (Grummer, 1993), ketosis (Grummer, 1993) and the occurrence of a displaced abomasum (Cameron et al. 1998). The monitoring of non-esterfied fatty acids has been identified as an effective method to determine the energy balance in dairy cattle (Kunz et al., 1985).

Metabolic disorders and disease

Metabolic disorders, such as displaced abomasum (DA), milk fever, retained fetal membrane, and ketosis are more prevalent during the transition period (Goff and Horst, 1997). Constable et al. (1992) associated an elevated incidence of DA during early lactation with depressed intake predisposing the dairy cow to an increased risk of a displaced abomasum due to low ruminal fill. These authors reported 57% of all displaced abomasums occur during the first two weeks of lactation.

Mastitis is also of concern during early lactation. Smith et al. (1985) reported clinical mastitis will most likely occur during the first month of lactation. Cows experiencing hypocalcemia can have reduced sphincter function at the teat opening allowing pathogens into the mammary gland (Kehrli et al., 1990). Included in the increased pathogen load is the degradation of the keratin plug beginning seven to ten days prior to calving.

Often, health disorders during the transition period interrelated. For example, Schukken et al. (1989) reported cows with retained fetal membranes are three times as likely to develop mastitis than those without. Furthermore, hypocalcemia results in decreased rumen motility and therefore predisposes the

animal to a displaced abomasum (Shaver, 1997). More generally, Curtis et al. (1985) found cows with a left DA had an increased incidence of metabolic disorders.

Periparturient immunosuppression is also of major importance, but is not well understood. Overton and Waldron (2004) speculated the etiology of impaired immune function to be multifactorial, related to the physiological changes during parturition and the onset of lactation. Mallard et al. (1998) reported the change in immune and innate host resistance begins three weeks prior to calving and without fully regaining mechanistic protection until three weeks into lactation. As a result of parturient hormone release, stress glucocorticoids are elevated (Roth and Kaeberle, 1982). Elevated glucocorticoids are known to decrease immune function. Overton and Waldron, (2004) stated "the consequence of immunosuppression is that cows may be hypersensitive to invading pathogens and therefore more susceptible to disease, particularly mastitis, during the periparturient period".

Endocrine changes

Hormonal control of gestation and parturition has a profound effect on the transitioning dairy cow. Near parturition, numerous hormones act in succession in the induction of calving. Plasma growth hormone, cortisol, and estrogen begin to increase during late gestation with a rapid increase just before calving and a decline after calving. Concurrently, plasma insulin and progesterone begin to decline at day 250 of gestation (Grummer, 1995). A review by Ingvartsen and

Andersen (2000) summarized the coordination of endocrine changes in the periparturient cow (Table 1.1).

Circulating concentrations of estrogen and progesterone are inversely related throughout gestation. Estrogen maintains a steady increase through gestation. Early gestation estrogen concentrations remain near 20 pg/ml and increases to approximately 300 by mid-gestation (Goff and Horst, 1997). Then, as progesterone declines on approximately day 250 of gestation, estrogen concurrently increases and peaks between 4000 and 6000 pg/ml (Keller et al., 1977). The estrogen surge in late gestation is partly in response to fetal cortisol.

The change in endocrine status results in important metabolic changes. Goff and Horst (1997) found increasing concentrations of estrogen near parturition acted as an immunosuppressant. At parturition and the day immediately after calving, the concentration of estrogen increases 15 - 30 ng/ml. Goff et al. (1989) found that cows which developed milk fever had a higher concentration of circulating plasma cortisol concentrations. Furthermore, these authors observed an increase of plasma cortisol from 11 to 28 ng/ml between one day prior and the day of calving.

Decline in DMI

On average, feed intake declines 30% in the final 17 days of gestation (Grummer, 1995). The decline in feed intake is related to a number of physiological and endocrine factors. With the increase in fetal growth during late gestation, rumen capacity declines. Rumen volume in late gestation is reduced to one third the normal available capacity when the fetus is not present (NRC,

2001). In addition to physical constraints on gut capacity, estrogen has been reported to have an inhibitory effect on dry matter intake and may be associated with changes in appetite and feeding behavior (Grummer et al., 1990). Johnson (1998) found the release of cytokines, in response to infectious diseases, also decrease appetite.

The maintenance of feed intake during the prepartum period has ramifications in the subsequent lactation. Dry matter intake on day one of lactation is positively correlated with intake on day twenty-one postpartum (Grummer, 1995). Futhermore, Mashek and Grummer (2003) reported a correlation between total prepartum DMI during late pregnancy to postpartum DMI and milk production. Therefore, the maintenance of high intake during the periparturient period have implications in the subsequent lactation. Decreased intakes during the periparturant period result in body weight and condition loss, increased fatty acid mobilization, increased metabolic disorders and reduced fertility (Hayirli and Grummer, 2004). To assist during this time of low intake and rapidly increasing milk production, grain supplementation is common to ensure adequate energy intake. Thus a significant dietary change occurs during the transition. The gestation diet contains modest amounts of grain and associatively moderate concentrations of NFC. Conversely, lactation diets contain a much higher concentration of grain with a concomitant increase of rapidly fermented carbohydrates (NFC). The higher levels of NFC coupled with the sudden switch of animals from the dry cow diet to a lactation cow diet places the animals at risk of developing ruminal acidosis. Nocek (1997) summarized graphically the ruminal

changes that occur during transition as the dietary concentrations of non structural carbohydrates increase (Figure 1.1). The figure also describes how this dietary switch can result in a predisposition to acidosis.

Ruminal acidosis is characterized by a reduced pH of rumen contents. Ruminal acidosis was categorized by Krause and Oetzel (2006) as either acute or subacute ruminal acidosis (SARA). Hibbard (1995) defined acute acidosis in feedlot steers when the rumen accumulates lactic acid, resulting in a ruminal pH of less than 5.0. SARA is defined as a series of depressed ruminal pH ranging from 5.2 - 5.6 (Cooper and Klopfenstein, 1996).

The effect of ruminal acidosis is multifaceted. The major derogatory effect of acidosis is reduced or inconsistent intake (Nocek, 1997). Enemark and Jorgensen (2001) reported that Danish dairy practitioners reported that poor feeding management including feed mixing and feed delivery practices were the primary cause of subclinical rumen acidosis in transition dairy cows.

Ruminal acidosis is also associated with an increase risk of laminitis. Donovan et al. (2004) induced acidosis by rapidly changing dietary NE_L concentrations during early lactation and reported the effects on hoof score. Hoof score was assessed in six zones of the hoof. Zone 1, white line at the toe; zone 2, abaxial white line; zone 3, abaxial wall-bulb junction; zone 4, sole bulb junction; zone 5, apex of the sole; zone 6, the bulb. Based on the number of hemorrhages observed, the zones were assigned a score from 0-5. A score of zero indicated no hemorrhages or discoloration and a score of 4 indicated a sole ulcer. Cows fed the dietary treatment with minor changes in NE_L had a higher

hoof score compared to the cows experiencing acidosis that was induced by dietary treatment. A result of dietary induced acidosis, the authors reported hoof scores elevated by more then one score greater during peak lactation then those exposed to minor changes in NE_L. This response is indicative of the release of histamines associated with early lactation acidosis (Nocek, 1997). Sole hemorrhages are a response to a feeding trauma that occurred six to eight weeks prior (Vermunt and Greenough, 1996).

Strategic use of dietary supplements including the feeding of ruminal buffers and direct fed microbials (such as lactic acid bacteria and yeast culture) have been investigated as an approach to ameliorate acidosis. A weak base, such as sodium bicarbonate, buffers against hydrogen ions of organic acids. Investigators have offered supplemental sodium bicarbonate as a free choice powder (Keunen et al., 2003) or diluted in the water source (Cottee et al., 2004). Regardless of sodium bicarbonate's ability to neutralize strong acids, the cows did not choose to consume the free-choice treatments. Conversely, when included in the TMR, sodium bicarbonate has the ability to improve intake (Hu and Murphy, 2005), and increased 4% FCM (Kennelly et al., 1999).

Several investigators have reported improved diet dry matter intake when yeast was added to the diets. Robinson (1997) suggested evidence from his research indicated that inclusion of yeast in the diets fed to lactating dairy cows increased diet digestibility which would increase energy available to the animal. Likewise, Dann et al. (2000) reported a similar result with an increased dry matter intake with the inclusion of yeast culture during early lactation.

Supplemental yeast culture

Yeast culture is produced through a fermentation process including *Saccharomyces cerevisiae* with liquid and grain ingredients (Diamond V Mills, 2006). These ingredients, which vary among yeast culture suppliers, and the anaerobic environment allow for proliferation of yeast cells. The dried products of the yeast proliferation are then supplemented to cattle diets by either top-dressing or premix inclusion. Concentrated yeast culture is of similar origin, however requires one quarter of the original product's normal inclusion rate while maintaining the beneficial production response.

The beneficial effects of yeast culture on ruminal fermentation have been investigated. The 2001 Dairy NRC cited the review of direct fed microbials published by Yoon and Stern (1995) that suggested six modes of action through which yeast culture has been found to improve the rumen environment. The modes of action included (NRC, 2001):

1. "stimulation of desirable microbial growth in the rumen,

2. stabilization of rumen pH

3. altered ruminal fermentation pattern and end product production,

4. increased nutrient flow postruminally,

5. increased nutrient digestion and

6. alleviation of stress through enhanced immune response."

Callaway and Martin (1997) reported yeast culture provides soluble growth factors that stimulate ruminal bacteria that utilize lactate and digest cellulose.

Martin and Nisbet (1992) found that it was the increased concentrations of malate as a result of yeast culture addition that was responsible for the support of ruminal bacteria that utilize lactate. Malate, can be directly converted into two molecules of pyruvate in the pyruvate/malate cycle. Malate may also be converted in the cytoplasm to oxaloacetate. Oxaloacetate can then enter the glycolytic pathway via phosphoenolpyruvate. Phosphoenolpyruvate is transformed by pyruvate kinase into two pyruvate molecules. Pyruvate is dehydrated into lactate.

Yeast culture was also reported to moderate ruminal pH through an increase of protozoa present in the rumen. Protoza decrease amylolytic bacteria resulting in a decrease in starch degradation (Plata et al., 1994). Thus, higher concentrations of protoza, which were reported to decrease amylolytic bacteria and starch degradation, may moderate ruminal pH (Nagaraja et al., 1992). These results suggest yeast would support the maintenance of a healthier ruminal pH. A desired benefit of yeast culture is the amelioration of rumen acidosis, but the response of ruminal pH has been variable (Enjalbert et al., 1999; Roa et al., 1997). Enjalbert et al. (1999) found no significant changes in ruminal pH when a corn silage based (67% forage: 32% concentrate) diet was fed to non-lactating dairy cows. In contrast, Roa et al. (1997) fed diets differing in fiber source with or without the inclusion of yeast culture. When Holstein steers were fed either 50% corn stalk or 50% alfalfa hay, the duration of ruminal time spent below pH of 6.2 was reduced ($P<0.05$) with the inclusion of yeast culture.

One important associative effect of increased pH is the concurrent increase in fiber fermentation (Miranda et al., 1996; Plata et al., 1994). This response could potentially have a positive impact on the transition of the rumen from a high fiber diet fed to non-lactating cows during late gestation to high NFC diets fed to early lactation cows. Microbial efficiency (defined as grams of bacterial N/kg of organic matter truly fermented) is also affected by ruminal pH (Shriver et al., 1986). Microbial efficiency has been reported to be the highest at pH 5.8 and decreased as pH approached 7.0. Yeast culture has been found to increase the amount of bacterial N flow (Erasmus et al., 1992). Also, Erasmus et al. (1992) found with yeast culture supplementation, an increase flow of methionine – one of the most limiting AA in lactating dairy cows. Lastly, Newbold and others (1996) reported that *Saccharomyces cerevisiae* respiratory activity protects anaerobic rumen bacteria from damage from oxygen.

Yeast culture's influence on decreased risk of ruminal acidosis, increased dry matter digestibility and increased feed intake would especially provide potential benefits to periparturient dairy cows.

Hypocalcemia

Extracellular calcium is required in numerous physiological activities including but not limited to muscle contraction, nerve transmission, skeletal tissue, bone formation and blood clotting (NRC, 2001). Furthermore, intracellular calcium serves as a second messenger and holds pivotal roles with many enzyme reactions (NRC, 2001). Hypocalcemia is the state of having insufficient readily available sources of calcium to meet physiological requirements of the

animal. During the periparturient period, many dairy cattle experience hypocalcemia as a result of inactivated metabolic control mechanisms during the onset of lactogenesis. Ramberg (1974) reported that during the first ten days in milk, cows are at the greatest risk of being in a negative Ca balance. This condition can result in impaired muscle function and is defined as parturient paresis with the clinical manifestation referred to as milk fever. Goff and Horst (1997) defined milk fever as a metabolic disorder in which Ca homeostatic mechanisms fail to maintain normal plasma Ca concentrations at the onset of lactation.

Calcium Requirements

Calcium requirements change based on the physiological state of the dairy cow. A non-lactating dairy cow requires 0.0154 g Ca / kg body weight for maintenance (Visek et al., 1953). Martz et al. (1990) reported that the requirement increases during lactation to 0.031g/kg body weight. It is during the transition from non-lactating to lactating that the dairy cow is exposed to the greatest risk of hypocalcemia. During early gestation, the cow requires just slightly more calcium to support development and growth of the uterus and fetus. Substantially more calcium is required during the final trimester of gestation, when the fetal skeleton begins to calcify. House and Bell (1993) defined this requirement with the following equation:

$$\text{Ca (g/day)} = 0.02456\ e^{(0.05581 - 0.00007\ t)t} - 0.02456\ e^{(0.05581 - 0.00007(t-1))(t-1)}$$

where t represents day of gestation.

The NRC (2001) reported the daily calcium requirement of 52 – 64 g for the early lactation (11 DIM) cow. As lactation progresses, the requirements for calcium change based on milk production. At ninety days in milk, the 2001 Dairy NRC recommended feeding a range of calcium (52.1 – 88 g) depending on daily milk production.

Calcium requirements increase slightly with an increased concentration of protein secreted in the milk. Therefore, some dairy breeds have a higher predisposition to milk fever. To counteract this, the NRC (2001) recommends 1.22, 1.45, and 1.37 g of absorbed Ca / kg of milk produced for Holstein, Jersey, and other breeds, respectively. Furthermore, the protein rich colostrum requires 2.1 g of absorbed Ca per kg. If, during this state of transition, a dairy cow produces 10 L of colostrum, she has exceeded her available calcium stores by nine fold in one milking (Horst et al., 1997). Rapid calcium removal associated with increased milk production in the absence of activated calcium homeostatic control mechanisms will result in either clinical or subclinical hypocalcemia for the early lactation dairy cow. Most cows develop some degree of hypocalcemia at calving (Goff et al., 1987).

Riond (2001) reported clinical symptoms of milk fever could include inappetence, inhabitation of urination and defecation, paresis, lateral recumbency and eventually coma and death. Subclinical hypocalcemia is commonly defined when plasma calcium concentrations are below 7.5 mg/100mL (Goff and Horst, 1997). Clinical milk fever is affirmed with levels of plasma calcium less than 5.5 mg/100mL. Sixty to seventy percent of clinical hypocalcemia cases are fatal if left

13

untreated (Hibbs, 1950). The common treatment for clinical milk fever is an intravenous injection of 8-10 g of calcium. During clinical milk fever, the homeostatic control mechanisms of calcium are not in fully functioning to capacity. This is a result low calcium demands during late gestation. In order to effectively prevent transitional hypocalcemia, the homeostatic control mechanisms of calcium must be activated prior to calving.

The homeostatic control mechanisms of calcium in the periparturient dairy cow

Vertebrates have the ability to synthesize vitamin D_3 through a photochemical conversion of 7-dehydrocholesterol. Vitamin D_3 may also be supplemented in the ration. The synthesized form is more readily mobilized into the extracellular fluid then the dietary supplement The most common circulating form of vitamin D_3 is hydroxycholecalciferol [25-(OH)D_3] which is a result of the hydroxylation of carbon 25 in the liver. Further hydroxylation occurs in the kidney to result in 1,25 dihydroxycholecalciferol [1,25 (OH)$_2$ D_3] which is the active form of vitamin D_3. This vitamin is under active homeostatic mechanisms responsible for the maintenance of ionized plasma Ca concentrations. The complex regulatory mechanisms of 25-hydroxyvitaminD-1α-hydroxylase and 25-hydroxyvitamin D-24R-hydroxylase enzymes have been summarized by Horst et al. (1994) in Figure 1.2.

While vitamin D status of the animal is important, the key variable is calcium form and availability. Ionized calcium is more readily available for transport into the extracellular fluids. At low blood pH as much as 50 percent of

14

plasma Ca is in the ionized form (NRC, 2001). Arterial calcium concentrations are under constant monitoring by the parathyroid gland. The stimulation of calcium adaptation mechanisms occur based on blood Ca and $1,25 (OH)_2D_3$ concentrations (Figure 1.3). If blood calcium concentrations drop below 10 mg/dL, the parathyroid gland releases parathyroid hormone (PTH). The PTH stimulates the hydroxylation of vitamin D_3 into the active form. This results in active absorption of calcium in the intestine and resorption of the bone. Likewise, if Ca concentrations increase above 10 mg/dL, then the release of PTH is inhibited and the animal will begin to store Ca in the skeleton. Bone tissue contains about ninety-eight percent of the calcium in the body (NRC, 2001). When necessary, these stores of calcium are mobilized and released into the extracellular fluids.

The active form of vitamin D_3 functions as a steroid hormone, responsible for the regulation of numerous genes. The most prevalent function in calcium metabolism is the $1, 25 (OH)_2 D_3$ cell surface receptors on the basolateral membrane. These receptors result in improved Ca^{2+} permeability, therefore increasing calcium absorption in the intestine (Combs, 1992). $1,25 (OH)_2 D_3$ accumulates in tissues with intracellular vitamin D receptors (VDR). The VDRs are responsible for tissue responsiveness to $1,25 (OH)_2 D_3$ (Horst et al., 1994).

As age increases, the probability of milk fever also increases. Hansard et al. (1954) reported efficiency of intestinal absorption of Ca declines with age in the bovine. Horst et al. (1978 and 1990) associated this change to a decline in intestinal VDR. Furthermore, tissue responsiveness PTH is also reduced (Goff et

al., 1991). Horst et al. (1978) reported cows with milk fever had higher blood concentrations of the active vitamin D_3 and PTH. This led Horst and Reinhardt (1983) to believe it is primarily a dysfunction or decline in vitamin D receptor numbers or sensitivity that allows hypocalcemia to occur. Goff et al. (1995) collected colon mucosa biopsies from periparturient aged Jersey cows to determine the response of 1,25 dihydroxyvitamin D receptors at calving. The results indicated a cow in late gestation has 3 to 4 fold higher concentration of 1,25 dihydroxyvitamin D receptors than non-lactating, non-pregnant cows. During parturition, the concentration of receptors decline by 70%. Early lactation cows regain the gestational concentration of 1,25 dihydroxyvitamin D receptors.

During early lactation, the absorptive capacity for calcium is minimal. Van't Klooster (1976) reported that the efficiency of Ca absorption improves 1.6 fold during the first eight days of lactation and then remains relatively constant. Intake of dietary Ca increases as a result of increased dry matter intake. Hibbs and Conrad (1983) suggested that most cows gained a positive Ca balance within 6 to 8 weeks after calving.

Economic ramifications of milk fever

The economic ramifications of milk fever extend beyond the periparturient period. With the onset of parturient paresis, costs associated are not limited to veterinary and labor costs. The productive life of a dairy cow diagnosed with milk fever is reduced (Payne, 1968). Guard in 1996 estimated the average cost per milk fever case for treatment and estimated production losses at $334.00. Milk fever plays a role in the increased incidence for other parturient and metabolic

disorders. It has been associated with increased incidence of dystocia, retained fetal membrane, mastitis, and displaced abomasum. These associated disorders are in large part due to calcium's role in muscle function. Curtis et al. (1983) found when recovering from milk fever, dairy cows have an 8-fold increased incidence in ketosis and mastitis compared to controls. Block (1984) reported a fourteen percent decline in milk yield for cows which experienced parturient paresis. Furthermore, Oetzel et al. (1988) reported a reduced incidence of retained fetal membranes.

Dietary Cation-Anion Difference

There have been a variety of attempts to control the factors associated with the incidence of milk fever (Block, 1984; Goff et al. 1989; Wang and Beede, 1992). More recently, there have been significant advancements in the prevention of milk fever through the induction of metabolic acidosis. Dietary cation – anion difference (DCAD) is a method of regulating the systemic acid base status of the dairy cow. The pivotal minerals involved in the balancing equation are associated by charge. Cation minerals are sodium (Na) and potassium (K) and anionic minerals are chloride (Cl) and sulfur (S). Block (1997) reported a table to assist in DCAD calculation for ingredients common to dairy rations (Table 1.2). Furthermore, he states "by knowing the percentages of the four minerals listed in the equation, any DCAD can be calculated" with the DCAD formula. The formulation used in the calculation of DCAD has been debated (Charbonneau et al., 2006). Most deviations from the base DCAD equation are a

result of discrepancies of mineral bioavailability (Table 1.3). Many however are based on the original equation first reported by Ender et al. in 1971:

$$\text{milliequivalents of } (Na^+ + K^+) - (Cl^- + SO_4^=) \text{ / kg of dry matter}$$

Bicarbonate (HCO_3^-, bicarb) is a key component in blood buffering capacity. Sodium and K^+ result in the increase of blood pH which results in the release of bicarb. Whereas, with Cl^- and $SO_4^=$ decline blood pH causing the sequestration of bicarb. When the equation is formulated to achieve a negative DCAD, negatively charged anions will begin to accumulate. The accumulation of anions results in a mild decline of blood pH and therefore the initiation of a mild metabolic acidosis. Anionic salts are a supplemental product added to the dairy cow ration to assist in increasing the negativity of the cation-anion balance.

Joyce et al., (1997) investigated the effects of feeding anionic salts prepartum in alfalfa based diets. Feeding anionic salts achieved a -7 DCAD, versus the control grass and alfalfa hay diets with +30 and +35 DCAD, respectively. Cows fed anionic salt experienced a decline in urine pH prepartum (-7 d) and a subsequent increase in serum ionized calcium at parturition. Cows fed anionic salts also experienced an improved appetite postpartum. Likewise, a change in metabolic disorders was noted with a decline in incidence of 38.5%. Other authors have reported elevated plasma Ca concentrations in cows fed anionic salt compared to control animals (Block, 1984; Goff et al.,1991). The increased blood calcium concentrations during a period of physiological stress have shown to be a result of increased bone mobilization (confirmed by elevated hydroproline levels) by Block (1984). Leclerc and Block (1989) later confirmed

these results by again documenting increasing concentrations of hydroxyproline with declining DCAD.

The concept of inducing metabolic acidosis is not associated with the removal of dietary calcium. The effectiveness of a negative DCAD in the prevention of milk fever was improved with elevated concentrations of dietary calcium (1.5% Ca) (Block, 1984; Goff et al., 1991; Oetzel et al., 1991). High DCAD results in the increased excretion of calcium in urine (Wang and Beede, 1992). Without high levels of dietary calcium, the low DCAD may induce hypocalcemia (Block, 1997). Goff and Horst (1997) examined the effects of strong cations and anions in an incomplete factorial design with two concentrations of dietary calcium (0.5 or 1.5%) on the incidence of milk fever in Jerseys. He found no significant effect of level of dietary calcium on the incidence of milk fever or the degree of hypocalcemia. The treatment with the highest incidence of milk fever (62.5%) occurred with dietary inclusions of 1.1% K, 1.3% Na and 1.5% Ca. These animals experienced an increased blood and urine pH, which resulted in a reduced concentration of plasma hydroxyproline. A decline in hydroxyproline indicates that dietary induced metabolic alkalosis causes a decline in bone resorption of calcium.

A good field indicator used to measure the efficacy of the negative DCAD diet on enacting the calcium homeostatic mechanisms is the frequent monitoring of urine pH (Oetzel and Vagnoni, 1998). Below 6, excessive acidification may have occurred (Jardon, 1995). A pH of 5.2 indicates severe acidosis and the kidney is no longer effective at regulating blood pH. Likewise, if the urine pH

exceeds 8, milk fever is likely to result. Beyond 8.3, the kidneys are once again unable to respond adequately and death may result.

Sources of anions

Goff et al. (2004) investigated six total sources of anions on non-pregnant, non-lactating Jersey cows. He reported anion effectiveness based on their ability to decrease blood and urine pH. Listed from the most to least effective as determined by the magnitude of blood pH decline are: hydrochloric acid, ammonium chloride, calcium chloride, calcium sulfate, magnesium sulfate, and sulfur. Across all experiments, the chlorides decreased biological pH 1.6 times greater then the sulfur substitutes. However, Oetzel and Barmore (1993) found the sulfur based anionic salts more palatable then those comprised of chloride. Specifically, finding magnesium sulfate promoting the highest level of intake followed by ammonium chloride and calcium chloride being the least palatable resulting in the lowest intake.

EXPERIMENTAL OBJECTIVES

There were two independent objectives of this thesis.

The first objective was to investigate the effects of sulfur based anionic salts fed during late gestation on calcium homeostasis, energy metabolism, health and production parameters of mature dairy cows.

The second objective was to determine the effects of feeding yeast culture and concentrated yeast culture to dairy cattle during the periparturient period on energy status and production of dairy cattle through week eleven postpartum.

Potential homeorhetic hormones[1]	Mid Pregnancy	Late Pregnancy	Lactogenesis Early lactation
Progesterone	↑	(↓)	↓
Placental lactogen		↑	↓
Estrogens		↑	↓
Prolactin	-	(↓)	↑
Somatotropin	-	(↓)	↑
Leptin	?	?	?
Homeostatic hormones[1]			
Insulin		↑	↓
Glucagon	-	-	-
CCK and somatostaatin	?	?	?
Tissue sensitivity			
Insulin	↑	↓	↓
Catacolamines		↑	↑
Tissue responsiveness			
Insulin		↓	↓
Catacolamines	↓	↑	↑
Liver[2]			
Gluconeogenesis			↑
Ketogenesis			↑
Adipose tissue[2]			
Lipogenesis	↑	↓	↓
FA esterification	↑	↓	↓
Lipolysis		↑	↑
Glucose utilization		↓	↓
Skeleton muscle[2]			
Protein synthesis		↓	↓
Protein degradation		↑	↑
Glucose utilization		↓	↓

↑: Increasing; ↓: Decreasing; ?: unknown in ruminants; -:no significant changes.

[1] Plasma hormone concentration changes.

[2] Changes in rate of metabolic processes.

Table 1.1. Changes in some homeorhetic and homeostatic hormones, tissue sensitivity, and responsiveness and effect in selected tissues in pregnancy and lactation. Adapted from Ingvartsen and Anderson (2000).
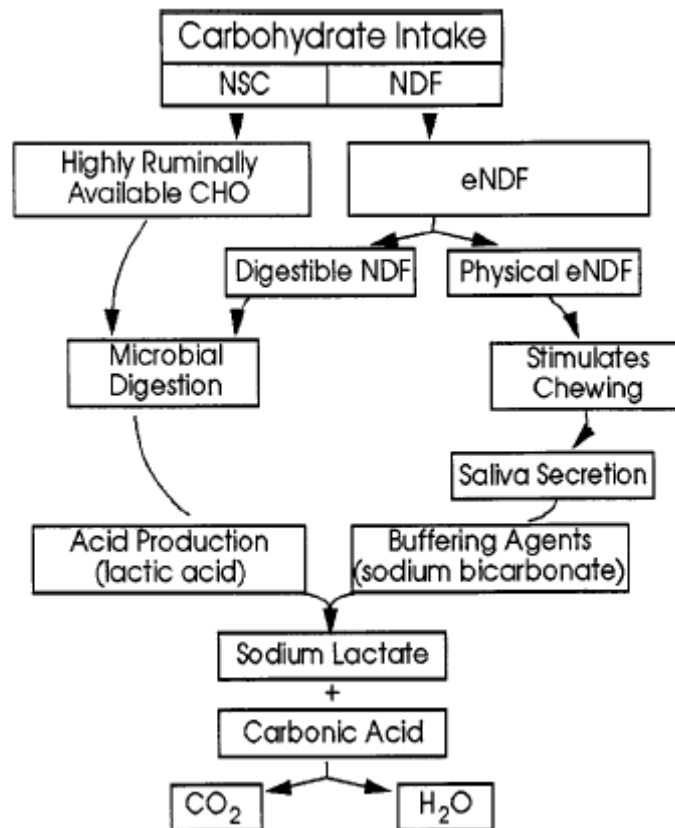
Figure 1.1. Ruminal changes that occur during transition as the dietary concentrations of fiber decrease and non structural carbohydrates increase (eNDF = effective neutral detergent fiber) Adapted from Nocek, 1997.

Table 1.2. Calculated DCAD of common ingredients in dairy cattle rations.[1,2]

Ingredient	% of DM				
	Na$^+$	K$^+$	Cl$^-$	S$^=$	DCAD[3]
Alfalfa hay (late vegetative)	0.15	2.56	0.34	0.31	431.1
Timothy hay (late vegetative)	0.09	1.6	0.37	0.18	232
Corn silage	0.01	0.96	--	0.15	156.4
Corn grain	0.03	0.37	0.05	0.12	18.8
Oats	0.08	0.44	0.11	0.23	-26.95
Barley	0.03	0.47	0.18	0.17	-23.4
Distillers' grain	0.1	0.18	0.08	0.46	-219.38
Soybean meal	0.03	1.98	0.08	0.37	266.37
Fish meal	0.85	0.91	0.55	0.84	-75.6

[1]Table adapted from Block (1997).

[2] From NRC for Na$^+$, K$^+$, Cl$^-$ and S$^=$

[3] Calculated as milliequilvalents of (Na$^+$ + K$^+$) – (Cl$^-$ + SO$_4^=$) kg^{-1} of DM

Table 1.3. Derivations of the dietary cation-anion difference equation as adapted from Block (1997).

Author	Year	DCAD equation
Ender et al.	1971	(Na + K) - (Cl + S)
Horst and Goff	1997	(Na + K + 0.38 Ca + 0.30 Mg) - (Cl + 0.6 S + 0.5 P)
Horst and Goff	1997	(Na + K + 0.15 Ca + 0.15 Mg) - (Cl + 0.2 S + 0.3 P)
National Research Council	2001	(Na + K + 0.15 Ca + 0.15 Mg) - (Cl + 0.6 S + 0.5 P)
Goff et al.	2004	(Na + K) - (Cl + 0.06 S)
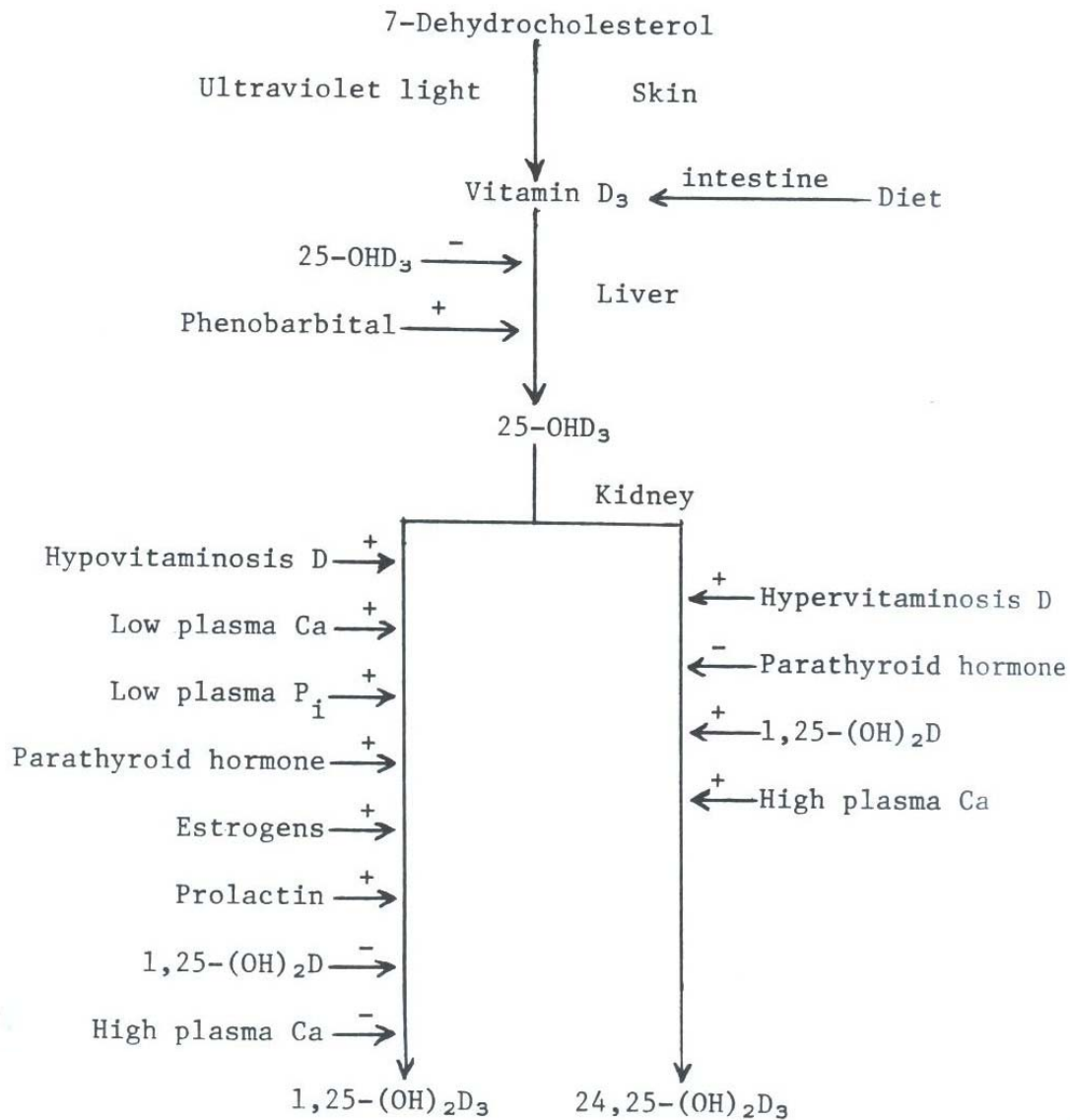
Figure 1.2. Regulatory mechanisms of kidney 25-hydroxyvitaminD-1α-hydroxylase and 25-hydroxyvitamin D-24R-hydroxylase enzymes as adapted from Horst et al. (1994).
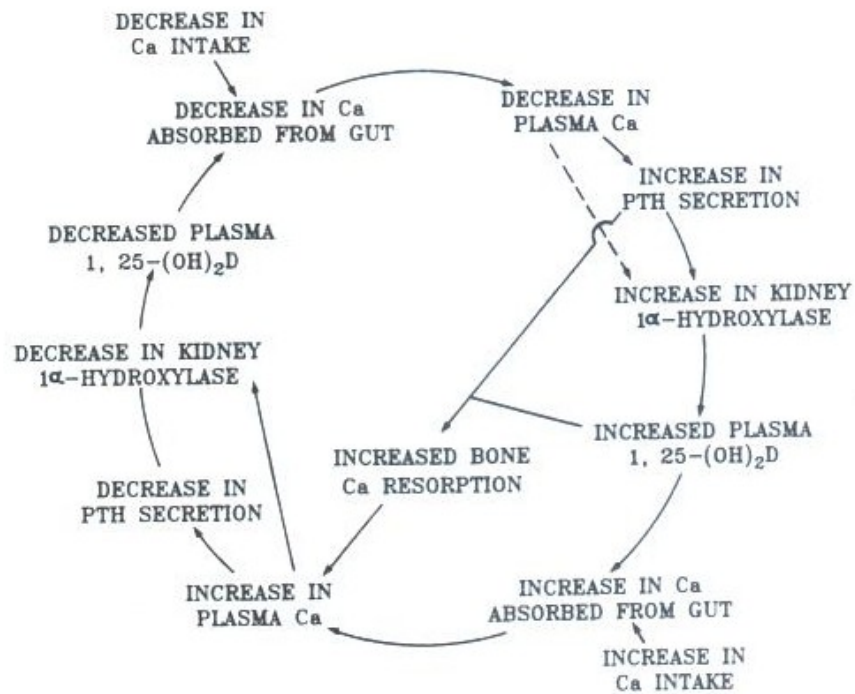
Figure 1.3. Overview of calcium adaptation mechanism. Dashed lines represent a response that occurs in rats but not in ruminants as adapted from Horst et al. (1994).

CHAPTER 2

THE EFFECTS OF YEAST CULTURE ON PRODUCTION PARAMETERS OF HIGH PRODUCING HOLSTEIN DAIRY CATTLE WHEN FED DURING THE TRANSITION FROM GESTATION TO EARLY LACTATION

Introduction

Proper nutrition is paramount to a successful transition (Ingvartsen, 2006) The rapid increase in feed intake after calving coupled with the change in diet composition (higher grain diet) creates a risk of ruminal acidosis (Kleen et al., 2003) that could result in decreased feed intake and DM digestibility with increased risk of digestive upsets, displaced abomasum and laminitis (Donovan et al., 2004). The benefits of a successful transition includes reduced incidence of metabolic diseases (clinical and subclinical), lower incidence of dystocia and improved reproductive tract involution and health as well as preventing the rapid and excessive mobilization of adipose tissue by supporting the rapid increase in feed intake during early lactation (Hayirli and Grummer, 2004). Numerous strategies for reducing ruminal acidosis have been presented with one strategy involving feeding yeast culture during the periparturient period through early lactation (Robinson, 1997; Wang et al., 2001).

The beneficial effects of yeast culture on ruminal fermentation have been investigated and potential modes of action were summarized by Yoon and Stern 1995. Research has shown that yeast culture provides soluble growth factors

that stimulate ruminal bacteria that utilize lactate and digest cellulose (Callaway and Martin, 1997). Yeast culture has also been reported to moderate ruminal pH through the increased number of protozoa (Plata et al., 1994). Higher concentration of protoza were reported to decrease amylolytic bacteria and starch degradation which may moderate ruminal pH (Nagaraja et al., 1992). However, the effects of yeast culture on rumen pH have been variable (Enjalbert et al., 1999; Giger-Reverdin et al., 2004; Roa et al., 1997).

Several investigators have reported improved diet dry matter intake when yeast culture was added to the diets. Robinson (1997) suggested evidence from his research indicated that yeast culture inclusion in the diets fed to lactating dairy cows increased diet digestibility which would increase energy availability to the animal. Dann et al. (2000) also have supporting evidence of an increased dry matter intake with the inclusion of yeast culture during early lactation.

Decreased risk of ruminal acidosis, increased dry matter digestibility and increased feed intake would provide important benefits to periparturient dairy cows. Numerous studies have focused on the benefits of feeding yeast culture to dairy cattle. However, to date there has been no research focusing on the effects of a concentrated yeast culture on dairy cattle. Therefore, the objectives of this study were to determine the effects of varied concentrations of supplemental yeast culture to multigravid and primigravid Holstein cows from three weeks prepartum to 77 d postpartum on dry matter intake (DMI), milk yield, milk composition, body weight (BW), body condition score (BCS), efficiency of lactation, and metabolic parameters.

Materials and Methods

One hundred and eighteen pregnant Holstein cows were assigned to one of three dietary treatments thirty days prior to expected day of calving were cared for according to a research protocol that was approved per the institutional guidelines of the University of Missouri-Columbia Animal Care and Use Committee. Twenty-three cows (C=15; YC=6; CYC=2) were removed due to health complications (Addendum 1). Only cows that completed the entire trial were included in the data analysis (36 primigravid and 59 mulitigravid animals).

Thirty days prior to expected day of calving, cows were moved to the freestall barn at the University of Missouri's Foremost Dairy Research Center (Columbia, MO) for the duration of the study. The first nine days were dedicated to training animals to the electronic feeding system (American Calan, Inc; Northwood, NH). Cows were blocked by expected day of calving and parity. Cows within blocks were then sorted to one of three treatments that were fed from approximately 28 days prior to calving through 77 days in milk (DIM). Treatments consisted of: 1) no supplemental yeast culture (Control; **C)** 56 g of yeast culture, (Diamond V XP™, Diamond V Mills, Inc., Cedar Rapids Iowa; **YC**), and 3) 14 g of concentrated yeast culture (Diamond V XPC™; **CYC)**. Ground corn in the premix was replaced to allow for the inclusion of the yeast culture treatments in the premix. Yeast culture treatments were designed to deliver equal concentrations of yeast with variable concentrations of carrier. Diets (Table 2.1) were formulated to meet or exceed NRC recommendations (NRC, 2001). Prepartum diets were fed until parturition. On the day of parturition (day 0), cows

were immediately switched to the lactation diet. The diets were mixed once daily as total mixed diets and delivered twice daily to individual animals at 0600 and 1430 hours. Feed was weighed and offered to maintain a five percent refusal. Refused feed was weighed and recorded prior to each feeding.

Samples of total mixed diet were collected once daily for each treatment. Samples were stored frozen (-20ºC) in sealed plastic bags. Daily samples were subsequently thawed and composited by week for analysis. Weekly composites were dried in a 55ºC forced air oven, ground through 2 mm screen (Wiley Mill, Thomas Scientific, Swedensboro, NJ), and analyzed for dry matter (DM; 105ºC), crude protein (CP; LECO Model FP-428 Nitrogen Determinator; LECO Corp., St. Joseph, MI), and acid and neutral detergent fiber (ADF and NDF, respectively; Fiber racks, Labconco, Kansas City, MO and ANKOM, Macedon, NY; Van Soest et al., 1991). Composition and chemical analysis of dietary treatments are summarized in Table 2.1.

Cows were milked twice daily at 0400 and 1600 hours. Milk production was measured and recorded at each milking from day of calving through day 77 of lactation. Milk samples were collected weekly at consecutive p.m. and a.m. milkings and then analyzed for butterfat, protein, urea nitrogen and somatic cell count (Mid-South Dairy Records, Springfield, MO). Values for milk composition were averaged to obtain a weekly mean. Four percent fat corrected milk (FCM) was calculated using the following equation (NRC, 2001):

$$4\% \text{ FCM} = (0.4 * \text{kg milk}) + (15 * \text{kg milk fat}).$$

Energy corrected milk was calculated using the following equation (NRC, 2001):

ECM = [(0.0929 * Milk fat %) +(0.0547 * milk protein %) + 0.192] * kg milk

Body weight (TruTest AG500; TruTest, San Antonio, TX) and body condition score (Wildman et al., 1982) were measured and recorded twice weekly by the same individual throughout the project. Blood samples were collected 21, 14, 7, 3 and 1 day prior to expected day of calving, on day of calving, on day 1, 3, 7, 10, and 14 postpartum, and then every third day through week 11 of lactation. Blood samples were collected at 0600 hours via coccygeal venipuncture into two evacuated tubes (one with and one without EDTA as an anticoagulant; Vacutainer®, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), The test tube containing whole blood and EDTA was refrigerated while the test tube containing only whole blood sample was allowed to clot at room temperature. Blood samples were centrifuged (RC3B Plus centrifuge, Sorvall Instruments, Newton, CT) for 21 minutes at 2,100 x g at 4ºC. Plasma and serum aliquots were collected and stored in three clear plastic tubes with snap tight caps at -20ºC until analyzed for serum non-esterified fatty acids (NEFA) and glucose concentrations.

Serum NEFA concentrations were determined using a NEFA C kit (Wako Chemicals USA, Inc., Richmond, VA). Colorimetric development was quantified on a TECAN rainbow plate reader (TECAN USA, Inc., Research Triangle Park, NC). Serum glucose concentrations were determined enzymatically using glucose oxidase and peroxidase method (Thermo Electron USA, Louisville, CO). Absorbance was quantified using Beckman DU-65 spectrophotometer (Beckman Instruments, Fullerton, CA).

All parameters containing repeated measures including: pre and postpartum dry matter intake, milk, FCM and ECM milk yields, lactation efficiency (kg FCM/kg DMI; kg ECM/kg DMI), milk composition and component yields, serum NEFA, glucose, body weight and body condition score and change over time were analyzed for statistical significance using the MIXED model procedure of SAS (SAS Institute, 2006) with block as the error term. Beyond the analysis of means, the GLM procedure (SAS Institute, 2006) was employed to analyze the treatment linear and quadratic polynomial contrasts. This procedure was used to determine any curvilinear response and or differences in the degree of slope. Significance was declared at $P \leq 0.05$ with trends at $0.05 < P \leq 0.10$.

Results and discussion

Body weight and body condition score (BCS) were not significantly different at the initiation of the project or on day of calving (Table 2.2). Cows had an average body weight of 714 kg prior to calving with a corresponding average BCS of 3.4. Dry matter intake pre-partum was not altered by the inclusion of yeast culture in the diet. Cows fed control, CYC or YC averaged 12.7, 12.1 or 13.0 kg of dry matter per day, respectively during the last 28 days of gestation. These results agree with Soder and Holden (1999) who reported no difference in feed intake with the inclusion of yeast in diets fed to dairy cows during late gestation. Similarly, Robinson (1997) reported no difference in feed intake pre-partum. In contrast, Dann and co-workers (2000) reported yeast culture increased feed intake during the last 7 day of gestation. The lack of response in the current study may reflect the higher level of fiber used in the pre-partum diets. In addition to lower NFC, the present study also included adequate long particle grass hay to encourage rumination.

Body weight change after calving was -33.5 kg for cows fed YC compared to -36.1 kg and -38.8 kg for Control and CYC, respectively, during the first 11 weeks of lactation. The decline in body weight is a direct response to negative energy balance associated with early lactation dairy cattle. Rastani et al. (2001) reported a similar decline while monitoring body composition change during early lactation. The nadir in body weight loss occurred during week 4 of lactation for all treatments. Body condition score declined from calving through week 5 for cows fed control diet. In contrast, cows fed YC and CYC continued to lose BCS until

34

week 9 of lactation (Figure 2.2). The decline in BCS resulted in a linear difference between CYC and C (P = 0.03). While the change in body condition score differs statistically, the difference is of little biological significance. Ferguson et al. (1994) found variation in body condition scoring reported by trained personnel was greater then 0.25 units. Therefore, the change in BCS described in the present study as significant is more precise then can be consistently measured.

Mean dry matter intake after calving was 19.3 kg per day and was not different due to dietary treatments (Table 2.2). Dry matter intake as a percentage of body weight exhibited a significant quadratic response (P < 0.002) due to dietary treatments as illustrated in Figure 2.3 and 2.3a. Cows fed CYC had numerically higher DMI as a percent of body weight during early lactation compared to cows fed C but the response over time was not significantly different. Cows fed YC had a different pattern of DMI as a percent of body weight during the study compared to cows fed control (P < 0.05) or CYC (P < 0.001). These results agree with the response reported by Dann et al. (2000) who found DMI was greater when yeast culture was included in the diets when fed pre-partum through early lactation. Similarly, Wang et al. (2001) reported that feeding yeast culture pre-partum appeared to result in a greater DMI in cows fed diets containing 21% forage NDF. The improvement in DMI could be a result of improved efficiency of fiber digestion. Williams et al. (1991) reported an improvement in forage degradation which may contribute to increased intake and therefore productivity when dairy cows are fed yeast culture.

Control cows produced 34.0 kg of milk per day, while cows fed YC produced 36.1 kg of milk per day. Cows fed CYC had an intermediate level of milk production (35.1 kg/d). Other investigators have also reported no differences in milk production due to the inclusion of yeast on the diet of cows during the periparturient period through early lactation (Robinson, 1997; Sader and Holden, 1999).

While milk production was not different, milk composition was altered by dietary treatment. In the present study, fat content of milk exhibited a linear trend to dietary treatment (P = 0.06). Cows fed CYC had higher fat test during early lactation compared to control cows (P < 0.04), with cows fed YC maintaining a milk fat percentage intermediate to the other two treatments. A higher fat test was measured for cows fed CYC and corresponded with higher feed intake during early lactation. This is in agreement with Putnam et al. (1997) who found elevated milk fat percentages during early lactation with inclusion of yeast culture in the diets. Improved fiber fermentation has been one response associated with feeding supplemental yeast culture (Doreau and Jouany, 1998), which could contribute to increased milk fat concentration as noted by Wang et al. (2001). Dann et al. (2000) found no significant differences in milk fat percentages measured during the transition period.

The linear response observed for milk fat percentage combined with trends in milk yield over time contributed to significant differences in linear and quadratic responses of cows for 4% fat corrected milk (Figure 2.4). A linear and quadratic difference (P < 0.04 and P = 0.0004, respectively) occurred among all

three experimental treatments. Treatment CYC had significantly different linear and quadratic responses over time compared to both control (P = 0.03 and P = 0.0012, respectively) and YC (P = 0.0009 and P = 0.0005, respectively. In addition to altering concentration of milk fat and FCM yield, yeast culture supplementation resulted in significant differences (P = 0.0001) in concentration of milk protein over time. Cows fed CYC were intermediate in concentration of milk protein during early lactation, with cows fed control diet sustaining the lowest level of milk protein during early lactation. Previous reports have found that cows fed yeast culture had similar milk composition as cows fed control diets (Soder and Holden, 1999; Dann et. al, 2000). The elevated milk protein percentage suggests an improved transition of the rumen microbial population as the diet changed from the low NFC to higher NFC fed in the lactation formulation.

Although unmeasured, the increase of milk protein could be attributed to improved microbial efficiency. Robinson (1997) concluded that yeast culture supplementation resulted in an increased net energy for lactation content of the basal diet due to increased digestion in the reticulorumen. Furthermore, Enjalbert et al. (1999) reported that yeast culture increased the concentration of propionic acid. This reported shift in fermentation and increased available energy could contribute to increased milk protein synthesis.

Milk urea nitrogen did not differ significantly over treatments (Table 2.2). However, somatic cell score (SCS) did differ linearly (Figure 2.6; P < 0.02) among treatments. Beginning week 5 of lactation, the cows receiving yeast culture maintained a statistically lower SCS. During this time, cows receiving YC

sustained a SCS value consistently lower then that cows fed the other treatments. Somatic cell count of cows receiving CYC was intermediate with cows fed C having the highest concentration of SCC in the milk. Improved mammary gland health is especially important for the transition dairy cow (Overton and Waldron, 2004). The mechanism through which YC might improve mammary gland health deserves further study.

The effect of yeast culture on non-esterfied fatty acid (NEFA) concentrations during transition has not previously been documented. In the present study, serum NEFA did not differ significantly due to treatments. Likewise, serum glucose was similar for all dietary treatment groups. These results agree with those previously reported by Putnam et al. (1997) and Piva (1993).

Summary

Supplementing transition dairy cows with yeast culture during late gestation through early lactation could improve the milk production during early lactation. In the current study, cows fed yeast culture exhibited a higher rate of improvement in DMI after calving. Concurrently, yeast supplementation resulted in significant quadratic responses in daily FCM yields. This response was associated with increased fat percentage which would be associated with improved fiber fermentation. Similarly, the quadratic response in milk protein percentage reflects a positive response to beneficial effects of yeast culture on feed intake and digestion. The yeast culture treatments similarly affected dry matter intake and 4% FCM yield during early lactation and outperformed the

control and could be used in the periparturient diets fed to dairy cattle to improve

animal performance during transition from late gestation to early lactation.
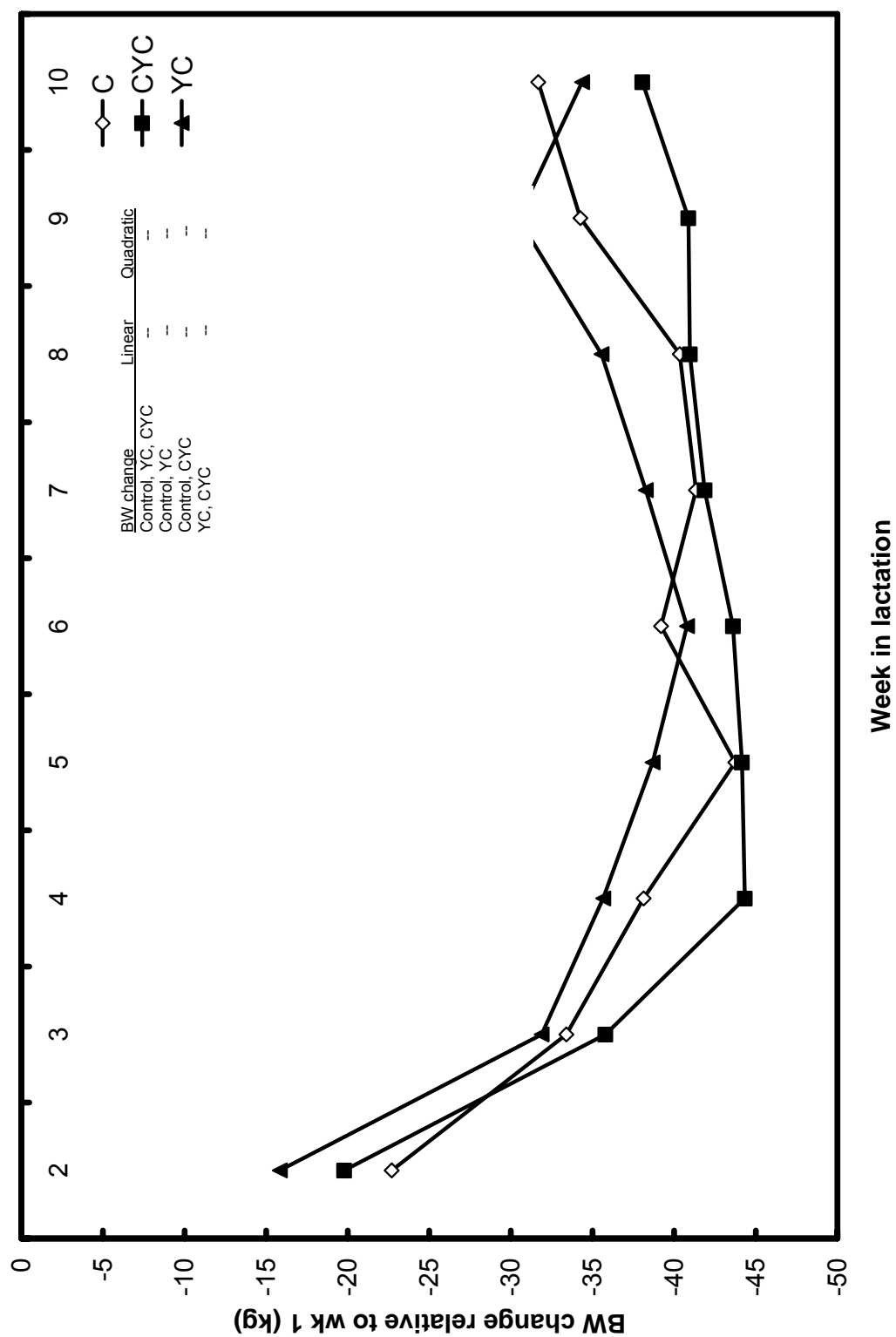
Figure 2.1. Body weight change relative to first week of lactation for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 6.33).

40

Figure 2.2. Body condition score change relative to first week of lactation for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 0.08).

Figure 2.3. Postpartum dry matter intake as a percent of body weight for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 0.001).
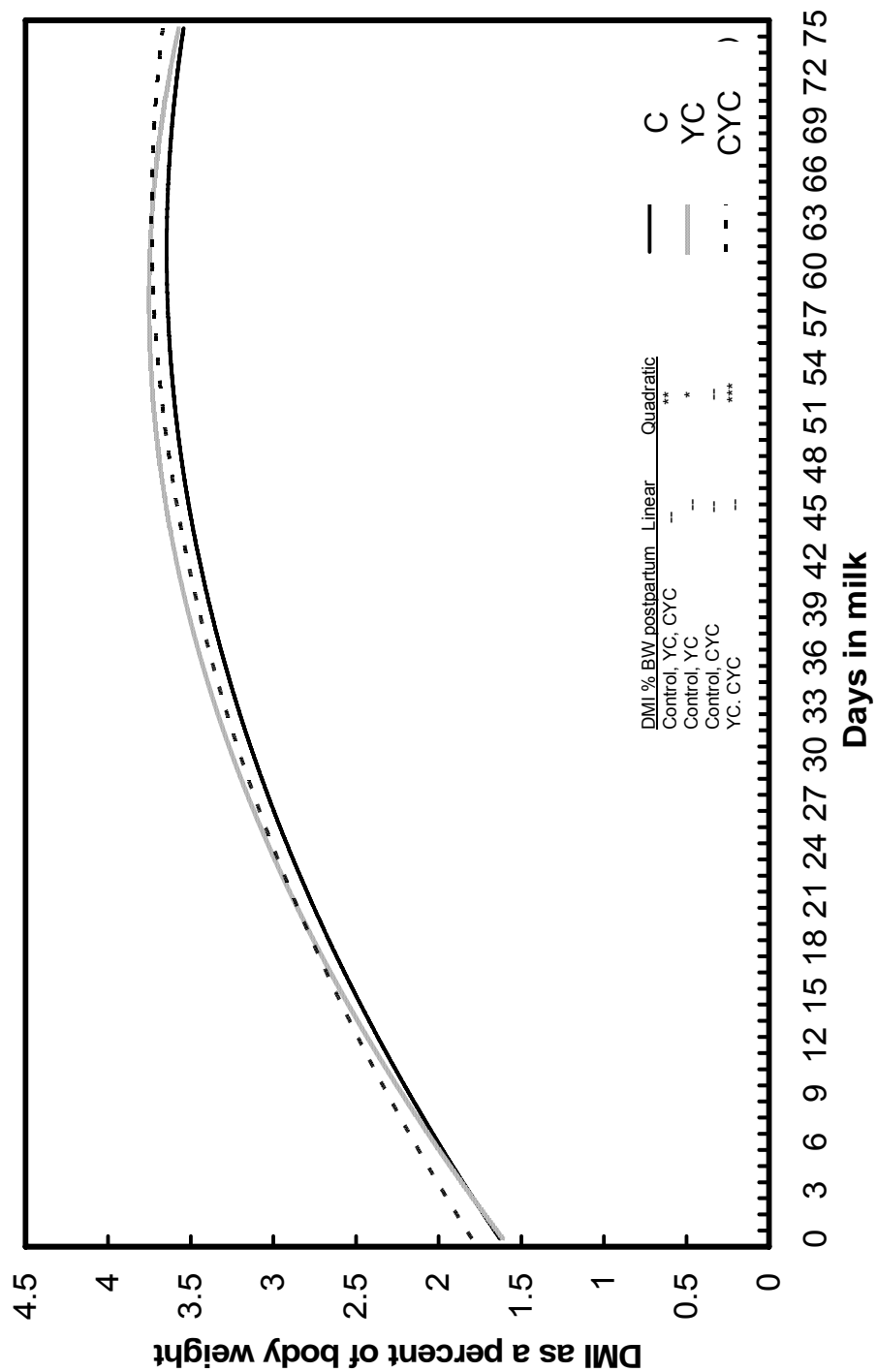
Figure 2.3a. Second order polynomial postpartum dry matter intake as a percent of body weight curve for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 0.001).
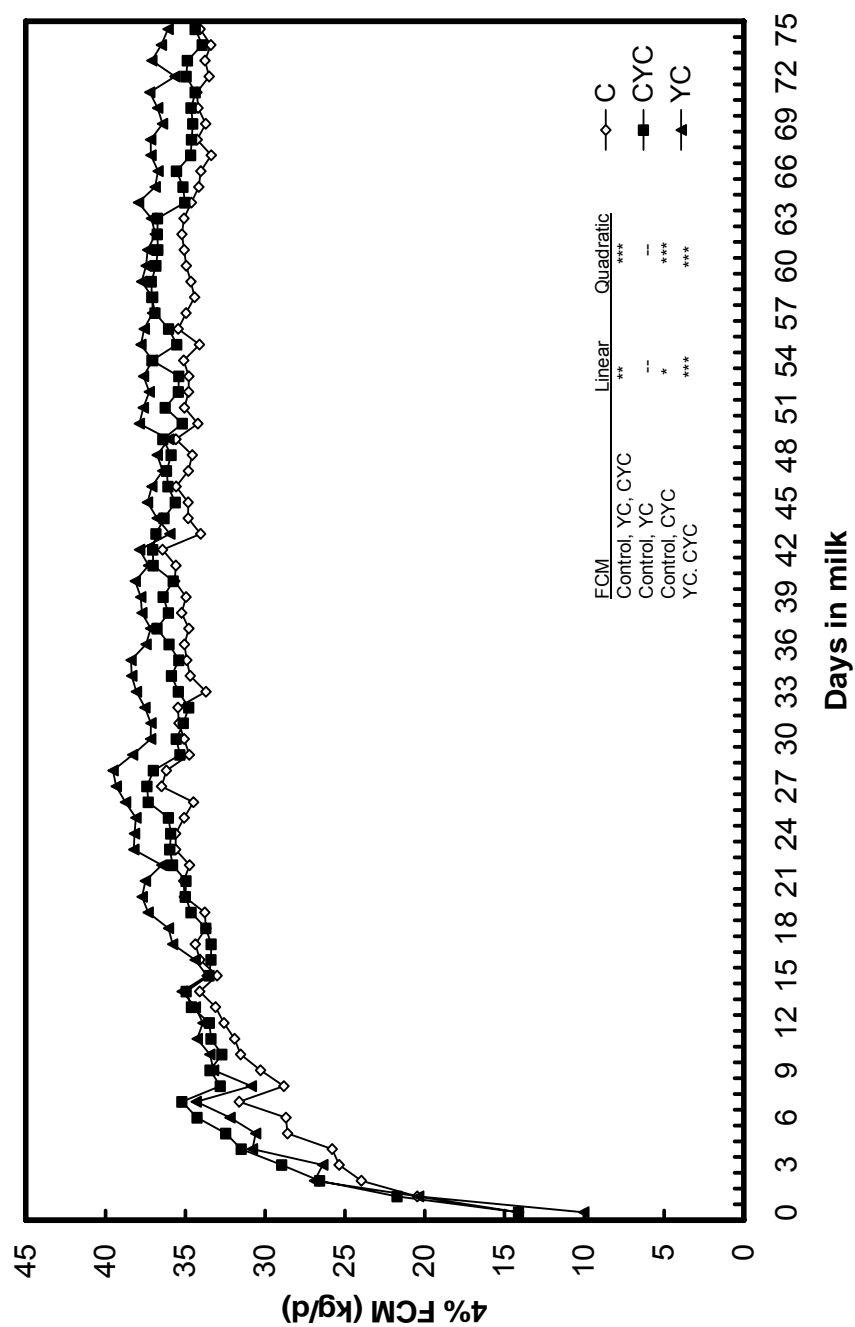
Figure 2.4. Four percent fat corrected milk yield for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 1.79).
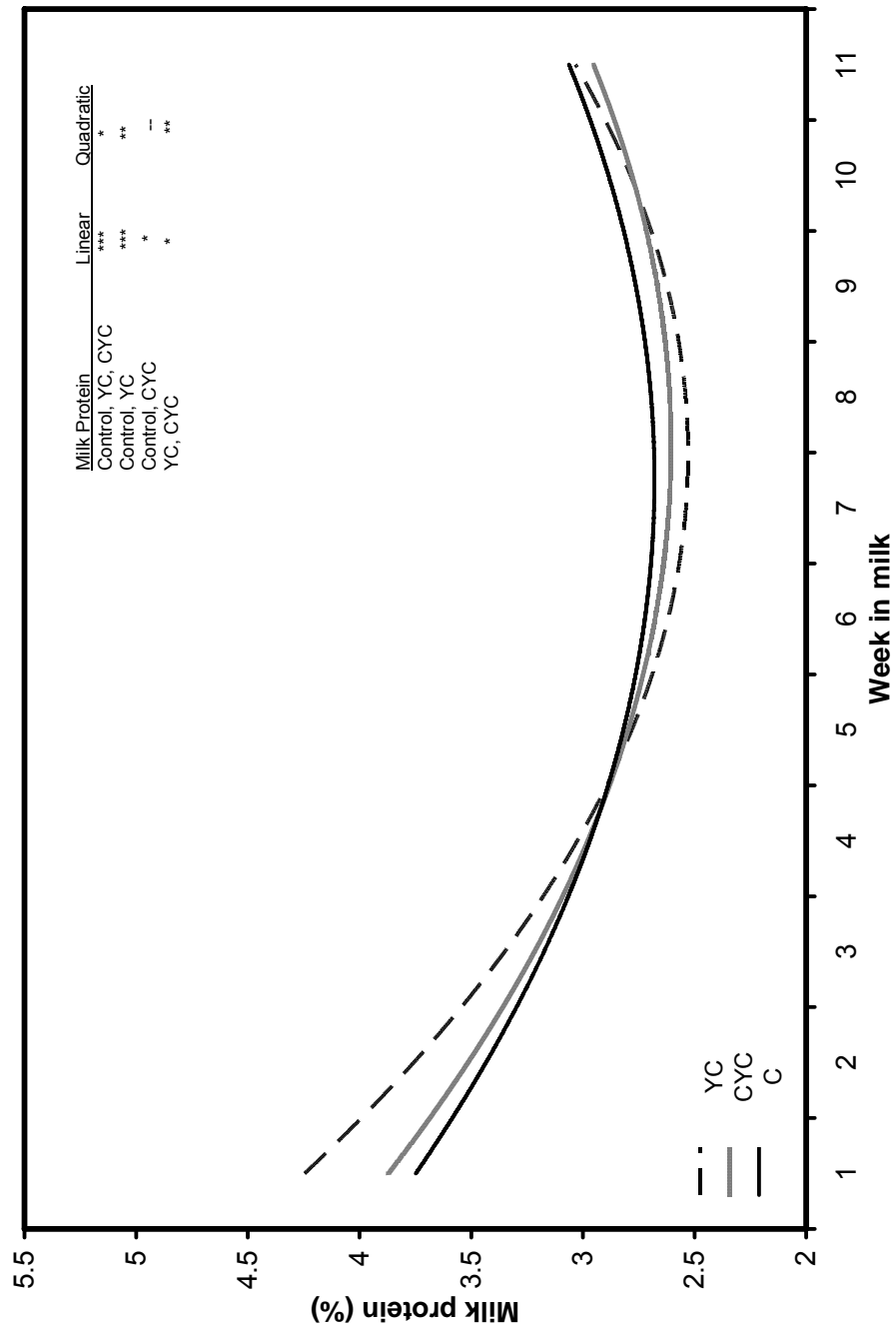
Figure 2.5. Concentrations of milk protein through week 11 for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 0.05).

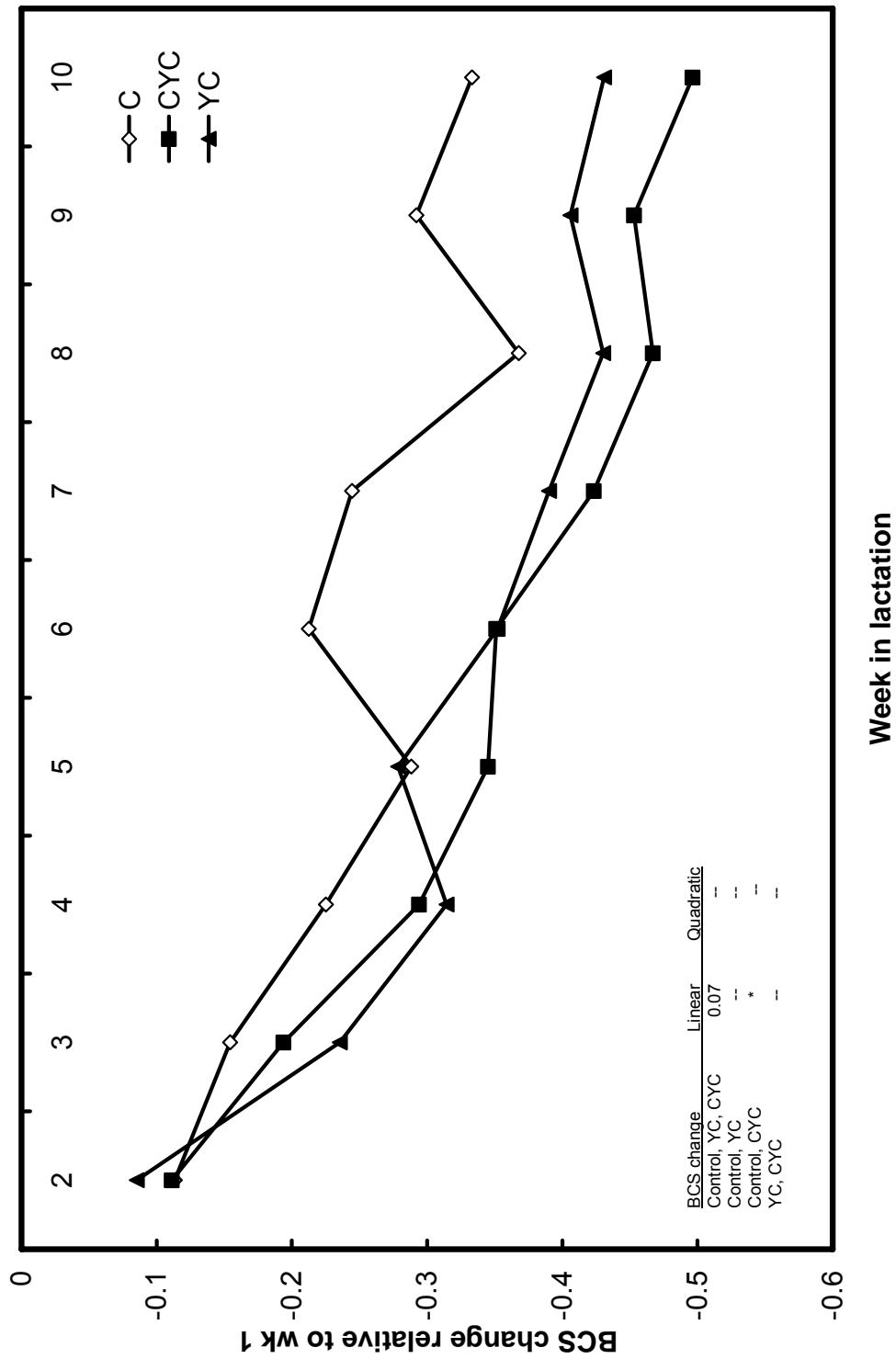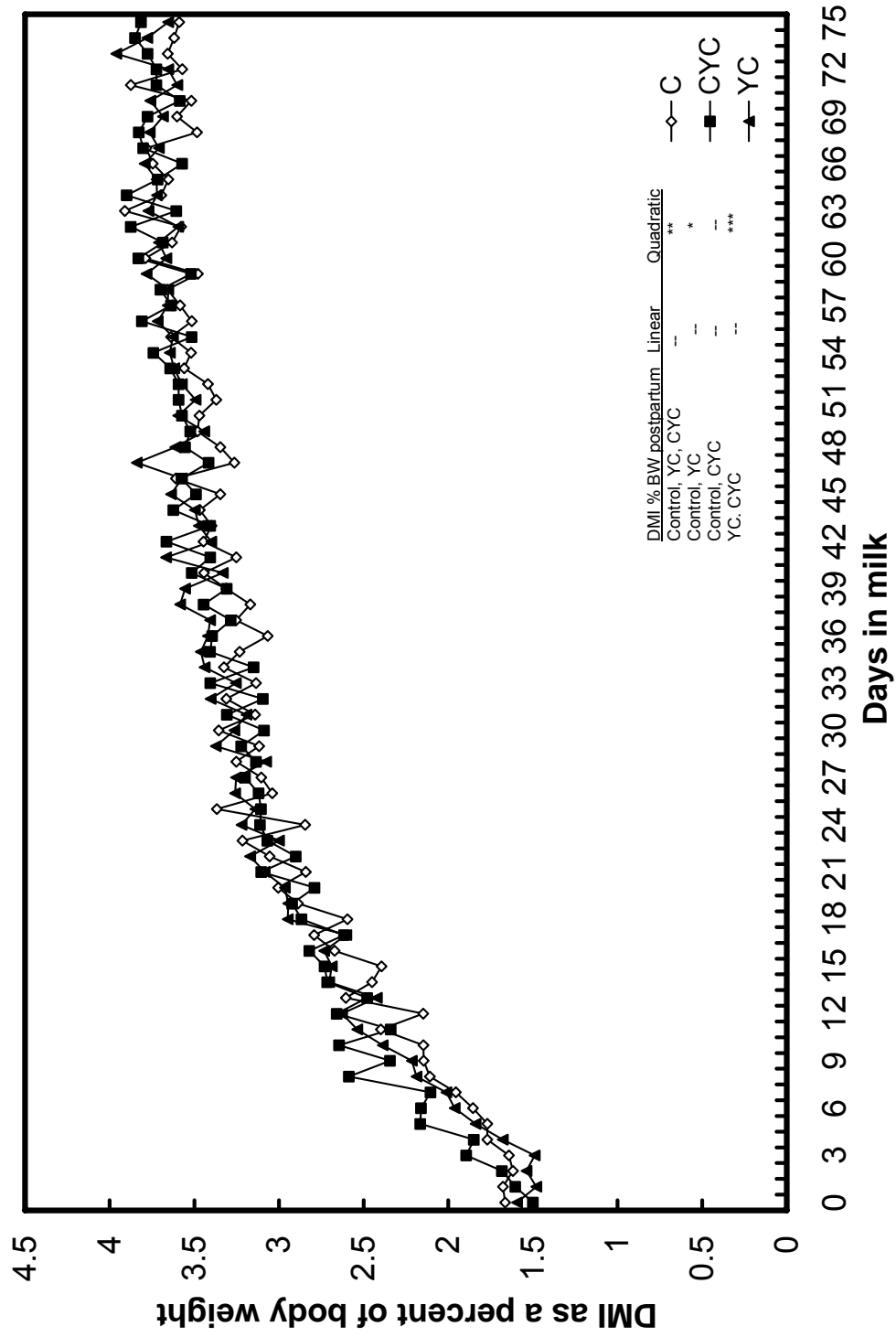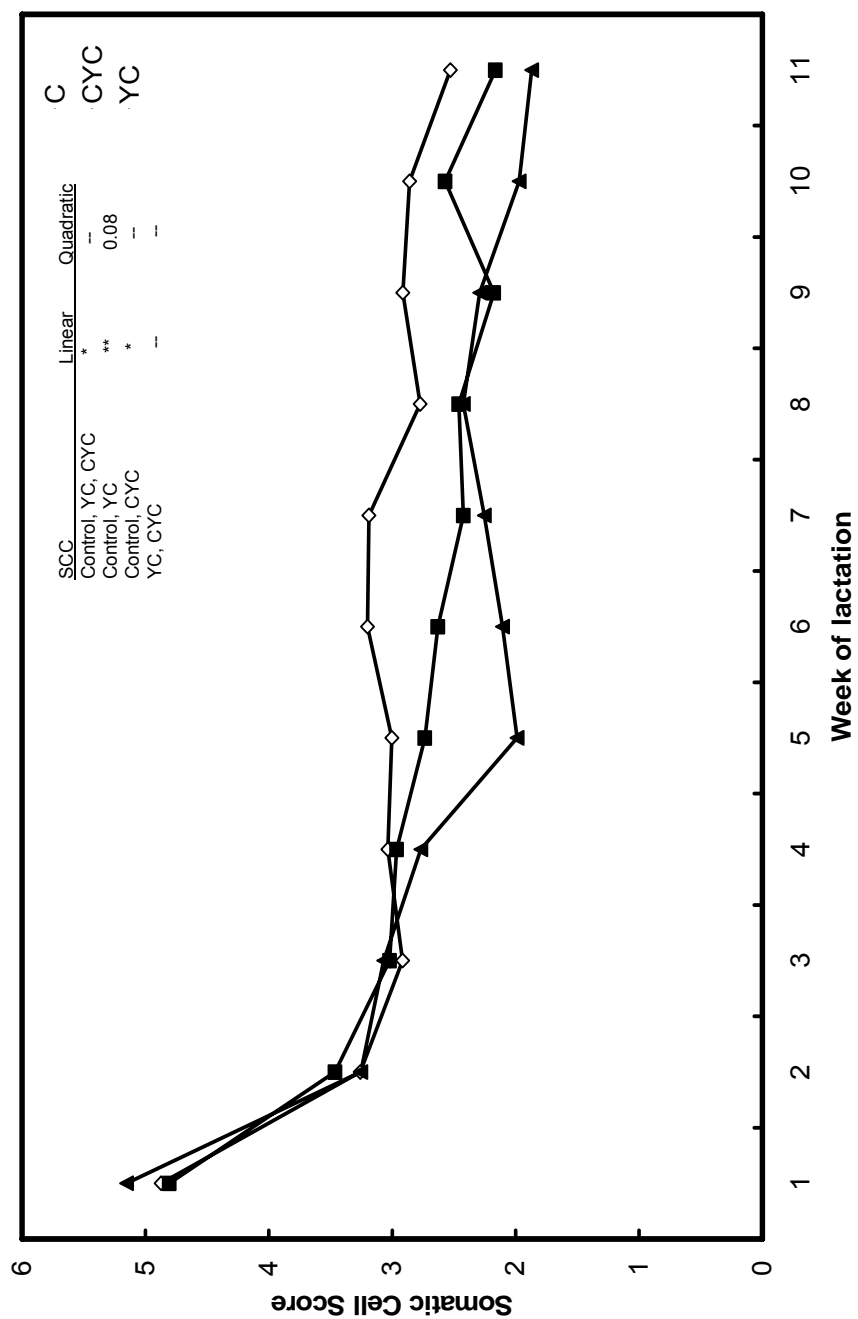Figure 2.6. Somatic cell linear score for Holsteins fed yeast culture during the transition from late gestation through early lactation (SE = 0.36).

Table 2.1. Composition and chemical analysis of yeast culture experimental diets.

	Dry diet [1]	Lactation Diet [1]
Ingredient	% of Dry Matter	
Grass hay	16.1	-
Corn silage	23.0	13.3
Alfalfa haylage	-	5.3
Alfalfa hay	-	19.2
SBM 48%	8.6	4.0
Soyhulls	30.5	6.5
Cracked corn	19.8	26.1
Roasted soybean meal [2]	-	4.4
Whole cottonseed	-	10.6
Wet brewers grain	-	8.0
Premix [3,4,5,6]	2.0	2.6
Chemical Analysis		
Dry matter	59.2	55.2
Crude protein	11.8	19.1
NDF	48.9	41.2
ADF	30.8	24.4

[1] Dry diet fed approximately 28 days prepartum through day of calving. Lactation diets fed on day of calving through 77 DIM.
[2] Roasted soybean meal manufactured by West Central, Ralston, IA.

[3] Yeast culture was included in premix so total diet delivered 14 and 56 g/cow/d CYC and YC, respectively. Both cultures were supplied by Diamond V Laboratories (Cedar Rapids, IA).

[4] Prepartum diet premix contained: 88,200,000 IU/kg vitamin A, 1,764,000 IU/kg vitamin D_3 and 46,746 IU/kg vitamin E, salt and trace minerals.
[5] Lactation diet premix contained: Dicalcium phosphate, dynamate, biotin, salt, limestone, 88,200,000 IU/kg vitamin A, 1,764,000 IU/kg vitamin D_3 and 46,746 IU/kg vitamin E, and trace minerals.
[6] Trace mineral included in premix contained: 12% Ca, 10% Fe, 8% Zn, 2% $CuSO_4$, 200 mg/kg Co, 10,0000 mg/kg I, and 600 mg/kg Se.

Table 2.2. Effect of yeast culture on body weight, body condition score, milk production and composition as well as blood metabolites of cows from late gestation through 77 days in lactation.

| Parameter | Treatments [1] | | | | |
	C	YC	CYC	SE	P-value
Body Weight (kg) [2]					
Prepartum	725.9	713.2	703.3	17.3	0.58
Week of calving	662.7	636.1	643.3	16.3	0.35
Postpartum	638.7	600.4	599.1	13.7	0.02
Body Condition Score (kg) [3]					
Prepartum	3.40	3.39	3.39	0.1	0.99
Week of calving	3.25	3.17	3.33	0.1	0.23
Postpartum	2.95	2.80	2.91	0.12	0.51
Dry Matter Intake (kg/d)					
Prepartum	12.7	13.0	12.1	0.57	0.33
Postpartum	19.3	19.2	19.3	0.74	0.99
Dry matter intake (%BW)					
Prepartum	1.70	1.84	1.75	0.08	0.31
Postpartum	3.08	3.18	3.18	0.1	0.71
Milk Yield (kg/d)	34.0	36.1	35.1	1.7	0.6
4% FCM (kg/d) [4]	33.4	35.8	34.7	1.8	0.57
4% FCM Efficiency [5]	1.73	1.86	1.80	0.08	0.79
ECM (kg/d) [6]	24.16	25.82	25.5	1.22	0.49
ECM efficiency [7]	1.25	1.34	1.32	0.05	0.35
Milk Fat (%)	4.01	3.97	4.01	0.08	0.92
Milk Protein (%)	3.00	3.03	2.97	0.05	0.68
Milk Urea Nitrogen (mg/dl)	16.88	16.96	16.92	0.47	0.99
Somatic Cell Score [8]	3.14	2.65	2.86	0.36	0.57
NEFA (µeq/l)	263.37	281.47	312.81	21.1364	0.16
Glucose (mg/dl)	69.29	69.27	66.93	1.8	0.34

[1] C, YC, CYC: Control; 56 g/d yeast culture; 14 g/d yeast culture concentrate.
[2] Prepartum and postpartum body weight recorded at ~21 days prior to calving and 70 DIM respectively
[3] Body Condition Score - Five point scale (Wildman et al., 1982). Prepartum and postpartum BCS recorded at ~21 days prior to calving and 70 DIM respectively

[4] 4% FCM = (0.4 * kg milk) + (15 * kg milk fat) (NRC, 2001).

[5] FCM efficiency = (kg FCM) / (kg DMI) (NRC, 2001).
[6] Energy corrected milk = [(0.0929 * Milk fat %) +(0.0547 * milk protein %) + 0.192] * Kg milk (NRC, 2001).

[7] ECM efficiency = (kg ECM) / (kg DMI) (NRC, 2001).

[8] Somatic cell score - scale 0-9

CHAPTER 3

THE EFFECTS OF SUPPLEMENTAL SULFUR BASED ANIONIC SALTS FED DURING THE PERIPARTURIENT PERIOD: IMPLICATIONS OF MILK PRODUCTION AND FEED INTAKE OF HIGH PRODUCING DAIRY COWS

Introduction

Calcium is required in numerous physiological activities including but not limited to muscle contraction, nerve transmission, skeletal tissue, bone formation blood clotting, and roles in enzymatic reactions (NRC, 2001). Hypocalcemia is the state of having insufficient readily available sources of calcium to meet the physiological demands. During the periparturient period, many dairy cattle experience hypocalcemia as a result of inactive metabolic control mechanisms during the onset of lactogenesis. This condition can result in impaired muscle function or tetany and is defined as parturient paresis or more commonly referred to as milk fever. Riond (2001) described clinical symptoms of milk fever with inappetence, inhabitation of urination and defecation, paresis, lateral recumbency and eventually coma and death. Subclinical hypocalcemia is commonly considered to occur at plasma calcium concentration less than 7.5 mg/100mL (Goff and Horst, 1997). Clinical milk fever can be affirmed by visually apparent impaired muscle function as described by Riond (2001) and confirmed by levels of plasma calcium less than 5.5 mg/100mL.

Guard (1996) estimated the average cost per milk fever case for treatment and estimated production losses at $334. Block (1984) found that cows that experienced parturient paresis produced 14% less milk during the subsequent lactation. Also, the productive life of a dairy cow diagnosed with milk fever is reduced (Payne, 1968). Milk fever plays a role in the increased incidence for other periparturient and metabolic disorders. Milk fever has been associated with increased incidence of ketosis (Curtis et al., 1983), dystocia, retained fetal membrane (Oetzel et al., 1988), mastitis, and displaced abomasum. These associated disorders are in large part due to calcium's role in muscle function.

Dietary cation – anion difference (DCAD) is a method of regulating the systemic acid base status of the dairy cow. Ender et al. (1971) defined the DCAD equation:

milliequivalents of $(Na^+ + K^+) - (Cl^- + SO_4^=)$ / kg of dry matter. When diets are formulated to achieve a negative DCAD, the accumulation of anions will result in a decline of blood pH and therefore the initiation of a mild metabolic acidosis. Plasma Ca was higher in cows fed anionic salts then the control animals (Block, 1984; Goff et al.,1991). Joyce et al. (1997) found cows fed anionic salts experienced a greater intake postpartum. Also, Joyce et al. (1997) the incidence of metabolic disorders declined 38.5%.

The concept of inducing metabolic acidosis is not associated with the removal of dietary calcium. The effectiveness of a negative DCAD in the prevention of milk fever was improved with high dietary calcium (1.5% Ca) (Block, 1984; Goff et al., 1991; Oetzel et al., 1991). High DCAD results in the

increased excretion of urinary calcium (Wang and Beede, 1992). Without high

levels of dietary calcium, the low DCAD may induce hypocalcemia (Block, 1997).

The objective of this experiment is to report the effects of feeding

supplement anionic salts during late gestation on calcium and energy

metabolism.

Materials and Methods

The animal use and care committee of the University of Missouri –
Columbia approved the care of twenty-six pregnant Holsteins employed to test
the effects of anionic salts when fed prepartum. These cows were blocked by
expected day of calving, lactation number, previous lactation milk yield, body
weight and body condition score. One of two dietary treatments was randomly
assigned to cows within pairs thirty days prior to expected day of calving.
Approximately thirty days prior to expected day of calving, the animals were
moved to the freestall barn at the University of Missouri's Foremost Dairy
Research Center (Columbia, MO) for the duration of the research trial. The first
nine days were dedicated to training the animals to the electronic feeding system
(American Calan, Inc; Northwood, NH). Animals were fed treatment diets starting
approximately 21 days prior to calving. Following parturition, the treatment diets
were abruptly terminated. A standard lactation diet was fed through day 42 in
milk to determine the treatment effects on the subsequent lactation. Treatments
were based on dietary cation and anion difference (DCAD) altered by the
inclusion of anionic salts (Dawe's Laboratories; Arlington Heights, IL). DCAD was
formulated using wet chemistry mineral analysis results of each feed ingredient
using (Ender et al., 1971):

$$DCAD = mEq (Na+K) – mEq (Cl+S)$$

Control **(C)** contained no anionic salts with a DCAD of +20 mEq/100g DM.
Whereas, the anionic treatment group **(A)** were fed 454 g of an anionic salt
(Dawe's Close Up Pellet) supplement per cow per day at a rate sufficient to lower

DCAD to -10 mEq/100g DM, with dietary Ca adjusted to achieve a daily intake of 150 grams per day. Premix ground corn was substituted to allow for the inclusion of the anionic salt treatment in the premix. Diets (Table 3.1) were formulated to meet or exceed NRC recommendations (NRC, 2001). Prepartum diets were fed until parturition. On the day of parturition (day 0), cows were immediately switched to the lactation diet. Ad libitum intake was supported by providing to total mixed diet to meet intake plus five percent. Diet was mixed once and delivered twice daily to individual Calan doors at 0600 and 1430. Feed offered and refused was recorded at each feeding.

Samples of total mixed diet were collected once daily for each treatment. Samples were stored frozen (-20ºC) in sealed plastic bags. Daily samples were subsequently thawed and composited by week for analysis. Weekly composites were dried in a 55ºC forced air oven and ground through 2 mm screen (Wiley Mill, Thomas Scientific, Swedensboro, NJ). Weekly composite TMR samples were analyzed for dry matter (105ºC), crude protein (LECO Model FP-428 Nitrogen Determinator; LECO Corp., St. Joseph, MI), acid and neutral detergent fiber (Fiber racks, Labconco, Kansas City, MO and ANKOM, Macedon, NY) (Van Soest, 1991). Composition and chemical analysis of dietary treatments are summarized in Table 3.1. Cows were milked twice daily at 0400 and 1600 hours. Milk production was measured and recorded at each milking from day of calving through day 42 of lactation. Milk samples were collected weekly at consecutive p.m. and a.m. milkings and then analyzed for butterfat, protein, urea nitrogen and somatic cell count (Mid-South Dairy Records, Springfield, MO). Values for milk

composition were averaged to obtain a weekly mean. Four percent fat corrected milk (FCM) was calculated using the following equation (NRC, 2001):

$$4\% \text{ FCM} = (0.4 * \text{Kg milk}) + (15 * \text{Kg milk fat}).$$

Body weight (TruTest AG500; TruTest, San Antonio, TX) and body condition score (Wildman et al., 1982) were measured and recorded twice weekly by the same individual throughout the project. Urine pH was monitored electronically (Oakton Acorn Meter Kit, Model no. 54, Eutech Instruments, Singapore) twice weekly as a tool to monitor health and the degree of metabolic acidosis achieved by the anionic salts. Blood samples were collected 21, 14, 10, 7, 3 days prior to expected day of calving, on day of calving, on day 1, 3, 7, 10, 14 and 21 days postpartum. Blood samples were collected at 0600 hours via coccygeal venipuncture into two evacuated tubes (Vacutainer®, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), one containing and one without EDTA as an anticoagulant. The test tube containing whole blood and EDTA was refrigerated while the test tube containing only whole blood sample was allowed to clot. Blood samples were centrifuged (RC3B Plus centrifuge, Sorvall Instruments, Newton, CT) for 21 minutes at 2,100 x g at 4ºC. Plasma and serum aliquots were collected and stored in clear plastic snap cap tubes at -20ºC until analysis. Plasma or serum were analyzed for nonesterfied fatty acids (NEFA), blood urea nitrogen (BUN), glucose, calcium, chlorine, potassium, sodium and phosphorous. Blood chemistry profile (maxi profile) was conducted on serum samples submitted to the animal health diagnostic laboratory at University of Missouri College of Veterinary Medicine.

All parameters containing repeated measures including: pre and postpartum dry matter intake, milk, FCM yield, milk composition and component yields, serum NEFA, glucose, body weight and body condition score over time were analyzed for statistical significance using the MIXED model procedure of SAS (SAS Institute, 2006) with block as the error term. Fixed effects were treatment, time, and treatment * time interaction. Significance was declared at P $\leq$ 0.05 with trends at 0.05 < P $\leq$ 0.10.

Results and Discussion

At the onset of the experiment twenty one days prior to expected day of calving, body weight (BW) and body condition score (BCS) were not different (Table 3.2). The cows averaged 689 and 683 kg prior to calving for control and anionic treatments, respectively. These weights corresponded with an average prepartum BCS of 3.04. Prepartum dry matter intake was not affected by the inclusion of sulfur based anionic salts (P = 0.8791). Both treatments averaged 14.1 kg/cow/d during gestation. These results agree with Block (1984) who reported no differences in prepartum dry matter intake with the inclusion of anionic salts during late gestation. Similarly, Chan et al. (2006) found no change in dry matter intake when anionic salts and varying levels of calcium were fed to the transition dairy cow. In contrast, Oetzel and Barmore (1993) reported a palatability problem with the chloride based anionic salts resulting in a reduced intake. The similar dry matter intake among treatments seen in this experiment support the observations of Oetzel and Barmore (1993) that sulfur based anionic salts are more palatable then those comprised of chlorides. However, Goff et al.

55

(2004) found the chloride based anionic salts possessed 1.6 times the acidifying effect then those sulfur based. The sulfur based anionic salts fed in this experiment resulted in a decreased urine pH relative to the control (6.77 versus 8.30; $P < 0.0001$; Figure 3.1). The acidifying response observed is similar to several previous anionic salt studies (Block, 1993; Tucker et al., 1991; Joyce et al., 1997). This response suggests, when fed in adequate concentrations, sulfur based anionic salts are effective in metabolic acidification. Gaynor et al. (1989) reported urine pH to be an excellent field indicator of metabolic alkalinity. Thus, the DCAD of -10 mEq/100gDM employed in the present study induced a mild metabolic acidosis.

During the first four weeks of lactation, treatment cow body weight was greater than controls ($P = 0.05$; Figure 3.2). The difference in body weight merged during weeks five and six of the trial, therefore overall treatment were not different for the overall study ($P = 0.35$). The curvature of the line indicates the negative energy balance found during the transition period was somewhat annulled for cows fed anionic salts. This response would be an advantage for the periparturient dairy cow (Grummer, 1995). Body condition score was not different due to the inclusion of anionic salts ($P = 0.37$; Table 3.2).

Cows fed anionic salts prepartum tended to have higher dry matter intake ($P = 0.11$) compared to control cows. However, a significant treatment by time difference was measured for dry matter intake ($P = 0.02$; Figure 3.3). Higher DMI was obtained by cows fed anionic salts suggesting that cows fed a diet with negative DCAD increased appetite. Hayirli et al., 1998 reported postpartum

56

intake was related to DMI prior to calving. In the present study DMI was similar during late gestation but was increased by prepartum dietary treatment.

Anionic salt fed cows produced numerically more milk than cows fed control diet prepartum (35.6 and 32.7 kg/cow/d respectively). There was a significant treatment by time difference in milk production with cows fed anionic salt having higher milk yield during the onset of lactation (P = 0.052; Figure 3.4). However, fat corrected milk yield did not differ (Table 3.2). Lack of response in FCM was due to the numerical differences in milk fat percentage (Figure 3.5). Likewise milk protein did not differ among treatments (Figure 3.6). This is in agreement with Joyce et al. (1997). Somatic cell scores did not differ between control and treatment, with an average score of 2.65 on a linear scale of 0-9 for both treatments (Figure 3.7; Table 3.2). Milk urea nitrogen tended to be higher for cows fed anionic salts then those on control (P = 0.10; Figure 3.8; Table 3.2). The more sensitive index, postpartum blood urea nitrogen (Table 3.4), was significantly greater for cows fed anionic salts. The differences in urea nitrogen (Figure 3.8) could be a response of the increased dry matter intake (Figure 3.3).

An additional factor that could influence urea balance is related to acid – base balance of the animal. In the case of metabolic acidosis, when lactate is present, a rise in NH_4^+ resulted in an increase of urea production (Meijer et al., 1990). The ornithine cycle produces urea as a mechanism of excess ammonia elimination. Although not tested in this experiment, others have shown the decline of HCO_3^- with a dietary source of anions (Moore et al., 2000; Oetzel et al., 1991). HCO_3^- is critical for the completion of the ornithine cycle. If the elevated

urea concentrations are a result of the ornithine cycle one might expect to see an increase in aspartate aminotransferase (AST). AST is the enzyme which catalyses the formation of aspartate from oxaloacetate. This is an early step in the ornithine cycle. Aspartate aminotransferase concentrations did not differ for cows fed anionic salts, therefore further research is needed with a more extensive blood profile including enzyme concentrations to better determine the definitive reason for elevated milk and blood urea nitrogen.

Anionic salt cows had improved energy status compared to control cows based on the differences of nonesterfied fatty acids during the transition period (Table 3.3 and 3.4). Kuntz et al. (1985) reported the monitoring of NEFA concentrations is an effective method of determining energy status in dairy cattle. NEFA concentrations peaked for both treatments during day one of lactation (Figure 3.9). The change in NEFA concentration during the periparturient period for this study, agree with the trends described by Ingvartsen and Anderson, 2000. On day one, concentrations of nonesterfied fatty acids averaged 175.0 µeq/l greater for cows fed control diet compared to cows receiving the anionic salt treatment. Postpartum NEFA concentrations differed between treatments (P = 0.05; Table 3.4). Moore et al. (2000) found no differences in NEFA concentrations when feeding DCAD at 0 or -15 mEq/100g DM to cows before calving. In the current study the larger difference in DCAD between treatments may explain the difference in NEFA response.

In addition to changes in NEFA, other metabolic indicators confirm the animal's response to dietary treatments. The observation of mild metabolic

acidosis measured by the low urinary pH was further supported with significant

differences in blood chloride (Figure 3.10) and blood carbon dioxide (Figure

3.11). Overtime, these parameters were significantly different on days 3 and 7 of

lactation. As a result of the induced metabolic acidosis, anionic salt

supplemented cows maintained a higher level of blood calcium on days 3, 7, and

14 (P = 0.0007, 0.0008 and 0.0015; Figure 3.12). The response in blood calcium

is a key metabolic response to the anionic salt treatment success. This response

agrees with several other investigators (Block, 1984; Goff et al., 1991; Oetzel et

al., 1991).

Control cows tended to have higher postpartum blood glucose

concentration versus cows fed anionic salts (P = 0.09, Table 3.4). This

association between blood calcium and glucose has been documented by Blum

et al. (1973). More recently, Larson et al. (2000) reported a similar relationship.

Postpartum blood potassium tended to be higher for cows fed anionic salts (P =

0.10, Table 3.4). The positive relationship between calcium and potassium was

similar to results also reported by Larson et al. (2000). The concentrations of

other minerals were found to be similar and are summarized in Tables 3.3 and

3.4.

Perhaps, the most unique portion of these results is the relationship of

anionic salts with alkaline phosphatase (ALP) and bilirubin. The prepartum blood

analysis indicated an elevated concentration of ALP for the control cows prior to

calving (P = 0.05; Table 3.3). Furthermore, control cows continued to have an

elevated ALP concentration postpartum over time. This difference was significant

59

during day of calving, numerically different for day 1 then merged on day 3 with the anionic salt treatment (Figure 3.13 and Figure 3.14). Concurrently, postpartum total bilirubin concentrations were consistently higher for control then anionic salt fed cows. A significant (P = 0.05) treatment difference was found for postpartum total bilirubin. Anionic salt treated cows averaged 0.2965 and 0.4483 for control (Figure 3.15).

This relationship might explain why cows experiencing hypocalcemia are predisposed to increased risk of other metabolic disorders and disease due to a compromised liver function. Grummer (1993) summarized the etiology of liver dysfunction associated with the increased level of NEFA. The elevated levels of ALP and bilirubin could be related to hepatic stress related to lipid metabolism during transition. With higher levels of feed intake during early lactation concurrent with lower levels of NEFA during transition, the metabolic benefit of feeding anionic salts appears to improve liver health. Furthermore, the overall health of the animals was also improved. As reported in Table 3.5, cows fed anionic salts during early lactation experienced a reduce rate and severity of health disorders. This improvement in animal health agrees with the responses of transition dairy cows as previously reported by Curtis et al. (1983) as well as Goff and Horst (1997) Prior to this report, there has been no published literature suggesting the relationship of ALP and bilirubin for the transition dairy cows fed anionic salts prepartum.

Summary

Anionic salts have been shown to be an effective feed additive for the prevention of hypocalcemia in the transition dairy cow. When anionic salts were fed prepartum, prepartum feed intake was not altered. Furthermore, the addition of anionic salts promoted an improved energy balance through increased dry matter intake early in lactation, and reduced mobilization of nonesterfied fatty acids. Finally, anionic salts have been shown to prevent liver damage during the periparturant period as measured by lower alkaline phosphatase and bilirubin.
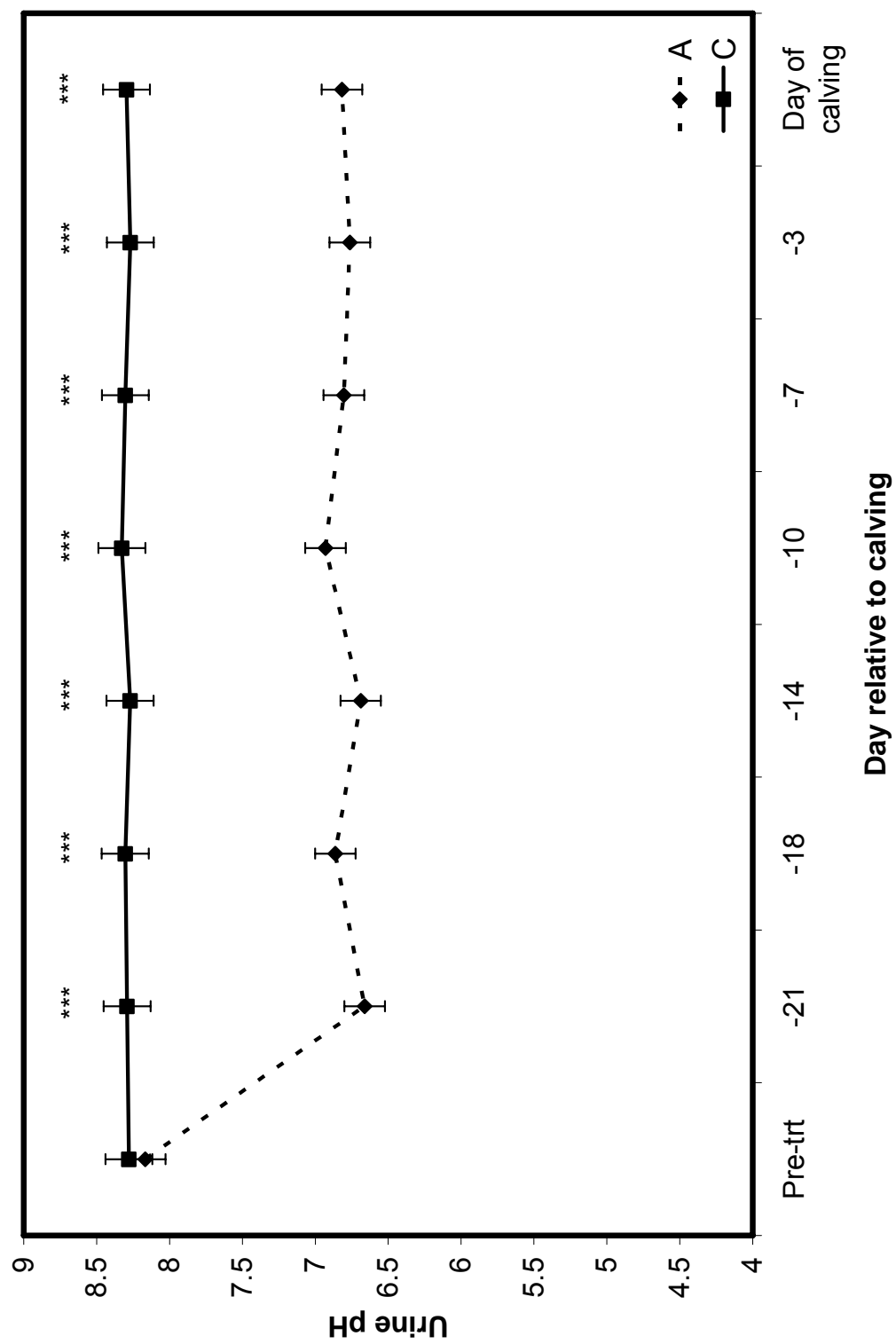
Figure 3.1. Prepartum urine pH for cows fed anionic salts during gestation (P < 0.0001; *** P < 0.0001)
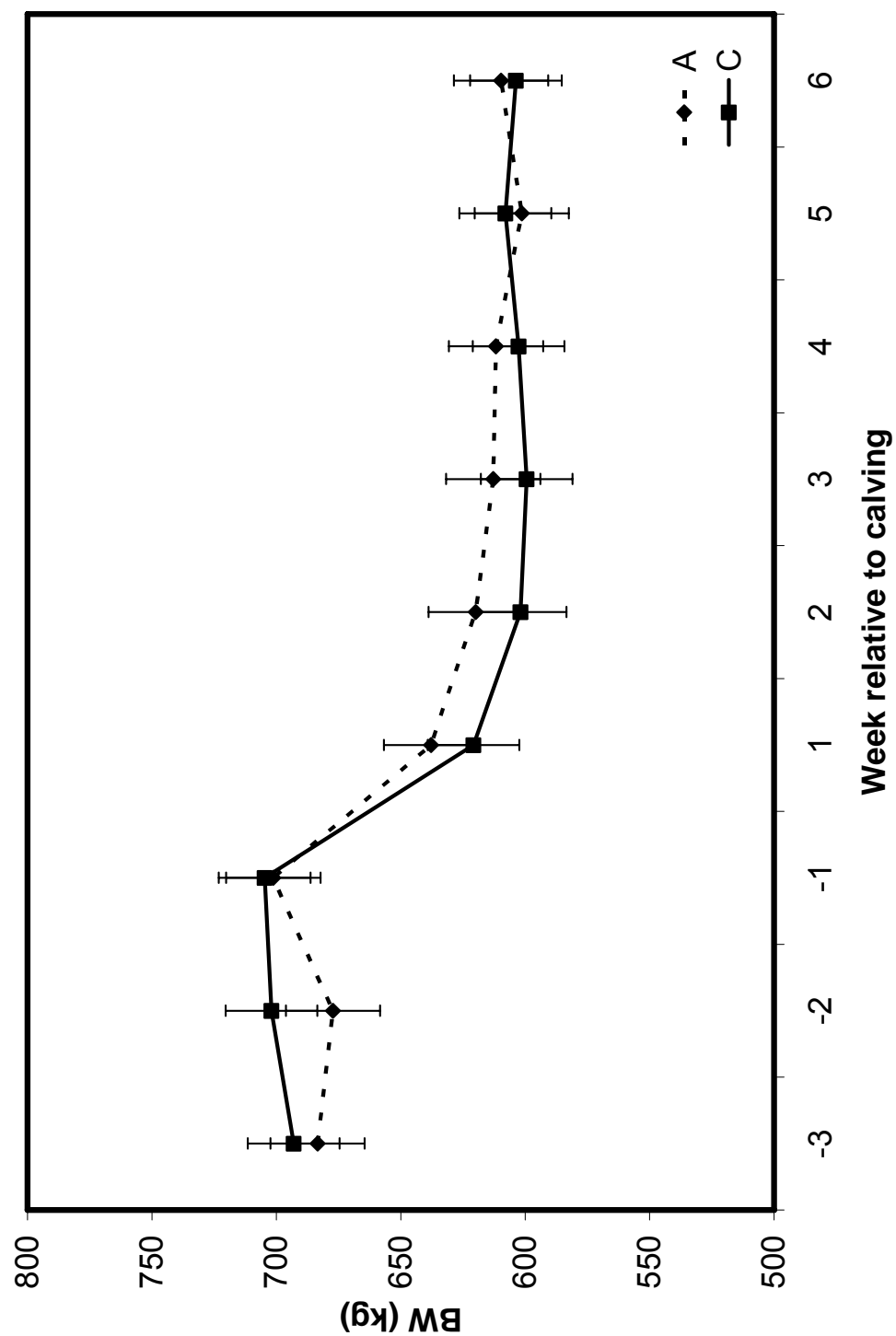
Figure 3.2. Weekly treatment means of body weight are illustrated for the entire experiment (P = 0.4713). Body weight analyzed during the post partum period was higher for cows fed anionic salt prepartum (P = 0.05)

63
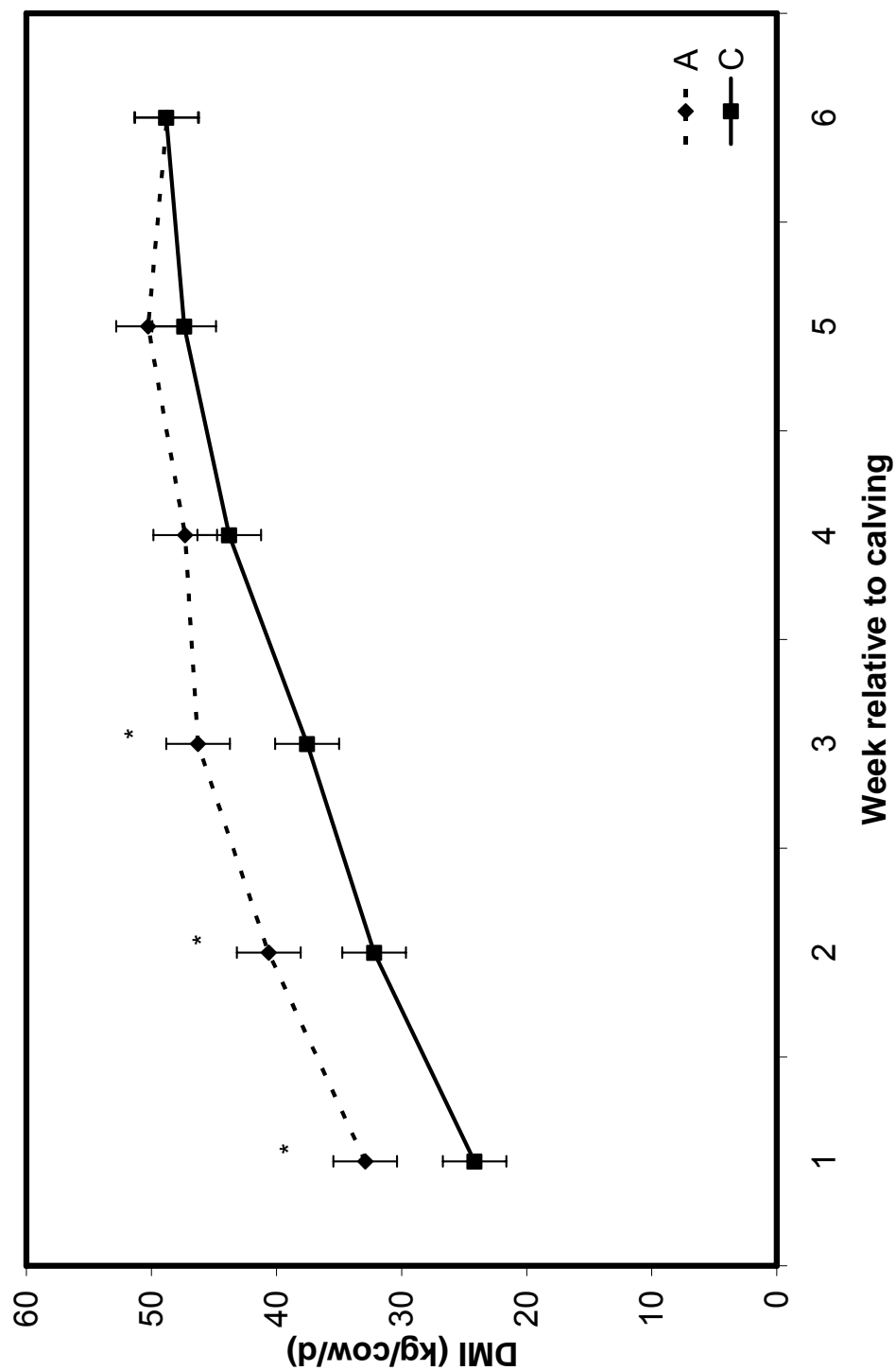
Figure 3.3. Weekly treatment means for postpartum dry matter intake for cows fed anionic salt during late gestation (P = 0.01; * P ≤ 0.05).
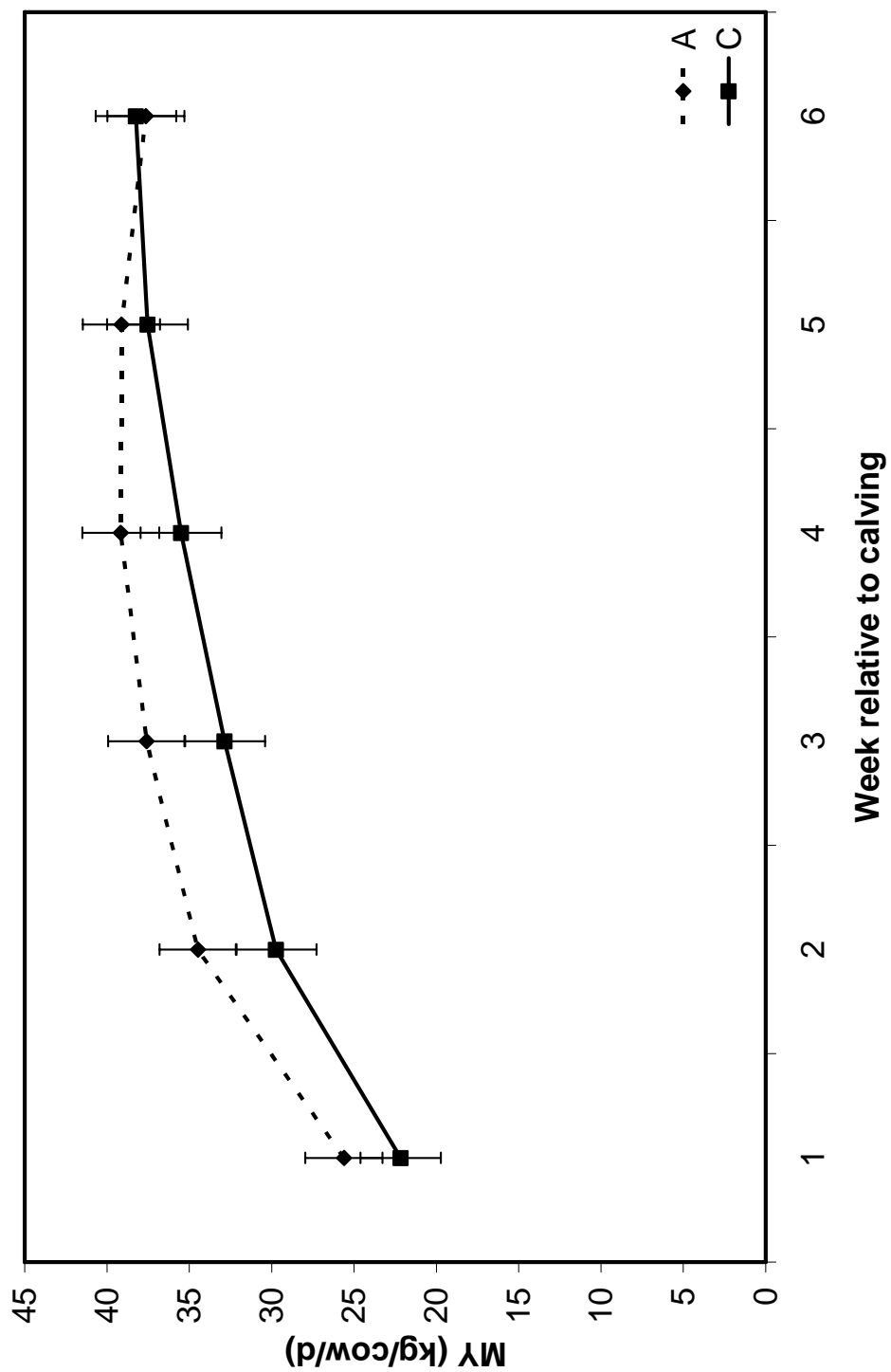
Figure 3.4. Weekly milk yield average for cows fed anionic salt during late gestation (P = 0.0520).
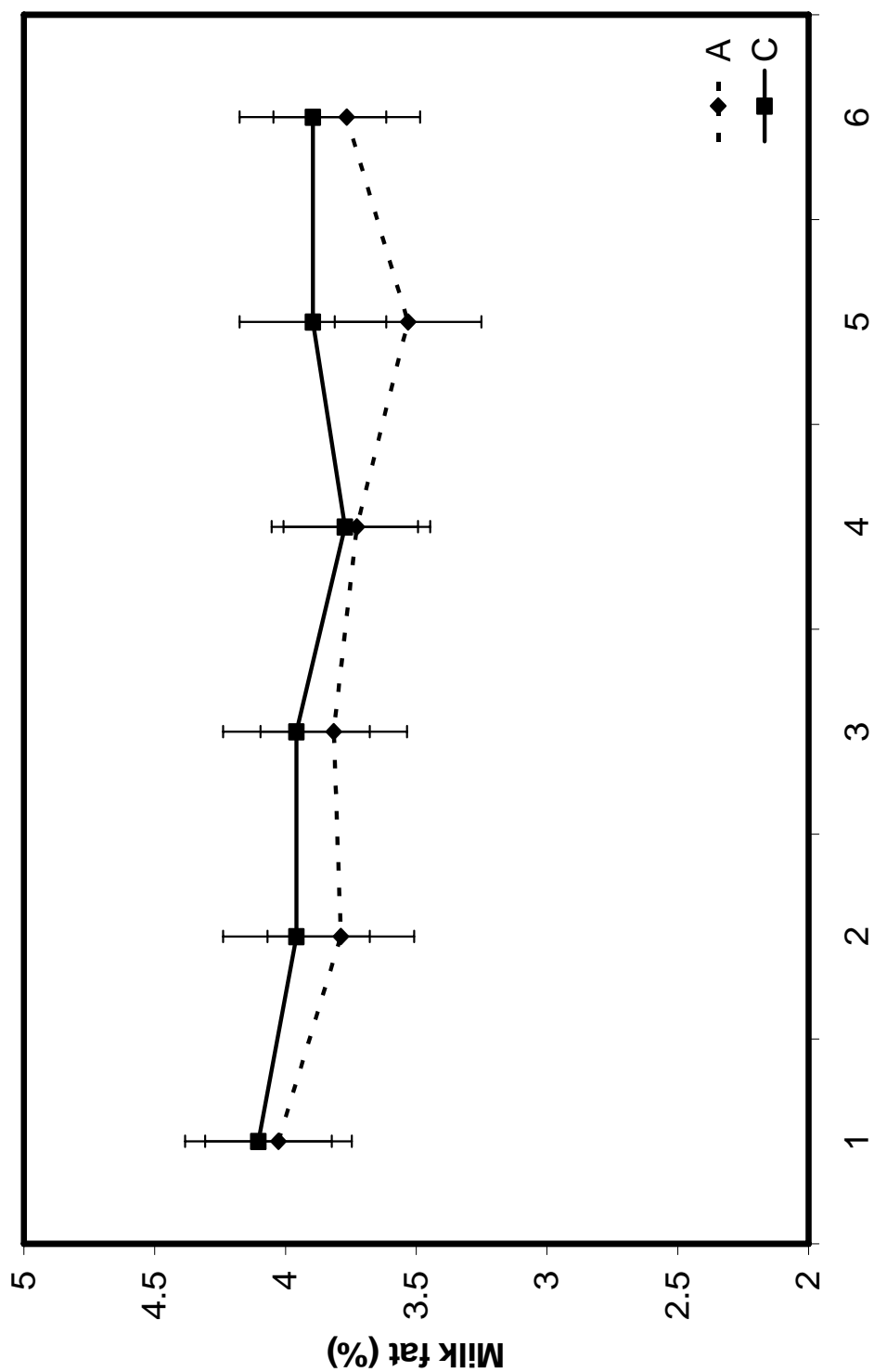
Figure 3.5. Treatment over time milk fat concentrations of dairy cows fed anionic salts or control diets approximately 21 days prior to calving (P = 0.9695).
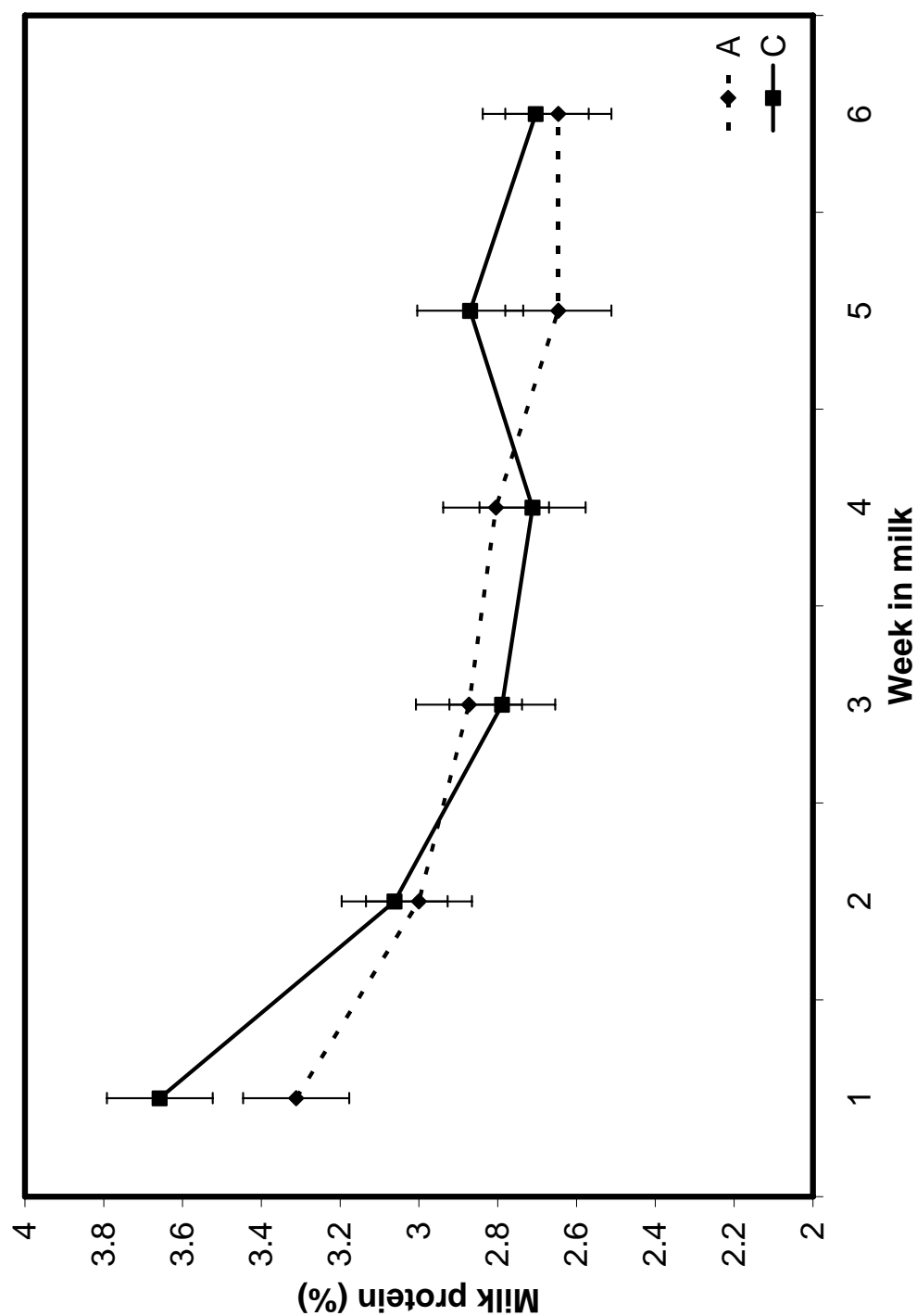
Figure 3.6. Treatment over time milk protein concentrations of dairy cows supplemented with anionic salts approximately 21 days prior to calving (P = 0.1515).

67
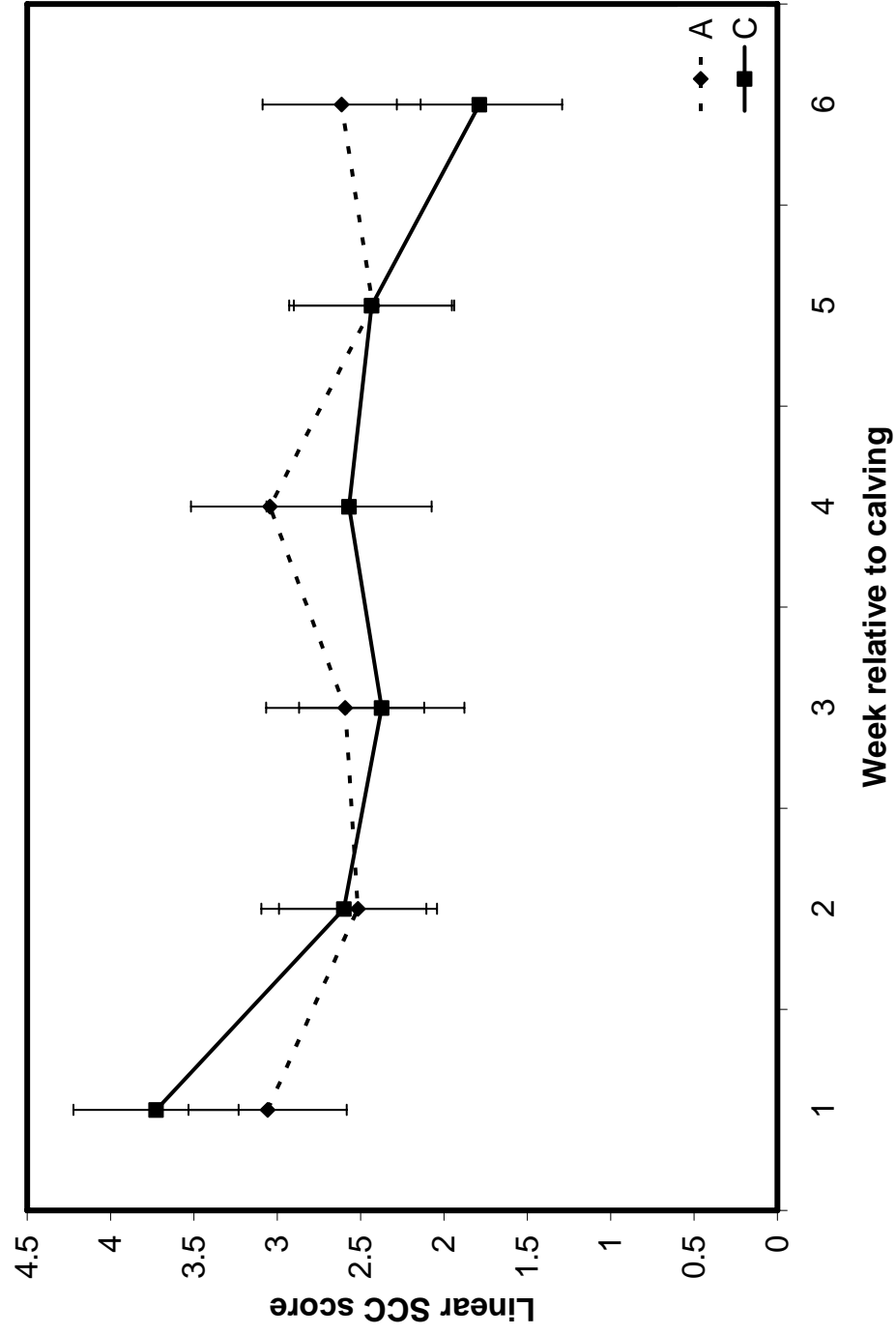
Figure 3.7. Treatment over time milk somatic cell count of dairy cows fed anionic salts or control diets approximately 21 days prior to calving (P = 0.4468).
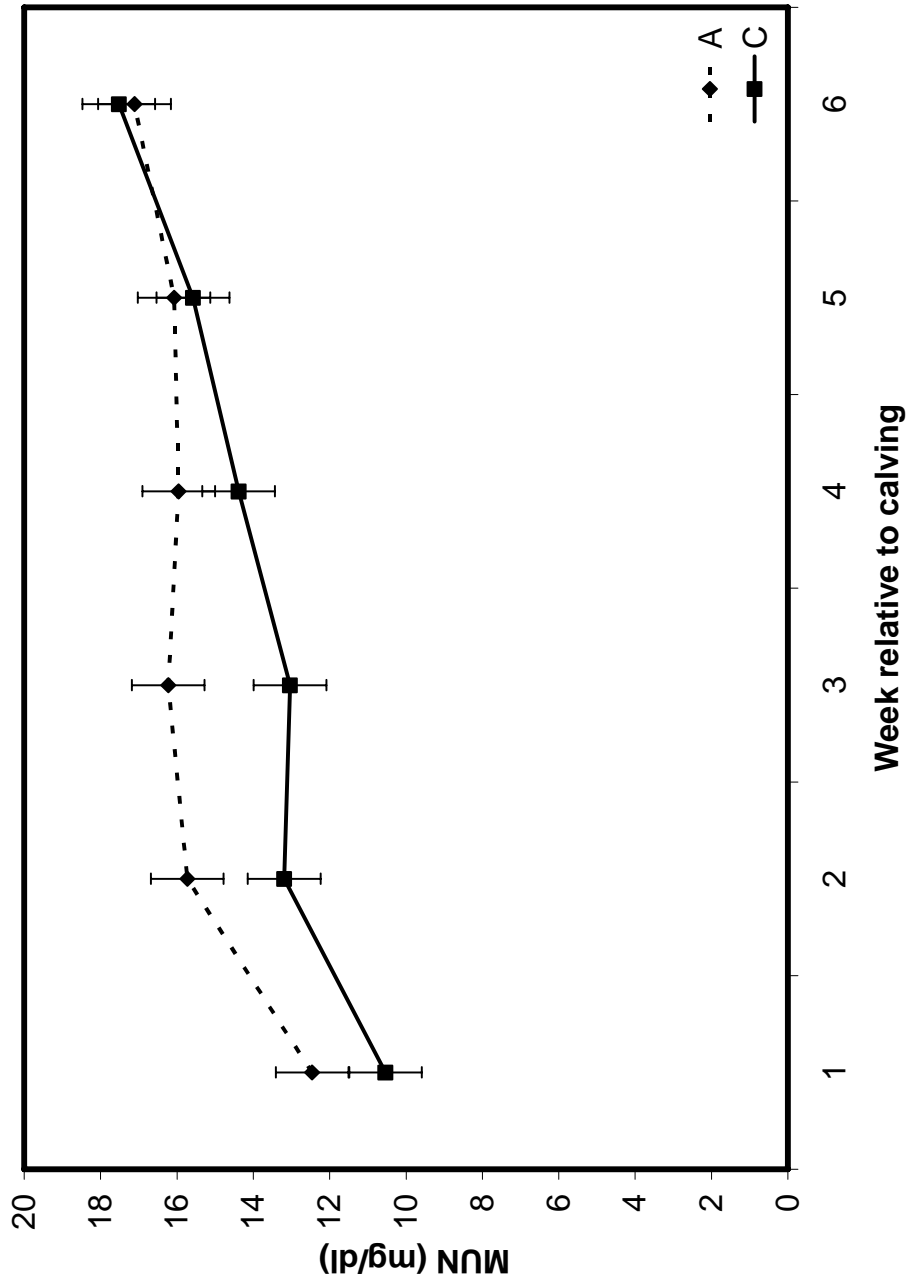
Figure 3.8. Milk urea nitrogen concentrations during early lactation for cows fed anionic salt during late gestation (P = 0.15)
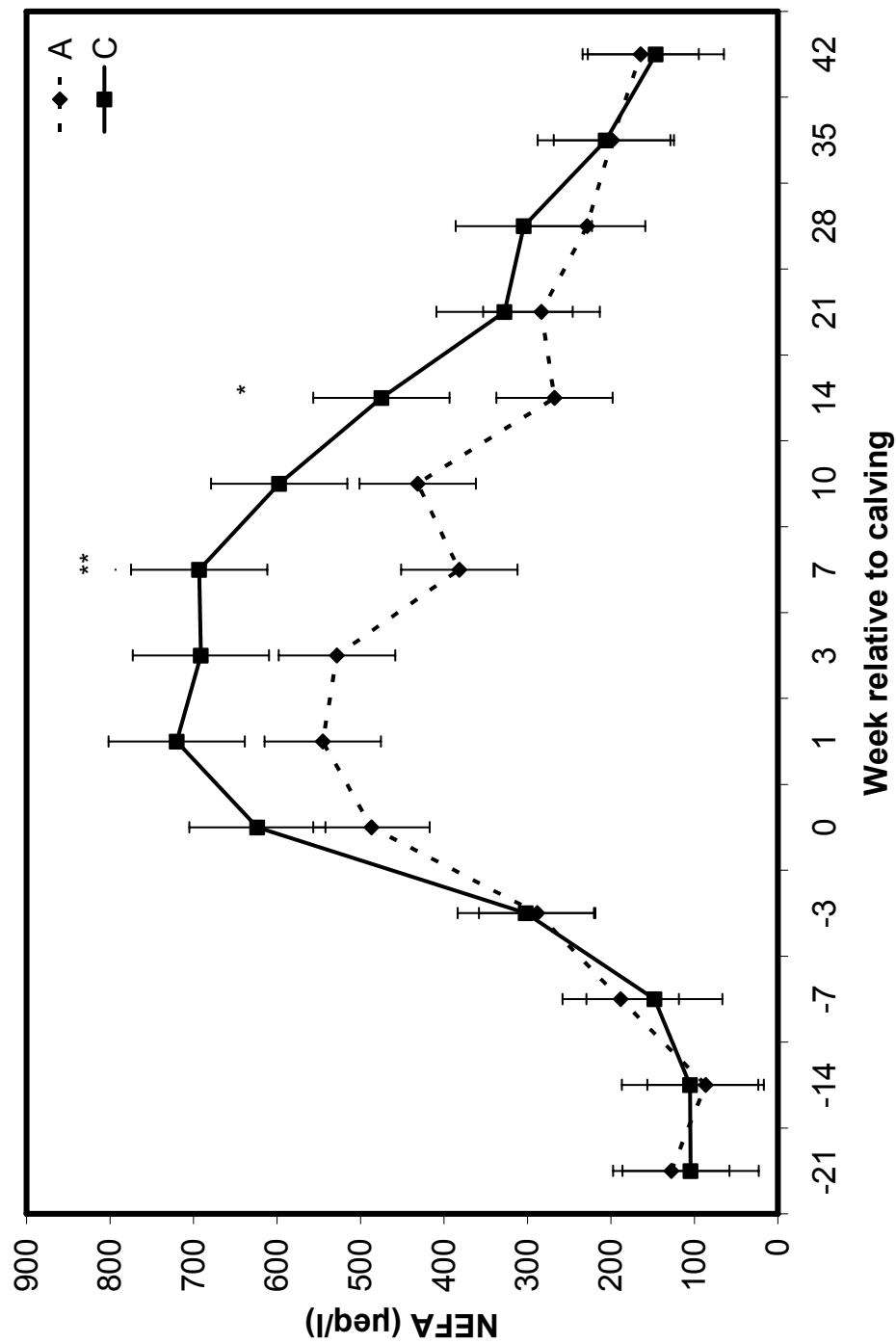
Figure 3.9. Serum non-esterified fatty acids concentrations by treatment over time for dairy cows fed anionic salt or control treatment diets twenty one days prior to calving (*P < 0.05, *** P < 0.001).

70
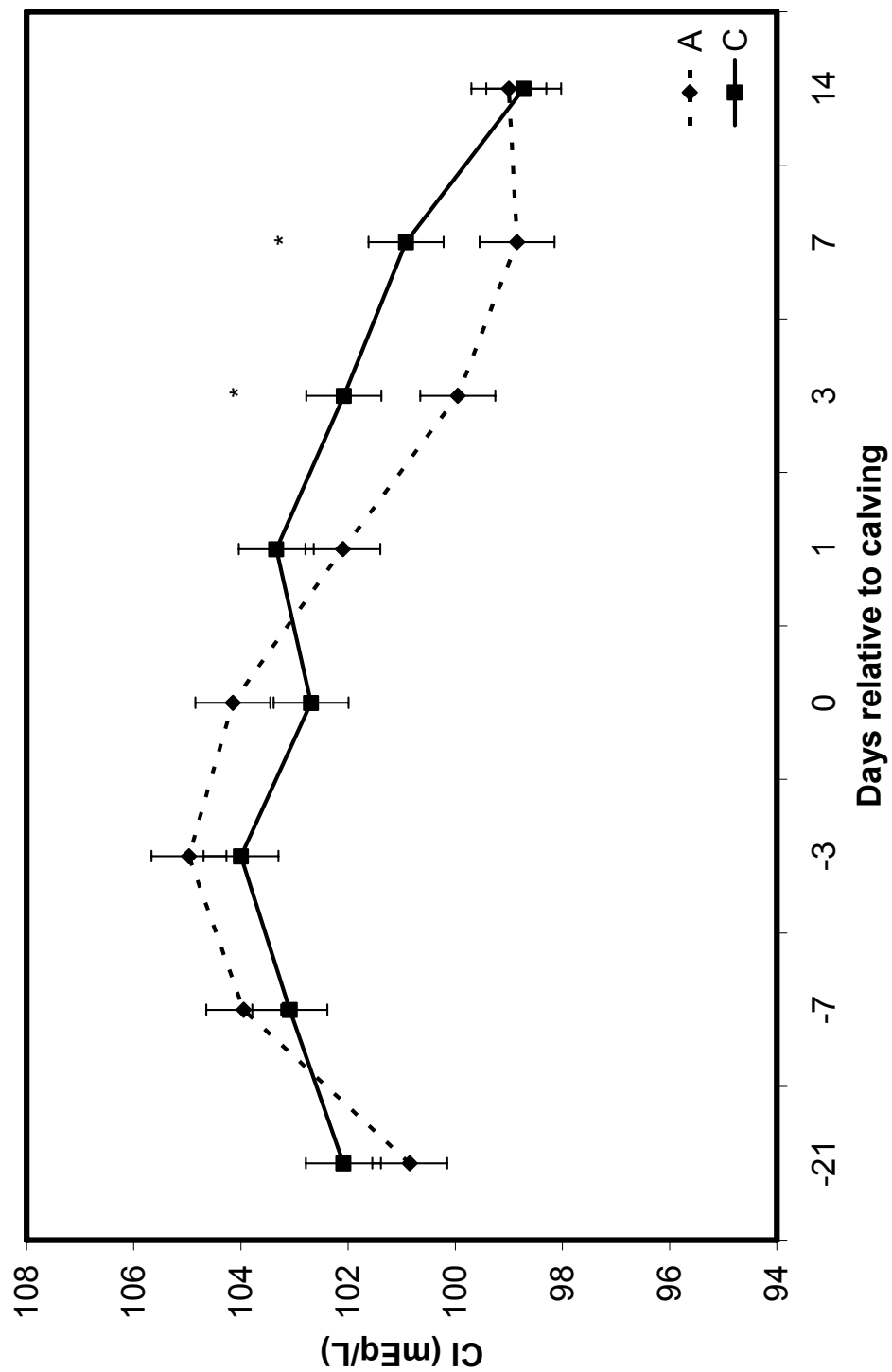
Figure 3.10. Blood chloride concentrations during the transition period for cows fed anionic salts during gestation (P = 0.02; * P ≤ 0.05).
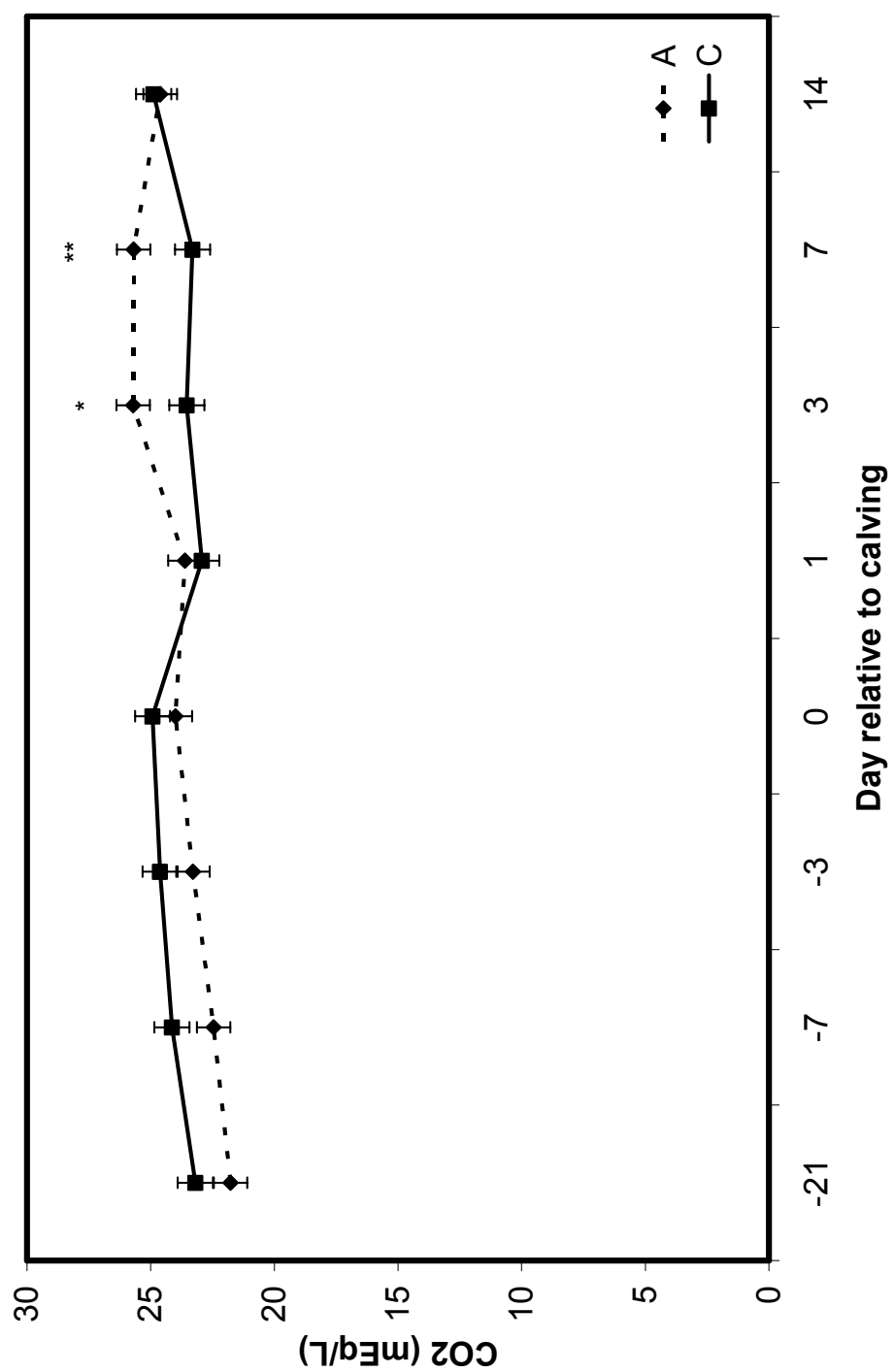
Figure 3.11. Blood CO_2 concentrations during the transition period for cows fed anionic salts during gestation ($P=0.002$; * $P \leq 0.05$, **$P \leq 0.01$).
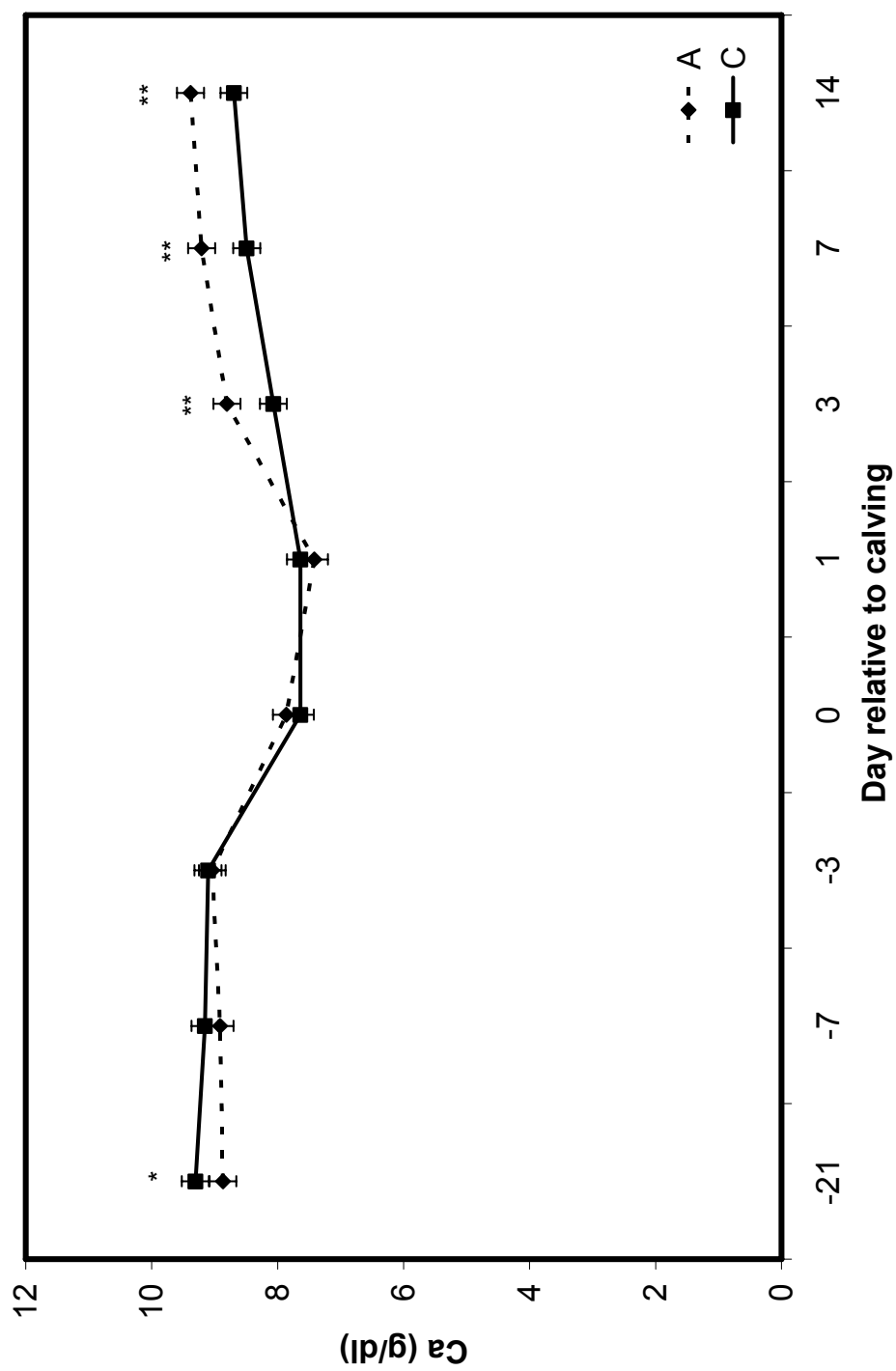
Figure 3.12. Blood Calcium levels by treatment over time to dairy cows fed anionic salt or control treatment diets twenty one days prior to calving (* P < 0.05, ** P < 0.01).
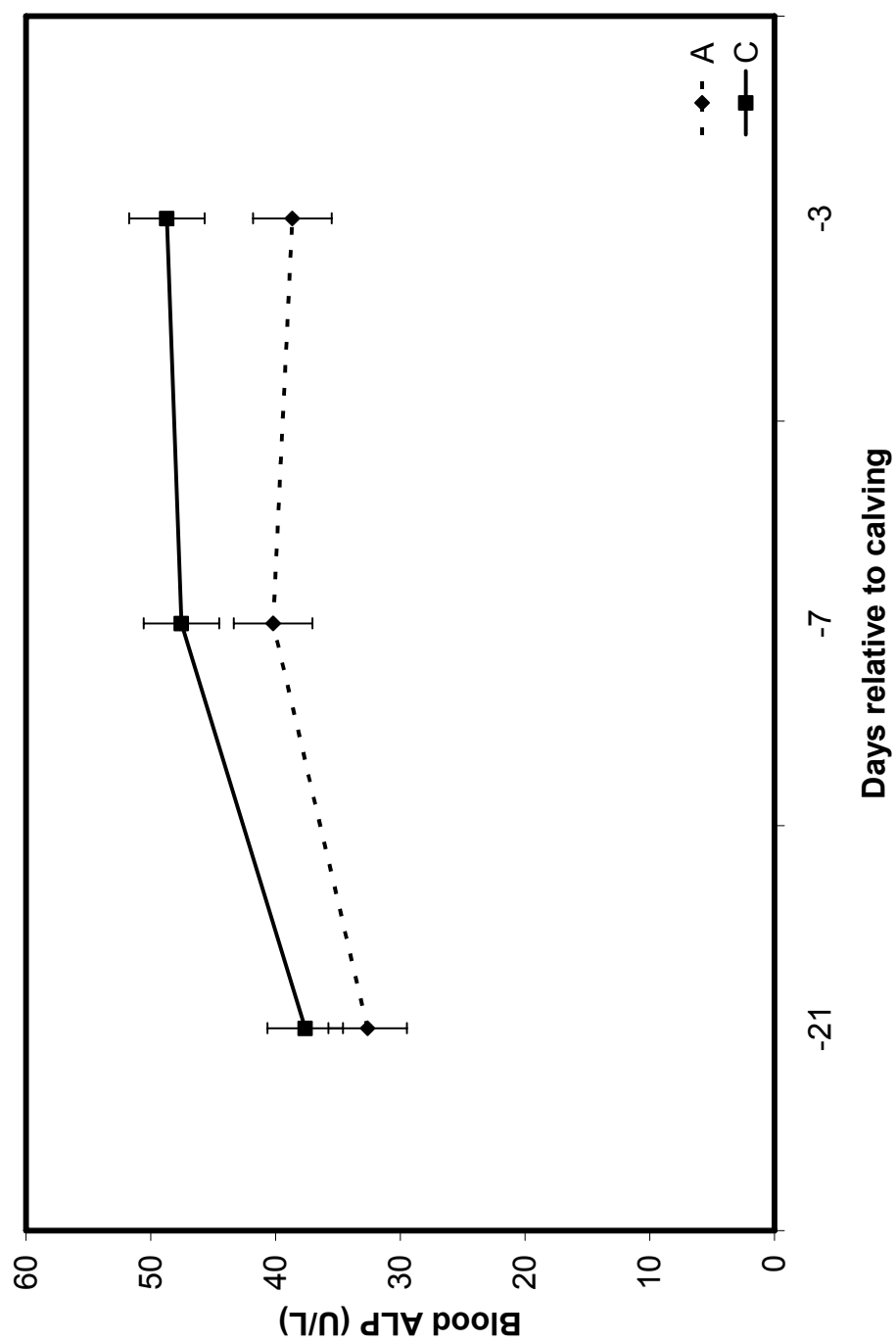
Figure 3.13. Prepartum blood alkaline phosphatase concentrations over time for cows fed anionic salt treatment prepartum (P = 0.5114).
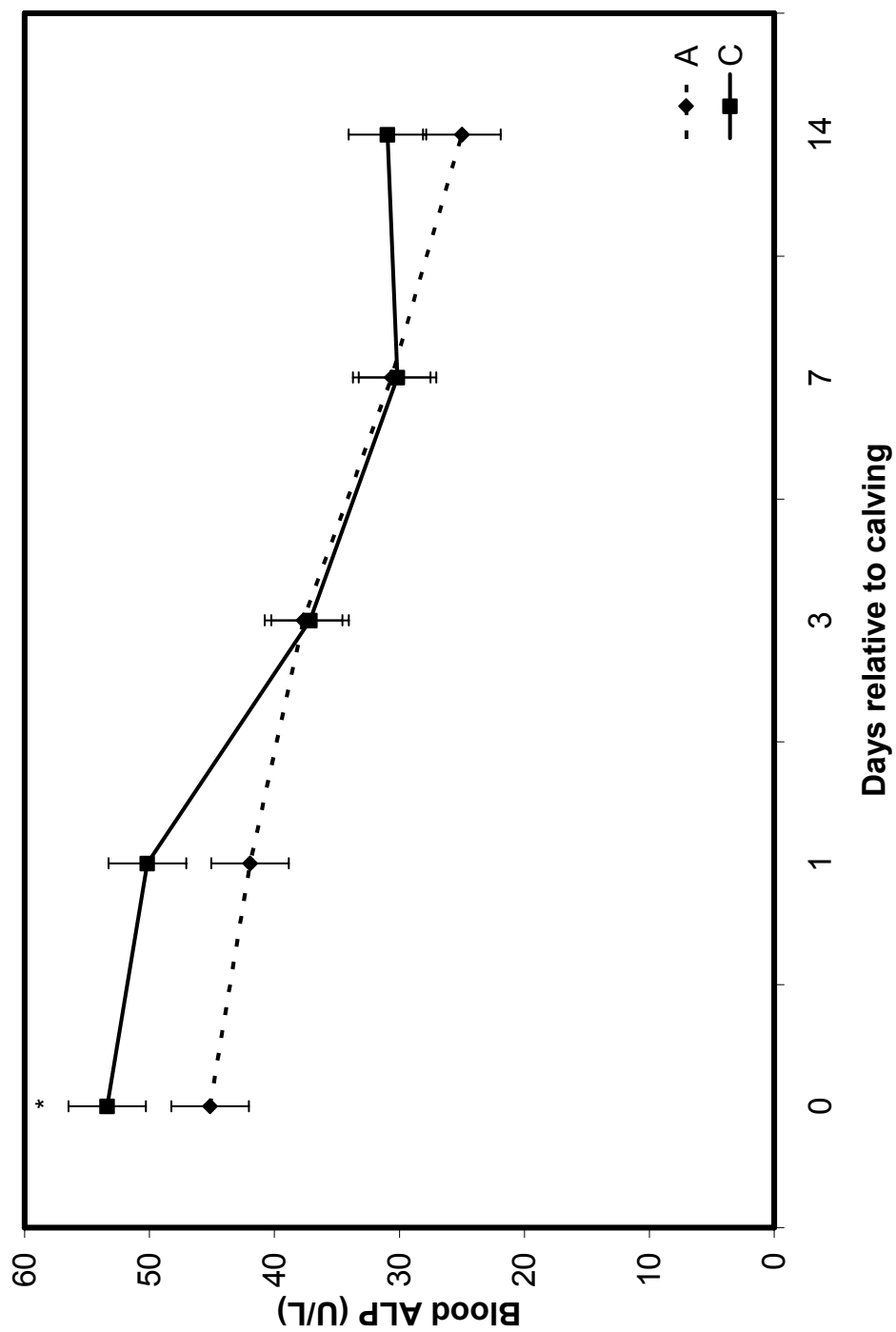
Figure 3.14. Postpartum blood alkaline phosphatase concentrations over time for cows fed anionic salt treatment prepartum (P = 0.02).
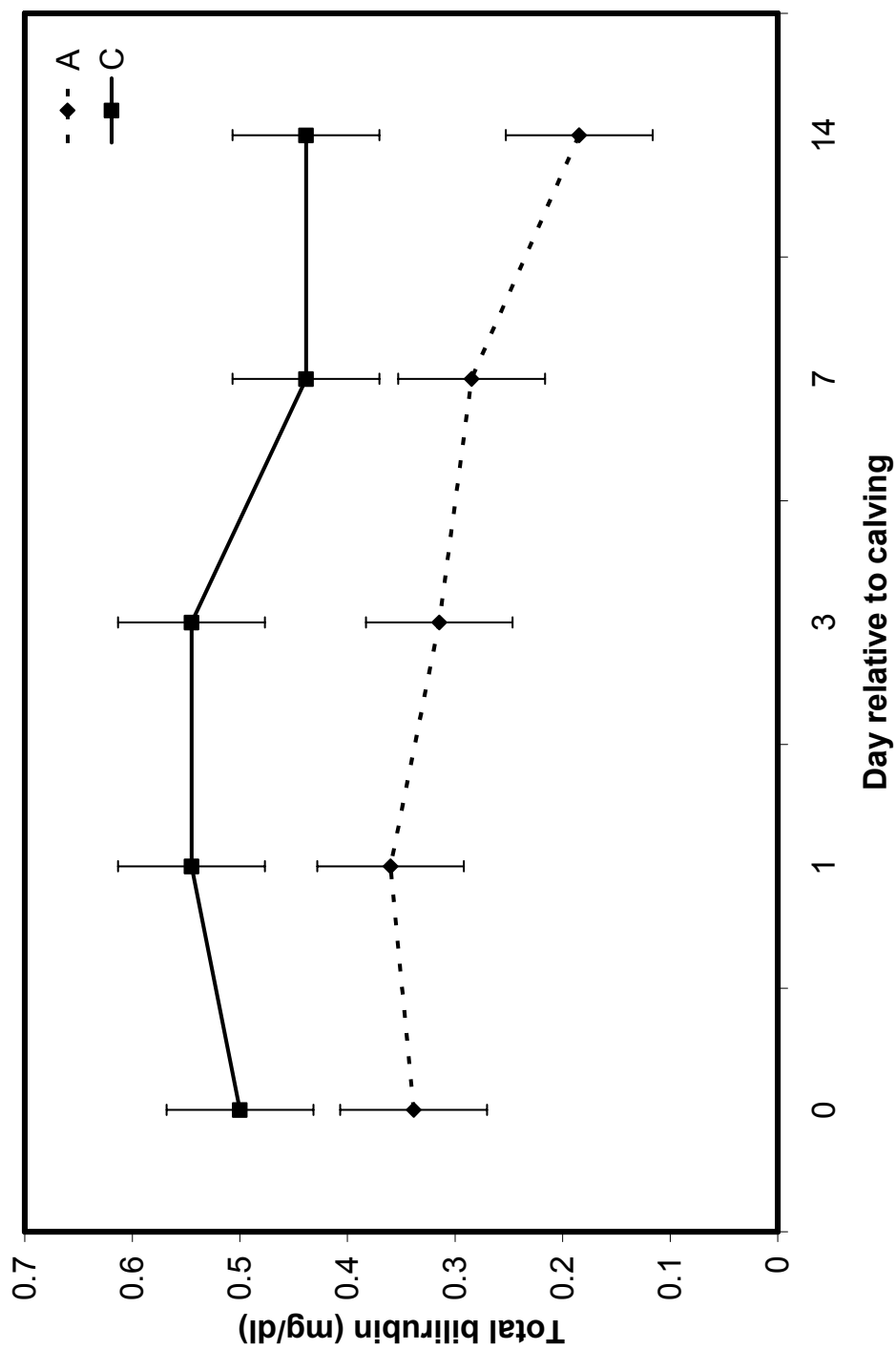
Figure 3.15. Postpartum total bilirubin concentrations over time for cows fed anionic salt treatment prepartum (trt*tm: P = 0.8753, TRT: P = 0.05).

76

Table 3.1. Composition and chemical analysis of prepartum anionic salts diet and postpartum lactation experimental diets.

	Pre-partum diets[1]		Lactation diet[1,2]
	Control	Anionic	
Ingredient		% Dry Matter	
Cracked Corn	21.3	18.3	24.86
Corn Silage	17.3	16.5	17.1
Corn Gluten	-	-	9.7
Grass Hay	14.8	18.0	-
SBM 48%	10.0	10.7	14.6
Soy hulls	26.3	22.0	9.8
Dicalcium Phosphate	-	1.5	0.6
Limestone	0.9	1.9	0.7
Salt	0.1	0.1	0.1
Diynamate	-	-	0.2
Potassium Chloride	-	-	0.3
Anionic salt Pellet [3]	-	4.1	-
Alfalfa Hay	8.9	6.9	15.2
Alfalfa Silage	-	-	6.7
Trace mineral + Vitamin [4,5]	0.4	-	0.14
Chemical Analysis			
Dry matter	62.57	62.76	58.03
Crude protein	13.41	13.61	18.98
NDF	60.04	59.25	35.71
ADF	36.27	34.88	22.47
Ash	7.29	9.03	9.01

[1]Dry diet fed approximately 28 days prepartum through day of calving. Lactation diets fed on day of calving through 42 DIM.

[2] Lactation diet included either cracked soybean or whole soybean. Protein concentrations were equal between postpartum diets.

[3] Anionic salt pellets were included in premix. Anionic salt pellet supplied by Dawe's Laboratories (Arlington Heights, IL).

[4] Prepartum diet premix contained: 88,200,000 IU/kg vitamin A, 1,764,000 IU/kg vitamin D3 and 46,746 IU/kg vitamin E, salt and trace minerals.

[5] Trace mineral included in premix contained: 12% Ca, 10% Fe, 8% Zn, 2% CuSO4, 200 mg/kg Co, 10,0000 mg/kg I, and 600 mg/kg Se.

Table 3.2. Effect of anionic salts when fed during late gestation on body weight, body condition score, milk production and composition as well as blood metabolites of cows from late gestation through 42 days in lactation.

Parameter	Treatments [1]		SE	P-value
	A	C		
Body Condition Score [2]				
Prepartum	3.06	3.02	0.06	0.6147
Postpartum	2.98	2.88	0.07	0.3739
Body Weight (kg) [3]				
Prepartum	683.4	689.2	20.3	0.8391
Postpartum	605.4	567.5	25.93	0.3466
Dry Matter Intake (kg/d)				
Prepartum	14.1	14.1	0.99	0.9849
Postpartum	44.4	39.0	2.2	0.1062
Milk Yield (kg/d)	35.6	32.7	2.2	0.3573
4% FCM (kg/d) [4]	33.7	31.3	2.5	0.5019
Milk Fat (%)	3.78	3.90	0.17	0.6179
Milk Protein (%)	2.88	2.97	0.09	0.5082
Milk Urea Nitrogen (mg/dl)	15.6	14.0	0.6	0.1027
Somatic Cell Score [8]	2.71	2.58	0.29	0.7624
Urine pH	6.77	8.30	0.07	<0.0001

[1] C: Control, A: Anionic salt; DCAD +20 and -10 mEq/100gDM respectively

[2] Body Condition Score - Five point scale (Wildman et al., 1982). Prepartum and postpartum BCS recorded at ~21 days prior to calving and 42 DIM respectively

[3] Prepartum and postpartum body weight recorded at ~21 days prior to calving and 42 DIM respectively

[4] 4% FCM = (0.4 * kg milk) + (15 * kg milk fat) (NRC, 2001).

[8] Somatic cell score - scale 0-9

Table 3.3. Effects of anionic salts fed during late gestation on blood metabolites of the prepartum dairy cow.

| Parameter | Treatment | | SE | P-value |
	A	C		
	Prepartum[1]			
Albumin (mg/dl)	3.20	3.30	0.05	NS
ALP (U/L)	37.16	44.63	2.48	0.05
Anion Gap	20.77	20.15	0.36	NS
AST (U/L)	55.46	56.65	3.19	NS
Calcium (mg/dl)	8.94	9.13	0.11	NS
Chloride (mEq/L)	103.25	103.01	0.30	NS
CPK (U/L)	108.25	99.88	16.75	NS
Creatinine (mg/dl)	0.90	0.95	0.05	NS
Direct bilirubin (mg/dl)	0.03	0.03	0.01	NS
GGT (U/L)	16.69	16.26	1.37	NS
Globulin (g/dl)	3.66	3.69	0.19	NS
Glucose (mg/dl)	56.26	58.09	1.11	NS
Magnesium (mg/dl)	2.17	2.17	0.05	NS
NEFA (µeq/l)	193.59	228.16	45.93	NS
Phosphorus (mg/dl)	6.23	5.52	0.13	0.002
Potassium (mEq/L)	4.56	4.52	0.05	NS
Sodium (mEq/L)	141.92	142.64	0.29	0.10
Total bilirubin (mg/dl)	0.16	0.20	0.03	NS
Total CO_2 (mEq/L)	22.45	23.96	0.45	0.03
Total protein (g/dl)	6.87	6.98	0.16	NS
Urea Nitrogen (mg/dl)	14.24	13.17	0.39	0.08

[1] Blood samples collected on 21, 7, 3 days prior to expected day of calving.
NS, Dietary treatments did not differ statistically

Table 3.4. Effects of anionic salts fed during late gestation on blood metabolites of the postpartum dairy cow.

	Treatment			
Parameter	A	C	SE	P-value
	Postpartum[1]			
Albumin (mg/dl)	3.31	3.19	0.06	NS
ALP (U/L)	36.08	40.36	2.56	NS
Anion Gap	21.44	21.32	0.34	NS
AST (U/L)	75.77	78.21	6.53	NS
Calcium (mg/dl)	8.54	8.13	0.09	0.01
Chloride (mEq/L)	100.79	101.56	0.31	NS
CPK (U/L)	179.29	199.81	28.84	NS
Creatinine (mg/dl)	0.92	0.91	0.04	NS
Direct bilirubin (mg/dl)	0.07	0.11	0.01	0.10
GGT (U/L)	18.61	17.89	1.41	NS
Globulin (g/dl)	3.56	3.60	0.20	NS
Glucose (mg/dl)	49.32	54.21	1.88	0.09
Magnesium (mg/dl)	2.20	2.13	0.05	NS
NEFA (µeq/l)	350.60	471.98	38.93	0.05
Phosphorus (mg/dl)	5.58	5.74	0.19	NS
Potassium (mEq/L)	4.87	4.70	0.07	0.10
Sodium (mEq/L)	142.03	142.07	0.46	NS
Total bilirubin (mg/dl)	0.30	0.45	0.05	0.05
Total CO_2 (mEq/L)	24.71	23.89	0.31	0.08
Total protein (g/dl)	6.87	6.78	0.16	NS
Urea Nitrogen (mg/dl)	16.10	14.14	0.51	0.02

[1] Blood samples collected on day of calving and 1, 3, 7, 14 days in milk
NS, Dietary treatments did not differ statistically

Table 3.5. Incidence of health disorder during the transition from late gestation to early lactation for cows on experimental diets

Health Data	Treatment	
	Control	Anionic Salt
Retained fetal membrane	8	4
Clinical metritis	7	1
Clinical milk fever	3	0
Clinical ketosis	4	2
Displaced abomasum	3	2

ID	TRT	Lact No.	Reason
			Addendum 1: Cows removed from yeast culture trial reported by treatment and cause of removal.
447	C	5	Cancer
548	C	5	Toxic mastitis
696	C	4	Prolapse uterus
726	C	2	Did not adjust to electronic feeding system
742	C	2	3 occurrences of displaced abomasum
752	C	1	Complications with cesarean section
793	C	2	Agalactia
861	C	1	Broken leg
879	C	1	Agalactia
910	C	1	Broken leg
913	C	1	Dystocia
931	C	1	Broken leg
933	C	1	Hock problem and frostbite on teats
936	C	1	Did not adjust to electronic feeding system
937	C	1	Toxic mastitis
768	CYC	2	Peritonitis
923	CYC	1	Chronic mastitis
775	YC	2	Aborted
807	YC	1	Broken leg
868	YC	1	Mastitis and Peritonitis
904	YC	1	Calving complications (pinched nerve)
906	YC	1	Mastitis
918	YC	1	Aborted

Totals:
15 Control
6 Yeast culture
2 Concentrated yeast culture

LITERATURE CITED

Bell, A. W., G. Slepetis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. J. Dairy Sci. 78:1954-1961.

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

Block, Elliot. 1993. Manipulation of dietary cation anion difference on nutritionally related production diseases and productivity of dairy cows. J. Dairy Sci. 76 (Suppl. 1):303.

Block, Elliot. 1997. Dietary cation-anion balance in dairy nutrition. Western Canadian Dairy Conference. Ch 17.

Blum, J. W., R. B. Wilson, and D. S. Kronfeld. 1973. Plasma insulin concentrations in parturient cows. J. Dairy Sci. 56:459-464.

Callaway, E. S., and S. A. Martin. 1997. Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

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

Cameron, R. E. B., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. Vandehaar, and R. S. Emery. 1998. Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high producing dairy herds. J. Dairy Sci. 81:132-139.

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

Combs Jr., G. F. 1992. Vitamin D. Pages 164-183 in The Vitamins. 2nd ed. Academic Press, San Diego.

Constable, P. D., G. Y. Miller, G. F. Hoffsis, B. L. Hull, and D. M. Rings. 1992. Risk factors for abomasal displacement in cattle. Am. J. Vet. Res. 53:1184-1192.

Cooper, R., and T. Klopfenstein. 1996. Effect of rumensin and feed intake variation on ruminal pH. Scientific update on rumensin/tylan/micotil for the professional feedlot consultant. Elanco Animal Health, Greenfield, IN.

Cottee, G., I. Kyriazakis, T. M. Widowski, M. I. Lindinger, J. P. Cant, T. F. Duffield, V. R. Osborne, and B. W. McBride. 2004. The effects of subacute ruminal acidosis on sodium bicarbonate supplemented water intake for lactating dairy cows. J. Dairy Sci. 87:2248-2253.

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

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Dann, H. M., J. K. Drackley, G. C. McCoy, M. F. Hutjens, and J. E. Garrett. 2000. Effects of yeast culture (Saccharomyces cerevisiae) on prepartum intake and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83(1):123-127.

Diamond V Mills. 2006. Subject: Yeast culture products. http://www.diamondv.com/products/xpc.html. Accessed June 28, 2005.

Donovan, G. A., C. A. Risco, G. M. D. Temple, T. Q. Tran, and H. H. van Horn. 2004. Influence of transition diets on occurrence of subclinical laminitis in Holstein dairy cows. J. Dairy Sci. 87(1):73-84.

Doreau, M., and J. P. Jouany. 1998. Effect of Saccharomyces cerevisiae culture on nutrient digestion in lactating dairy cows. J. Dairy Sci. 81(2):3214-3221.

Ender, F., I. W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. The solution of the aetiology and the prevention of milk fever by dietary means. Z. Tierphysiol. 28:233– 256.

Enemark, J. M. D., and R. J. Jorgensen. 2001. Subclinical rumen acidosis as a cause of reduced appetite in newly calved dairy cows in Denmark: Results if a poll among Danish dairy practitioners. Vet. Quarterly. 23(4):206-210.

Enjalbert, F., J. E. Garrett, R. Moncoulon, C. Bayourthe and P. Chicoteau. 1999. Effects of yeast culture (Saccharomyces cerevisiae) on ruminal digestion in non-lactating dairy cows. Anim. Feed Sci. and Tech. 76(3-4):195-206.

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Ferguson, James O., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77: 2695-2703.

Gaynor, P. J., F. J. Mueller, J. K. Miller, N. Ramsey, J. P. Goff, and R. L. Horst. 1989. Parturient hypocalcemia in Jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. J. Dairy Sci. 72:2525-2531.

Giger-Reverdin, S., D. Sauvant, J. Tessier, G. Bertin, and P. Morand-Fehr. 2004. Effect of live yeast culture supplementation on rumen fermentation in lactating dairy goats. S. African J. Anim. Sci. 34(Suppl.1):59-61.

Godden, S. M., S. C. Stewart, J. F. Fetrow, P. Rapnicki, R. Cady, W. Weiland, H. Spencer, and S. W. Eicker. 2003. The relationship between heard rbST-supplementation and other factors and risk for removal for cows in Minnesota Holstein dairy herds. Pages 55-64 in Proc. Four-State Nutr. Conf. LaCrosse, WI. MidWest Plan Service publication MWPS-4SD16.

Goff, J. P., R. L. Horst, and T. A. Reinhardt. 1987. The pathophysiology and prevention of milk fever. Vet. Med. 82:943.

Goff, J. P., M. E. Kehrli, Jr., and R. L. Horst. 1989. Periparturient hypocalcemia in cows: prevention using intramuscular parathyroid hormone. J. Dairy Sci. 72:1182.

Goff, J. P., R. L. Horst, F. J. Mueller, J. K. Miller, G. A. Kiess, and H. H. Dowlen. 1991. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever. J. Dairy Sci. 74:3863-3871.

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

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

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

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

Grummer, R. R., S. J. Bertics, D. W. LaCount, J. A. Snow, M. R. Dentine, and R. H. Stauffacher. 1990. Estrogen induction of fatty liver in dairy cattle. J. Dairy Sci. 73:1537-1543.

Grummer, R. R. 1993. Etiology of lipid related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820-2833.

Guard, C. 1996. Fresh cow problems are costly: culling hurts the most. Page 100 in Proc. 1994 Annu. Conf. Vet., Cornell Univ., Ithaca, NY.

Hansard, S. L., C. L. Comar, and M. P. Plumlee. 1954. The effects of age upon calcium utilization and maintenance requirements in the bovine. J. Anim. Sci. 13:25-36.

Hayirli, A., R. R. Grummer, E. Nordheim, P. Crump, D. K. Beede, M. J. VandeHaar, and L. H. Kilmer. 1998. A mathematical model for describing dry matter intake of transition cows. J. Dairy Sci. 80(Suppl. 1):296 (Abstr.).

Hayirli, A., and R. R. Grummer. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: a review. Canadian J Anim. Sci. 84:337-347.

Hibbard, B., J. P. Peters, S. T. Chester, J. A. Robinson, S. F. Kotarski, W. J. Croom Jr. and W. M. Hagler Jr. 1995. The effects of slaframine on salivary output and subacute and acute acidosis in growing beef steers. J. Animal Sci. 73:516-525.

Horst, R. L., H. F. DeLuca, and N. A. Jorgensen. 1978. The effect of age on calcium absorption and accumulation of 1,25-dihydroxyvitamin D3 in intestinal mucosa of rats. Metabolic Bone Disease and Related Research. 1:29– 33.

Horst, R. L. and T. A. Reinhardt. 1983. Vitamin D. Metabolism in ruminants and its relevance to the periparturient cow. J. Dairy Sci. 66:661.

Horst, R. L., J. P. Goff, and T. A. Reinhardt. 1994. Calcium and vitamin D metabolism in the dairy cow. J. Dairy Sci. 77:1936-19.

Horst, R. L., J. P. Goff, and T. A. Reinhardt. 1990. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. J. Endocrinol. 126:1053-1057.

Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1994. Calcium and vitamin D metabolism in the dairy cow. J. Dairy Sci. 77:1936-1951.

Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1997. Strategies for preventing milk fever in dairy cattle. J. Dairy Sci. 80:1269-1280.

House, W. A. and A. W. Bell. 1993. Mineral accretion in the fetus and adnexa during late gestation in Holstein cows. J. Dairy Sci. 73:2999-3010.

Hibbs, J. W. 1950. Milk fever (parturient paresis) in dairy cows: a review. J. Dairy Sci. 33:758.

Hibbs, J. W., and H. R. Conrad. 1983. The relationship of calcium and phosphorus intake and digestion and the effects of vitamin D feeding on the utilization of calcium and phosphorus by lactating dairy cows. Pp. 1–23 in Research Bulletin 1150, Ohio State University, Ohio Agr. Res. and Dev. Center, Wooster, Ohio.

Hu, W. P., and M. R. Murphy. 2005. Statistical evaluation of early and mid lactation dairy cow responses to dietary sodium bicarbonate addition. An. Feed Sci. and Tech. 119:43-54.

Ingvartsen, K. L. 2006. Feeding and management related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Animal feed science and technology. 126:175-213.

Ingvartsen, K. L. and J. B. Anderson. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Jardon, P. W. 1995. Using urine pH to monitor anionic salt programs. Food Animal. 860-862.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest. Anim. Endocrinol. 15:309-319.

Joyce, P. W., W. K. Sanchez, and J. P. Goff. 1997. Effects of anionic salts in prepartum diets based on alfalfa. J. Dairy Sci. 80:2866-2875.

Kehrli, M. E., J. P. Goff, J. A. Harp, J. R. Thurston, N. L. Norcross. 1990. Effects of preventing periparturient hypocalcemia in cows by parathyroid hormone administration on hematology, conglutinin, immunoglobulin, and shedding of Staphylococcus aureus in milk. J. Dairy Sci. 73:2103-2111.

Keller, H. F., B. P. Chew, R. E. Erb, and P. V. Malven. 1977. Estrogen dynamics and hormonal differences associated with lactational performance of cows induced to lactate. J. Dairy Sci. 60:1617-1623.

Kennelly, J. J., B. Robinson, G. R. Khorasani. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early lactation Holstein cows. J. Dairy Sci. 82:2486-2496.

Keunen, J.E., J. C. Plaizier, I. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2003. Short communication: effects of subacute ruminal acidosis on free-choice intake of sodium bicarbonate in lactating dairy cows. J. Dairy Sci. 86:954-957.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): a review. Zentralblatt fur Veterinarmedizin – Reihe A. 50(8):406-414.

Krause, K. M., and G. R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds. Anim. Feeding Sci. and Tech. 126(3-4):215-236.

Kunz, P. L., J. W. Blum, I. C. Hart, H. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food intake, performance and blood hormones and metabolites in dairy cows. Anim. Prod. 40:219-231.

Larson, T., G. Moller, and R. Bellio. 2000. Evaluation of clinical and clinical chemical parameters in periparturient cows. J. Dairy Sci. 84:1749-1758.

Leclerc, H. and E. Block. 1989. Effects of reducing dietary cation-anion balance for prepartum dairy cows with specific reference to hypocalcemic parturient paresis. Can. J. Anim. Sci. 69:411.

Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. Lacey Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J. Dairy Sci. 81:585.595.

Martin, S. A., and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75: 1736-1744.

Mashek, D. G., and R. R. Grummer. 2003. Feeding pre-fresh transition cows: should we maximize feed intake or minimize feed intake depression? J. Dairy Sci. 86(Suppl. 1):11 (Abstr.).

Martz, F. A., A. T. Belo, M. F. Weiss, R. L. Belyea, and J. P. Goff. 1990. True absorption of calcium and phosphorus from alfalfa and corn silage when fed to lactating cows. J. Dairy Sci. 73:1288-1295.

Meijer, A. J., W. H. Lamers, and Robert A. F. M. Chamuleau. 1990. Nitrogen metabolism and ornithine cycle function. Physiological Reviews. 70(3): 701-748.

Miranda, R. L. A., M. G. D. Mendoza, J. R. Barcenagama, M. S. S. Gonzalez, R. Ferrara, C. M. E. Ortega, and P. M. A. Cobos. 1996. Effect of Saccharomyces cerevisiae or aspergillus oryzae cultures and NDF level on parameters of ruminal fermentation. Anim. Feed Sci. and Tech. 63(1-4):289-296.

Moore, S. J., M. J. VandeHaar, B. K. Sharma, T. E. Pilbeam, D. K. Beede, H. F. Bucholtz, J. S. Liesman, R. L. Horst, and J. P. Goff. 2000. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. J. Dairy Sci. 83:2095-2104.

Nagaraja, T. G., G. Towne, and A. A. Beharka. 1992. Moderation of ruminal fermentation by ciliated protozoa in cattle fed a high-grain diet. Appl. Environ. Microbiol. 58:2410-2414.

Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. British J. Nutrition. 76:249-261.

Noeck, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005-1028.

NRC. 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. National Academy Press, Washington, DC.

Oetzel, G. R., J. D. Olson, C. R. Curtis and M. J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302.

Oetzel, G. R., M. J. Fettman, D.W. Hamar and J.D. Olson. 1991. Screening of anionic salts for palatability, effects on acid-base status, and urinary calcium excretion in dairy cows. J. Dairy Sci. 74:965.

Oetzel, G. R. and J. A. Barmore. 1993. Intake of a concentrate mixture containing various anionic salts fed to pregnant, nonlactating cows. J. Dairy Sci. 76:1617-1623.

Oetzel, G. R., Vagnoni. 1998. D. B. Effects of dietary cation-anion difference on the acid-base status of dry cows. J. Dairy Sci. 81(6): 1643-1652.

Overton T. R., and M. R. Waldron. 2004. Nutritional Management of transition dairy cows: strategies to optimize metabolic health. J. Dairy Sci. 87(E. Suppl.):E105-E119.

Payne, J. M. 1968. Outlook on milk fever. Outlook Agric. 5:266.

Plata, F., G. D. Mendoza, J. R. Barcenagama, and S. Gonzalez. 1994. Effect of a yeast culture (Saccharomyces cerevisiae) on neutral detergent fiber digestion in steers fed oat straw diets. Anim. Feed Sci. and Tech. 49(3-4):203-210.

Piva, G. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Putnam, D. E., C. G. Schwab, M. T. Socha, N. L. Whitehouse, N. A. Kierstead, and B. D. Garthwaite. 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci. 80:374-384.

Ramberg, C. F., Jr. 1974. Kinetic analysis of calcium metabolism in the cow. Fed. Proc. 33:183– 187.

Rastani, R. R., S. M. Andrew, S. A. Zinn, and C. J. Sniffen. 2001. Body composition and estimated tissue energy balance in Jersey and Holstein cows during early lactation. J. Dairy Sci. 84: 1201-1209.

Riond, Jean-Luc. 2001. Animal nutrition and acid-base balance. Eur. J. Nut. 40:245-254.

Roa, M. L., J. R. Barcenagama, S. Gonzalez, G. Mendoza, M.E. Ortega, and C. Garcia. 1997. Effect of fiber source and a yeast source (Saccharomyces cerevisiae(1026)) on digestion and the environment in the rumen of cattle. Anim. Feed Sci. and Tech. 64(2-4):327-336.

Robinson, P. H. 1997. Effect of yeast culture (Saccharomyces cerevisiae) on adaptation of cows to diets postpartum. J. Dairy Sci. 80(6):1119-1125.

Roth, J. A., and M. L. Kaeberle. 1982. Effects of glucocorticoids on the bovine immune system. JAVMA 180:894-901.

Salway, J. G. and D. K. Granner. 2004. Metabolism at a glance. 3rd ed. Blackwell Publishing, Malden, MA.

SAS Institute. 2006. User's Guide Version 9.1: Statistics. SAS Institute, Cary, NC.

Schukken, Y. H., H. N. Erb, and J. M. Scarlett. 1989. A hospital-based study of the relationship between retained placenta and mastitis in dairy cows. Cornell Vet. 79:319-326.

Shaver, R. D. 1997. Nutritional risk factors in the etiology of left displaced abomasum in dairy cows: a review. J. Dairy Sci. 80:2449-2453.

Shriver, B. J., W. H. Hoover, J. P. Sargent, R. J. Crawford, Jr., and W. V. Thayne. 1986. Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. J. Dairy Sci. 69(2): 413-419.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 73:1531-1553.

Soder, K. J., and L. A. Holden. 1999. Dry matter intake and milk yield and composition of cows fed yeast prepartum and postpartum. J. Dairy Sci. 82:605-610.

Tucker, W. B., Z. Xin, R. W. Hemken. 1991. Influence of calcium chloride on systemic acid base status and calcium metabolism in dairy heifers. J. Dairy Sci. 74:1401-1407.

Van Soest, P. J., J. B. Robertson, B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van't Klooster, A. T. 1976. Adaptation of calcium absorption from the small intestine of dairy cows to changes in the dietary calcium intake and at the onset of lactation. Zeitschrift fuer Tierphysiology. 37:169– 182.

Vermunt, J. J., and P. R. Greenough. 1996. Sole haemorrhages in dairy heifers managed under different underfoot and environmental conditions. Br. Vet. J. 152:57–73.

Visek, W. J., R. A. Monroe, E. W. Swanson, and C. L. Comar. 1953. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutr. 50:23– 33.

Wang, C. and D.K. Beede. 1992 Effects of ammonium chloride and sulfate on acid-base status and calcium metabolism of dry Jersey cows. J. Dairy Sci. 75:820.

Wang, Z., M. Eastridge, and X. Qui. 2001. Effects of forage neutral detergent fiber and yeast culture on performance of cows during early lactation. J. Dairy Sci. 84(1):204-212.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Bowman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495.

Williams, P. E. V., C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae,* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumens of steers. J. Anim. Sci. 69:3016-3026.

Yoon, I. K., and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. Austral. Asian J. Anim. Sci. 8:533– 555.

VITA

Reagan J. Vogel was born August 29, 1981, in Sedalia, Missouri to Ray Vogel and Tutti Howard. Reagan is the youngest of three daughters, siblings are Cindy Rippie and Rachel Stewart. She attended LaMonte High School where she was senior class president in 1999. Reagan entered the University of Missouri-Columbia as a self labeled "pre-vet" student, and quickly realized her passion towards improving the health of animals through nutrition, rather then treating the ailed. Therefore, she sought out and completed her B.S. in Animal Science in May 2003. During her undergraduate education, she was actively involved as the president of the University YMCA and Habitat for Humanity.

On October 14, 2006 Reagan was wed to her friend, Neal I. Bluel.