DIETARY CATION ANION DIFFERENCE AND ACIDIFIED COPRODUCTS: EFFECTS ON PERIPARTUM DAIRY COWS

by

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Abstract

The transition from gestation to lactation requires numerous physiological and metabolic adaptations in order for the body to maintain relative homeostasis. For the modern dairy cow, the difficulty to meet these challenges is increased many-fold due to the large demand for energy and metabolites placed on the body by the high producing mammary gland. Milk fever or periparturient hypocalcemia can be defined as a failure of the calcium homeostatic mechanisms to maintain serum calcium around the time of calving. Though clinical cases may only arise in ≈ 5% of transition cows, subclinical rates are much higher. Animals suffering from even subclinical milk fever are much more susceptible to numerous other transition disorders. Preventing milk fever by formulation of the prepartum ration may be accomplished by decreasing the dietary cation anion difference (DCAD) which can be defined as the balance between positively and negatively charged ions in the diet. An experiment was designed to test 2 diets containing t products designed to deliver supplementary anions to the diet versus a control ration with no added anions. Total serum calcium and incidence of postpartum health disorders were not affected by prepartum dietary treatment. Though DCAD was drastically different between the control ration and the 2 anionic diets, the concentration of the strong cation potassium was low across all treatments which presumably prevented hypocalcemia with the onset of lactation. Though our diets contained low concentrations of potassium, many diets used by dairymen contain forages that are high in potassium and thus might benefit from the addition of anions.

An experiment of an unrelated nature was conducted to observe the effects of 2 diets containing wet corn gluten feed (46 or 56% of DM) as the primary energy substrate and tallgrass prairie hay (14 or 20% of DM) as the sole source of physically effective fiber versus a control ration containing alfalfa and corn silage. The 20% tallgrass prairie hay diet resulted in milk components and efficiencies similar to those of the control ration, but production and income over feed cost did not match that of the control ration in this situation.

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Dedication

I dedicate this work to the man that is my hero, my best friend, my #1 fan, and my Dad. You will never know how much I am truly grateful for all the sacrifices you have made and continue to make for me. You are the example that any father should strive to model himself after. Your love and guidance made me the person I am today; it's time for you to take a break, crack open a cold one, and let me do the work for a while.

CHAPTER 1 - Literature Review

Introduction

The transition period demands of the modern dairy cow enormous and in some cases insurmountable physiological adaptations. Energy and metabolite demands multiply with the onset of lactation and the cow's inability to immediately meet these needs can cause an array of metabolic diseases, most of which are highly correlated with one another. Most high producing multiparous dairy cows will experience some degree of hypocalcemia around the time of calving, but only clinical cases are typically detected. Unfortunately, ramifications of sub-clinical periparturient hypocalcemia may reach far into the lactation of affected cows because of the possibility of developing other metabolic diseases (Charbonneau et al., 2006); because subclinical hypocalcemia is much more common than clinical periparturient hypocalcemia (milk fever) it may be just as detrimental to herd health and performance (Horst et al., 1994). Animals suffering from milk fever are highly susceptible to other transition disorders such as retained placenta, metritis, mastitis, ketosis, and displaced abomasum (Curtis et al., 1983; Curtis et al., 1985). In addition, milk production may suffer long after the transition period has passed (Block, 1984).

Prevention of hypocalcemia in the transition dairy cow has been the subject of much research. Traditional methods used to prevent hypocalcemia have limited calcium intake during the dry period by formulating low calcium diets. Although effective at reducing the severity and incidence of milk fever, this strategy proves difficult when using typical feedstuffs found on dairies for transition cow nutrition. Another well proven and more feasible option is lowering the dietary cation-anion difference (DCAD; meq [(Na + K) - (Cl +S)]/100 g DM) in the diet during the last 21 days before expected calving. Although it is possible to achieve this goal without using feed additives, many types of forage have to be limited or excluded because of their high potassium content as a result of fertilization. To achieve a negative DCAD balance, anionic salts [$MgSO_4$, $MgCl_2$, NH_4Cl, $(NH_4)_2SO_4$, $CaCl_2$, and $CaSO_4$] have been incorporated in close-up rations. By feeding prepartum diets with a negative DCAD, calcium homeostasis is improved at calving and the onset of lactation thereby decreasing incidence rates of milk fever (Horst et al., 1994; Goff and Horst, 2003; Overton and Waldron, 2004)

Anionic salts, while improving postpartum calcium status when fed in the close up period, decrease feed intake at a time when energy status of the animal is key (Oetzel and Barmore, 1993). The need for a more palatable supplement that still has the ability to decrease DCAD in the diet led to the creation of acidified coproducts (**ACP**). ACP are either treated with hydrochloric acid to add chloride ions or contain the additional anions as a result of their processing. All, however, are marketed as having less negative effects on feed intake compared to feeding anionic salts.

Etiology of Hypocalcemia and Parturient Paresis

Hypocalcemia in the periparturient dairy cow is caused by a failure of the homeostatic mechanisms that are in place to regulate serum calcium to normal physiological levels when faced with the onset of lactation (Horst et al., 1994). This condition is commonly referred to as milk fever or periparturient paresis in cases where the degree of hypocalcemia is severe enough to cause paralysis. Production of colostrum requires an enormous amount of calcium; if a cow produces 10 L of colostrum she will lose approximately 23 g of calcium in a single milking, which is an amount 9 times greater than the entire amount of plasma calcium present in the cow (Horst et al., 1997). During the dry period calcium requirements are minimal; fetal and fecal drains are only 10 to 12 g of calcium/d and the mechanisms in place to replace calcium lost from the plasma pool are relatively inactive (Ramberg et al., 1984). Due to the overwhelming demands that are placed on the cow at the time of calving, nearly all high producing dairy cows will undergo some degree of hypocalcemia within the first two days of parturition and subsequent onset of lactation (Ramberg et al., 1984). The earliest known description of milk fever was published in agricultural literature by Eberhardt in 1793, and early treatments for the condition included drenching with compounds such as alum, niter, cream of tartar, strong beer, and even bleeding of cows to remove as much as 10 pints of blood (Murray et al., 2008). The first effective treatment was suggested by Schmidt in 1897. He theorized that a viral infection in the udder was the causative factor of milk fever and prescribed a potassium iodide injection into udder via the teat canal (Hibbs, 1950). This method proved effective, of course, because it ceased lactation, thereby removing the calcium stress from the animal. Later others would discover that inflation of the udder with air or oxygen yielded similar results (Hibbs, 1950). Little (1932) observed that cows with clinical signs of milk fever were deficient of serum calcium by as much

as 50% relative to normal levels and that the severity of symptoms seemed to correlate with increased deficiency. As a result of these observations, milk fever (now recognized as hypocalcemia) was treated with infusions of calcium borogluconate, calcium chloride, and calcium gluconate; calcium borogluconate is still utilized as an effective treatment today (Murray et al., 2008). The correct pathogenesis for milk fever had yet to be elucidated, but veterinarians concluded that the hypocalcemic state of the animals was caused by a deficiency in bioavailable dietary calcium and prescribed dietary supplementation of large amounts of calcium in late gestation (Murray et al., 2008). This theory, though intuitive, would prove to be incorrect as research progressed and showed that increased dietary calcium in late gestation actually increased milk fever incidence as described below. Greig (1935) was the first to implicate lack of parathyroid gland function as a possible cause for the condition. Parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are both key constituents of calcium regulation (Horst et al., 1994). Many endocrine disorders are caused by lack or excess hormone production, and it was thought that a deficiency in PTH or $1,25\text{-}(OH)_2D$ was the primary reason for the hypocalcemia observed (Horst et al., 1994). Normal levels of these hormones in hypocalcemic animals suggests otherwise, however (Mayer et al., 1969; Horst et al., 1979). Further investigation into $1,25\text{-}(OH)_2D$ levels in hypocalcemic animals found cows that relapsed after treatment for hypocalcemia had lower levels of $1,25\text{-}(OH)_2D$ before and during the development of milk fever but had similar levels of PTH (Goff et al., 1989). Additionally it was observed that the relapsing cows took 24-48 hours longer produce the levels of $1,25\text{-}(OH)_2D$ required to induce a recovery.

PTH stimulates the activation of the enzyme 1α-hydroxylase in the kidney, this enzyme converts 25-hydoxycholecalciferol ($25\text{-}(OH)D_3$) into 1α,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$), the active form of vitamin D that stimulates increased dietary calcium absorption and bone resorption (Murray et al., 2008). The current theory for the pathogenesis of milk fever rests on the hypothesis that bone and kidney tissue responses to PTH are blunted in postpartum cows and that metabolic alkalosis is responsible for this unresponsiveness (Goff and Horst, 2003).

Normal physiological levels of plasma calcium range from 8.5 to 11.5 mg/dL and a normal decrease in plasma calcium of 2 mg/dL (ex. 10 to 8 mg/dL) is expected at calving (Niedermeier et al., 1949). Hypocalcemia may be classified as subclinical (7.5 to 8.5 mg/dL) or clinical (5.0 to 6.0 mg/dL) depending on the respective total serum calcium levels or by observed

symptoms (Jorgensen, 1974). Animals suffering from clinical hypocalcemia exhibit symptoms such as hypophagia, tetany, inhibition of urination and defecation, lateral recumbency, and eventual coma and death if their condition goes untreated (Horst et al., 1997).

The correlation between periparturient diseases has been well recognized and demonstrated (Erb et al., 1981; Dohoo and Martin, 1984; Curtis et al., 1985; Grohn et al., 1989). For instance, a multiparous cow suffering from parturient paresis is 7.2 times more likely to suffer from vet assisted dystocia, 4 times more likely to have a retained placenta, 23.6 times more likely to suffer from complicated ketosis, and 5.4 times more likely to contract mastitis as a cow without clinical hypocalcemia (Curtis et al., 1985). Some of these correlations are clear and some are not; for example the cows suffering from parturient paresis may lack the plasma calcium required for the smooth muscle contractions that expel the placenta, but the connection between parturient paresis and mastitis is less clear. Ducusin et al. (2003) demonstrated that an in vitro decrease in extracellular calcium concentrations is accompanied by a decrease in phagocytosis by polymorphonuclear leukocytes (PMNs) as well as a decrease of intracellular calcium concentration within the PMNs. Likewise, they observed that in blood samples obtained from cows diagnosed with parturient hypocalcemia PMNs had lower intracellular calcium concentrations accompanied by a decrease in phagocytosis by the PMNs as compared to clinically sound postpartum cows. Because phagocytosis by PMNs is a key host defense mechanism against pathogens, these findings may explain why cows that are diagnosed with milk fever are more susceptible to infectious conditions such as metritis and mastitis.

Estimates for incidence rates of clinical parturient paresis range from 6.6% (Curtis et al., 1985) to 5.3% (Grohn et al., 1989) in cows with > 2 lactations. Horst et al. (1997) also points out that milk fever is economically detrimental; it can reduce the productive life of a dairy cow by 3.4 years with an average cost of each milk fever case being estimated at $334. This figure only takes into account the cost of treatment of milk fever and the revenue lost due to decreased production; with the increased chance of succumbing to another disorder as demonstrated above, this cost could increase dramatically (Horst et al., 1997).

Predisposing Factors for Milk Fever

Milk fever does not strike randomly and without bias. There are several factors that can predispose a cow to being affected by milk fever including age, diet, and even breed. These

factors can act alone or in conjunction with one another to increase the severity of milk fever of the affected animal. Aging results in milk production increases which inherently increases calcium demand. Epidemiology studies agree that incidence of milk fever strongly increases with lactation number / age (Curtis et al., 1985; Grohn et al., 1989; Dohoo and Wayne Martin, 1984). In addition to increases in milk production, aging also results in a decrease in the ability to reabsorb calcium from bone, a decrease in the active transport of calcium across the gut wall, and impaired synthesis of the important steroid hormone $1,25(OH)_2D_3$ (Horst et al., 1978). All of these factors help explain why animals of an increased age may have an impaired ability to respond to increased calcium needs. Increasing age has also been shown to decrease the number of intestinal $1,25(OH)_2D_3$ receptors (Horst et al., 1990; Johnson et al., 1995). Rationale for the negative effects associated with the decrease of $1,25(OH)_2D_3$ receptors in the gut is explained below.

Cows of certain breeds have been found to be more vulnerable to milk fever; Channel Island, Swedish Red and White, and Jersey breeds in particular (Horst et al., 1997). Though this observation is quite widely accepted, the underlying reason has yet to be completely elucidated. Limited research has shown that the concentration of $1,25(OH)_2D_3$ receptors in the gut is lower in Jersey cattle compared to Holstein cattle of similar age and point of gestation (Goff et al., 1995). With the onset of lactation and the subsequent decrease in plasma concentration of calcium as a result of colostrum synthesis, $1,25(OH)_2D_3$ synthesis increases in order to enhance bone resorption and intestinal absorption. However, a decreased number of receptors for the hormone to bind to could result in impaired function in a time of calcium stress (Horst et al., 1997).

Several prepartum dietary factors have been linked to increased risk of peripartum hypocalcemia; most notably, feeding high levels of calcium and feeding alkalogenic diets (diets higher in cation concentration relative to anions). Jorgensen (1974) observed that cows with high calcium intake were at an increased risk of developing parturient paresis as compared to cows consuming less calcium. This agrees with others (Boda, 1956; Goings et al., 1974; Green et al., 1981; Kichura et al., 1982). Curtis et al. (1984) did not observe a connection between dietary calcium concentration and incidence rates of milk fever; however, they noted that a majority of the cows in the data set were being fed high levels of calcium prepartum (200 – 500% of NRC requirements) and that this meant the power of calcium to contribute to the

discrimination analysis was decreased or completely masked. Goff and Horst (1997a) observed that dietary calcium alone had no effect on plasma calcium levels at the time of calving, but cows fed higher levels of dietary potassium (2.1% and 3.1%) had significantly decreased levels of plasma calcium peripartum. They also showed an interaction between calcium and potassium. The group fed high levels of calcium and high levels of potassium had a low incidence of milk fever as compared to the low calcium and high potassium group. These results would suggest that calcium level does not act alone as a dietary factor to increase the incidence of milk fever or as a causative factor at all in certain diets.

A 500-kg cow in late gestation requires approximately 31g/d of calcium to meet her maintenance and fetal development requirements; these demands can be met with passive absorption of dietary calcium across the gut wall (Horst et al., 1994). This means that the acquisition and conservatory homeostatic mechanisms (bone resorption, active gut uptake, and renal reabsorption) may be inactive during this time if >100 g of Ca/d is fed (Ramberg et al., 1984; Horst et al., 1994). Feeding a diet that is low in calcium (≤ 20 g/d) forces the cow to activate her homeostatic mechanisms to meet calcium requirements (Horst et al., 1994). By prematurely activating these mechanisms the cow is prepared to combat the large calcium sink that occurs with the onset lactation and thus is able to maintain normal physiological levels of plasma calcium (Goings et al., 1974; Green et al., 1981). This strategy is not easy to achieve with the use of many forages typically utilized on the dairy farm, including alfalfa, as they contain high amounts of calcium as compared to forages such as grass hays. These feedstuffs are also high in other minerals, such as the strong cation potassium, that have been implicated as causative factors for milk fever.

The DCAD Theory

Ender et al (1971) were the first to suggest that the alkalo-acid property of the diet and not the calcium content was the principal factor for predisposing animals to milk fever. They observed in multiple experiments that milk fever could be induced by feeding diets alkalogenic in nature, and, in contrast, milk fever could be prevented by feeding diets treated with mineral acids, regardless of calcium content. Early research involving acidifying the diet as a way to alter calcium metabolism in the body took place in rat models (Barzel and Jowsey, 1969; Newell and

Beauchene, 1975). It was concluded that acidification of the diet using hydrochloric or sulfuric acids is effective for promoting bone mobilization, however, acids can present a danger to the user and the animals if not correctly handled and implemented, and would not be a plausible tool for dairy producers (Block, 1984;Oetzel et al., 1988). It was instead proposed by Block (1984) to increase the concentration of acidogenic minerals (anions) in the diet in relationship to the alkalogenic ions (cations) as opposed to supplementing acid directly to the ration. He concentrated on the balance of four minerals in the diet: sodium and potassium, strong cations, and chloride and sulfur, strong anions. A number was derived from the milliequivallent (meq) values of the respective minerals using the equation [(Na + K) – (Cl + S)] / 100 g of dietary DM to use as a basis for evaluating DCAD. To calculate meq/100 g of DM you must first ascertain the milligrams of the mineral of interest in the diet and multiply it by the valence number that mineral carries (sodium, potassium, and chloride = 1, sulfur = 2). This number is divided by the respective atomic weight of the mineral of interest (sodium = 23 g, potassium =39 g, chloride =35.5 g, and sulfur = 32 g) and the resulting quotient is milliequivallents / 100 g DM. In order to formulate a diet with an excess of anions $CaCl_2$, $Al_2(SO_4)_3$ and $MgS0_4$ were added to the ration. Over a 2-year switchback design, Block (1984) observed a 47.4% overall incidence of milk fever in cows being fed a prepartum diet with a DCAD of +33.05 meq/100 g DM and a 0% incidence of milk fever in cows being fed a prepartum diet with a DCAD of -12.85 meq/100 g DM. Moore et al. (2000) reported that ionized serum calcium levels of 4.35 mg/dL on the day of calving for cows fed prepartum diets with a -15 meq/100 g DM DCAD, whereas fed diets with a 0 meq/100 g DM and a +15 meq/100 g DM DCAD were 3.85 and 3.67 mg/dL, respectively. Ionized calcium levels of < 4 mg/dL are considered atypical and hypocalcemic. Moore et al. (Moore et al., 2000) also concluded that feeding of anionic salts to heifers was fruitless because milk fever is negligible in heifers and anionic diets may actually be harmful because of negative effects on energy balance. Oetzel et al. (1988) achieved similar results using ammonium salts [NH_4Cl and $(NH_4)_2SO_4$] to decrease DCAD; serum ionized calcium values were greater on the day of calving for cows receiving the negative DCAD diet (-7.5 meq/ 100 g DM) versus the positive DCAD diet (+18.9 meq/100 g DM). Furthermore, a high (105 g Ca/d) or low level (53 g Ca/d) of calcium was fed along with the positive or negative DCAD, and it was observed that when calcium was high and DCAD was negative, the odds ratio of cows developing hypocalcemia was decreased 10 fold, but there was no benefit for low calcium and negative

DCAD (Oetzel et al., 1988). These results suggest that if a negative DCAD diet is to be fed prepartum then it is advantageous to formulate the ration so that calcium intake is high (100~ g/d). It is important to note that the low calcium levels that were fed were still higher than the levels of calcium intake (<20 g/d) reported to decrease the incidence of milk fever by Green (1981). Dissention remained as to the level of calcium to be fed with a decreased DCAD. Lean (2006) criticized Oetzel (2000) for recommending a calcium intake of 150 g/d (1.1 to 1.5% of DM) for diets of approximately -15 meq/ 100 g DM DCAD, citing that his meta-analysis (Oetzel, 1991) showed the highest incidence of milk fever occurred at a calcium level of 1.16% DM. Current research shows that the optimal level of calcium to be fed is dependent on the DCAD of the ration (Oba et al., 2009) and would agree with Oetzel et al. (1988). Oba et al. (2009) observed that feeding a high calcium diet with a low DCAD decreased recovery time to an EDTA challenge compared to a low calcium, low DCAD combination. Inversely, low calcium, high DCAD diet decreased the recovery time to the EDTA challenge compared to high calcium, high DCAD.

Contrary to some findings (Horst et al., 1994; Oetzel and Barmore, 1993) addition of anionic salts did not have a significant negative impact on prepartum (Block, 1984; Oetzel et al., 1988; Moore et al., 2000) DMI in some studies. Addition of anionic salts to the diet of pregnant nonlactating cows to achieve a DCAD of -6.3 to -4.0 meq/100 g DM has decreased DMI compared to a control diet (DCAD 20.3 meq/ 100 g DM, Vagnoni and Oetzel, 1998). The variability between these studies may be explained by the variability of DMI observed in peripartum animals. Studies that show a significant decrease in DMI for anion supplemented diets (Oetzel and Barmore, 1993; Vagnoni and Oetzel, 1998) were conducted in pregnant nonlactating animals rather than peripartum cows whose intakes are measured relative to day of parturition. DMI deceases by 30% 1 or 2 days before parturition (Goff and Horst, 1997b); this reduction can be variable from cow to cow and thus contributes to larger standard errors in the measurements of DMI for close-up animals. Intake of pregnant nonlactating animals would theoretically have less variability than close-up animals and therefore lower standard errors which allow for clearer detection of significance. Though DMI seems to be variable between these studies it is widely agreed upon that anionic salts used to decrease DCAD are unpalatable to cattle if inclusion rate is too high regardless of actual DCAD. In general, inclusion rates of anionic salts should be kept below or equal to 2 equivalents/ day to avoid potential toxic effects

(Oetzel et al., 1991). Complete anion supplementation from ammonium salts is generally not good practice; rather a mix of sulfate and ammonium salts is preferred (Horst et al., 1994).

Monitoring of the efficacy of DCAD modifications is best done on farm with the use of urine pH testing. Urine pH of Holstein cows should be between 6.2 and 6.8 and between 5.8 and 6.3 in Jersey cows for maximum effect on milk fever incidence (Goff and Horst, 2003). A urine pH of >7 is not associated with effective maintenance of serum calcium when anion supplementation is used (Moore et al., 2000). A urine pH of < 5.5 would dictate excessive anion supplementation and that an uncompensated metabolic acidosis has been inflicted on the animal which can be detrimental to DMI (Goff and Horst, 2003).

The expected result of decreasing DCAD is a mild, compensated metabolic acidosis (Oetzel, 1991; Goff and Horst, 1997a; Charbonneau et al., 2006; Kurosaki, N. , Yamato O., 2007) which is accompanied by decreased plasma bicarbonate, decreased urinary pH and higher urinary net acid excretion (Charbonneau et al., 2006). The Strong Ion Difference (SID) theory explains how the concentration of cations and anions in the diet can affect the acid-base balance of the body. For any aqueous solution, such as plasma, the SID = Σ (Strong Cations) – Σ (Strong Anions). Strong Ions that are present in diet are absorbed in the digestive tract and then released into the blood and thus are the primary effectors of the blood SID (Goff and Horst, 2003). The SID theory as proposed by Stewart (1983) states that two equations must remain satisfied simultaneously in an aqueous solution: 1.) # Moles cations = # Moles anions, and 2.) $[H^+]$ x $[OH^-] = 1 \times 10^{-14}$. This explains why pH is can be dictated by the number of cations or anions in the solution, since pH is the negative log of $[H^+]$ and $[H^+]$ in a solution must be changed according to the ions present in the solution in order for electrical neutrality to remain. For example, if chloride anions were added to a solution, $[H^+]$ would increase and $[OH^-]$ would decrease in order to maintain electrical neutrality of the solution. As a result of the increase in $[H^+]$, the pH of the solution would decrease. Inversely, if potassium cations were added to a solution, $[H^+]$ would decrease and $[OH^-]$ would increase to retain electroneutrality, and the pH would increase as result of the decrease in $[H^+]$. Supplementation of chloride in the diet to decrease DCAD essentially causes hyperchloremic acidosis by decreasing the SID in the plasma pool.

Mild Metabolic Acidosis and its Effects on Calcium Metabolism

Research has focused on altering acid-base status as a means to influence calcium metabolism with a number of objectives, including diminishing the effects of osteoporosis in humans, preventing hypocalcemia in dairy cows, and even promoting antler growth for deer. Calcium balance is affected by a plethora of factors including urinary calcium excretion, gastrointestinal absorption, and calcium mobilization from bone (Beck and Webster, 1976). It has been well documented from both in vitro and in vivo research that a decrease in pH activates osteoclasts, which results in a net loss of calcium from bone (Frings-Meuthen et al., 2008).

When blood pH is approximately 7.35, PTH and its receptor (located on the surface of bone and tissue cells) interact efficiently and PTH fulfills its role to stimulate the target cells (Goff and Horst, 2003). However when blood pH becomes more alkaline, the PTH receptor undergoes a conformational change that impairs its interaction with PTH (Bushinsky, 2001). Beck and Webster (1976) found that the baseline levels of both total and ionized serum calcium for thyroparathyroidectomized rats in acidotic, neutral, and alkalotic states were not different. However, when PTH was injected, total and ionized serum calcium concentrations were increased for both the acidotic and neutral rats, but not alkalotic rats. Serum calcium in acidotic rats, however, was greater than neutral suggesting that the effects of PTH are diminished in cases of metabolic alkalosis and that it would be advantageous to inflict mild metabolic acidosis when increased serum or ionized calcium would be beneficial.

The question as to the specific source of the calcium increase was still not clearly answered however. PTH acts on all three sources for control of calcium metabolism: absorption from the gut via changes in activation of Vitamin D, absorption of calcium in the distal tubules of the kidney, and increased osteolysis and osteoblast-initiated recruitment of osteoclasts (Reece, 2009). In fact, acidosis has been found to decrease the absolute tubular reabsorption rate of calcium by the kidney and increase the amount excreted in the urine.(Beck and Webster, 1976) Frings-Meuthen et al. (2008) observed that decreased blood pH was accompanied by an increase in urinary calcium excretion as well as an increase in C-and N-terminal telopeptide of type I collagen (CTX, NTX). Both CTX and NTX are markers for bone resorption. The increase in urinary calcium excretion may be related to the increase in serum calcium. Another explanation

may be that the decreased pH directly decreases tubular resorption but still increases bone resorption and gut absorption.

More recently, in vitro experiments have found that inducing metabolic acidosis can act independently of PTH to increase bone resorption (Frick and Bushinsky, 2003). This action is facilitated by an increased expression of RANKL, a ligand on the surface of osteoblasts that interacts with RANK receptors on the surface of osteoclasts. The RANK/RANKL interaction initiates a differentiation cascade which results in maturation of osteoclasts, an increase in bone-resorbing activity of the mature osteoclasts, and a decrease in osteoclastic apoptosis. Administration of indomethacin, a cyclo-oxygenase 1 &2 inhibitor, reversed the acid induced increase in RANKL expression as well as the increase in calcium efflux. These results suggest that the increase in prostaglandin E_2 synthesis from osteoblasts that is also associated with acidemia is the causative factor for an increase in RANKL expression (Frick and Bushinsky, 2003).

Apparent calcium absorption from the gut has been found to increase, decrease, or remain unchanged in cows fed acidogenic diets; however, apparent Ca absorption is hard to interpret because the intestine is a principal site of calcium excretion (Vagnoni and Oetzel, 1998). In a rat model, utilizing labeled calcium as a marker for intestinal Ca absorption, intestinal calcium absorption did not increase in the case of mild metabolic acidosis (Gafter et al., 1980).

Collectively, these data indicate that a mild metabolic acidosis can increase serum calcium levels and that this increase is apportioned between bone resorption (either dependent or independent of PTH) and an increase in uptake of dietary calcium. However, gut absorption may be dependent on dietary calcium concentration. In the case of osteoporosis, the effect of removing calcium from bone would be extremely detrimental, but in the dairy cow, increasing the labile calcium pool in the periparturient period could be could be advantageous for preventing the onset of periparturient hypocalcemia.

Regulation of Blood pH

Blood pH regulation is one of the principal roles of acid-base homeostasis (Vagnoni and Oetzel, 1998). The high efficacy of the systems in place to ensure stable and neutral blood pH (7.35-7.45) renders it difficult and detrimental to alter the pH in order to achieve desired effects. The body compensates for the change in concentration of hydrogen ions in many ways. Heisey

and Adams (2002) explain the complex relationship of compensatory mechanisms that are activated when pH is lowered. The system with the most rapid response is the chain of reactions that occur in the lung. Alveolar ventilation can increase quickly in response to decreased arterial pH; this decreases the partial pressure of CO_2 and increases the [HCO_3^-] and pH, but does not fully compensate for the decreased pH. This is the primary compensatory mechanism in place to account for acute respiratory acidosis; however chronic respiratory acidosis must be compensated for by mechanisms in the renal system, which is also the system that the body relies on to alleviate metabolic acidosis. The renal system uses its ability to regulate the plasma HCO_3^- concentration in order to counteract the condition of metabolic acidemia. In cases of acidosis the kidney retains bicarbonate in order to increase the buffering ability in the plasma by increasing plasma [HCO_3^-].

Acidified Coproducts

Data reporting reductions in DMI when anionic salts were fed (Horst et al., 1994; Oetzel and Barmore, 1993) spurred commercial interest in an alternative means of decreasing DCAD. Two such commercially available products are Bio-Chlor® (Church & Dwight Co. Inc, Princeton, NJ) and SoyChlor® (West Central Cooperative, Ralston, IA). Both are marketed primarily as palatable anion sources, but Bio-Chlor is also promoted as a protein supplement. Ingredients in Bio-Chlor include dried corn fermentation solubles, processed grain by-products from monosodium glutamate production, and natural and artificial flavors, whereas SoyChlor primarily contains SoyPLUS (high bypass soybean meal), dried distillers grains without solubles, beet pulp, and chloride added as hydrochloric acid and calcium chloride.

There is no published data to date that compares the effects of these two ACP supplements; however, some research has compared them to anionic salts. Vagnoni and Oetzel (1998) compared diets containing Bio-Chlor, $MgSO_4 \cdot 7H_2O + NH_4Cl$, or $MgSO_4 \cdot 7H_2O + CaCl_2 \cdot 2H_2O + CaSO_4$ to decrease DCAD to -5.1, -4.0, and -6.3 meq/ 100 g DM, respectively versus a control ration with a DCAD of 20.3 meq/ 100 g DM. Inclusion of anions reduced DMI compared the control ration, but DMI of the diet containing Bio-Chlor was the lowest. They reasoned that the decrease in DMI may be correlated with the degree of metabolic acidosis inflicted; urine pH values were 8.23, 6.37, 6.89, and 7.20 for Control, Bio-Chlor, $MgSO_4 \cdot 7H_2O + NH_4Cl$, and $MgSO_4 \cdot 7H_2O + CaCl_2 \cdot 2H_2O + CaSO_4$, respectively. Vagnoni and Oetzel (1998)

concluded that Bio-Chlor was the most efficacious of the anion sources examined for inducing a compensated metabolic acidosis.

Ramos-Nieves et al. (2009) investigated the effects of prepartum diets low in potassium with or without the inclusion of supplemental anions provided by SoyChlor. Urine pH was lower for cows fed the diet containing SoyChlor than for the control diet (6.7 versus 8.2), signifying that the DCAD of -15 meq/100 g DM for the SoyChlor diet was more effective at inducing a mild compensated metabolic acidosis than the control diet with a DCAD of +11 meq/ 100 g DM. No differences were detected for the incidence rates of hypocalcemia (clinical or subclinical) as defined by serum calcium levels. Total plasma calcium during the first 24 hours postpartum tended to be higher for cows fed SoyChlor suggesting a more rapid response to calcium demand. DMI for the last 21 days prepartum was lower for the low DCAD diet containing SoyChlor, but no negative effects on health or energy balance postpartum were observed.

Though these studies both show decreased intake associated with ACP, additional investigation is required to ascertain whether the palatability of these supplements or the mild metabolic acidosis induced by the low DCAD causes the depression of intake. Urine pH values for cows supplemented with ACP show that the level of acidification shown to positively affect calcium metabolism is possible with their use.

Conclusion

The transition period for the dairy cow has become an area of intense research as many have realized the potential for increased performance throughout the lactation and productive life of the cow if she is able to make the transition to lactation without difficulty. The obscure nature of hypocalcemia (aside from clinical cases) and the high correlation of milk fever with multiple other transition disorders renders prevention a critical control point in the dry cow program of any dairy. Though low calcium diets are effective in preparing the cow's system to combat the increased demand placed on her by colostrum synthesis, formulating diets to levels low enough to ensure efficacy is extremely challenging. Decreasing DCAD to induce a mild compensated metabolic acidosis that increases bone and renal tissue sensitivity to PTH offers a plausible strategy for maintaining serum calcium levels peripartum. Reducing DCAD with the use of

anionic salts is indeed efficacious in doing so; however intake of these rations has been shown by some to be compromised. Commercially available ACP are also effective at decreasing DCAD, but claims of improved palatability and intake are yet to be consistently proven. Monitoring a DCAD program with the use of urine pH measurements seems to be the best way to ascertain whether or not proper level of acidification is being achieved.

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CHAPTER 2 - Effects of acidified coproducts and prepartum DCAD on serum calcium, postpartum health and performance when fed to prepartum transition dairy cows.

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Abstract

Two products designed to deliver supplemental anions were evaluated for their effects on total serum calcium, post-partum health events, DMI, and performance of transition dairy cows relative to a control diet that did not contain supplemental anions. Diets differed in dietary cation-anion difference (DCAD) and anion source. Treatments were diets including BIO-CHLOR® (BC, DCAD +2.5 meq/100 g DM; n=14), SoyChlor® (SC, DCAD -0.2 meq/100 g DM; n=15), and control (CON, DCAD +18.8 meq/100 g DM; n=13**)**. Treatments began 21 d prior to expected calving and continued through parturition; upon calving, all animals received the same diet. Milk yield was measured through 21 days in milk and milk samples were collected daily between 5 and 21 days in milk. Data were analyzed using mixed models with repeated measures. Prepartum DMI was 9.0, 8.5, and 7.5 kg/d for CON, BC, and SC treatments, respectively. Prepartum intake tended to be lower for SC than CON ($P = 0.09$), but postpartum intake and milk yield were similar among treatments. Milk protein, lactose, and urea nitrogen concentrations were highest for SC and lowest for BC with CON being intermediate ($P < 0.05$). Postpartum plasma glucose tended to be greater for cows fed CON vs. the anion supplemented diets ($P = 0.08$; 67, 57, and 64 mg/dL for CON, BC and SC, respectively). Serum calcium concentrations did not differ among dietary treatments and only tended to be different ($P = 0.07$) over time; values were not indicative of clinical hypocalcemia. With limited sample size, no significant effects of treatment were detected for incidence of postpartum health disorders or plasma BHBA concentration. Although DMI tended to be depressed in the prepartum period by SC, this intake depression was not accompanied by negative effects on performance or health in the postpartum period. Results suggest that cows were not adequately stressed to cause hypocalcemia and/or that DCAD values near 0 were insufficient to improve postpartum health and performance.

Introduction

Decreasing the DCAD of prepartum diets has a positive influence on calcium metabolism (Charbonneau et al., 2006; Block, 1984; Joyce et al., 1997) and can decrease the incidence of milk fever (Block, 1984; Horst et al., 1997; Charbonneau et al., 2006; Lean et al., 2006). However, meta-analysis has also shown that decreasing the DCAD to a level resulting in urine pH < 7.0 can result in a decrease in DMI (Charbonneau et al., 2006). Overton and Waldron (2004) concluded that feeding strategies that support increased energy supply during the close up period are optimal for peripartum metabolic health. A strategy that results in greater DMI depression during the close up period would likely prove detrimental to the health and performance of the cow.

To address suspected palatability issues experienced with anionic salts, products containing supplemental anions were designed with the goal of decreasing DCAD but with more acceptable palatability and thus less negative effects on DMI. Because these products have been formulated using coproducts from the milling and production of various other products they are referred to as acidified coproducts (ACP). Published research comparing the effects of ACP to anionic salts or effects among different ACP in transition cows is limited. However, Vagnoni and Oetzel (1998) observed the lowest DMI for dry cows fed a TMR containing an ACP compared to anionic salts and a control diet. However, net acid excretion was highest and urine pH was lowest for ACP, suggesting relative degree of acidification was greatest. This begs the question as to whether the metabolic acidosis inflicted by a decreased DCAD or palatability of the ration is to blame for the effects on intake observed.

Our objective was to compare rations containing 1 of 2 commercially available ACP versus a control ration without the addition of anions and observe effects on peripartum serum calcium, DMI, postpartum health disorders, and early lactation performance when fed to prepartum dairy cows. We hypothesized that diets containing ACP to would maintain serum calcium levels at calving and positively influence heath and production postpartum.

Methods and Materials

Experimental Design and Treatments

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Twenty-nine multiparous and 16 primiparous pregnant Holstein cows were selected from the Kansas State University Dairy Teaching and Research Unit herd, blocked by parity, and randomly assigned to 1 of 3 treatments beginning 21 d before expected calving. Treatment diets (Table2.1) differed in DCAD and source of anionic supplement and were CON (target DCAD +10 meq/100 g DM; n = 9 cows n = 4 heifers), BC (target DCAD -10 meq/100 g DM; n = 8 cows n = 6 heifers), and SC (target DCAD -10 meq/100 g DM; n = 9 cows n = 6 heifers). Treatment diets were fed for ad libitum intake beginning 21 d prior to expected calving date through parturition. After parturition, all cows received a common lactation TMR (Table 1) and were fed for ad libitum intake. Prepartum diets were mixed daily using a 113 kg batch mixer and were fed once daily at 1400 h. Wheat straw particle size was reduced using a bale processor set for an average cut length of 7.6 cm. Dry matter content of the prepartum diets was lowered to ≈50% by addition of water to the diets at mixing time. The lactation TMR was mixed daily in a TMR wagon and was fed at 0700 h and 1400 h. Cows were housed in a tie-stall facility from the beginning of the study through 14 days in milk, and then were moved to a free-stall facility, where they continued to receive the identical ration until 21 d postpartum.

Data and Sample Collection and Analysis

Cows were milked 3 times daily at 0200 h, 1000 h, and 1800 h; milk yield was recorded at each milking through 21 days in milk and milk samples were collected at all milkings between 5 and 21 days in milk. Samples were analyzed for true protein, fat, lactose, urea nitrogen, and somatic cells at the Heart of America DHIA, Manhattan, KS, and analyzed values for each milking were composited by day. Samples of feed ingredients were collected weekly and composited by month for analysis. Feed ingredient samples were analyzed using standard wet chemistry methods (Dairy One, Ithaca, NY) for dry matter, CP, NDF, ADF, EE, Ca, P, Mg, K, Na, Cl, and S. DCAD was calculated as [(Na + K) – (Cl + S)] meq/100 g. Dry matter was

determined by drying samples in a forced air oven for at 105°C for 8 h. Crude protein concentration was determined by oxidation and detection of N_2 using a Leco FP-528 combustion analyzer (Leco Corp, St Joseph, MI, AOAC 990.03). Neutral detergent fiber concentration was determined (Van Soest et al., 1991) using an ANKOM A200 filter bag technique (ANKOM Technology, Macedon, NY). Acid detergent fiber concentrations were determined using an ANKOM A200 filter bag technique with solutions the same as AOAC 973.18 (C; AOAC, 2000). Crude fat concentration was determined by ether extract (AOAC 2003.05). Concentrations of Ca, P, Mg, K, and Na were determined using a Thermo IRIS Advantage HX (Thermo Fisher Scientific Inc., Waltham, MA) or Intrepid Inductively Coupled Plasma Radial Spectrometer after microwave digestion. Chloride ion concentration was determined using a Brinkman Metrohm 716 Titrino Titration unit (Riverview, FL) and sulfur concentration using near infrared reflectance spectroscopy (NIRS, AAOC 989.03).

Blood samples for total calcium analysis were collected from coccygeal vessels into serum Vaccutainer tubes (Becton Dickinson and Company, Franklin Lakes, NJ) at 1300 h beginning 7 d prior to expected calving and continuing through 5 d in milk. Serum samples were allowed to clot at room temperature for 60 min and then centrifuged at 2,500 × g for 5 min; serum was removed and frozen at -20°C until analysis. Additionally, on d 5, 10 and 21 postpartum blood samples were collected at 1300 h into EDTA-containing Vacutainers (Becton Dickinson and Company) and centrifuged at 2,500 × g for 5 min within 10 min of collection and plasma was removed and frozen at -20°C until analysis. Serum calcium was determined by flame atomic absorption spectroscopy according to (Bowers and Rains, 1988), and commercial assay kits were used to quantify glucose (Wako Chemicals USA, Richmond, VA) and beta-hydroxybutyrate (Pointe Scientific, Canton, MI) in plasma samples.

Displaced abomasum, retained placenta, metritis, mastitis, and milk fever were diagnosed according to Kelton et al. (1998). Clinical ketosis was identified using urinary ketone reagent strips (Reli On®, Bayer Healthcare LLC, Mishawaka, IN). Cows that tested moderate or greater on the colorimetric scale for two consecutive days were designated as clinical cases. Animals diagnosed with clinical ketosis were orally treated with Keto Gel™ (Jorgensen Laboratories, Inc., Loveland, CO) or propylene glycol. Though urine pH data was not gathered for analysis, initial tests were performed to ensure that proper acidification was being achieved in anionic treatments. Body condition scores were evaluated by 2 trained investigators and body weights

were recorded on days -21 and -7 relative to expected calving and days 1, 10, and 21 after calving.

Statistical Analysis

A total of 45 animals were included in the study; however, 3 (all multiparous) were removed due to unrelated health events (pyrexia, bovine respiratory disease). Data were analyzed using the MIXED procedures of SAS (version 2001; SAS Institute Inc., Cary, NC) with repeated measures over time by modeling the fixed effects of treatment, parity, day relative to parturition, day by treatment interaction, parity by treatment interaction, and day by parity by treatment interaction, as well as the random effect of animal. Prepartum and postpartum DMI data were analyzed separately. When treatment effects were significant, means were separated by Student's *t* test. Treatment differences were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$. Incidence of periparturient disorders were tested by Fisher's exact test.

Results

Chemical analysis of feed ingredients revealed that diets closely matched targets for NDF, NFC, and CP concentrations (Table 2.1). However, DCAD values were substantially more cationic than the target values of +10 and -10 meq/100 g DM. Differences in DCAD values can be attributed to greater than expected potassium concentrations in corn silage and grain mixes. Even though target DCAD values were not achieved, the difference between the diets was maintained, and there was approximately 20 meq/100 g DM difference between the control and ACP diets.

Pre- and postpartum means for DMI, milk yield, and milk composition are shown in Table 2.2. Prepartum DMI tended to be different ($P = 0.09$) among treatments with means of 9.0, 8.5, and 7.5 kg/d DM for CON, BC, and SC, respectively; however, no difference was observed between anion-supplemented diets and CON. Postpartum DMI through 14 days in milk did not differ by parity, treatment. Milk yield, energy-corrected milk, fat yield, fat %, protein yield, lactose yield, and somatic cell count were not altered by treatment through 21 days in milk. However, lactose % ($P = 0.02$), protein % ($P = 0.03$) and milk urea nitrogen ($P < 0.01$) were significantly different among treatments; (Table 2.2). A significant day x parity x treatment interaction was detected for fat % and can be viewed in Figures 2.1 and 2.2 for primiparous and

multiparous cows, respectively. Fat % was greater on d 15 postpartum for primiparous cows consuming CON prepartum ($P < 0.05$) and was greater on d 5, 11, 16, 17, and 21 for multiparous cows consuming BC prepartum ($P < 0.05$).

Body weight was not affected by treatment ($P > 0.10$), however there was a tendency for a day x parity x treatment interaction detected for BCS ($P = 0.07$) which is plotted in Figures 2.3 & 2.4 for primiparous and multiparous cows, respectively.

Mean plasma glucose concentrations (Table 2.4) on d 5, 10, and 21 postpartum tended to be different among treatments ($P = 0.06$) and greater for cows fed CON ($P = 0.08$). There was no significant effect of time on postpartum plasma glucose, but there was a significant treatment x parity x day effect ($P < 0.01$) which is plotted in Figures 2.5 & 2.6 for primiparous and multiparous cows, respectively. Plasma BHBA concentrations (Table 2.4) analyzed on d 5, 10, and 21 postpartum were not different between treatments but were different over time ($P < 0.01$) with means of 9.98, 6.99, and 6.09 mg/dL for d 5, 10, and 21 prepartum, respectively. Peripartum serum calcium concentrations did not differ among treatments and surprisingly, only tended to be different over time ($P = 0.07$). Serum calcium means were 8.11, 7.71, and 8.04 mg/dL for CON, BC, and SC, respectively. Serum calcium concentrations from d -7 prepartum through d 5 postpartum for prepartum dietary treatments are shown in Figure 2.7.

There were no differences in frequency of postpartum health disorders as tested by Fisher's exact test (Table 2.5). The most prevalent disorder was ketosis; it occurred in 7, 6, and 4 cows for CON, BC and SC, respectively. Only 2 cows suffered from milk fever during the study, with 1 case each for CON and BC and no cases for SC. Retention of fetal membranes 24 h after parturition occurred in 2 cows from CON and 1 from SC.

Discussion

Though it was unfortunate that the target DCAD values were not met, the margin of difference was maintained between the DCAD treatment factors and thus, the validity of the experiment was not compromised. Dietary cation-anion difference values for BC and SC did not differ greatly from values used in other experiments; Vagnoni and Oetzel (1998) offered a ration to dry cows containing Bio-Chlor® as an anion source to decrease the DCAD to -5.1 meq/100 g DM; this feeding level decreased urine pH to 6.37, thus validating the acidogenic properties of the diet. Peterson et al. (2005) offered diets with a DCAD of -1.30 and -2.00 meq/100 g DM that

contained SoyChlor® as an anion source; this level was sufficient to maintain serum calcium levels at calving above the threshold of clinical hypocalcemia. The DCAD level of +2.5 meq/100 g DM for BC and -0.2 meq/100 g DM for SC was sufficient to maintain peripartum serum calcium levels at levels greater than the threshold for clinical hypocalcemia (5.0-6.0 mg/dL) throughout the peripartum period. Surprisingly, CON (DCAD +18.8 meq/100 g DM) did not cause greater occurrences of hypocalcemia. A possible explanations for this observation may be the low concentrations of potassium in the diets; Ramos-Nieves et al. (2009) formulated prepartum diets low in potassium with (DCAD -15 meq/100 g DM) or without (DCAD +11 meq/100 g DM) the addition of anions and concluded that the supplementation of anions to the low potassium diets did not change the incidence of clinical or subclinical hypocalcemia. Potassium concentrations of our prepartum diets (Table 2.1) were less than the 1.29% DM of Ramos-Nieves et al. (2009) leading us to believe that the lack of a significant decrease in serum calcium for CON may be attributed to the beneficial effect that low potassium prepartum diets have on calcium homeostasis. Kurosaki et al. (2007) observed that a DCAD of + 1.2 meq/100 g DM prevented the onset of milk fever in multiparous cows and successfully decreased urine pH to 6.8-7.0 in the peripartum period compared to a non-anion supplemented group (urine pH 7.9-8.1). Research has yet to elucidate an optimal prepartum DCAD, but these findings suggest that a DCAD level of ≈ -5 to +2.5 meq/100 g DM may be sufficient to attenuate the onset of clinical hypocalcemia at the time of calving.

Prepartum DMI for SC tended to be lower than CON ($P = 0.09$), but was only numerically less than BC; SC also had the lowest DCAD. Though no data were collected that would elucidate direct differences in acidogenic properties of the diets, the degree of acidosis inflicted by a lowered DCAD has been suggested to be correlated with the extent to which DMI is reduced (Vagnoni and Oetzel, 1998), based on evidence that anion salts that were the least acidogenic (Oetzel et al., 1991) also had minimal negative effects on intake as compared to other, more acidogenic anionic salts (Oetzel and Barmore, 1993). Decreases in DMI for diets containing supplementary anions have traditionally been associated with palatability issues as a result of the anionic salts included in the diets (Horst et al., 1997). Dry matter intake was lower for all treatments than what was observed for diets with similar DCAD (Vagnoni and Oetzel, 1998; Moore et al., 2000), and no difference in parity was observed as was in (Moore et al., 2000). All prepartum diets contained wheat straw at 38% of diet DM which could have

promoted satiety. Dann et al. (2006) fed diets to far-off cows (dry-off to 25d prior to expected calving) containing wheat straw at 26% of DM and 1.30 Mcal/kg NEL and observed a DMI of 10.4 kg DM/d . Our diets contained nearly 12% more wheat straw which could explain the lower intakes we observed.

Calcium intakes varied among treatments and were 75.6, 68.0, and 46.5 g/d for CON, BC, and SC, respectively. Prepartum calcium intake has been linked to milk fever (Boda, 1956; Goings et al., 1974; Green et al., 1981; Kichura et al., 1982). However the levels consumed by all treatments were less than those associated with negative effects on calcium metabolism and greater than those associated with positive effects (Horst et al, 1994).

Surprisingly, milk protein % and MUN differences coincided with slight differences in crude protein of the prepartum diets. For protein % and MUN, BC was less than SC ($P < 0.05$) and CON was intermediate. The prepartum dietary CP was greatest for SC and least for BC, with CON again intermediate. Although the CP values only differed by 0.3% of DM, it is interesting that the significant production effects correlated with the differences in CP of the prepartum rations. Postpartum intake did not differ among treatments or parity, but intake for SC was numerically the greatest. Intake differences, though not significant, seem to be the only explanation for the differences in these production parameters. Significant differences among treatments in peripartum production for cows fed diets that differ in DCAD and/or anion source is lacking (Block, 1984; Joyce et al., 1997; Moore et al., 2000; Roche et al., 2003; Ramos-Nieves et al., 2009). Block (1984) reported that cows fed cationic diets vs. anionic diets produced 6.8% less milk in a 305 d lactation ($P < 0.05$) and when the cationic group was separated into animals that were diagnosed with milk fever and those who were healthy, 305 d milk yield was 14% greater ($P < 0.05$) for animals fed the anionic diet prepartum vs. those who were fed the cationic diet and diagnosed with milk fever.

Total body weight tended to be increased ($P = 0.08$) by a lower prepartum DCAD, which did not agree with Ramos-Nieves et al. (2009), who observed that multiparous cows consuming a low DCAD diet had lower body weight 2 wk postpartum ($P < 0.05$) compared to cows consuming a control ration with a positive DCAD. Postpartum DMI was not different between treatments in that study (Ramos-Nieves et al., 2009), consistent with our observations.

Plasma glucose measurements taken on d 5, 10 and 21 postpartum showed a tendency for a treatment and DCAD effect. Few studies investigating effects of prepartum DCAD level or

source have reported postpartum glucose levels. However, Ramos-Nieves et al. (2009) observed that postpartum glucose did not differ between cows fed prepartum diets with a low DCAD or a control DCAD, but means were numerically lower than what we observed postpartum; 53.4 and 51.0 mg/dL for control and low DCAD, respectively vs. 66.8, 57.1, and 63.8 mg/dL for CON, BC, and SC, respectively. Interestingly, the tendencies for differences in postpartum glucose in our study did not correlate with numerical differences in postpartum DMI, but did correlate with numerical differences in ECM yield. No treatment-related differences in postpartum BHBA levels were detected; however, BC had the lowest mean postpartum plasma glucose and also had greatest numerical postpartum plasma BHBA concentrations.

Total serum calcium was not different by different among treatments, parity, or DCAD and only tended to be different by day (Table 2.4). Block et al. (1984) reported that total serum calcium levels were greater for cows fed an anionic diet (DCAD = - 12.9 meq/100 g DM) versus a cationic diet (DCAD = +33.1 meq/100 g DM) on d -3,-2,-1,0,1, and 3 relative to calving ($P <$ 0.05). Oetzel et al.(1988) observed similar results when feeding prepartum diets supplemented with ammonium salts to decrease DCAD to – 7.5 meq/100 g DM. Similar to our results, Ramos-Nieves et al. (2009) did not observe a difference in mean peripartum total serum calcium between treatments with differing DCAD. Interestingly, there was only a tendency ($P = 0.07$) for an effect of time on total serum calcium from d -7 prepartum through d 5 postpartum, suggesting that animals did not undergo a large calcium stress at parturition or were under the influence of a preventive factor regardless of DCAD treatment. As stated above, feeding low potassium diets prepartum with or without an ACP to decrease DCAD did not have an effect on peripartum serum calcium levels (Ramos-Nieves et al., 2009), which was consistent with our observations. Ramos-Nieves et al. (2009) did, however, observe an effect of anion supplementation on peripartum levels of phosphorus and the occurrence of hypophosphatemia. Unfortunately plasma phosphorus was not measured in this study. The low concentration of the strong cation potassium in the diets presumably prevented a state of metabolic alkalosis, which has been shown to cause the parturient dairy cow's inability to respond to the increased calcium demand by blunting tissue response to PTH (Horst et al., 1997; Goff and Horst, 1997).

No differences were detected in the incidence of postpartum health. With the number of animals included in this study, the power to detect differences in such variables is relatively low and numerical differences between treatments were not great. The most prevalent disorder,

ketosis, affected 53% of cows in CON, 42% of cows consuming BC, and 27% of cows in SC. These rates are certainly higher than the rates for occurrence of ketosis observed in other trials (Goff and Horst, 1997; Joyce et al., 1997; Moore et al., 2000). Close-up period feeding has been suggested to have limited effects on peripartum metabolism as compared to feeding in the far-off period (Dann et al., 2006); hence the lower than expected DMI close-up should not have caused the increased ketosis rates. Postpartum DMI was, however, unexplainably low and is most likely the underlying cause of the high ketosis rates. This may be attributed to environmental effects experienced by the cows in the tie-stall barn, as it was observed that ketosis cases were often resolved upon movement out of the tiestall barn at 14 d postpartum. Another possible explanation for the high ketosis rates may be that the transition from the relatively low fermentable prepartum rations with 38% wheat straw to the lactating ration containing high quality alfalfa and an increased amount of wet corn gluten feed shocked the ruminal flora and may have caused ruminal acidosis thereby decreasing intakes and increasing rates of ketosis. However intuitive this theory may seem, recent research by Stebulius et al. (2009) demonstrated no difference in the postpartum rumen microbial population between cows fed either a moderate grain diet (37.6% NDF) or a high forage diet (45.5% NDF) prepartum and then switching to a common postpartum ration.

Conclusion

Feeding prepartum diets containing supplemental anions provided by an ACP did not increase total serum calcium levels at the time of calving compared to a control ration containing no supplemental anions. Additionally, dietary DCAD had few effects on productivity or postpartum health. Though the DCAD of the control ration was higher than normal recommendations for close-up rations, the dietary concentration of the strong cation potassium was relatively low. The low dietary concentration of potassium likely decreased the metabolic alkalosis associated with decreased tissue sensitivity to PTH thus allowing calcium levels to remain normal for the peripartum period across all treatments. Close-up diets that are inherently high in potassium might benefit from the addition of anions to decrease DCAD.

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Table 2.1 Ingredients and chemical composition of experimental diets.

	CON	BC	SC	Lactating
Ingredient (% of DM)				
Corn silage	22.7	22.7	22.7	23.8
Wheat straw	37.8	37.8	37.8	-
Wet corn gluten feed	11.2	11.2	11.2	32.4
Alfalfa hay	-	-	-	12.1
Rolled corn	10.2	10.1	10.0	19.4
Soybean hulls	4.6	4.3	1.5	1.8
Soybean meal	9.5	4.9	8.0	-
SoyBest®	-	-	-	6.2
Blood Meal	0.8	0.8	0.8	-
Bio-Chlor®	-	5.3	-	-
SoyChlor®	-	-	5.8	-
Limestone	1.0	1.0	-	1.6
Magnesium Oxide	0.3	0.3	-	0.8
Molasses	1.5	1.5	1.5	-
Micronutrient premix[1]	0.5	0.5	0.5	2.5
Chemical composition (% of DM)				
DM, % as-fed	49.7	49.6	49.5	57.4
CP	15.0	14.9	15.2	18.3
ADF	30.8	31.0	31.1	14.0
NDF	49.4	49.7	49.1	30.1
NFC	30.2	29.5	29.8	44.5
EE	2.1	2.1	2.3	3.2
NE_L, Mcal/kg (NRC 1989)	1.21	1.21	1.23	1.72
Ca	0.84	0.80	0.62	0.76
P	0.34	0.34	0.34	0.50
Mg	0.36	0.37	0.34	0.31
K	1.26	1.21	1.21	1.43
Na	0.09	0.14	0.10	0.32
Cl	0.19	0.64	0.80	0.26
S	0.20	0.26	0.21	0.28
DCAD, mEq/100 g DM	+18.8	+2.5	-0.2	+25

[1] CON, BC, and SC micronutrient premix consisted of 12.1% trace mineral salt, 3.7% 4-Plex (Zinpro Corp., Eden Prairie, MN), 7.3% selenium premix (0.06%), 5.0% vitamin A (30,000 IU/g), 5.0% vitamin D (30,000 IU/g), 64.3% vitamin E (44 IU/g), 0.7% EDDI (20,000 mg/lb), and 3.6% Rumensin 80.Lactating micronutrient premix consisted of 5.2% trace mineral salt, 24.1% Menhaden fish meal, 27.5 % sodium bicarbonate, 3.1% MFP (Novus International, Inc., St. Charles,MO), 1.9% 4-Plex (Zinpro Corp.), 1.1% selenium premix (0.06%), 0.5% vitamin A(30,000 IU/g), 0.2% vitamin D (30,000 IU/g), 10.3% vitamin E (44 IU/g), 0.03% EDDI (20,000 mg/lb), 0.22% Rumensin 80, 17.2% XP Yeast (Diamond V Mills, Cedar Rapids, IA), and 8.6% Reashure (Balchem Corp, New Hampton, NY).

Table 2.2 Effects of treatments on dry matter intake and performance.

	Treatment				*P*		
Item	CON	BC	SC	SEM	Day	Parity	Trt
Prepartum DMI (kg/d)	9.0	8.5	7.5	0.6	< 0.01	0.67	0.09
Postpartum DMI (kg/d)[1]	11.5	12.1	13.3	1.4	< 0.01	0.21	0.62
Milk yield (kg/d)[2]	31.9	33.9	34.6	2.4	< 0.01	< 0.01	0.70
Energy-corrected milk (kg/d)	35.3	37.6	37.2	2.3	0.08	< 0.01	0.74
Fat yield (kg/d)	1.32	1.46	1.34	0.08	0.13	< 0.01	0.41
Fat %	4.33	4.46	4.01	0.2	< 0.01	0.03	0.27
Protein yield (kg/d)	1.01	1.01	1.13	0.09	< 0.01	< 0.01	0.46
Protein %	3.18[ab]	2.99[a]	3.29[b]	0.1	< 0.01	0.55	0.03
Lactose yield (kg/d)	1.52	1.59	1.68	0.13	< 0.01	< 0.01	0.62
Lactose %	4.70[ab]	4.66[a]	4.87[b]	0. 1	< 0.01	0.08	0.02
SCC	162	131	107	68	0.98	0.40	0.86
MUN	8.82[ab]	8.57[a]	9.11[b]	0.12	< 0.01	0.19	< 0.01

[a,b]Means within a row that do not share a superscript differ ($P < 0.05$)

[1]Through 14 days in milk

[2]Through 21 days in milk

Table 2.3 Effects of treatments on body weight and BCS.

	Treatment				*P*		
Item	CON	BC	SC	SEM	Day	Parity	Trt
Weight (kg)[1]	621	683	671	22	< 0.01	< 0.01	0.14
BCS[1]	3.10	3.15	3.20	0.08	< 0.01	0.04	0.69

[1]Weight and BCS collected on d -21,-7,1,10 and 21 relative to calving

Table 2.4 Effects of treatments on plasma glucose, BHBA, and total serum calcium.

	Treatment				*P*		
Item	CON	BC	SC	SEM	Day	Parity	Trt
Glucose (mg/dL)[1]	66.78	57.09	63.76	3.07	0.59	< 0.01	0.06
BHBA (μM)[1]	7.72	7.93	7.42	0.97	< 0.01	0.27	0.91
Calcium (mg/dL)[2]	8.11	7.71	8.04	0.70	0.07	0.73	0.95

[1] d 5, 10, and 21 postpartum

[2] d -7 prepartum through d 5 postpartum

Table 2.5 Postpartum health disorders[1].

	Treatment		
Item	CON	BC	SC
Cows on treatment	13	14	15
Ketosis	7	6	4
Displaced abomasum	3	1	2
Retained placenta	2	0	1
Metritis	1	0	0
Mastitis	1	1	0
Milk fever	1	1	0

[1]No significant treatment effects detected using Fisher's exact test.

Figure 2.1a Fat % in primiparous cows fed different prepartum diets from days 5 to 21 postpartum. Fat % was greater on day 15 for cows that consumed SC than cows that consumed BC and CON ($P < 0.05$). Treatment × parity × day interaction ($P < 0.05$).

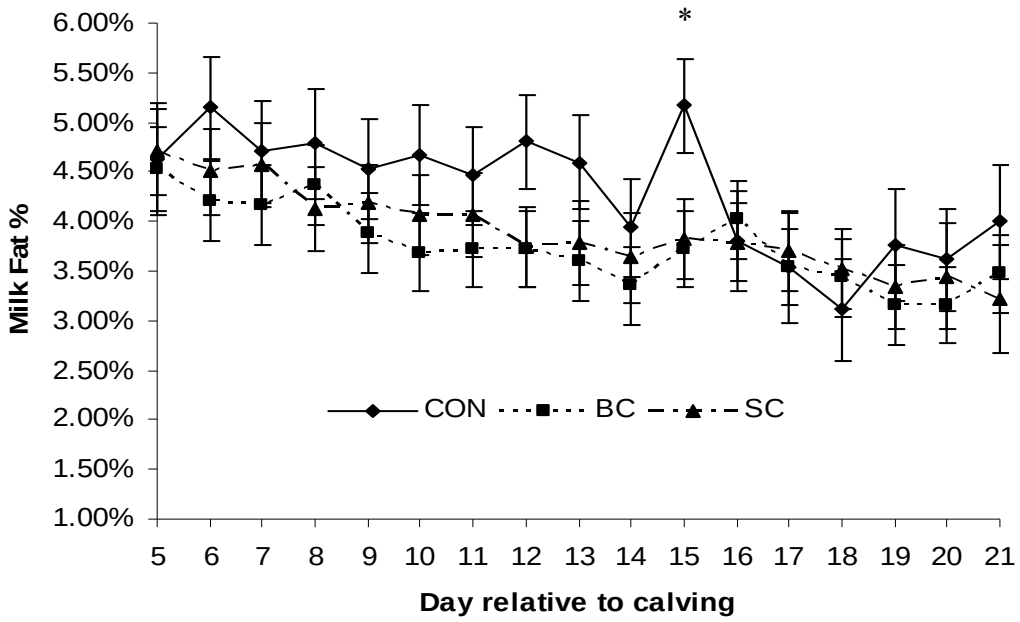


Figure 2.2 Fat % in multiparous cows fed different prepartum diets. Fat % was greater on day 5, 11, 16, 17, and 21 for cows that consumed BC than cows that consumed SC and CON ($P < 0.05$). Treatment × parity × day interaction ($P < 0.05$).

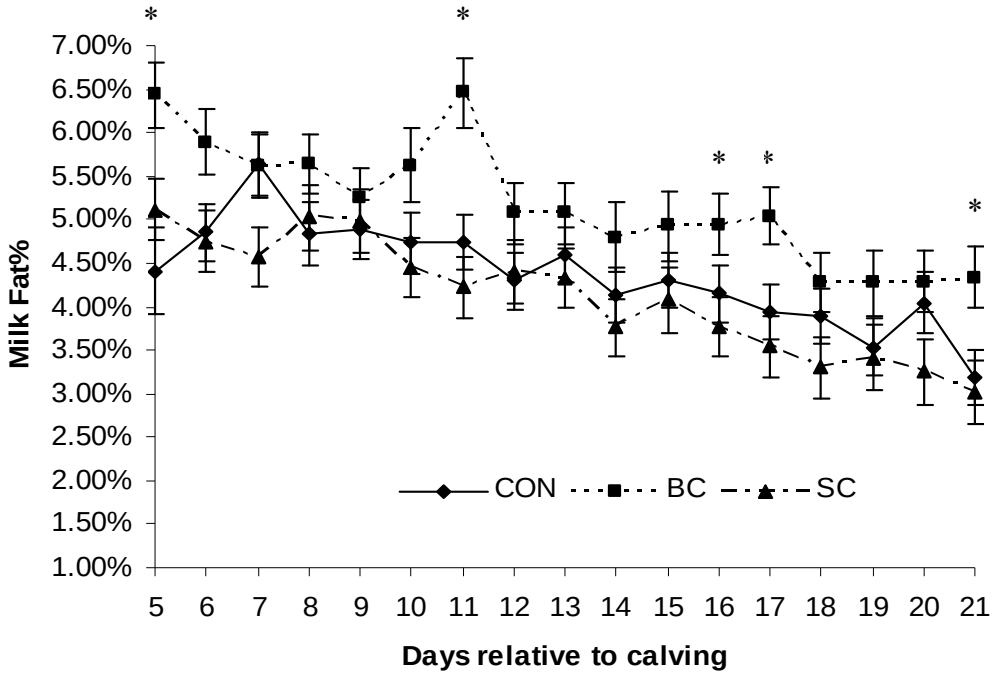


Figure 2.3 BCS of primiparous cows fed different prepartum diets. BCS was assessed on days 21 and 7 prior to expected calving and on days 1, 10 and 21 postpartum. Treatment × parity × day interaction ($P < 0.05$).

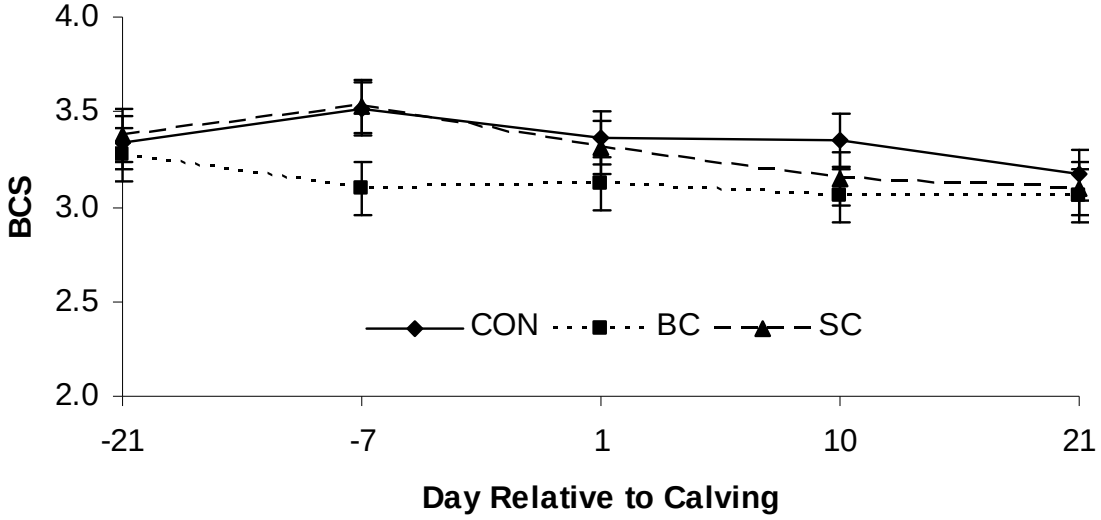


Figure 2.4 BCS of multiparous cows fed different prepartum diets. BCS was assessed on days 21 and 7 prior to expected calving and on days 1, 10 and 21 postpartum. Treatment × parity × day interaction ($P < 0.05$).

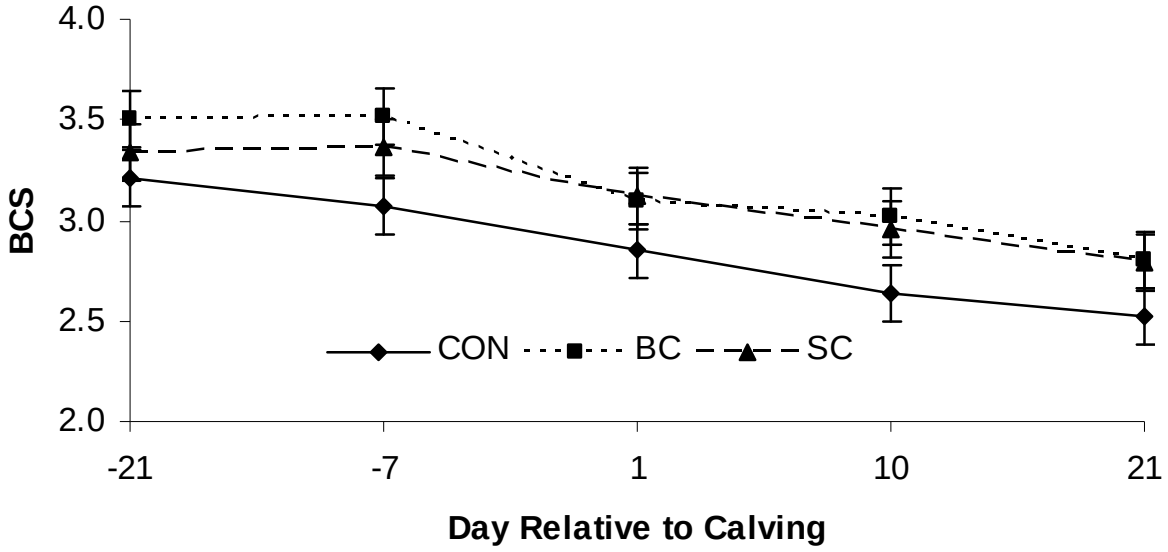


Figure 2.5 Postpartum plasma glucose in primiparous cows fed different prepartum diets. Plasma glucose was measured on days 5, 10, and 21 postpartum. Plasma glucose concentrations were greater for cows that consumed SC prepartum on d 10 postpartum than for cows that consumed BC and CON prepartum ($P < 0.05$). Plasma glucose concentrations were greater for cows that consumed BC prepartum on d 21 than for cows that consumed SC and CON prepartum. Treatment × parity × day interaction ($P < 0.05$).

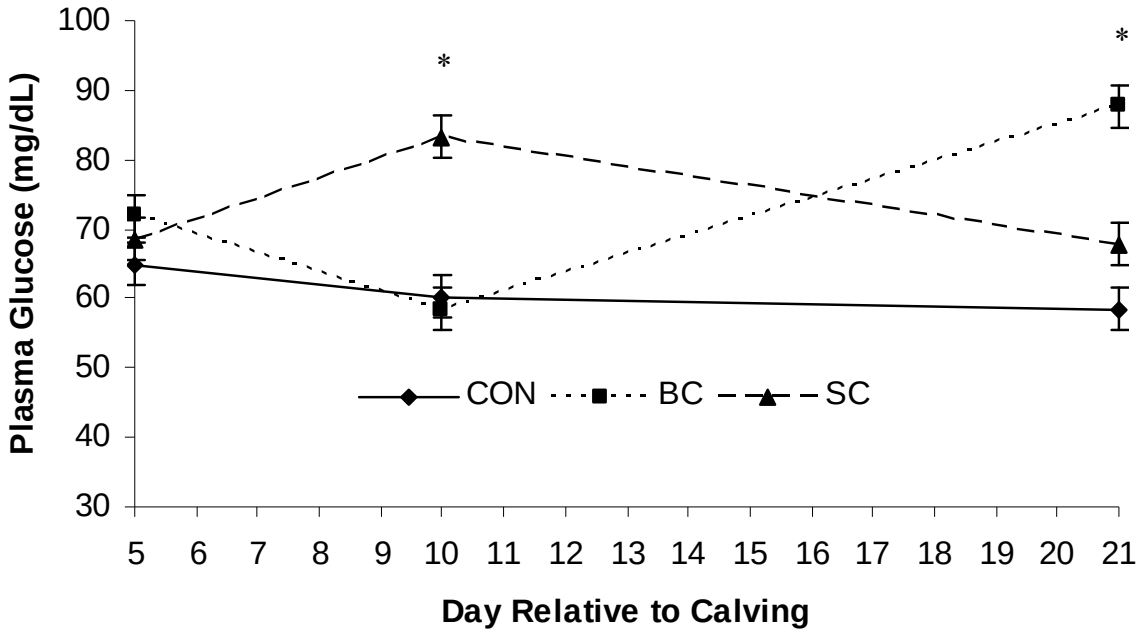


Figure 2.6 Postpartum plasma glucose in multiparous cows fed different prepartum diets. Plasma glucose was measured on d 5, 10, and 21 postpartum. Treatment × parity × day interaction ($P < 0.05$).

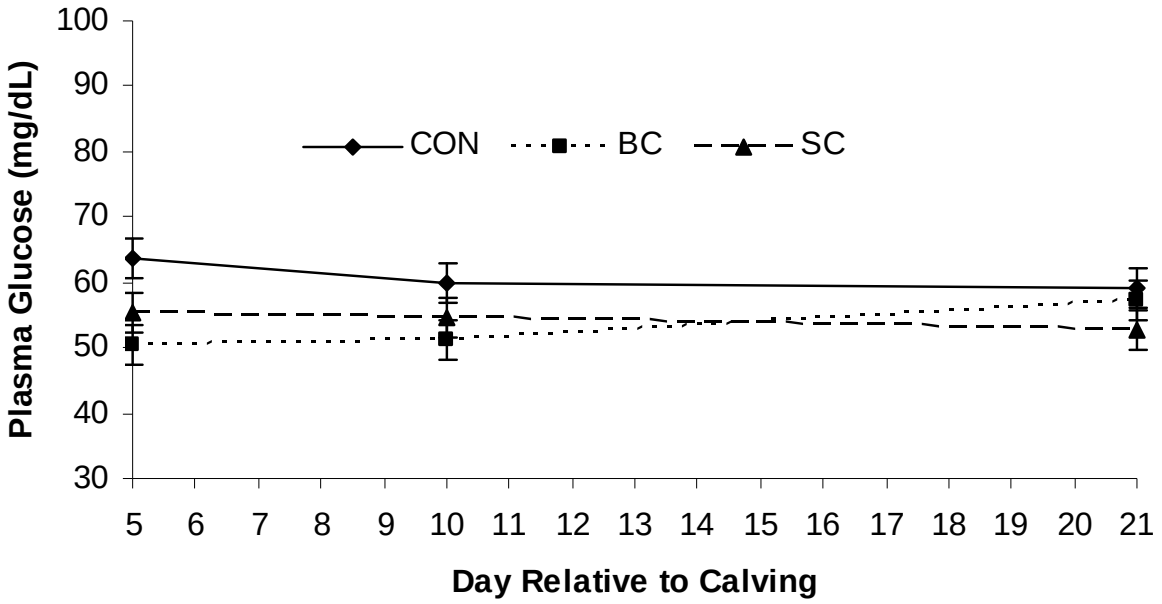
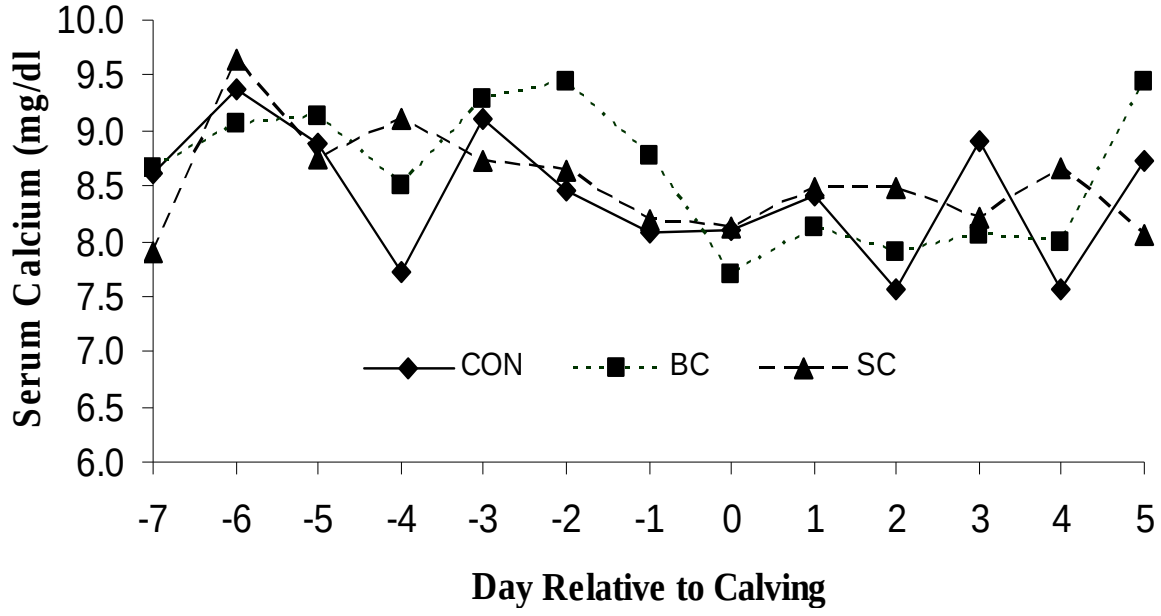


Figure 2.7 Peripartum total serum calcium concentrations of cows fed different prepartum diets. Serum calcium concentration was measured beginning 7 days prior to calving through 5 days postpartum. Time tended to decrease serum calcium concentrations ($P = 0.07$).



CHAPTER 3 - Effects of varying rates of tallgrass prairie hay and wet corn gluten feed on productivity of dairy cows.

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Abstract

Productivity of lactating dairy cows fed diets with wet corn gluten feed (Sweet Bran, Cargill Inc.; **WGCF**) as the primary energy substrate and prairie hay as the primary source of physically effective NDF (**peNDF**) was assessed versus a control diet. Forty-eight Holstein cows, 100-250 days in milk, were randomly assigned to 1 of 6 pens and pens were randomly assigned to treatment sequence in a replicated 3x3 Latin square. Treatments were a control ration with 18% alfalfa, 18% corn silage, 33% WCGF, and 15% forage NDF (**CON**); a diet with 20% tallgrass prairie hay, 46% WCGF, and 13% forage NDF (**TPH20**); and a diet with 14% tallgrass prairie hay, 56% WCGF, and 9% forage NDF (**TPH14**). However, midway through period 2, TPH14 was discontinued due to numerous cases of diarrhea among cows on that treatment. Data from period 2 for TPH14 pens was discarded and the pens which had been assigned to TPH14 for period 3 were randomly assigned to the other treatments. Data were analyzed with mixed models using random effects of period and pen and the fixed effect of treatment. Dry matter intake was not altered by treatment. Least square mean milk yields were 36.2, 34.6, and 35.6 kg/d for CON, TPH20 and TPH14, respectively; milk yield was significantly greater for CON than TPH20 ($P = 0.03$). Milk fat concentration was lowest for TPH14 ($P < 0.01$), with means of 3.47, 3.40, and 2.82 % for CON, TPH20, and TPH14, respectively. Fat yield was significantly greater for CON compared to TPH14 ($P<0.01$) but was not different from TPH20. Milk urea nitrogen was the greatest for TPH20 and least for CON ($P < 0.01$) with TPH14 being intermediate, consistent with differences in dietary protein. Efficiencies, expressed as energy corrected milk divided by DMI, were 1.45, 1.40, and1.30 for CON, TPH20, and TPH14, respectively, and were not significantly different. These data suggest that TPH14 did not provide adequate peNDF to support normal rumen function in midlactation dairy cows; however, TPH20 offered a feasible diet for use on dairies where high-NDF grass hay and WCGF are available.

Introduction

Low milk prices or small profit margins lead dairyman to search for opportunities to reduce input costs. Often the first area of interest is feed cost, since this often represents the largest variable cost for dairy operations. Novel diet formulation methods using atypical feedstuffs or uncommon inclusion rates may be a way to decrease ration costs. Additionally, in circumstances in which supplies of typical feedstuffs may not be sufficient for a production year, a ration that includes alternative feed ingredients that are readily available and does not severely compromise performance may be useful.

Wet corn gluten feed (WCGF), a coproduct of the wet-milling process, is a high-fiber, low-lignin feedstuff that was been repeatedly demonstrated as a viable option for inclusion into lactating dairy cattle rations (Wickersham et al., 2004; Mullins et al., 2009b). Though the fiber in WCGF is highly digestible, the effective NDF % can be variable depending on the method being used to estimate it. Allen and Grant (2000) calculated the effective NDF (**eNDF**) % of WCGF to be 32.90% or 5.71% based on change in milk fat concentration and ruminal pH, respectively, and physically effective NDF (**peNDF**) to be 4.80%, based solely on rumination activity. Regardless of the variance of these figures, peNDF must be supplied by other fiber sources to prevent ruminal acidosis and/or milk fat depression. However, WCGF, due to the nature of its origin, is quite low in rapidly fermentable carbohydrates such as starch compared to other high energy feedstuffs, so the risk of ruminal acidosis is decreased (Wickersham et al., 2004). Taking this into account, a diet with high inclusion rates of WCGF may be able to be formulated with lower peNDF.

Sources of peNDF in the diet have traditionally come from forages such as alfalfa or corn silage and in many cases a combination of both. In a survey conducted in the summer of 1995 by Mowrey and Spain (1999), 62% of the lactating dairy cattle across the United States were fed alfalfa hay and 61% were fed some type of corn forage. However, replacing a portion of alfalfa hay, corn silage, and corn grain with WCGF had been shown to increase DMI, energy corrected milk (**ECM**) yield and efficiency expressed as ECM/DMI (VanBaale et al., 2001). Mullins et al. (2009) fed diets that included 0, 7, 14, or 21% of DM as alfalfa hay as well as 31% of DM as WCGF and observed only a tendency for an effect on ECM but no effect on ECM/DMI, milk fat %, or milk fat yield. The absence of effect on milk fat suggests that alfalfa hay inclusion or exclusion in WCGF based diets did not affect the rumen environment in such a

way as to alter milk fat synthesis. Furthermore, economic analyses were carried out to assess whether or not the additional milk yield gained by including alfalfa could be justified, and it was concluded that when milk:feed cost ratios were low, alfalfa hay inclusion at 21% of DM may not be profitable compared to 0% inclusion rates.

Tallgrass prairie hay (**TPH**), a mixture of many grass species native to the central plains region, is a relatively inexpensive forage fiber source that is typically fed to beef cattle or far-off dry dairy cows with a low energy requirement. According to Olson et al. (2008) TPH contains on average 67.4% NDF, 15.2% acetyl bromide lignin and 3.9% CP and thus, depending on processing TPH may be used as a good source of peNDF in a ration.

The nature of TPH and WCGF may to serve to complement each other in lactating dairy cow rations. However, no published research has shown the effects of such a diet compared to a ration containing common ingredients such as alfalfa hay and corn silage. Our objectives were to compare diets containing varying amounts of TPH and WCGF to a control ration and observe effects on productivity of lactating dairy cows.

Materials and Methods

Animals, Design, and Diets

Twenty-one primiparous and 27 multiparous lactating Holstein cows (167 ± 47 DIM, 1.8 ± 0.97 lactations, mean ± SD) were selected from the Kansas State University Dairy Teaching and Research Center herd and randomly assigned to 1 of 6 free-stall pens. Pens were assigned to treatment sequence in a replicated 3×3 Latin square design that was balanced for carryover effect of treatment. Treatment periods were 21 d, with 17 d of diet adaptation and 4 d of sampling; feeding of treatments began in September and continued through November 2009. Cows were fed fresh TMR blended in a TMR wagon daily at 0930 h and milked 3 times daily at 0600 h, 1300 h, and 2000 h.

Treatments were a ration containing 18% of DM alfalfa hay and 18% of DM corn silage (CON), a ration containing20% of DM TPH (TPH20), and a ration containing 14% of DM TPH (TPH14; Table 3.1.). Rations were formulated to be isocaloric and isonitrogenous, with varying amounts and sources of forage NDF; however, chemical analysis showed that rations were not completely isonitrogenous.

Midway through period 2, feeding of TPH14 was discontinued due to visual observations of diarrhea in > 25% of cows consuming that diet. The 2 pens on TPH14 switched to the CON ration for the remainder of period 2 and pens allocated to TPH14 in period 3 were instead assigned to either TPH20 or CON.

Data and Sample Collection and Analysis

Feed offered and refused for each pen was recorded on the final 4 d of each treatment period except in the case of inclement weather. TMR samples were also gathered on these days, composited by period and particle size analyzed using a 4 compartment Penn State Particle Separator. After separation, each fraction was dried for 48 h in a 55° C forced-air oven and then weighed to determine particle size distribution on a DM basis. Physically effective NDF was calculated as the proportion of particles retained on the top 2 sieves multiplied by the NDF content of the diet (Yang and Beauchemin, 2009).

Samples of corn silage, alfalfa hay, TPH, WCGF, cottonseed and grain mixes were gathered on d 18 and d 21 of each period and composited and then dried for 48 h in a 55°C forced-air oven before laboratory analysis. All samples were ground to pass through a 1-mm screen utilizing a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and dry matter content was determined by drying at 105°C in a forced-air oven for 12 h. Crude protein content was estimated by determination of elemental nitrogen content (Leco Analyzer, Leco Corp, St. Joseph, MI). Neutral detergent fiber concentration was quantified according to Van Soest et al., (1991); method A by using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Crude fat was determined by ether extraction (AOAC, 2000; method 920.9). Ash content was measured following 8 h of incineration at 500°C by a muffle furnace.

Milk samples were collected for each cow at every milking during the last 4 d of each sample period. Samples were composited over a 48-h period and analyzed for fat, true protein, and lactose with a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN), somatic cells were counted using a flow cytometer laser (Somacount 500; Bentley Instruments, Chaska, MN) and urea N was determined using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 analyzer; Bentley Instruments, Chaska, MN) at Heart of America DHIA (Manhattan, KS). Body weight was measured on d 21 of each period immediately following the milking at 1300 h.

Economic Analysis

Prices of alfalfa hay, corn silage, dry rolled corn, soybean meal, and whole cotton seed were obtained from the Penn State Feed Price list (June 15, 2010). Price of WCGF was obtained from the University of Missouri By-Product Feed Price Listing (June 19, 2010) with freight costs added for transportation from the point of origin to the Kansas State University Dairy in Manhattan, KS. Vitamin and mineral mix cost was fixed across both treatments at $0.83/kg DM. Ration costs were multiplied by the DM intakes for each respective treatment to produce actual cost per cow/d. The milk price of $0.30/ kg was multiplied by the milk yields for each respective treatment to produce income per cow/d.

Statistical Analysis

During period 2, two cows were removed from the study due to health problems associated with TPH14. Two additional cows were removed from the study as a result of health issues unrelated to treatment. Dry matter intake was adjusted using number of cows within pen to account for inequality of animal number within pen.

Data were analyzed using JMP (version 6.0, SAS Institute, Cary, NC) according to the model below:

$$Y_{ilk} = \mu + T_i + P_j + N_k + e_{ijk}$$

where μ is the overall mean, T_i is the fixed effect of treatment (i= 1 to 3), P_j is the random effect of period (j= 1 to 3), N_k is the random effect of pen (k= 1 to 6), and e_{ijk} is the residual error. The random effects of cow nested within pen and period by pen interaction were also used in the model when analyzing milk parameters. Significant effects were declared at $P < 0.05$ and tendencies at $P < 0.10$.

Results and Discussion

Diet Composition and Particle Size

Diets were formulated to be isocaloric and isonitrogenous; however crude protein levels fluctuated between diets due to differences in nitrogen concentration of the respective grain mixes (Table 3.1). Milk urea nitrogen was greatest for cows that consumed TPH20 and least for CON, 17.0 and 13.9 mg/dL ($P < 0.004$), respectively. Not surprisingly, these differences

coincided with the differences in dietary crude protein, however minimum target values for MUN of 10 mg/dL recommended by Jonker et al. (1999) were met, suggesting that protein limitation of milk synthesis or components was not a factor.

Physically effective NDF values, calculated according to Yang and Beauchemin (2009) were 15.8, 11.9, and 11.6% of diet DM for CON, TPH20, and TPH14 and were greater for CON compared to TPH20 ($P < 0.05$). As described in the methods, TPH14 was discontinued midway through period 2 because of numerous cases of diarrhea and gastro-intestinal tract abnormalities, which is a common result of a lack of adequate peNDF in the diet. However, peNDF values for TPH20 and TPH14 were not different; suggesting that perhaps the method used to calculate peNDF for the diets was not adequate for rations of this nature.

Particles > 19.0 mm (% of DM) were 18.8%, 14.7%, and 9.1% for CON, TPH20, and TPH14 (Table 3.2), respectively, but were not significantly different. Percent of particles retained on the middle screen was greatest for CON and least for TPH20 ($P < 0.05$, 27.2% vs. 16.0%). Percentage of particles retained on the lower sieve was greatest for TPH20 and least for CON ($P < 0.05$).

Dry Matter Intake and Performance

Dry matter intakes were not different ($P > 0.10$) among any of the treatments (Table 3.4). This observation was consistent with Schroeder (2003) who observed no change in DMI when WCGF was increased in the diet. Dry matter intake is controlled by a complex set of factors that possess the ability to out-weigh each another depending on the nature of the diet being consumed. Dry matter intake of diets with greater amounts of peNDF as a result of a greater amount of large feed particles, as was the case for CON, are more likely to be limited by physical regulation mechanisms. This type of regulation occurs when the time required for chewing or distention within the gastrointestinal tract, more specifically, within the reticulo-rumen, limit feed intake (Allen, 2000). However, in the case of TPH20 and TPH14 where peNDF was lower, a significant increase in intake was not observed. This may be explained by the differences in retention time of the forage included in the diet. Allen (2000) suggests that compared to perennial species such as those found in tallgrass prairie, alfalfa particles possess a much higher fragility and a shorter buoyancy period, both of which would decrease retention time and therefore distention and satiety.

Milk yield (Table 3.4) was greatest for CON and least for TPH20 ($P < 0.05$) with TPH14 remaining intermediate. Efficiency was not different among any treatments ($P > 0.10$). Milk fat yield and concentration (Table 3.5) were greatest for CON and least for TPH14 ($P < 0.05$); however, TPH20 was not different from CON. It has been shown that feeding diets containing levels of peNDF as low as 9.6% can maintain milk component yield and concentration equal to that of a diet containing 12.7% peNDF (Yang and Beauchemin, 2007). The ability of the diets with high inclusion rates of WCGF but with low forage NDF and peNDF concentrations to maintain acceptable milk fat production can likely be attributed to the lower starch content of WCGF which may limit the occurrence of ruminal acidosis which leads to milk fat depression. This supports the definitions of peNDF and eNDF as proposed by Mertens (1997) where not all eNDF is peNDF and eNDF relates to the sum of the ability of a feed to replace forage or roughage in a diet so that the concentration of fat in milk produced by cows eating the ration is maintained and peNDF only relates to the ability of the fiber to stimulate chewing and influence the consistency of the ruminal mat. By this definition, eNDF of CON and TPH20 did not differ because milk fat concentration and yield were not significantly different. Although chewing behavior and ruminal characteristics were not measured in this study, peNDF, measured using particle size separation of the diet, was significantly different between CON and PH20. This variation between eNDF and peNDF of these diets may well be explained by the increased amount of WCGF in the PH20 diet and its physical and chemical attributes as a highly digestible fiber source that can limit the rate of production of fermentation acids, but due its small particle size, provides little physical effectiveness to the diet (Allen and Grant, 2000).

Though use of milk fat to measure the effectiveness of the fiber in rations encompasses a far greater set of variables within the ration, it is a *post hoc* method of determination and thus is not feasible for many field situations. For our diets, peNDF, as calculated according to Yang and Beauchemin (2009), was not a good predictor of eNDF because only a 3% difference in peNDF between TPH20 and TPH14 resulted in a large difference in milk fat production and overall cow health. In an attempt to account for this we alternately calculated peNDF by multiplying the proportion of particles on the top 2 sieves by the forage NDF concentration rather than total dietary NDF. Though not significantly different from one another ($P > 0.05$), most likely due to a high standard error associated with TPH14, physically effective forage NDF was 21% greater for TPH20 suggesting that perhaps in diets with a large amount of a non forage fiber source, this method may better represent true physical effectiveness.

Milk urea nitrogen was greatest for TPH20 and least for CON ($P < 0.05$) which coincided with differences in dietary crude protein content. Milk protein yield and concentration were not different between the treatments ($P > 0.10$) suggesting that the differences in dietary crude protein did not lead to limitation of milk protein synthesis. Though, there were differences in particle size between TPH20 and CON there were few effects on milk components, which suggests that particle size was sufficient to promote a healthy rumen environment.

Economic Analysis

Because WCGF and TPH are relatively low cost feedstuffs, an economic analysis was conducted to determine if the decreased cost of TPH20 would result in an increased income over feed cost (IOFC, Table 3.6). Because TPH14 did not prove to be a viable option for ration formulation it was not included in the analysis. Cost per kg of DM and feed cost per cow per day were lower for TPH20 than CON ($0.178 vs. $0.189 and $4.41 vs. $4.72). However, IOFC was $0.21 per cow/d greater for CON because of greater milk yield. Table 3.7 shows the potential income differential of feeding TPH20 versus CON. According to Table 3.7, feeding TPH20 would become more profitable than CON when the feed cost margin per cow/d between TPH20 and CON reached $0.35. The potential income differential of feeding TPH20 is greatest when milk prices are low and feed cost margins between the diets are high.

Proximity to a source for WCGF can drastically influence price per kg of DM because of transportation costs. Therefore, farms closer to the point of origin may see less expensive ration costs. Even though feeding TPH20 is not always profitable because of decreased milk yield, fluctuating commodity prices, milk price and proximity to point of origin of WCGF may make it profitable for some producers to feed a ration similar to TPH20.

Conclusion

Though TPH14 apparently did not supply adequate peNDF or forage NDF to the diet, TPH20 offered a feasible option for lactating dairy cows and resulted in component yield and efficiency similar to that of CON. Use of a diet similar to TPH20 may sometimes be economically feasible in a location where WCGF and TPH are readily available. Additionally, in an emergency situation where supplies of other feedstuffs are limited or exhausted, TPH20 could serve as an auxiliary option for dairy producers.

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Table 3.1 Ingredient and nutrient composition of experimental diets.

	Treatment[1]		
Item	CON	TPH20	TPH14
Ingredient, % of DM			
Corn silage	17.6	-	-
Alfalfa hay	17.7	-	-
Prairie hay	-	19.2	13.8
WCGF[2]	33.0	46.1	56.0
Cottonseed	7.3	7.5	7.5
Corn grain	16.6	17.5	15.6
Soybean meal (48%)	1.0	2.6	-
SoyBest®[3]	4.1	4.2	4.2
Limestone	1.2	1.6	1.7
Magnesium oxide	0.1	0.1	0.1
Sodium bicarbonate	0.8	0.8	0.8
Trace mineral salt	0.5	0.1	0.1
Salt	0.03	-	-
Micronutrient premix[4]	0.13	0.13	0.13
Nutrient, % of DM			
DM, % (as fed)	62.7	60.7	61.5
CP	16.5	18.0	18.6
NE_L (Mcal/kg)	1.7	1.6	1.7
NDF	34.5	38.3	37.0
Forage NDF	15.3	12.9	9.3
Ether extract	3.6	4.1	3.7
Starch	20.8	13.9	12.1
Ash	10.9	8.9	9.5
Physically effective fiber[5]			
$peNDF^{8.0}$	15.8 ± 1.0^{a}	11.9 ± 1.0^{b}	11.6 ± 2.7^{ab}
$peFNDF^{8.0}$	7.0 ± 0.38^{a}	4.0 ± 0.38^{b}	3.1 ± 1.0^{b}

[1]CON = Control, TPH20 = Tallgrass prairie hay 20%, TPH14 = Tallgrass prairie hay 14%
[2]Wet corn gluten feed (Sweet Bran, Cargill, Inc., Blair, NE).
[3]SoyBest®, West Point, NE
[4]Micronutrient premix consisted of 30.2% Se premix (0.06%), 34.9% 4-Plex (Zinpro Corp. , Eden Prairie, MN), 23.3% Vitamin E (44 IU/g), 9.3% Vitamin A (30,000 IU/g), 2.32% Vitamin D (20,000 IU/g)
[5]$peNDF^{8.0}$ was calculated as the proportion of particles retained on the top 2 sieves of a Penn State particle separator multiplied by the total dietary NDF concentration (Yang et al., 2009).
$peFNDF^{8.0}$ was calculated as the proportion of particles retained on the top 2 sieves of a Penn State particle separator multiplied by the total dietary forage NDF concentration (Yang et al., 2009).
[a,b]Means within a row with different superscripts are different by Tukey's HSD ($P < 0.05$)

Table 3.2 Composition of corn silage, alfalfa hay, WCGF, and tallgrass prairie hay.

	Ingredient			
Nutrient[1]	Corn Silage	Alfalfa Hay	WCGF	Tallgrass Prairie Hay
DM	36.9	89.5	58.5	88.5
NDF	43.2	43.6	37.5	67.5
CP	8.0	18.3	22.9	6.6
EE	2.9	1.1	2.6	1.7
Ash	5.2	11.4	5.8	7.3

[1]All nutrients except DM are expressed as a percentage of diet DM

Table 3.3 Particle size separation (% of DM).

	Treatment[1]			
% DM retained on sieves	CON	TPH20	TPH14	SEM
19.0 mm	18.8	14.7	9.1	6.3
8.0 mm	27.2[a]	16.0[b]	21.7[ab]	4.7
1.18 mm	43.1[b]	61.6[a]	55.4[ab]	7.8
Pan	10.9	7.7	9.2	5.3

[1]CON = Control, TPH20 = Tallgrass prairie hay 20%, TPH14 = Tallgrass prairie hay 14
[a,b]Means within a row with different superscripts differ by Tukey's HSD ($P < 0.05$)

Table 3.4 Effect of treatments on dry matter intake and performance.

Item	Treatment[1]			*P*-Value
	CON	TPH20	TPH14	
No. of observations	53	53	15	
DMI, kg/d	24.9 ± 0.85	24.8 ± 0.85	27.12 ± 1.31	0.24
Milk, kg/d	36.3 ± 1.0[a]	34.6 ± 1.0[b]	35.6 ± 1.3[ab]	0.02
ECM, kg/d	36.3 ± 0.72[a]	34.7 ± 0.73[b]	33.3 ± 1.3[b]	0.03
ECM/DMI	1.45 ± 0.04	1.40 ± 0.04	1.30 ± 0.09	0.31
BW Change, kg/21 d	7.2 ± 3.8	13.4 ± 4.3	6.0 ± 5.0	0.71

[1]CON = Control, TPH20 = Tallgrass prairie hay 20%, TPH14 = Tallgrass prairie hay 14
[a,b]Means within a row with different superscripts differ by Tukey's HSD ($P < 0.05$)

Table 3.5 Effect of treatments on milk component yield and concentration.

Item	Treatment[1]			*P*-Value
	CON	TPH20	TPH14	
Milk Fat, kg/d	1.23 ± 0.03[a]	1.16 ± 0.03[ab]	1.02 ± 0.06[b]	0.009
Milk Fat, %	3.47 ± 0.13[a]	3.40 ± 0.13[a]	2.82 ± 0.19[b]	0.005
Milk Protein, kg/d	1.20 ± 0.03	1.15 ± 0.03	1.23 ± 0.07	0.66
Milk Protein, %	3.35 ± 0.05	3.37 ± 0.05	3.37 ± 0.10	0.88
Milk Lactose, kg/d	1.73 ± 0.05	1.68 ± 0.05	1.72 ± 0.07	0.24
Milk Lactose, %	4.82 ± 0.04	4.85 ± 0.05	4.87 ± 0.11	0.74
SCC	260 ± 76	198 ± 76	190 ± 140	0.62
MUN mg/dL	13.9 ± 0.89[b]	17.0 ± 0.89[a]	16.5 ± 1.12[ab]	0.004

[1]CON= Control, TPH20=Tallgrass prairie hay 20%, TPH14=Tallgrass prairie hay 14%.
[a,b]Means within a row with different superscripts are different by Tukey's HSD ($P < 0.05$).

Table 3.6 Economic analysis of CON and TPH20.

	Diet[1]	
Item	CON	TPH20
$/kg feed DM	$0.189	$0.178
Feed Cost/c/d	$4.72	$4.41
Income/c/d	$11.12	$10.60
IOFC[2]	$6.40	$6.19

[1]CON= Control, TPH20=Tallgrass prairie hay 20%
[2]Income over feed cost

Table 3.7 Potential income differential of feeding TPH20 across different milk prices and feed costs per cow / day.

		Potential difference in feed cost per cow / day between CON and TPH20								
		$0.20	$0.25	$0.30	$0.35	$0.40	$0.45	$0.50	$0.55	$0.60
Milk price ($/kg)	$0.20	-$0.14	-$0.09	-$0.04	**$0.01**	**$0.06**	**$0.11**	**$0.16**	**$0.21**	**$0.26**
	$0.22	-$0.17	-$0.12	-$0.07	-$0.02	**$0.03**	**$0.08**	**$0.13**	**$0.18**	**$0.23**
	$0.24	-$0.21	-$0.16	-$0.11	-$0.06	-$0.01	**$0.04**	**$0.09**	**$0.14**	**$0.19**
	$0.26	-$0.24	-$0.19	-$0.14	-$0.09	-$0.04	**$0.01**	**$0.06**	**$0.11**	**$0.16**
	$0.28	-$0.28	-$0.23	-$0.18	-$0.13	-$0.08	-$0.03	**$0.02**	**$0.07**	**$0.12**
	$0.30	-$0.31	-$0.26	-$0.21	-$0.16	-$0.11	-$0.06	-$0.01	**$0.04**	**$0.09**
	$0.32	-$0.34	-$0.29	-$0.24	-$0.19	-$0.14	-$0.09	-$0.04	**$0.01**	$**0.06**
	$0.34	-$0.38	-$0.33	-$0.28	-$0.23	-$0.18	-$0.13	-$0.08	-$0.03	**$0.02**
	$0.36	-$0.41	-$0.36	-$0.31	-$0.26	-$0.21	-$0.16	-$0.11	-$0.06	-$0.01
	$0.38	-$0.45	-$0.40	-$0.35	-$0.30	-$0.25	-$0.20	-$0.15	-$0.10	-$0.05
	$0.40	-$0.48	-$0.43	-$0.38	-$0.33	-$0.28	-$0.23	-$0.18	-$0.13	-$0.08

Values within grid reflect the potential income differentials of feeding TPH20 across different milk prices and ration cost differences. Values were calculated as (TPH20 Income – CON Income) + Potential difference in feed cost.