ANALYSIS OF A LAB PROCEDURE AND PREDICTIVE MODEL FOR RUMINAL BUFFERS AND A PROPHYLACTIC TREATMENT PROGRAM FOR UDDER EDEMA

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ANALYSIS OF A LAB PROCEDURE AND PREDICTIVE MODEL FOR RUMINAL BUFFERS AND A PROPHYLACTIC TREATMENT PROGRAM FOR UDDER EDEMA

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CHAPTER I

INTRODUCTION

To maintain high levels of milk production of dairy cattle, one must feed an energy dense diet. Many producers and nutritionists increase energy density by increasing the proportion of concentrate in the diet. However, concentrates are digested more rapidly than fibrous feedstuffs and do not stimulate saliva production as well as forages. Digestive upsets may occur if the animal is unable to neutralize the acid produced by high concentrate diets. Many different compounds currently are being promoted as buffering compounds. An in vitro procedure to rapidly and accurately screen these compounds for effective buffering capacity would be useful. Three culture procedures are used currently: 1) continuous; 2) semi-continuous; and, 3) batch. The continuous and semi-continuous procedures that effectively imitate ruminal changes are quite complex and time consuming. The batch culture approach is less complex and less time consuming, but may imitate post-feeding responses accurately. Results from all three procedures can be influenced by the type of diet consumed by the cow supplying the rumen fluid for the in vitro incubations. In addition, the quantity of rumen fluid added must be measured accurately (i.e., foam production must be minimized). Further, the cow supplying the rumen fluid should be consuming a diet typical of that currently fed in the industry. When incubated, the in vitro changes should simulate typical ruminal fermentation patterns. The objectives of the first study were to determine: 1) if a batch culture procedure adequately simulates

the release rates of ruminal buffers, 2) the optimum substrate for rumen fluid incubations and the impact of the source of the rumen fluid (from high or low concentrate diets), and 3) the procedure that imitates most closely the changes observed postfeeding in vivo, based upon temporal changes in pH, BC, BVI, and VFA production as the vital indicators.

Dietary buffers have been fed to assist the animal's natural buffering system when high concentrate diets are fed. Several researchers (26, 29, 78) have attempted to determine the need for buffers based on the innate buffering capacity of individual dietary ingredients. Tucker et al. (63) attempted to predict the need for buffers based on the diet's buffer value index (BVI). However, in a study by Le Ruyet et al. (39), a high dietary BVI was associated with a low ruminal BVI. However, their results may have been influenced by use of two forages and separation of dietary ingredients. The objective of the second study was to examine the influence of dietary BVI on the ruminal environment of cows consuming diets with various BVI achieved by altering the ratio of sorghum silage to concentrate in the diet.

Udder edema is a prevalent and potentially serious problem for dairy cows. Characterized by buildup of fluid between cells in the mammary gland, edema is not well understood. Grain has been implicated as a cause of udder edema; however, excessive salt intake and genetically superior animals have been correlated positively with udder edema. One frequent recommendation is to limit the salt intake during the dry period (last 45 - 60 days postpartum). Unfortunately, edema still persists in many herds. With the new antibiotic residue laws now being enforced, prevention programs, rather than treatment programs, should be developed. One feed ingredient, calcium chloride, has been reported to reduce udder edema slightly when fed prepartum. The objectives of the third trial were to examine: 1) the relationship between udder edema and mineral concentrations in plasma and urine, and 2) the effects of feeding calcium chloride prepartum on mineral concentrations in plasma and urine.

CHAPTER II

REVIEW OF LITERATURE

Ruminal Buffers

Diets for dairy cattle have undergone numerous changes as nutritionists attempt to meet the nutritional demands of high producing dairy cattle. Milk production per cow has increased steadily through genetic improvement and better management. The number of cows per farm also has increased; this has reduced the feasibility of manually feeding each cow individually. Automated computer feeders allow dairy producers the flexibility of individually feeding and topdressing concentrate to cows, without having to manually feed each cow. However, the success of computer feeders has been mixed; some producers are not able to devote the time necessary to update the computer as milk production or diet composition changes. Historically, lactating cows were allowed to graze forages and were supplemented with a minimum of concentrate. Currently, ensiled forages are displacing grazed forage; this decreases the labor requirements of the dairy producer, and permits one to monitor dry matter intake more precisely and to mechanize the feeding program. Ensiled forages may constitute the entire forage portion of the diet. In addition, many different by-product feeds currently are available to alter extent of ruminal degradation of protein, and alter the non-fiber carbohydrate portion of the diet. The concentrate portion of the diet also has increased in an attempt to increase the caloric density of the diet.

Need for Ruminal Buffers

These feeding strategies enhance the possibility for adverse changes in ruminal pH and digestibility. The potential exists for negative effects of pH on fiber digestibility (46) as the concentrate portion of a diet fed to high producing dairy cows increases. As ruminal pH fluctuates, the bacterial population within the rumen fluctuates between starch and fiber digesting species. A depression in fiber digestibility decreases ruminal acetate concentrations, and depresses fat content of the milk (30). Minimizing the fluctuations in ruminal fluid pH helps to maintain a stable environment for ruminal microbes and prevents the depression in milk fat percentage associated with high concentrate diets. Decreasing the digestibility of any portion of the diet decreases the amount of energy available from the diet. Concentrate feeding depresses ruminal pH by two mechanisms. First, concentrates are digested rapidly, resulting in rapid production of lactic acid and volatile fatty acids which depress ruminal pH. Second, high concentrate diets often contain insufficient amounts of effective fiber to stimulate rumination and production of saliva. In addition, if the silage is chopped too finely and no further fiber sources (such as long-stemmed hay) are included in the diet, the effectiveness of the fiber to stimulate rumination may be inadequate. If rumen contents are not agitated, lactic acid may build up around the ruminal epithelium from the digestion of small particles, resulting in the development of parakeratosis. This condition is characterized by abnormal papillae development and has been associated with an increased incidence of liver abscesses (20). Parakeratosis is alleviated partially by addition of fibrous feedstuffs and may be prevented by gradual adaptation of animals to high concentrate diets.

Temporal In Vivo Changes

Postprandial changes in ruminal fluid pH have been characterized extensively. Ruminal fluid pH tends to be lowest 4 to 8 h after feeding, when ruminal volatile fatty acid concentration is highest (18). The use of ensiled forages poses potentiai feeding problems because 1) lactic acid is present in the silage, being formed during the fermentation process, and 2) moisture content of the feed is inversely related to saliva production. In addition, the nutrient availability of silage is reduced if the silage is not properly harvested and stored. Dry matter intake associated with ensiling forages has been depressed 4 to 50% (60). This may partially be due to the unpalatability associated with feeding an acidic diet. Shaver et al. (59, 60) increased the pH of corn silage using sodium bicarbonate and increased dry matter intake by 17% when compared to unbuffered corn silage (60). Kilmer et al. (33) also reported a trend towards increased dry matter intake when cows consumed buffered diets compared to unbuffered diets. Diets containing sufficient effective fiber modulate changes in ruminal pH because 1) they increase salivary flow, and 2) release of the rapidly digested cell contents is modulated by the presence of fibrous cell walls. In contrast, high concentrate diets are digested rapidly and may significantly depress ruminal pH. Increasing the feeding frequency of concentrates may help minimize the fluctuations in ruminal fluid pH. Kaufman (30) fed cows 14 times/d and noted less fluctuation in ruminal fluid pH compared to a typical twice/d feeding schedule. In addition, increasing the feeding frequency increased the acetate:propionate ratio. However, the additional labor requirements and/or the expense associated with automated feeding equipment to feed numerous times per day may not prove feasible economically. Thus, it becomes necessary to supplement the diet with buffers to neutralize ruminal acid.

The addition of dietary buffers may allow twice daily feeding while minimizing the deleterious effects associated with fluctuations in ruminal fluid pH. Maximum

ruminal cellulolytic activity occurs between pH 6.4 and 6.8 (18). Feeding high concentrate diets may depress ruminal pH to 6.0 or less, depressing fiber digestion, decreasing passage rate and reducing dry matter intake. *Streptococcus bovis* is one of the major lactic acid producers in the rumen. Because the growth rate of *Streptococcus bovis* is slowed above pH 6, maintaining ruminal pH above 6 would inhibit the production of lactate (19, 52) and reduces the incidence of acidosis. Abrupt diet alterations may change the ruminal fermentation pattern, affecting ruminal pH, ruminal volatile fatty acid production, and ruminal buffering capacity. During the first few weeks postpartum, it is necessary to rapidly change the diet from high fiber to low fiber to increase the energy density of the diet to meet the energy demands associated with lactation. Addition of dietary buffers may prove beneficial in preventing the depression in ruminal pH and low milk fat percentage and in increasing the dry matter intake during the early postpartum period. The addition of dietary buffers to grass-legume based diets also has increased milk protein concentration (23). Because propionate is positively correlated with milk protein production (61), the addition of dietary buffers to high concentrate diets may increase both milk fat and protein production.

Common Buffering Compounds

Several different feed additives are available to help alleviate the depressed ruminal pH and other problems associated with feeding a high concentrate diet. The addition of $NaHCO_3$ (77), MgO (18), K_2CO_3 (77), Na_2CO_3 (18) , $KHCO_3$ (18, 77), and limestone (31) have increased either ruminal fluid pH, dry matter intake, and/or milk fat percentage. However, all these compounds cannot be classified as buffers according to the criteria defined by Erdman (18). By definition, a true buffer stabilizes

a solution by regulating hydrogen ion concentration. A buffer must be water soluble (18). Limestone and MgO are not readily soluble in water. Further, a buffer must be a weak acid or base or salt thereof (18). To be effective, the buffer's maximum effectiveness or equivalence point (pKa) must be near the physiological pH of the solution being buffered (18). MgO does not have a defined pKa, although it does raise ruminal fluid pH and milk fat percentage (18). Because in vivo studies to determine the buffering efficacy of a compound are costly and time consuming, in vitro procedures are needed to rapidly and accurately screen compounds.

Culture Procedures

Several procedures are available to evaluate ruminal fluid fermentation in vitro. The continuous culture system has received extensive evaluation (11, 19, 70, 77); semicontinuous cultures also have been used (6, 31, 34). These procedures have been used primarily to determine nutrient digestibility, although Keyser et al. (31) used a semicontinuous culture to study the effects of different grades of limestone on ruminal fluid pH. In addition, they (31) used a batch culture approach to evaluate the effects of limestone on changes in ruminal fluid pH during 6 h of incubation. Kone (34) used a semicontinuous culture to investigate the effects of ionophores and isoacids on rumen fermentation. Russell and Hino (52) utilized batch and continuous cultures to investigate the effects of pH on lactate production. Because ruminal fluid pH tends to be lowest 4 to 8 h after feeding, the effects of buffers on ruminal fluid pH should be determined during this period. Although the batch culture approach is less complex than continuous or semicontinuous culture, it appeared to imitate postfeeding ruminal acid concentration reasonably well (31). Nonetheless, results of in vitro measurements of the acid-neutralizing capacity of different limestone sources were not consistent with

results of in vivo studies run concurrently. However, the medium (31) used may not have been similar enough to what the ruminal microorganisms were adjusted to consuming, preventing proper substrate breakdown in the in vitro procedure.

Tucker et al. (63) and Le Ruyet and Tucker (40) used batch cultures to investigate the effects of buffers on ruminal acid-base status. Ruminal fluid was obtained from cows fed typical concentrate:forage diets and was incubated for different lengths of time with different buffer additions. Results from both studies were consistent with in vivo studies utilizing ruminal buffers (62). Thus, the in vitro batch culture procedures yield results similar to in vivo results, but the rumen fluid used for the incubations should come from cows consuming a typical concentrate:forage diet. The impact of different concentrate:forage ratios fed to the cow supplying the rumen fluid still needs to be determined.

The response of in vitro incubations of ruminal fluid to dietary buffers may be affected by the type of diet fed to the cows supplying the ruminal fluid. Herod et al. (26) used a batch culture approach with ruminal fluid from cows fed an all concentrate with or without added alfalfa hay (72:28 ratio, DM basis); this fluid was incubated with ground, extruded, cooked corn grain to evaluate the influence of several buffering compounds on pH and buffering capacity (BC) after 6 h of incubation. They reported that, compared with the grain and hay diet, the low initial pH of ruminal fluid from the all concentrate diet reduced the effectiveness of the buffers. Forages contribute effective fiber to diets, stimulating salivary flow and rumination. The lack of response in alfalfa-based diets to dietary buffers (18) would suggest that buffers need not be included when forages constitute a majority of the diet. Eickelberger et al. (15) reported no advantage of buffer supplementation of diets fed to early lactation cows when high quality alfalfa was the primary forage source. However, the inclusion of $NaHCO_3$ to corn silage based diets increased dry matter intake by .5 kg/d and fat-corrected milk by 1.1 kg/d (18). Well-made corn silage is approximately 50% grain;

this suggests that the fiber contributed by corn silage may be overstated if corn silage is considered 100% roughage. In addition, significant amounts of organic acids are produced in the fermentation and preservation process of silage, and may depress ruminal pH shortly after silage is consumed. Diets containing a large proportion of concentrates tend to depress ruminal fluid pH shortly after consumption, due to rapid fermentation of starch and sugars. High fiber feeds are digested less rapidly and would depress ruminal fluid pH several hours after consumption, if at all.

Buffering Capacity

Buffering capacity (BC) is defined as resistance to change in pH, and is calculated as milliequivalents of acid or base required to change pH from 4 to 9 (18, 29, 78), 5 to 7 (5, 18, 19, 27, 39, 62, 63), or 5.5 to 7 (21). When 30 ml of rumen fluid is used, BC, in liters is calculated as: $BC = [(\text{milliliters of } 1N\ HCl) + (\text{milliliters of } 1N\ NaOH)] \times 10^3/30$. Physiologically, it is more accurate to use pH 5 to 7 because ruminal pH seldom reaches pH extremes of 4 or 9. Ruminants consuming a forage based diet typically have a ruminal pH of 6.5 to 7, whereas ruminants fed a high concentrate diet may have ruminal pH between 5 to 5.5.

In addition to the type of diet fed, individual feedstuffs may have an innate buffering capacity. In a continuous culture system, high protein feeds required less base to maintain pH at 6.5 when compared to concentrate feeds (11). Soybean meal even required addition of acid to maintain pH at 6.5 (11). Ammonia, which is produced during the catabolism of proteins, acts as a base and helps stabilize pH by combining with free hydrogen ions. Jasaitis et al. (29) found that forages and high protein feeds had an inherent buffering capacity (BC) three to fourfold higher than concentrates. Fadel (21) found that forages had a higher inherent buffering capacity

and that heating increased the buffering capacity of feedstuffs; concentrates tended to have the lowest BC. Heating may decrease the digestibility of feeds, reducing the possibility of rapid fermentation and subsequent acid production. Total dietary cations and total dietary ash (21) were correlated with BC of the diet. Herod et al. (26) found that oxides and hydroxides alone or in combination were poor buffers. Even though these compounds prevented a depression in ruminal fluid pH, they raised pH more than one pH unit immediately after being added. Bicarbonate and carbonate were more effective in minimizing the depression in ruminal fluid pH and did not significantly change ruminal fluid pH immediately when added.

Buffer Value Index

Because alterations both in pH and in BC of ruminal fluid are important to the maintenance of a viable ruminal fermentation, Tucker et al. (63) developed a buffer value index (BVI) to evaluate the effects of buffers on ruminal fluid acid-base status; this index is related directly to BC but inversely to hydrogen ion concentration. The formula is: BVI = ((((antilog$_{10}$(-STPH)) - (antilog$_{10}$(-SAPH)))/(antilog$_{10}$(-STPH)) + ((SABC - STBC)/STBC)) x 10) + 100, where STPH = a standard pH of 6, SAPH = the ruminal fluid sample pH, SABC = the ruminal fluid sample BC (milliequivalents per liter), and STBC = a standard BC of 50 meq/L. This index has been used to appraise the acid-base status of diets. Le Ruyet et al. (39) investigated the effects of dietary ADF and BVI of the diet on the acid-base status of the rumen. Ruminal pH has been correlated positively with level of ADF in the diet (18). Le Ruyet et al. (39) noted that increasing the dietary ADF percentage increased ruminal fluid BVI, but a high dietary BVI inexplicably reduced ruminal fluid pH and BVI. Reducing diet ADF by one percentage unit decreases ruminal pH by .0564 (18). Thus,

increasing dietary ADF would increase ruminal fluid BVI. These paradoxical results were encountered with diets containing multiple fiber sources, which may have allowed sifting of ingredients and selective consumption of the diet.

In summary, dietary buffers have shown positive responses in high concentrate diets for dairy cows by neutralizing ruminal acid, and allowing for increased fiber digestion and dry matter intake. Several compounds are used frequently as dietary buffers, but the response of these compounds is influenced by the type of diet consumed by the cow. Attempts have been made to determine whether dietary buffers are needed based on the innate buffering capacity of individual dietary ingredients. A buffer value index was developed to account for simultaneous changes in ruminal fluid pH and buffering capacity. Because of the variability associated with these methods, more research is needed to accurately determine the need for dietary buffers.

Udder Edema

Udder edema, characterized by excessive accumulation of fluid in the intercellular tissue spaces, causes swelling. Udder edema occurs approximately two to three weeks before parturition, peaks at calving (10), and disappears one to three weeks postpartum. Animals afflicted with severe cases of udder edema are more susceptible to mastitis, trauma, teat injury, and shorter herd life due to damage of the udder ligaments. Severe udder edema may rupture the ligaments supporting the udder (13, 45), resulting in a pendulous udder with teats that strut laterally. This poses management problems to the dairyman in attaching the milking unit and in obtaining complete milkout.

Severe prepartum udder edema in heifers may limit genetic improvement in a dairy herd because primiparous cows typically represent superior genetics in a dairy

herd. Severe udder edema is more prevalent in first calf heifers (16, 22, 24, 25). Hayes and Albright (24) indicated higher culling rates for animals exhibiting severe edema, because of mastitis and pendulous udders. As the severity of udder edema increased, the amount of scar tissue also increased; scar tissue may hinder the animal from expressing full genetic potential for milk production. Hence, severe and prolonged udder edema may have a significant negative impact on the profitability and genetic advancement of a dairy enterprise.

Several different management practices have been recommended to control udder edema; these include restricting prepartum grain feeding and salt intake, increasing the amount of exercise, prophylactic treatments such as feeding a negative dietary cation-anion balance, and the use of diuretics. With the new drug residue laws in effect, it is important to limit the use of drugs to treat udder edema and emphasize prophylaxis.

Mechanism of Udder Edema

The formation of lymph is due to an interaction between two forces. The relationship is stated by *Starling's law of ultrafiltration*: $C_{fm} = K_f[(H_c - H_i) - (O_c - O_i)]$, where C_{fm} = rate of fluid movement across the capillary; K_f = filtration coefficient of capillary wall; H_c = capillary hydrostatic pressure; H_i = interstitial fluid hydrostatic pressure; O_c = plasma colloid osmotic pressure; and O_i = interstitial fluid colloid osmotic pressure (56). Hydrostatic pressure in the arterial end of the capillary, due to capillary blood pressure, is offset partially by tissue hydrostatic pressure. Constricting blood flow through either the external pudendal vein or the cranial superficial epigastric vein (milk vein) increases the venous blood pressure within the mammary gland. As capillary pressure exceeds tissue hydrostatic pressure, fluid is

forced into the interstitial spaces. Blood leaves the udder primarily by either the cranial superficial epigastric vein or the external pudendal veins. If blood flow remains constant, constriction of either vein should decrease mammary blood flow and increase capillary hydrostatic pressure. Compared to control cows, udder edema developed earlier and was more severe at parturition in Jersey cows afflicted with rectovaginal constriction (3). A decrease in mammary blood flow at parturition in cows afflicted with rectovaginal constriction was correlated with increased cranial superficial epigastric vein pressure, even though jugular vein pressure was not different from normal cows (3, 71). Mammary blood flow was lower at parturition in Holstein cows with a history of developing udder edema (2).

Capillary osmotic pressure, which partially offsets the capillary hydrostatic pressure, is due primarily to plasma proteins, of which 80% is due to the albumin fraction of the protein. The volume of fluid and the concentration of protein are higher in blood than in the tissue spaces (74). As blood travels from the arterial end to the venous end of the capillary, capillary hydrostatic pressure forces fluid and crystalloids out, which increases the capillary protein concentration and, thus, the capillary osmotic pressure. Capillary hydrostatic pressure is greatly reduced at the venous end, even though the tissue hydrostatic pressure remains the same. The increased capillary osmotic pressure, coupled with the reduced capillary hydrostatic pressure, causes a net movement of fluid into the bloodstream. If the net filtration of fluid out of the bloodstream exceeds that being reabsorbed, fluid accumulates in the tissue spaces. The excess fluid not absorbed by the lymph system results in edema.

Rating Systems

Edema tends to develop in a specific pattern, settling first around the base of the udder, and then extending ventrally along the abdominal wall until it reaches the brisket. It also develops vertically until it reaches the thighs and vulva (severe edema). The scale typically utilized has been one (no edema) to five (very severe edema) (2, 10, 12, 13, 22, 25, 36, 50, 53, 58, 76). A scale of zero (none) to four (extreme) was utilized by Emery et al. (16), while a ten point system was utilized by Malven et al. (44). Because of the variation within these scoring systems and their subjective nature, Tucker et al. (65) statistically evaluated the precision and accuracy of a ten point rating system (0 = no edema, 10 = severe edema).

Effects of Grain Feeding

Heifers that calve at approximately 24 months of age are not physically mature and need additional nutrients for growth as well as for milk production. To allow for growth of first and second lactation cows, maintenance allowances are increased by 20 and 10%, respectively (47). Yet, dairymen are reluctant to increase the amount of concentrate fed to heifers, because they believed feeding grain during the prepartum period will increase the incidence and severity of udder edema.

Several studies have been conducted to investigate the effects of prepartum grain feeding on the incidence and severity of udder edema. Schmidt and Schultz (57) fed three levels of grain during an 8-wk dry period to Holstein, Jersey, Guernsey, and Brown Swiss cows. All cows were fed 12 to 13 kg corn silage daily and were given free choice access to good quality mixed hay. A 16% crude protein concentrate, consisting of ground oats, ground corn, wheat bran, corn gluten feed and soybean oil

meal, was fed at either 0, 2.7, or 6.8 kg/cow daily. High levels of concentrate were not correlated with higher edema scores, but udder edema ratings and production were positively correlated.

Hemken et al. (25) fed two different concentrate mixes to cows and heifers beginning 40 to 50 days before calving. Concentrate A was an 18.7% crude protein mix with 1.71 Mcal NE_L/kg, consisting of ground oats, wheat bran, linseed oil meal and molasses. Concentrate B contained 18.7% crude protein and 1.93 Mcal NE_L/kg; this mix consisted of corn and cob meal and soybean oil meal. Groups I and II were fed concentrate A at 2.7 and 7.3 to 8.2 kg/d, respectively, while group III was fed 7.3 to 8.2 kg/d of concentrate B. The only significant difference was higher edema ratings for heifers than for cows.

Greenhalgh and Gardner (22) fed cows and heifers a basal ration consisting of corn silage and alfalfa hay, supplemented with a 15.4% crude protein grain mix consisting of yellow shelled corn, oats, wheat bran, soybean meal, linseed meal, steamed bone meal, and trace mineralized salt. Cows supplemented with an average of 4.1 kg/d of grain during the 6 wk prepartum period did not exhibit significantly higher edema scores than cows receiving no grain during the prepartum period. Heifers supplemented with an average of 2.7 kg/d of grain for 6 wk prepartum exhibited more edema than cows, but they did not exhibit more edema than heifers receiving no grain during the prepartum period.

Emery et al. (16) fed two levels (none vs some) of grain to cows and heifers beginning 21 days prepartum. The grain mix consisted primarily of ground corn, oats, soybean oil meal, and a trace mineralized salt mix. Even though udder edema scores were higher in heifers consuming grain prepartum, the results are difficult to separate from the increased production also noticed from supplemented heifers. High pedigree heifers had 11% more cases of edema than heifers selected for low genetic potential (16); this supports work by Shanks et al. (58). In contrast, Wautlet et al. (76) reported

no difference in severity of udder edema between Holstein heifers selected for low vs high milk production. Emery et al. (16) suggested that the increased nutrient supply raised the intramammary pressure sufficiently to hinder venous and lymphatic drainage, which results in the formation of edema.

Due to the differences in diets fed in the individual trials, significant trends in udder edema due to prepartum grain feeding are difficult to detect. The studies reported above disprove the theory that grain feeding for any length during the prepartum period will consistently increase the incidence and severity of udder edema in primiparous and multiparous cows. These studies do, however, show a trend towards increased udder edema in primiparous heifers vs multiparous cows.

Season

Hayes and Albright (24) and Conway et al. (10) reported that edema was more severe in fall and winter months. Animals typically are more confined during winter months and, thus, exercise less. Because the walls of lymph vessels are thin, lymph movement is aided by muscle contractions, the mechanical action of the visceral organs, and by the skin. Lamb et al. (36) reported that prepartum udder edema scores were decreased in heifers exercised 1.6 km/d, 5 d/wk for 4 wk prepartum. However, edema scores at calving and duration of postpartum edema was not affected. Dentine and McDaniel (12), Wautlet et al. (76), Erb and Grohn (17), and Malven et al. (44) reported no differences in udder edema due to seasonal effects.

Age at First Calving

Age at first calving has been correlated positively with severity of udder edema. Hayes and Albright (24) reported an increase of 152% in reported cases of severe edema as age at first calving increased beyond 26 months in Holsteins and Guernseys. This is supported by Malven et al. (44) and Dentine and McDaniel (12) who noticed increased edema severity in Holstein heifers as age at first calving increased. Heifers that calve at 24 mo would tend to be smaller and exhibit less udder edema than heifers that calve at an older age. However, Wautlet et al. (76) reported that udder edema was more severe in smaller animals. Breeding for smaller body size generally is not practiced in the dairy industry, but the genetic study (76) suggests that direct selection for smaller size might increase the incidence of severe edema.

Length of Gestation

Malven et al. (44) and Wautlet et al. (76) reported that edema was less prevalent as calf size increased in larger heifers. However, udder edema was more severe in smaller heifers who gave birth to larger calves. Longer gestation also is associated with increased udder edema (44); this seems contradictory because heavier birth weight usually is associated with longer gestation length. Malven et al. (44) noticed a positive relationship between udder edema and plasma estrone and estradiol-17α; however, the relationship was negative for plasma estradiol-17β and progesterone. Plasma prolactin was not related to edema score.

Protein Replacement

Feeding colostrum is an excellent method of passively transferring antibodies to the newborn calf. Colostrum contains 14% protein and 6% immunoglobulins whereas whole milk contains only 3.1 and .09%, respectively. Larson and Hays (37) reported a drop in blood protein concentration near the time of parturition, due chiefly to reduced amounts of β_2 and γ_1 globulins. This is supported by other studies (38, 42). A decrease in blood protein concentration would increase the difference between the blood hydrostatic pressure and blood osmotic pressure, resulting in an increased flow of water and crystalloids into the interstitial fluid. By intravenously injecting bovine serum albumin, they increased blood osmolality and slightly decreased udder edema. However, one animal died due to respiratory difficulties associated with the treatment; the cost of the blood protein replacements makes this treatment approach impractical.

Vestweber and Al-Ani (74) measured serum and interstitial fluid concentrations of total protein, albumin, globulin, sodium, potassium, chloride, calcium, and inorganic phosphorous and compared concentrations of these respective constituents between cows with or without udder edema. In contrast to results of Larson and Hayes (37), serum concentrations of total protein, albumin, globulin, and calcium were not significantly different between control and affected cows two weeks before parturition, at parturition, or two weeks postpartum. Interstitial fluid concentrations of total protein, albumin, globulin, and calcium were significantly lower than the serum concentrations, however. Differences between serum and interstitial concentrations of sodium, potassium, chloride, and inorganic phosphorus were not significant. Lymph is similar to blood except that it contains no red blood cells and only one-half the protein. Lymph protein content is related inversely to the rate of lymph flow.

Mineral Interactions

The addition of sodium, chloride, and potassium to prepartum diets has been suggested to cause udder edema (4, 10, 28, 48, 50, 54). Increasing the sodium content of the diet increases excretion of sodium and plasma volume. The increased plasma volume would decrease plasma osmotic pressure and increase plasma hydrostatic pressure, thus increasing extracellular fluid. However, Vestweber et al. (72) suggested that excessive NaCl is not a major factor in udder edema. If increased dietary concentrations of these nutrients were responsible for increased likelihood of udder edema, increased excretion of these specific nutrients would be detected as the body attempted to equalize intake and excretion of sodium. Because renal clearance of creatinine is quite constant, comparing renal clearance of electrolytes and creatinine should reflect renal absorption or secretion of the electrolyte. Renal clearance of sodium and chloride was not significantly different between control cows and cows affected with udder edema.

The dietary cation-anion balance is a relatively new concept in the field of nutrition. It is calculated by subtracting anion milliequivalents (meq) from cationic milliequivalents. Cation-anion balance equals meq ((Na + K) - (Cl + S)) per 100 g diet DM (66). Feeding negative DCAB diets for an extended period of time would tend to produce a metabolic acidosis (75). As blood pH decreases, H^+ concentration increases. Blood HCO_3^- combines with excess H^+ in a natural buffering response. Tucker et al. (67, 68) reported that HCO_3^- decreased as dietary $CaCl_2$ was increased from 1.0 to 1.5%. As available HCO_3^- decreases, the respiratory and renal systems attempt to minimize the change in the pCO_2/HCO_3^- ratio by increasing the respiration rate, thereby decreasing pCO_2 (51). In a natural buffering response, calcium is released from bone, increasing the pool of available calcium. In order to maintain

plasma calcium, less of the filtered calcium is reabsorbed, resulting in increased urine calcium output.

Calcium chloride has recently been investigated as a method of reducing edema, increasing feed intake, and improving milk yield. In addition to mobilizing calcium, a low DCAB diet increases calcium absorption from the intestine (43), which may be beneficial in preventing parturient paresis. Kiess et al. (32) fed primiparous Holstein heifers different amounts of chloride and sodium. Chloride, supplemented at four times the required dietary DM concentration (47) as calcium or ammonium salts without added sodium, lowered the dietary DCAB by twenty eight meq/100 g diet DM. Sodium, supplemented also at four times the required dietary DM concentration (47) without added chloride, raised the DCAB by thirty meq/100 g diet DM. The low DCAB did not affect plasma concentrations of calcium, magnesium, potassium or sodium (32). Feeding a low DCAB diet prepartum resulted in more rapid regression of udder edema postpartum (69). Although they (32) did not measure urinary mineral concentrations to detect an increase in the readily available pool of calcium, urinary calcium concentration needs to be measured. Neither Tucker et al. (69) or Kiess et al. (32) rated the severity of udder edema daily. Instead, Kiess et al. (32) measured the decrease in udder floor after removal of 12 h of milk. Because the relationship between udder floor area and edema has not been established, no direct effects of feeding a low DCAB on udder edema could be determined.

Lema et al. (41) fed heifers a diet containing 1.5% $CaCl_2$ (DM basis) for three wk prepartum. Udder edema was scored daily on a ten point scale (65). While no blood and urine samples were taken, they detected a slight decrease in udder edema during the first week of feeding $CaCl_2$. Calcium is excreted by the kidneys in a manner similar to sodium (35); i.e., calcium excretion increases urine volume. Feeding $CaCl_2$ initially should increase plasma calcium concentrations, resulting in a decrease in parathormone (35). Renal excretion of calcium should increase (67, 68),

increasing urine volume and decreasing plasma volume. Capillary hydrostatic pressure would decrease and plasma osmotic pressure would increase, resulting in net absorption of interstitial fluid and a decrease in edema. If the reduction in udder edema due to feeding $CaCl_2$ lasts for only a few days, feeding for a short period of time and then removing and refeeding it may be a better approach to reduce udder edema than feeding $CaCl_2$ continuously for the entire three week prepartum period.

Effects of a negative DCAB on dry matter intake have been difficult to interpret. Feeding a negative DCAB diet during the postpartum period to lactating cows decreased DMI 7% when compared to a positive DCAB (64), while feeding a negative DCAB diet during the prepartum period to heifers decreased DMI 9%. Other studies (7, 49) have reported no decrease in DMI during the prepartum period due to feeding a negative DCAB. Depression of DMI with a negative DCAB may be associated with palatability problems. Removal of the acidic portion of the diet would increase the palatability of the diet and increase DMI (41).

The effects of dietary cation-anion balance on milk production have been variable. A positive DCAB increased actual milk production 8% compared to a negative DCAB (64). Milk production of heifers fed a negative DCAB during the prepartum period was lower than heifers fed a positive DCAB during the prepartum period (41). In a study investigating the effects of DCAB on the occurrence of milk fever (7), cows fed a positive DCAB during the prepartum period produced less milk during the next lactation than did cows fed a negative DCAB during the prepartum period. This effect was attributable to the fact that cows consuming the positive DCAB during the prepartum period had a higher incidence of milk fever. In herds where milk fever is not a problem, the potential positive effects of increased postpartum DMI may outweigh the decreased milk production from feeding a negative DCAB during the prepartum period.

Treatment

The new drug residue laws in effect for milk increase the need for alternative therapies which do not involve the use of antibiotics. Massaging the udder and alternately applying hot and cold packs help to stimulate blood flow. The physical action of massaging the udder forces the lymph fluid towards the supra mammary lymph gland. Udder supports help protect the udder from abrasions, reduce the possibility of teat injury, and help ease the strain on the supporting ligaments of the udder. Theoretically, prepartum milking would ease the strain on the ligaments and stimulate mammary blood flow. However, this approach has not proven to be beneficial (1).

Diuretics are not antibiotics and have been used to treat severe, prolonged cases of udder edema. These drugs act on the proximal tubule and the distal nephron, disrupting the absorption of crystalloids and water. Furosemides inhibit the absorption of Na^+ and Cl^- by the thick ascending limb of the Loop of Henle, thus preventing water from being absorbed. Vestweber et al. (73) detected a decrease in the cranial superficial epigastric venous blood pressure within 5 min after IV administration of 500 mg of furosemide. Serum calcium and sodium increased while serum potassium decreased. A decrease in urine pH was associated with an increase in urine chloride, potassium, and sodium.

Thiazide diuretics, of which hydrochlorothiazide is a member, inhibit the Na^+-Cl^- symport in the distal nephron, increasing the Na^+ load to the collecting duct. Increased plasma aldosterone levels, stimulated by decreased plasma Na^+, increase K^+ secretion in the collecting duct, which may lead to hypokalemia (35). Because of the high electrical resistance and the tight junctions in the collecting duct, reabsorption of chloride becomes hindered. Administering 250 mg of hydrochlorothiazide caused a

mean increase in serum chloride while decreasing serum potassium and sodium. Urine chloride, potassium, and sodium increased, while urine pH decreased (72).

Acetazolamide inhibits the enzyme carbonic anhydrase, which catalyzes the hydration of CO_2. It also inhibits Na^+ reabsorption in the proximal tubule (35), which increases the delivery of Na^+ to the distal nephron. Vestweber et al. (72) administered 500 mg of acetazolamide IV and noticed increases in serum chloride and urine sodium, whereas serum potassium, sodium, and phosphorous concentrations decreased.

Summary

The exact mechanism of udder edema development is not fully understood. The common belief that prepartum grain feeding increases the severity of udder edema has been disproved. Restriction of mammary blood flow and the subsequent effects on hydrostatic and osmotic pressure can partially explain udder edema. Salt has also been implicated, but recent results suggest that salt is not a major cause of udder edema. Feeding a negative dietary cation-anion balance has shown promise in reducing udder edema, increasing dry matter intake, and increasing milk production. Even though diuretics have proven repeatedly to increase urinary output, metabolic disturbances associated with diuretics require close supervision, and these drugs should be used for only short time periods.

CHAPTER III

EVALUATION OF BATCH CULTURE APPROACHES THAT CAN BE USED TO SCREEN RELEASE RATES OF RUMINAL BUFFERS

Abstract

Our objectives were to evaluate in vitro characteristics of ruminal fluid from several diets and from several substrates that can be used to evaluate release rates of ruminal buffers. Ruminal fluid was collected from three cows fed diets of concentrate and sorghum silage in three ratios: 70:30, 60:40, and 50:50 (DM basis). Ruminal fluid was incubated in a shaking water bath with either purified corn starch, the same grain mix that was fed to the cow providing the ruminal fluid for incubation, or the TMR fed to the cow providing the ruminal fluid. In addition to this substrate, incubation flasks received either .5 g of a 2:1 mixture of $NaHCO_3$ and MgO or no buffer. A flask representing each substrate and buffer combination was removed every hour for 5 h and analyzed for pH, buffering capacity, buffer value index, and VFA content. Each of the substrates yielded temporal alterations in ruminal fluid acid-base status similar to those observed previously in vivo. However, because ruminal fluid acidity tended to develop more rapidly with TMR as a substrate, we recommend that TMR should be used as the substrate in a batch culture approach to evaluate release rates of ruminal buffers. Addition of .5 g of a 2:1 mixture of $NaHCO_3$ and MgO

yielded an increase in ruminal fluid pH similar to that observed previously in vivo. Ruminal fluid acidity was highest for fluid from cows consuming the 70:30 diet; however, the high gas content of this fluid prevented accurate volume measurement for the incubations. Based on handling characteristics and temporal acid generation, we suggest that donor cows be fed a 60:40 grain to forage ratio to provide ruminal fluid for batch culture incubation; a combination of 75 ml of this fluid, .5 g of this TMR, and .5 g of the test buffer provided a model acceptable for rapidly screening the release rates of ruminal buffers in vitro.

Introduction

Several procedures are available to evaluate ruminal fluid fermentation in vitro. The continuous culture system has received extensive evaluation (11, 70); semicontinuous cultures also have been used (6). These procedures were used primarily to determine nutrient digestibility, although Keyser et al. (31) used a semicontinuous culture to study the effects of different grades of limestone on ruminal fluid pH. In addition, they (31) used a batch culture approach to evaluate the effects of limestone on changes in ruminal fluid pH during 6 h of incubation. The batch culture approach is less complex than continuous or semicontinuous culture, but it appeared to imitate postfeeding ruminal acid concentration reasonably well (31). Nonetheless, in vitro measurements of the acid-neutralizing capacity of different limestone sources were not consistent with in vivo studies run concurrently.

Herod et al. (26) used a batch culture approach with ruminal fluid from cows fed either an all concentrate or a concentrate plus alfalfa hay diet (72:28 ratio, DM basis); this fluid was incubated with ground, extruded, cooked corn grain to evaluate the influence of several buffering compounds on pH and buffering capacity (BC) after

6 h of incubation. They reported that, compared with the grain and hay diet, the low initial pH of ruminal fluid from the all concentrate diet reduced the effectiveness of the buffers.

Because alterations both in pH and in BC of ruminal fluid are important to the maintenance of a viable ruminal fermentation, Tucker et al. (63) developed a buffer value index (BVI) that simultaneously accounts for changes in both pH and BC. The objective of the present study was to evaluate batch culture procedures for rapidly screening the release rates of ruminal buffers. The influence of substrate and of ruminal fluid source (high or low concentrate diet) on these release rates was examined. An additional objective was to utilize comparative analysis of temporal alterations in pH, BC, BVI and VFA production to determine the procedure that imitates most closely the changes observed postfeeding for these variables in vivo. The purpose for developing this procedure was to allow rapid evaluation of release rates of different buffering compounds, enhancing our efforts to develop a controlled release buffer that releases its buffering capacity and acid-neutralizing potential during the interval postfeeding in which ruminal fluid acidity is highest.

Materials and Methods

Animals, Feeding, and Experimental Design

Three ruminally cannulated Holstein cows (18 $\pm$ 10 DIM) were fed three diets in a 3 x 3 Latin square study with 3-wk experimental periods to provide ruminal fluid to evaluate in vitro systems designed to screen rapidly the release rates of ruminal buffers. Cows were housed individually in pens and had free access to dirt exercise lots; cows were milked twice per day (0300 and 1500 h). The three different TMR

(Table 1) contained concentrate and sorghum silage (70:30, 60:40, or 50:50 ratio, DM basis), and cows were fed twice daily (0550 and 1750 h). Orts were recorded daily. Samples of the TMR were collected weekly and frozen for nutrient analyses by a commercial laboratory (Northeast DHIA, Ithaca, NY). Dry matter content of the sorghum silage, determined weekly via toluene distillation, was used to maintain a constant ratio of ingredients and nutrients in dietary DM.

Sample Collection, Incubation, and Analysis

Beginning 3 d before the end of each experimental period, ruminal fluid was collected from one of the three cows each day. Ruminal fluid (4 L) was collected from the ventral sac of the rumen with an electric vacuum pump 2 h after the 0550-h feeding; the fluid was filtered through four layers of cheesecloth and collected in a 4-L Erlenmeyer flask. Treatments were fermentation additions added in a 2 x 4 factorial arrangement. These factors were the amount of buffer added (0 or .5 g of a 2:1, weight/weight, mixture of $NaHCO_3$ and MgO) and the type of substrate added to the fluid (no substrate, .5 g of purified corn starch, .5 g of the grain mix consumed by the cow from which ruminal fluid was collected, or .5 g of the TMR consumed by the cow from which ruminal fluid was collected).

At the beginning of the study, representative samples of the three grain mixes and the three TMR were collected. These grain mixes were dried for 48 h at 60°C. The TMR samples were lyophilized. After drying, the samples were ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to pass through a 1-mm screen and stored in a desiccator until used.

Ruminal fluid (75 ml) was dispensed into each of six, 125-ml Erlenmeyer flasks; each of these six flasks contained the same substrate and buffer combination.

TABLE 1

INGREDIENT AND NUTRIENT COMPOSITION OF EXPERIMENTAL DIETS (DM BASIS) FOR COWS SUPPLYING RUMINAL FLUID FOR IN VITRO INCUBATIONS

	Grain:forage ratio		
	50:50	60:40	70:30
		(%)	
Ingredient			
Forage sorghum silage	50.01	40.03	30.02
Ground shelled corn	23.44	34.45	44.66
Soybean meal, 44% CP	23.95	22.87	22.58
Limestone	.75	.91	1.06
Dicalcium phosphate	.91	.88	.82
Dynamate®[a]	.39	.36	.41
Trace-mineralized salt	.53	.49	.43
Vitamin A premix[b]	.01	.01	.01
Vitamin E premix[c]	.01	.01	.01
Nutrient			
DM, %	38.8	40.9	48.0
CP, %	14.6	16.0	16.5
NE_L, Mcal/kg	1.43	1.58	1.65
Ca, %	.59	.73	.75
P, %	.32	.46	.47
Mg, %	.32	.31	.31
S, %	.26	.28	.30
K, %	1.31	1.27	1.22
Na, %	.20	.20	.20

[a]Double sulfate of K and Mg, Pitman-Moore, Inc., Mundelein, IL.
[b]Supplied 30,000,000 IU of vitamin A / kg of premix.
[c]Supplied 500,000 IU of vitamin E / kg of premix.

One of the six flasks was analyzed immediately for pH and BC; the remaining flasks were incubated in a shaking water bath at 39°C. An incubation was begun every 7.5 min until each of the eight buffer and substrate combinations was added to the water baths. A flask representing each substrate and buffer combination was removed each 60 min for 5 h after incubation was initiated. A 5-ml aliquot of ruminal fluid, dispensed into a polyethylene snap-cap tube containing 50 mg of crystalline metaphosphoric acid, was frozen for VFA analysis by GLC (AutoSystem GC; Perkin-Elmer, Norwalk, CT). A capillary column (.53 mm) coated with acidified polyethylene glycol was utilized for the analysis; He served as the carrier gas. Ruminal fluid pH (model 950 pH-ion analyzer; Fisher Scientific, Pittsburgh, PA) was recorded following 30 s of equilibration. Buffering capacity, defined as the resistance to change in pH from pH 7 to 5, was determined by titrating a 30-ml aliquot of ruminal fluid with continuous stirring from its initial pH to a pH of 5 with 1*N* HCl and titrating a second 30-ml aliquot from its initial pH to a pH of 7 with 1*N* NaOH. When the initial pH was higher than 7, we recorded only the volume of acid required to reduce the pH from 7 to 5. Buffering capacity was converted to milliequivalents per liter as follows: BC = ((milliliters of 1*N* HCl) + (milliliters of 1*N* NaOH)) x $10^3/30$. The BVI of the ruminal fluid was calculated according to the formula of Tucker et al. (63) as: BVI = ((((antilog$_{10}$(-STPH)) - (antilog$_{10}$(-SAPH))) / (antilog$_{10}$(-STPH)) + ((SABC - STBC) / STBC)) x 10) + 100, where STPH = a standard pH of 6, SAPH = the ruminal fluid sample pH, SABC = the ruminal fluid sample BC (milliequivalents per liter) and STBC = a standard BC of 50 meq/L.

Statistical Analysis

Data were analyzed via SAS General Linear Models ANOVA (55) with the following model:

$$Y = \mu + C_h + P_i + D_j + CD_{hj} + B_k + S_l + I_m + BS_{kl} + BI_{km} + SI_{lm} + DS_{jl} + DI_{jm} + DB_{jk} + E_{hijklm},$$

where

Y = dependent variable,

μ = mean,

C = cow (h = 1, 2, 3),

P = period (i = 1, 2, 3),

D = diet (j = 1, 2, 3),

B = buffer (k = 0, 1),

S = substrate (l = 0, 1, 2, 3),

I = incubation interval (m = 0, 1, 2, 3, 4, 5), and

E = residual error.

The test term for cow, period, and diet was the cow by diet interaction; buffer, substrate, incubation interval, and their interactions were tested using the residual error. Statistical significance was declared at $P < .05$ unless noted otherwise.

Results and Discussion

Acid-Base Status

Diet Effects. Although the mean effect of diets throughout the incubation interval was not significant, the diet by incubation interval interaction was an important

source of variation for ruminal fluid H^+ and BVI (Table 2). Acid content of the ruminal fluid increased during incubation, presumably as a result of substrate fermentation. Ruminal fluid acidity increased sharply for fluid from cows consuming a 70:30 grain to forage ratio (Figure 1); temporal changes for the 50:50 and 60:40 diets were more moderate. Diet by incubation interval variation was not significant for ruminal fluid BC (Table 2, Figure 1). Because BC was similar for fluid from the three diets, changes in ruminal fluid BVI (Table 2, Figure 1) were dictated primarily by changes in ruminal fluid H^+; BVI dropped more sharply with incubation of fluid from cows fed the 70:30 diet than from cows fed the 60:40 and 50:50 diets.

We intended to characterize the rapidity of fermentation-induced changes in ruminal fluid acid-base status occurring in batch culture for ruminal fluid from low, intermediate, and high grain diets. This information is essential to the selection of appropriate proportions of grain and forage to feed to cows used as sources of ruminal fluid for in vitro evaluation of the release rates of buffers. Dietary buffers have been more beneficial for cows consuming diets with high than with low grain content (18); ideally, the acidity and BC of incubated fluid should follow patterns occurring in cows consuming high grain diets. However, the fluid also should have a low enough gas content to allow accurate measurement of fluid volume. In the present study, ruminal fluid from the 70:30 diet yielded the sharpest increase in ruminal fluid acidity upon incubation; however, the high gas content of this fluid caused excessive foaming. When a graduated cylinder was used to measure 75 ml of fluid for filling the batch culture flask, foam interfered with accurate measurement of the fluid. This problem did not occur for fluid from the 60:40 or 50:50 diets. Although not apparent in our study, fluid from cows fed the 60:40 diet should yield more acid than from cows fed the 50:50 diet. Hence, we think that among the diets tested in our study, the 60:40 grain to forage ratio provided the most acceptable combination of handling and

TABLE 2

MEAN SQUARES FOR INDICATORS OF RUMINAL FLUID ACID-BASE STATUS

Source[a]	df	pH		H^+, neq/L		BC[c], meq/L		BVI[d]	
		MS[b]	*P*	MS	*P*	MS	*P*	MS	*P*
Main plots									
Cow	2	3.770	.214	15,647,487	.399	3747	.462	1097	.368
Period	2	.678	.603	5,689,399	.646	1156	.736	507	.557
Diet	2	3.392	.233	23,217,292	.309	183	.946	2359	.213
Cow by diet	2	1.028		10,401,435		3217		638	
Subplots									
Buffer	1	66.550	<.001	238,370,713	<.001	96,128	<.001	46,380	<.001
Substrate	3	.622	<.001	2,849,087	<.001	205	.014	278	<.001
Incubation interval	5	.684	<.001	6,760,557	<.001	73	.273	601	<.001
Buffer by substrate	3	.014	.333	1,465,463	<.001	108	.130	106	<.001
Buffer by incubation interval	5	.211	<.001	4,504,726	<.001	265	<.001	333	<.001
Substrate by incubation interval	15	.038	<.001	168,895	.577	40	.787	19	.391
Diet by substrate	6	.038	.005	238,085	.278	20	.909	25	.222
Diet by incubation interval	10	.017	.159	389,430	.028	64	.343	44	.008
Diet by buffer	2	.119	<.001	13,075,054	<.001	652	<.001	996	<.001
Residual	373	.012		189,982		57		18	

[a]Main plot variables (cow, period, and diet) were tested against cow by diet interaction; all other variables were tested against residual error.
[b]Type I mean squares.
[c]BC = buffering capacity.
[d]BVI = buffer value index.

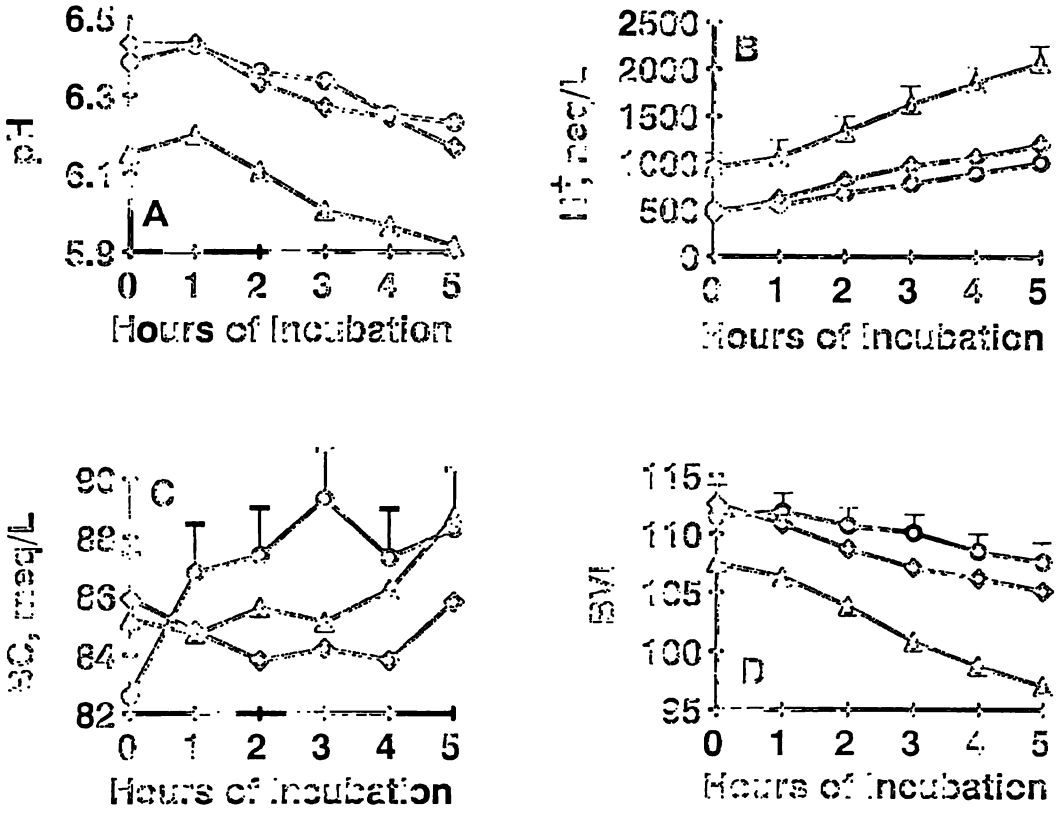

Figure 1. Ruminal Fluid pH (A), H+ (B), Buffering Capacity (BC) (C), and Buffer Value Index (BVI) (D) During 5 h of Incubation with Different Diets. o = Ruminal Fluid from Cows Fed a 50:50 Grain to Forage Diet (DM Basis), ◇= Ruminal fluid from Cows Fed a 60:40 Diet, and △= Ruminal Fluid from Cows Fed a 70:30 Diet. Vertical Bars Represent Standard Errors.

fermentation characteristics for evaluating temporal release rates of buffers in batch culture.

Buffer Effects. Addition of .5 g of buffer to our batch culture flasks dramatically affected all measures of ruminal fluid acid-base status (Table 2). However, several buffer interactions were evident. A buffer by incubation interval interaction was identifiable for all measures of acid-base status. Ruminal fluid acidity increased sharply during incubation for unbuffered flasks, but it was lower and fairly stable with buffer addition (Figure 2). Herod et al. (26) reported that a 2:1 mixture of $NaHCO_3$ and MgO increased ruminal fluid pH in batch culture by 2 to 3 pH units; our increase was more moderate, ranging from .5 to .9 units throughout the incubation interval. Our batch cultures provided a consistent increase in ruminal fluid acidity from 0 to 5 h of incubation in the unbuffered flasks. This pattern is similar to that observed in vivo; Erdman (18) reported that ruminal fluid acidity typically is highest from 4 to 8 h postfeeding.

Ruminal fluid BC (Figure 2) was stable for buffered flasks but tended to increase with incubation for unbuffered flasks. The reason for this increase is not clear, but it may be related to the increase in VFA concentrations; VFA provide some BC at a pH of 5. Ruminal fluid BVI (Figure 2) decreased by 13 units for unbuffered flasks during incubation, but it dropped by only 2 units when buffer was present. Increased stability of ruminal fluid BVI with dietary buffers also was observed in vivo (62). In the present study, all indicators of acid-base status in buffered flasks were remarkably constant during incubation.

Diet by buffer interaction was significant for all measures of acid-base status (Table 2). Ruminal fluid acidity (Figure 3) increased sharply for unbuffered flasks as the grain to forage ratio fed to the fluid source cows was increased from 60:40 to 70:30; the acidity increase for the corresponding ratios in the buffered flasks was less dramatic. Ruminal fluid BC (Figure 3) for unbuffered flasks was highest for 70:30,

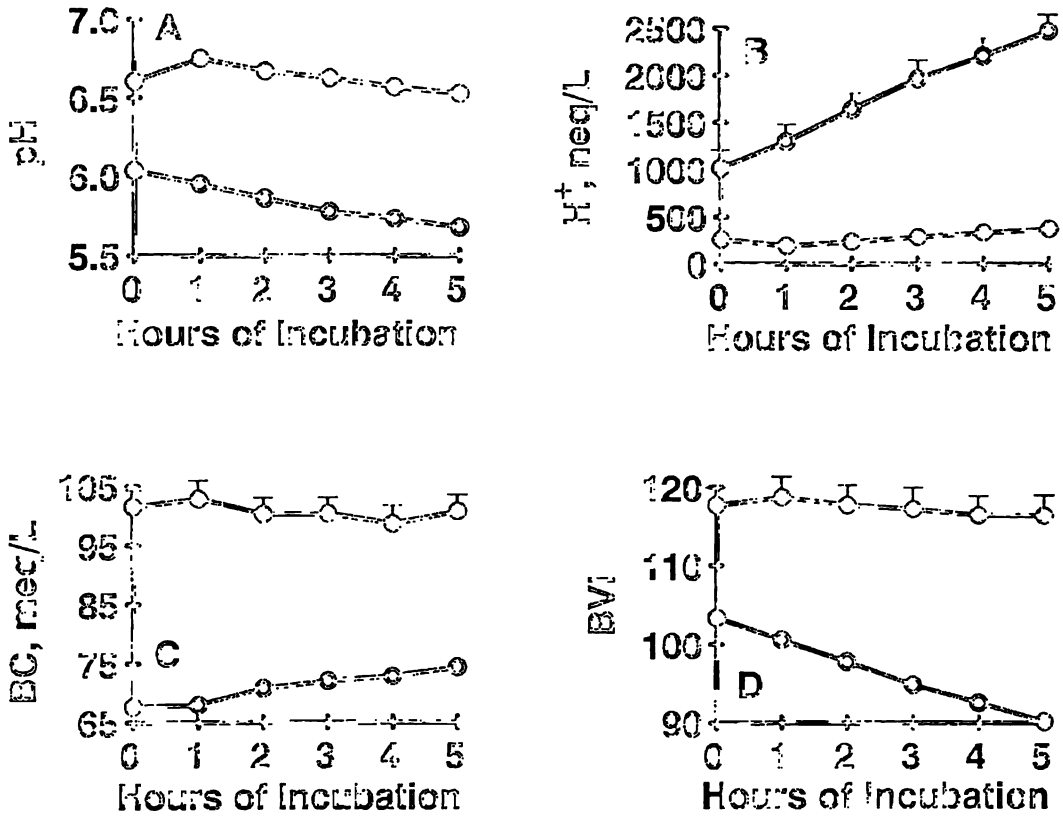

Figure 2. Ruminal Fluid pH (A), H+ (B). Buffering Capacity (BC) (C), and Buffer Value Index (BVI) (D) During 5 h of Incubation with Different Buffer Additions. o = Unbuffered Ruminal Fluid; o = Ruminal Fluid with 6.7 g of a 2:1 Mixture of NaHCO3 and MgO/L of Fluid. Vertical Bars Represent Standard Errors.

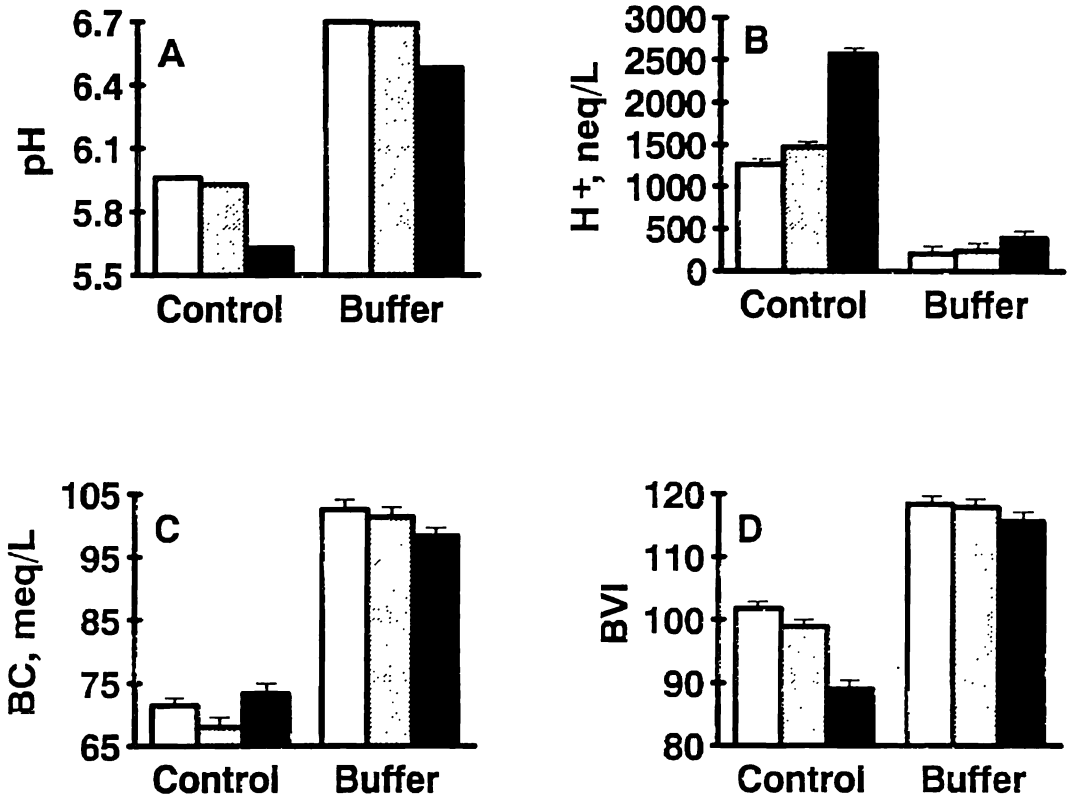

Figure 3. Ruminal Fluid pH (A), H+ (B), Buffering Capacity (BC) (C), Buffer Value Index (BVI) (D) Averaged for Entire Incubation Interval with Different Diets. Open Bar = Ruminal Fluid from Cows Fed a 50:50 Grain to Forage Diet (DM Basis), Shaded Bar = Ruminal Fluid from Cows Fed a 60:40 Diet, and Solid Bar = Ruminal Fluid from Cows Fed a 70:30 Diet. Control = Ruminal Fluid with No Buffer; Buffer = Ruminal Fluid with 6.7 g of a 2:1 Mixture of NaHCO3 and MgO/L of Fluid. Vertical Bars Represent Standard Errors.

intermediate for 50:50, and lowest for 60:40 grain to forage ratios; with buffer addition, BC also tended to decrease with increasing grain in the diet. No explanation for this is apparent. Herod et al. (26) also observed diet by buffer interactions, classifying some compounds as good buffers when incubated with ruminal fluid from a low grain diet, but poor buffers when incubated with fluid from a high grain diet.

Substrate Effects. Acidity at 5 h of incubation was highest for ruminal fluid incubated with TMR, followed by grain, starch, and no substrate (Figure 4). Ruminal fluid BC was approximately 2.5 meq/L lower for starch than for the other substrates (Figure 4). No explanation for this is apparent, but the difference was very small. Ruminal fluid BVI was dictated primarily by differences in H^+ for the four substrates; BVI was highest for no substrate, followed by starch, grain, and TMR (Figure 4).

Although substrate was a significant source of variation for each measure of acid-base status, the buffer by substrate interaction was significant for H^+ and BVI (Table 2, Figure 5). For the unbuffered flasks, ruminal fluid acidity decreased as the similarity of the substrate to the source diet decreased; i.e., acidity was highest for TMR, followed by grain, starch, and no substrate. Addition of buffer to the flasks, however, yielded similar effects on ruminal fluid acidity regardless of substrate employed. This suggests that the acid-neutralizing capacity of the buffer increased with the acid content of the ruminal fluid. Perhaps this is attributable to more thorough dissolution of the MgO portion of the buffer under acidic conditions. The response of ruminal fluid BVI (Figure 5) was opposite that of H^+ for unbuffered flasks, but buffer addition elevated BVI similarly for all flasks, regardless of substrate.

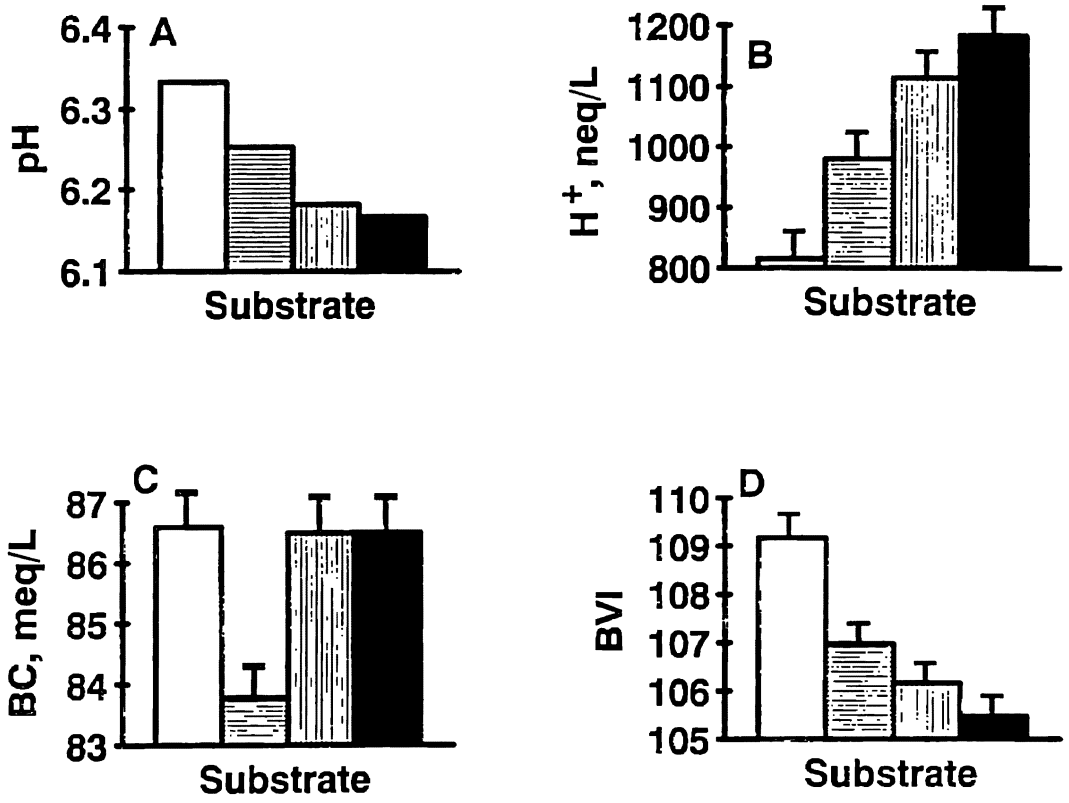

Figure 4. Ruminal Fluid pH (A), H+ (B), Buffering Capacity (BC) (C), and Buffer Value Index (BVI) (D) Averaged for Entire Incubation with Different Substrates. Open Bar = No Substrate, Horizontal Hatch = 6.7 g of Purified Corn Starch/L of Ruminal Fluid, and Vertical Hatch = 6.7 g of Grain Mix/L of Ruminal Fluid; Solid Bar = 6.7 g of TMR/L of Ruminal Fluid. Vertical Bars Represent Standard Errors.

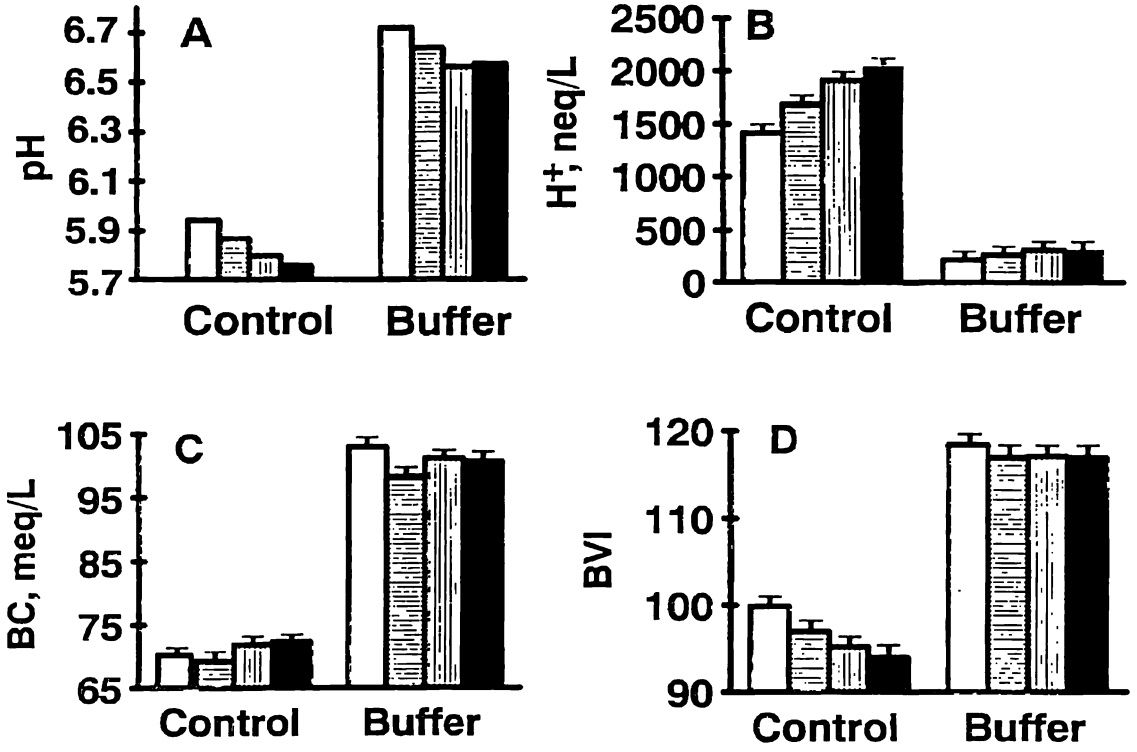

Figure 5. Ruminal Fluid pH (A), H+ (B), Buffering Capacity (BC) (C), and Buffer Value Index (BVI) (D) Averaged for Entire Incubation Interval with Different Substrates and Buffer Additions. Open Bar = No Substrate, Horizontal Hatch = 6.7 g of Purified Corn Starch/L of Ruminal Fluid, Vertical Hatch = 6.7 g of Grain Mix/L of Ruminal Fluid, and Solid Bar = 6.7 g of TMR/L of Ruminal Fluid. Control = Ruminal Fluid with No Buffer; Buffer = Ruminal Fluid with 6.7 g of a 2:1 Mixture of NaHCO3 and MgO/L of Fluid. Vertical Bars Represent Standard Errors.

Volatile Fatty Acids

Diet Effects. Although the effect of diets throughout the incubation interval was not significant, the diet by incubation interval interaction was an important source of variation for all VFA (Table 3). Ruminal fluid acetate was highest for the diet with the 50:50 grain to forage ratio throughout the incubation interval (Figure 6). Acetate is a major end product of cellulose digestion; hence, the high acetate concentration for 50:50 likely is the result of the high fiber content of this diet. The high initial acetate concentration for the 50:50 diet may be attributable to our collection of ruminal fluid from the cow at 2 h postfeeding; this allowed time for fiber fermentation to occur in vivo before we collected the fluid for in vitro incubation. Ruminal fluid propionate (Figure 6) displayed a pattern similar to that of acetate, except that propionate concentration for the 70:30 diet increased to that of the 50:50 diet by 3 h of incubation. Ruminal fluid acetate to propionate (A:P) ratio (Figure 6) was highest for the 50:50 diet from 2 to 5 h of incubation; this response was attributable more to a high acetate concentration for 50:50 than to high propionate concentrations for the 60:40 and 70:30 diets. Total VFA concentration was highest for the 50:50 diet throughout the incubation interval (Figure 6); this was unexpected, and the reason for this is unclear.

Buffer Effects. Mean effects of buffer throughout the incubation interval on VFA were significant only for A:P ratio (Table 3); the A:P ratio was reduced (2.52 vs. 2.57) by addition of buffer to the flasks. Although this reduction was small, it again was opposite to in vivo results in which A:P ratio typically was increased by dietary buffer (18). A diet by buffer interaction was significant for acetate and total VFA (Table 3). Addition of buffer appeared to increase acetate concentration compared with the unbuffered flasks in fermentation with ruminal fluid from cows fed the 50:50 diet but to reduce acetate for fluid from those fed 60:40 and 70:30 diets (Figure 7). This trend also was evident for total VFA (Figure 7); no explanation is available. The

TABLE 3

MEAN SQUARES FOR RUMINAL FLUID VOLATILE FATTY ACIDS

Source[a]	df	Acetate (A) MS[b]	*P*	Propionate (P) MS	*P*	A:P ratio MS	*P*	Total VFA MS	*P*
Main plots									
Cow	2	1,153	.684	83.4	.653	1.1223	.435	3,020.3	.296
Period	2	2,561	.494	848.5	.156	3.0342	.222	8,307.1	.133
Diet	2	10,234	.196	575.1	.215	1.4663	.371	14,733.3	.079
Cow by diet	2	2,497		157.2		.8631		1,269.7	
Subplots									
Buffer	1	179	.094	2.2	.640	.2243	.031	49.4	.559
Substrate	3	241	.011	39.7	.009	.0659	.249	731.7	.002
Incubation interval	5	1263	<.001	230.7	<.001	.0943	.082	4531.8	<.001
Buffer by substrate	3	176	.042	25.5	.060	.0008	.997	463.7	.023
Buffer by incubation interval	5	104	.148	20.3	.080	.0272	.724	299.9	.068
Substrate by incubation interval	15	52	.654	6.6	.833	.0312	.830	117.3	.664
Diet by substrate	6	138	.045	23.6	.034	.0565	.316	415.2	.010
Diet by incubation interval	10	205	<.001	50.2	<.001	.2474	<.001	452.3	.001
Diet by buffer	2	294	.010	18.8	.160	.0055	.891	437.1	.050
Residual	373	64		10.2		.0478		144.4	

[a]Main plot variables (cow, period, and diet) were tested against cow by diet interaction; all other variables were tested against residual error.
[b]Type I mean squares.

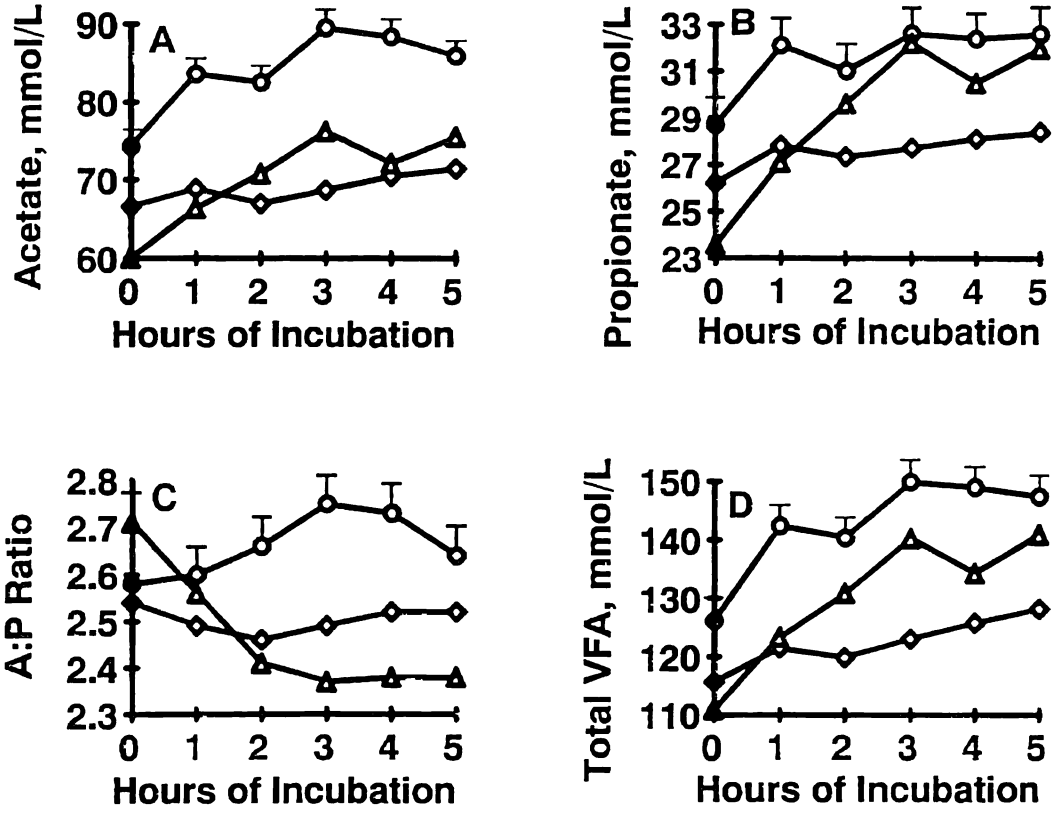

Figure 6. Ruminal Fluid Acetate (A), Propionate (B), Acetate to Propionate (A:P) Ratio (C), and Total VFA (D) During 5 h of Incubation with Different Diets. o = Ruminal Fluid from Cows Fed a 50:50 Grain to Forage Diet (DM Basis), ◇ = Ruminal Fluid from Cows Fed a 60:40 Diet, and △ = Ruminal Fluid from Cows Fed a 70:30 Diet. Vertical Bars Represent Standard Errors.

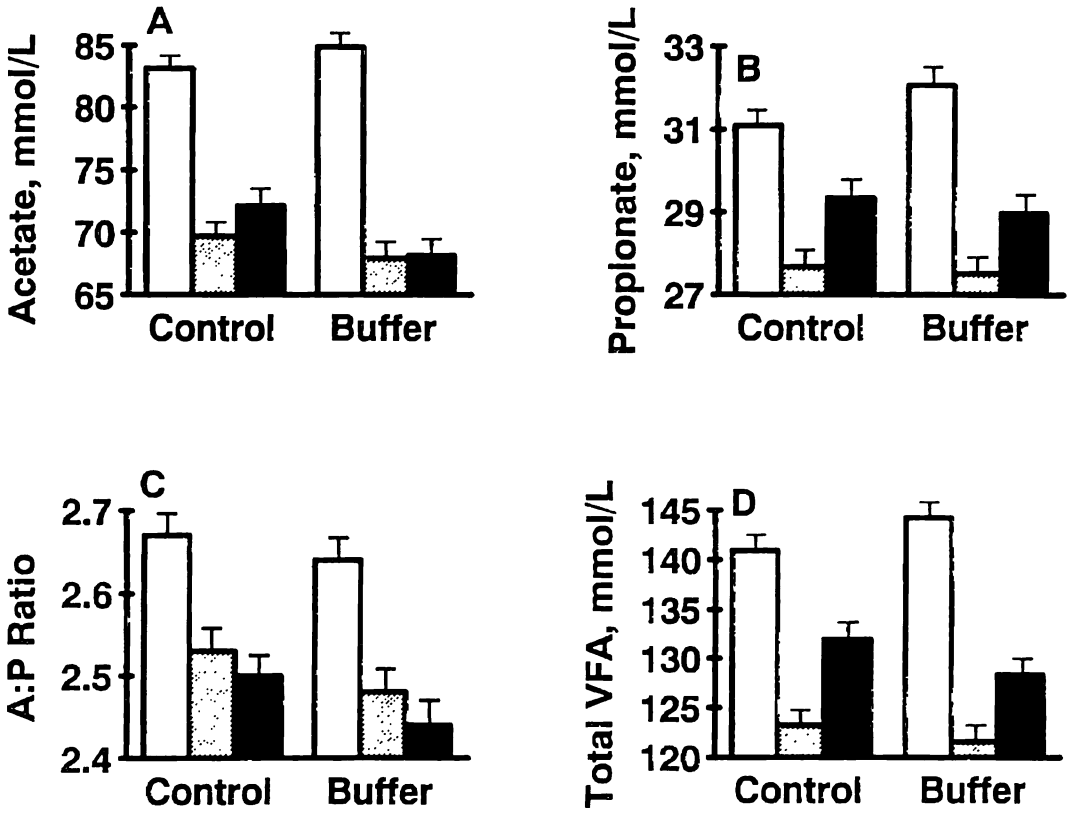

Figure 7. Ruminal Fluid Acetate (A), Propionate (B), Acetate to Propionate (A:P) Ratio (C), and Total VFA (D) Averaged for Entire Incubation Interval with Different Diets. Open Bar = Ruminal Fluid from Cows Fed a 50:50 Grain to Forage Diet (DM Basis), Shaded Bar = Ruminal Fluid from Cows Fed a 60:40 Diet, and Solid Bar = Ruminal Fluid from Cows Fed a 70:30 Diet. Control = Ruminal Fluid with No Buffer; Buffer = Ruminal Fluid with 6.7 g of a 2:1 Mixture of NaHCO3 and MgO/L of Fluid. Vertical Bars Represent Standard Errors.

pattern for propionate was similar to that for total VFA. With or without buffer, the A:P ratio was decreased by a higher proportion of grain in the diet or as a substrate.

Substrate Effects. Our objective was to evaluate substrates differing in degrees of similarity to the diets fed to cows serving as the source of ruminal fluid for our incubations. Substrate was a significant source of variation for all measures of VFA except A:P ratio (Table 3). However, the buffer by substrate interaction (Table 3) was significant for acetate, total VFA, and propionate ($P = .06$). In the unbuffered flasks, addition of starch or grain to the flasks increased VFA content more sharply than for addition of TMR (Figure 8); the opposite was true for the buffered flasks. This conflicts with substrate effects on H^+. No explanation is apparent. All substrates tested in our study readily provided substrate for in vitro ruminal fermentation. However, because the TMR tended to produce the highest concentrations of VFA, we suggest that it should be used for evaluating release rates of buffers in vitro. Fluid containing TMR as a substrate is difficult to pipet unless the TMR has been ground finely enough to pass through a .5 mm screen.

Summary

In summary, .5 g of purified corn starch, .5 g of the grain mix consumed by the cow providing ruminal fluid for incubation, or .5 g of the TMR consumed by the cow providing ruminal fluid for incubation were added to 75 ml of ruminal fluid for batch culture incubation; each of these substrates yielded temporal alterations in acid-base status of ruminal fluid that were similar to those observed in vivo. However, because ruminal fluid acidity tended to develop more rapidly with TMR as a substrate, we recommend that TMR be used as the substrate in a batch culture approach to evaluate ruminal buffers. Addition of .5 g of a 2:1 mixture of $NaHCO_3$ and MgO yielded an

increase in ruminal fluid pH similar to that observed in vivo. Based on a combination of handling characteristics and temporal acid generation, we suggest that donor cows be fed a 60:40 grain to forage ratio to provide ruminal fluid for batch culture evaluations of release rates of ruminal buffers.

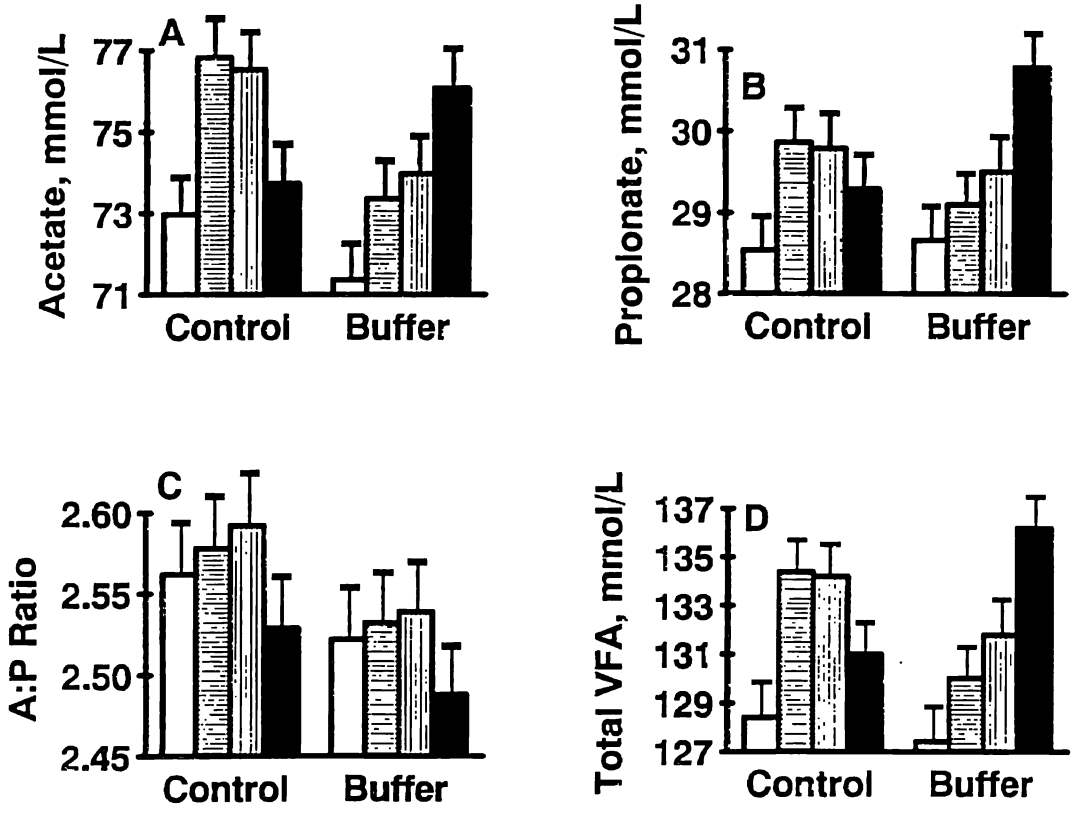

Figure 8. Ruminal Fluid Acetate (A), Propionate (B), Acetate to Propionate (A:P) Ratio (C), and Total VFA (D) Averaged for Entire Incubation Interval with Different Substrates and Buffer Additions. Open Bar = No Substrate, Horizontal Hatch = 6.7 g of Purified Corn Starch/L of Ruminal Fluid; Vertical Hatch = 6.7 g of Grain Mix/L of Ruminal Fluid, and Solid Bar = 6.7 g of TMR Per Liter of Ruminal Fluid. Control = Ruminal Fluid with No Buffer; Buffer = Ruminal Fluid with 6.7 g of 2:1 Mixture of NaHCO3 and MgO/L of Fluid. Vertical Bars Represent Standard Errors.

CHAPTER IV

INFLUENCE OF DIETARY BUFFER VALUE INDEX ON THE RUMINAL MILIEU OF LACTATING DAIRY COWS FED SORGHUM SILAGE AND GRAIN

Abstract

The objective of this study was to evaluate the influence of dietary buffer value index on ruminal fluid pH, buffering capacity, and buffer value index in lactating cows. Three Holstein cows averaging 18 $\pm$ 10 DIM were used in a 3 x 3 Latin square with 3-wk experimental periods. Diets contained grain:sorghum silage ratios of 50:50, 60:40, and 70:30 (DM basis). These by analysis had buffer value indexes of -74, -41, and -7. These values are 250 to 400 units higher than the cumulative buffer value index of individual ingredients. Milk fat content tended to be highest for milk from cows fed the 50:50 concentrate to forage diet; milk protein production was highest for cows fed the 70:30 concentrate to forage diet; milk yield, 4% FCM, milk fat yield, protein content and milk fat content were not affected by dietary buffer value index. Dietary buffer equivalents were calculated to be only 11% of total buffering equivalents available to the cow and dietary acid equivalents were only 15% of total acid production in the rumen. Compared to ruminal acid production and salivary buffering in the rumen, dietary acid and dietary buffer contributions to the acid-base balance of the cow are minor quantitatively. Ruminal fluid pH, hydrogen ion

concentration, buffer value index, buffering capacity and total VFA were not significantly affected by dietary buffer value index. Hence, dietary acid-base status alone appears inadequate as a predictor of the need for adding buffers to the diet of lactating cows.

Introduction

Because the diet may influence the ruminal milieu, recent studies have focused on the effect of acid-base status of the diet on acid-base status in the rumen. Jasaitis et al. (29) found that forages and high protein feeds had inherent buffering capacities (BC) three to fourfold higher than concentrates. Concentrations of total dietary cations and total dietary ash were correlated with BC of the diet. They suggested that evaluation of the pH and BC of the diet be used to predict the need for supplementing the diet with buffers to control acid-base balance of the rumen.

Tucker et al. (63) developed a buffer value index (BVI) to evaluate the effects of buffers on ruminal fluid acid-base status; this index is related directly to BC but inversely to hydrogen ion concentration (H^+; acidity). This index has been used to appraise the acid-base status of diets. Le Ruyet et al. (39) investigated the effects of dietary ADF and BVI of the diet on the acid-base status of the rumen. They noted that increasing the dietary ADF percentage increased ruminal fluid BVI, but a high dietary BVI inexplicably reduced ruminal fluid pH and BVI. Because these paradoxical results were encountered with diets containing multiple fiber sources, we decided to evaluate the influence of dietary BVI on ruminal fluid BVI using diets containing a single forage.

The objective of our study was to examine the influence of dietary BVI on the ruminal environment of cows consuming these diets. Various dietary BVI were

achieved by altering the ratio of sorghum silage to corn grain in the diet. Well fermented silage, with a pH of 4, should immediately depress ruminal fluid pH. Supplemented grain should depress ruminal fluid pH for several hours post-feeding due to starch fermentation.

Materials and Methods

Animals, Feeding, and Experimental Design

Three ruminally-cannulated Holstein cows (653 $\pm$ 13 kg) were used in a 3 x 3 Latin square with 3-wk experimental periods. Animals were housed individually in pens and had free access to dirt exercise lots; cows were milked twice daily (0300 and 1500 h). Diets were TMR (Table 4) with analyzed BVI of -74, -41, and -7. These diets, containing concentrate and sorghum silage (70:30, 60:40, or 50:50 ratio, DM basis), were fed twice daily (0550 and 1750 h). Orts were recorded daily. Samples of the TMR were collected weekly and frozen for subsequent nutrient analyses at a commercial laboratory (Northeastern DHIA, Ithaca, NY). Period three was extended by two weeks to allow time for the cow consuming the 60:40 diet to recover from mastitis. Effects of diet on milk yield and milk composition were difficult to detect due to depressed feed intake and milk production by this cow.

Diet Formulation and BVI Determination of Feedstuffs

Sorghum silage, two concentrates (ground shelled corn and soybean meal), and four mineral sources (limestone, dicalcium phosphate, trace mineralized salt, double

TABLE 4

INGREDIENT AND NUTRIENT COMPOSITION OF DIETS (DM BASIS) WITH DIFFERENT BUFFER VALUE INDEX VALUES

Ingredients	Diet[a] 50:50	60:40	70:30
	(%)		
Ingredient			
Forage sorghum, silage	50.01	40.03	30.02
Ground shelled corn	23.44	34.45	44.66
Soybean meal, 44% CP	23.95	22.87	22.58
Limestone	.75	.91	1.06
Dicalcium phosphate	.91	.88	.82
Dynamate®[b]	.39	.36	.41
Trace mineralized salt	.53	.49	.43
Vitamin A premix[c]	.01	.01	.01
Vitamin E premix[d]	.01	.01	.01
Nutrient[e]			
DM	38.8	40.9	48.0
CP	14.6	16.0	16.5
NE_L, Mcal/kg	1.43	1.58	1.65
ADF	22.9	19.2	15.5
NDF	32.8	28.2	23.7
Ca	.59	.73	.75
P	.32	.46	.47
Mg	.32	.31	.31
S	.26	.28	.30
K	1.31	1.27	1.22
Na	.20	.20	.20
Cl	.41	.38	.34
pH	4.76	4.85	4.97
H^+, neq/L	17,538	14,125	10,715
BC[f] meq/L of solution containing 16.7 g of feed DM	4.67	3.67	3.00
BC[g], meq/kg of DM	280.2	220.2	180.0
BVI, calculated	-487.7	-377.4	-265.6
BVI, analyzed	-74.5	-40.5	-6.6

[a]Grain to forage ratio.
[b]Double sulfate of potassium and magnesium, Pitman-Moore, Inc. Mundelein, IL.
[c]Supplied 30,000,000 IU of Vitamin A per kg of premix.
[d]Supplied 500,000 IU of Vitamin E per kg of premix.
[e]Analyzed nutrient content except for NE_L, which was estimated from ADF.
[f]Buffering capacity.
[g]Buffer value index.

sulfate of Mg and K) were combined to prepare three diets (Table 4). To facilitate diet formulation, the nutrient content of the sorghum silage was determined prior to feeding; tabular nutrient contents (47) were utilized for concentrate and mineral supplements. Dry matter content of sorghum silage, determined weekly via toluene distillation, was utilized to maintain a constant ratio of ingredients and nutrients in diet DM. The BVI calculations for the TMR were determined from individual feedstuffs using the procedures detailed by Le Ruyet et al. (39).

Sample Collection, Incubation and Analysis

The first 2 wk of each experimental period were utilized for adaptation; feed intake, milk yield, and milk composition were calculated from the last week of each period. Milk yield was measured daily throughout the study; milk samples were collected weekly during consecutive p.m. and a.m. milkings for analysis of fat, protein, lactose, and SNF content via infrared spectrophotometry (Multispec 2, Multispec Limited, Wheldrake, York, Engl.). Milk component concentrations were calculated as weighted averages according to the average a.m. and p.m. milk yield for the week.

Ruminal fluid (150 ml) was collected on d 13 of each period from the ventral sac of the rumen with an electric vacuum pump immediately before the 0550 h feeding, and every 30 min thereafter for 12 h. However, the 9.5 h sample during period two was missed for all cows. After being filtered through four layers of cheesecloth, 100 ml of ruminal fluid was transferred to a polyethylene snap-cap vial for immediate analysis of acid-base status. An additional 5-ml aliquot of ruminal fluid was dispensed into a polyethylene snap-cap tube containing 50 mg crystalline metaphosphoric acid; this mixture was frozen for VFA analysis by GLC (AutoSystem GC; Perkin-Elmer,

Norwalk, CT). Ruminal fluid pH, determined with a pH meter (model 950 pH-ion analyzer; Fisher Scientific, Pittsburgh, PA), was recorded following 30 s of equilibration. The BC, defined as the resistance to change in pH from 7 to 5, was determined by titrating a 30-ml aliquot of ruminal fluid with continuous stirring from its initial pH to a pH of 5 with 1*N* HCl and titrating an additional 30-ml aliquot from its initial pH to a pH of 7 with 1*N* NaOH. If the initial pH was higher than 7, we recorded only the volume of acid required to reduce the pH from 7 to 5. Buffering capacity was converted to meq/L as follows: BC = ((ml 1*N* HCL) + (ml 1*N* NaOH)) x 10^3/30. The BVI of the ruminal fluid was calculated according to the formula of Tucker et al. (63) as: BVI = ((((antilog$_{10}$(-STPH)) - (antilog$_{10}$(-SAPH))) / (antilog$_{10}$(-STPH)) ÷ ((SABC - STBC) / STBC)) x 10) + 100, where STPH = a standard pH of 6, SAPH = the ruminal fluid sample pH, SABC = the ruminal fluid sample BC (milliequivalents/L) and STBC = a standard buffering capacity of 50 meq/L.

Statistical Analysis

Ruminal fluid data were analyzed via general linear models ANOVA of SAS (55) with the following model:

$$Y_{hijk} = u + C_h + P_i + B_j + CB_{hj} + S_k + BS_{jk} + E_{hijk},$$

where

Y = dependent variable,

u = mean,

C = cow (h = 1, 2, 3),

P = period (i = 1, 2, 3),

B = BVI content of the diet (j = 1, 2, 3),

S = sampling time (k = 0, .5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0), and

E = residual error.

Main plot variables were tested by the cow by diet interaction; sub-plot variables were tested using residual error. Milk data were analyzed via the following model:

$$Y_{hij} = u + C_h + P_i + B_j + E_{hij}.$$

Linear and quadratic contrasts were employed to evaluate dietary BVI effects on milk and ruminal fluid variables. Statistical significance was declared at $P < .05$ unless noted otherwise.

Results and Discussion

Performance

Milk yield tended to increase as dietary grain, and thus energy density of the diet, was increased (Table 5). This supports the results of Le Ruyet et al. (39) . Although the mean effect of diet was not significant, milk protein yield increased linearly ($P<.06$) with increasing concentrate to forage ratio. Higher starch diets tended to decrease the acetate:propionate ratio (Figure 9). Thomas and Martin (61) reported that ruminal propionate concentration and milk protein synthesis were correlated positively. Thus, the increase in milk protein concentration may be in response to an increase in the supply of energy and propionate. Performance responses may have been compromised by mastitis in one cow at the beginning of period three.

TABLE 5

LEAST SQUARES MEANS FOR DAILY DRY MATTER INTAKE, AND YIELD AND COMPOSITION OF MILK FROM COWS FED DIETS CONTAINING GRAIN:FORAGE AT 50:50, 60:40, AND 70:30.

	Diet					
	50:50	60:40	70:30	SE	Effect[a]	*P*
DMI, kg	20.0	19.8	24.3	1.9	NS[b]	
NE_L, Mcal	28.6	31.3	40.1	3.0	NS	
Milk yield, kg	34.4	35.8	38.0	1.6	NS	
4% FCM, kg	32.7	33.5	35.3	2.5	NS	
Milk/NE_L, kg/Mcal	1.20	1.14	.96	.06	NS	
Milk fat, %	3.72	3.53	3.54	.26	NS	
Milk fat, kg	1.26	1.28	1.34	.13	NS	
Milk protein, %	3.11	3.22	3.29	.07	NS	
Milk protein, kg	1.07	1.14	1.25	.03	L	.056
Lactose, %	4.98	4.33	5.12	.39	NS	
Lactose, kg	1.71	1.60	1.95	.19	NS	
Milk SNF, %	8.77	8.23	9.06	.31	NS	
Milk SNF, kg	3.01	2.98	3.44	.23	NS	

[a]L = Linear response to dietary buffer value index.
[b]$P > .10$.

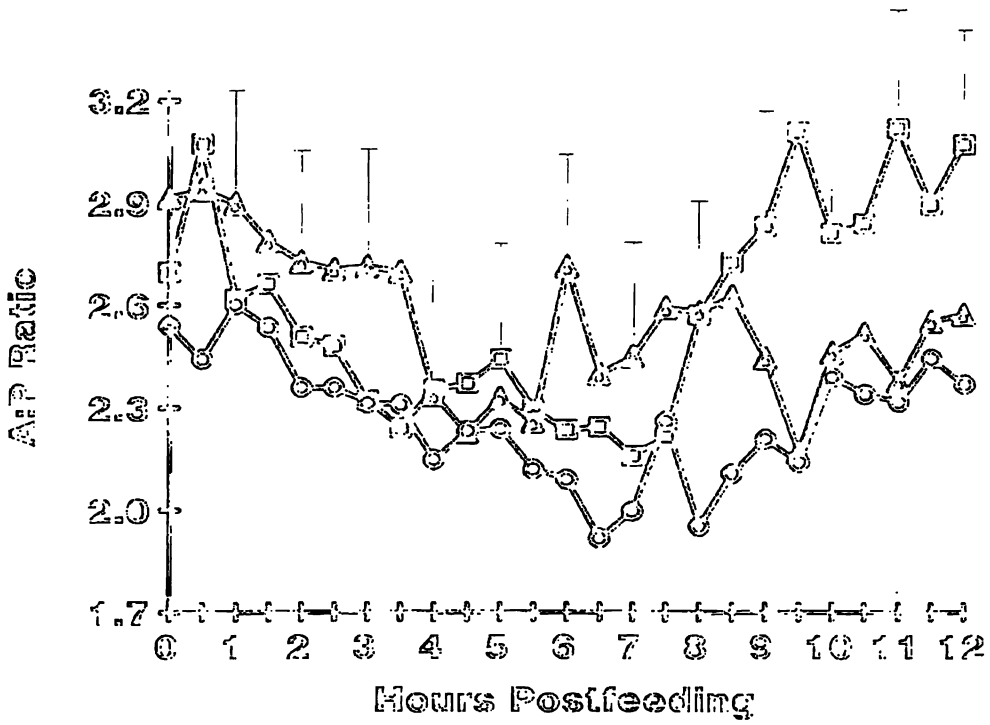

Figure 9. Ruminal Fluid Acetate:Propionate Ratio for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratios: 50:50, (□); 60:40, (△); 70:30, (○). Vertical Bars Represent Standard Errors.

Dietary Acid-Base Status

Analyzed dietary BVI were markedly lower than BVI values calculated from individual diet components (Table 4), but tended to decrease as the proportion of silage in the diet increased. Le Ruyet et al. (39) found similar relationships between calculated and analyzed dietary BVI. However, they (39) used alfalfa hay in their formulations, which may have allowed individual ingredients in the TMR to segregate. In addition, their samples were frozen for 4 mo before analysis. Our analyses were conducted after the samples were refrigerated for 5 h. More study is required to determine the influence of mixing, freezing or refrigerating, and storage time on the relationship between individual ingredient and total dietary H^+ and BC. Individual dietary ingredients with a low pH correspondingly had calculated BVI which were low. BVI was much higher when analyzed than calculated from individual ingredients, indicating that BVI values are not additive. The overestimate from summing ingredients was greater for diets containing more silage. Ignoring the BC, except in the pH range of 5.0 to 7.0, may be responsible for this discrepancy.

In the present study, the 50:50 diet had the lowest calculated and analyzed BVI. Because sorghum silage is acidic, diets containing larger quantities of sorghum silage have lower calculated and analyzed BVI.

Ruminal Acid-Base Status

Jasaitis et al. (29) suggested that dietary acid-base status may affect ruminal acid-base status. Because silage is an acidic feed, high silage diets should depress ruminal fluid pH soon after consumption. Ruminal fluid pH of cows fed the 50:50 diet decreased from 7 to 6.4 (increase of 298 nM in H+) within .5 h after feeding (Figure

10). High concentrate diets should yield a higher initial pH until starch fermentation begins. The ruminal pH of cows fed the 70:30 decreased from an initial pH of 6.7 to 6.4 within .5 h after feeding. This initial response likely is attributable to the consumption of silage; however, pH continued to decrease due to starch fermentation. The pH of all three diets increased slightly from 2.5 h to 4 h postfeeding but decreased again from 4 h to 8.5 h postfeeding.

Sampling time was an important source of variation for ruminal fluid pH, H^+, BC and BVI (Table 6). The diet by sampling time interaction was not significant. Denton (14) demonstrated that routine activities that precede feeding may increase salivary flow. The cows in this trial were milked 9 h postfeeding. At this time, ruminal fluid pH increased .7 units for the 70:30 diet and .5 units for the 60:40 diet (Figure 10). An increased secretion of salivary buffer may explain this increase in pH because VFA concentration in ruminal fluid did not decrease during this period (Figure 10). Salivary secretion should increase ruminal fluid BC; this was detected for two of the diets (Figure 11). Le Ruyet et al. (39) showed that a decrease in H^+ increased BVI. An increase in BC from saliva production also will increase BVI of the rumen.

The impact of ruminal VFA production and salivary buffer secretion on ruminal fluid acid-base status may be more important than dietary BVI. Salivary flow into the rumen is a function of DMI and dietary DM content. Higher dietary forage increases salivary $NaHCO_3$ production (18), while increased dietary grain decreases the flow rate of saliva and the pH of ruminal fluid. Total equivalents of BC available to neutralize dietary and ruminal acid would be dependent on BC of the diet and total salivary buffer secretion. If we assume that half of the dietary DM was fermented in the rumen to VFA at ratios equivalent to those found in the rumen (60:28:12; Figure 12), 50 to 60 moles of acid would be produced in the rumen. This compares with only 8 moles of acid present in the silage consumed. Thus, the contribution of diet to total ruminal acid is approximately 15% (Table 7).

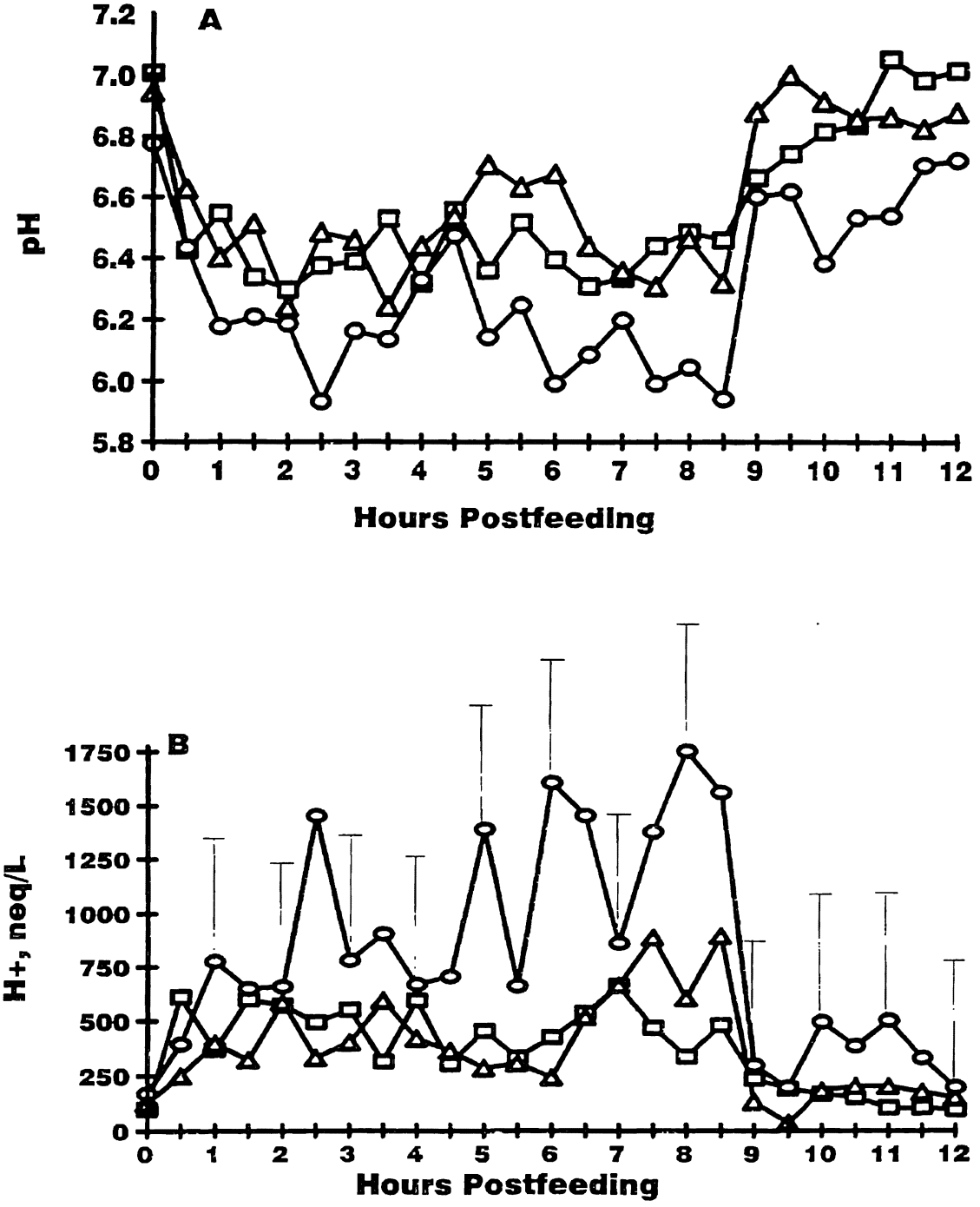

Figure 10. Ruminal Fluid pH (A) and H+ (B) for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratio: 50:50, (□); 60:40, (△); 70:30, (○). Vertical Bars Represent Standard Errors.

TABLE 6

MEAN SQUARES FOR INDICATORS OF RUMINAL FLUID ACID-BASE STATUS

Source[a]	df	pH		H^+, neq/L		BC[c]		BVI[d]	
		MS[b]	*P*	MS	*P*	MS	*P*	MS	*P*
Cow	2	.467	.855	1,413,574	.794	206.8	.359	212.7	.757
Period	2	1.170	.702	2,627,157	.675	81.7	.586	258.1	.719
Diet	2	2.074	.570	4,961,807	.524	116.8	.498	584.7	.531
Cow by diet	2	2.751		5,458,775		115.7		661.6	
Sampling time	24	.464	<.001	603,768	.003	74.1	.002	80.9	<.001
Diet by sampling time	48	.041	.980	186,528	.943	29.8	.673	19.4	.941
Residual	141	.069		277,464		33.5		28.8	

[a]Main plot variables (cow, period, and diet) were tested by the cow by diet interaction; sub-plot variables were tested by the residual error.

[b]Type I mean squares.

[c]Buffering capacity.

[d]Buffer value index.

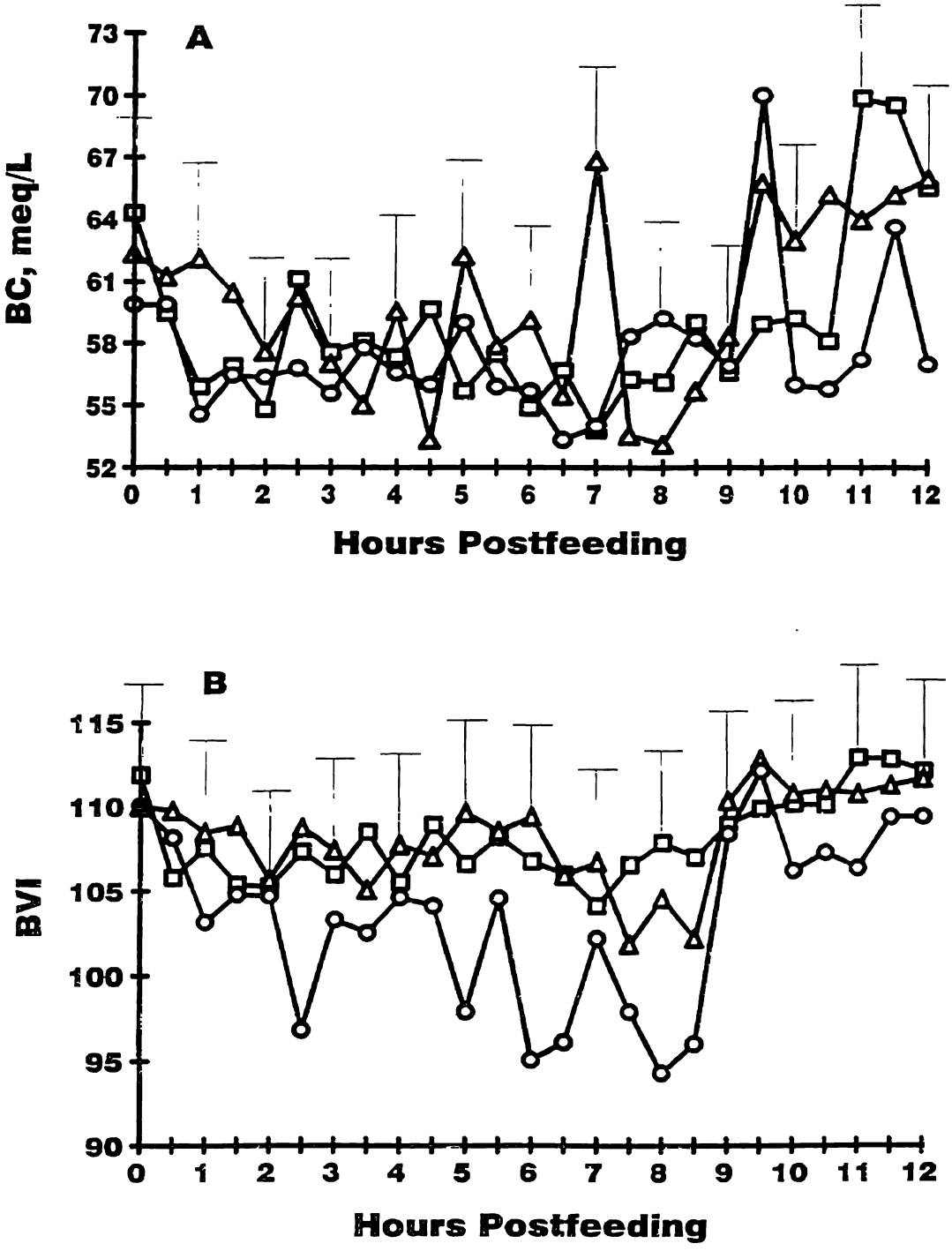

Figure 11. Ruminal Fluid Buffering Capacity (BC) (A) and Buffer Value Index (BVI) (B) for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratio: 50:50, (□); 60:40, (△); 70:30, (○). Vertical Bars Represent Standard Errors.

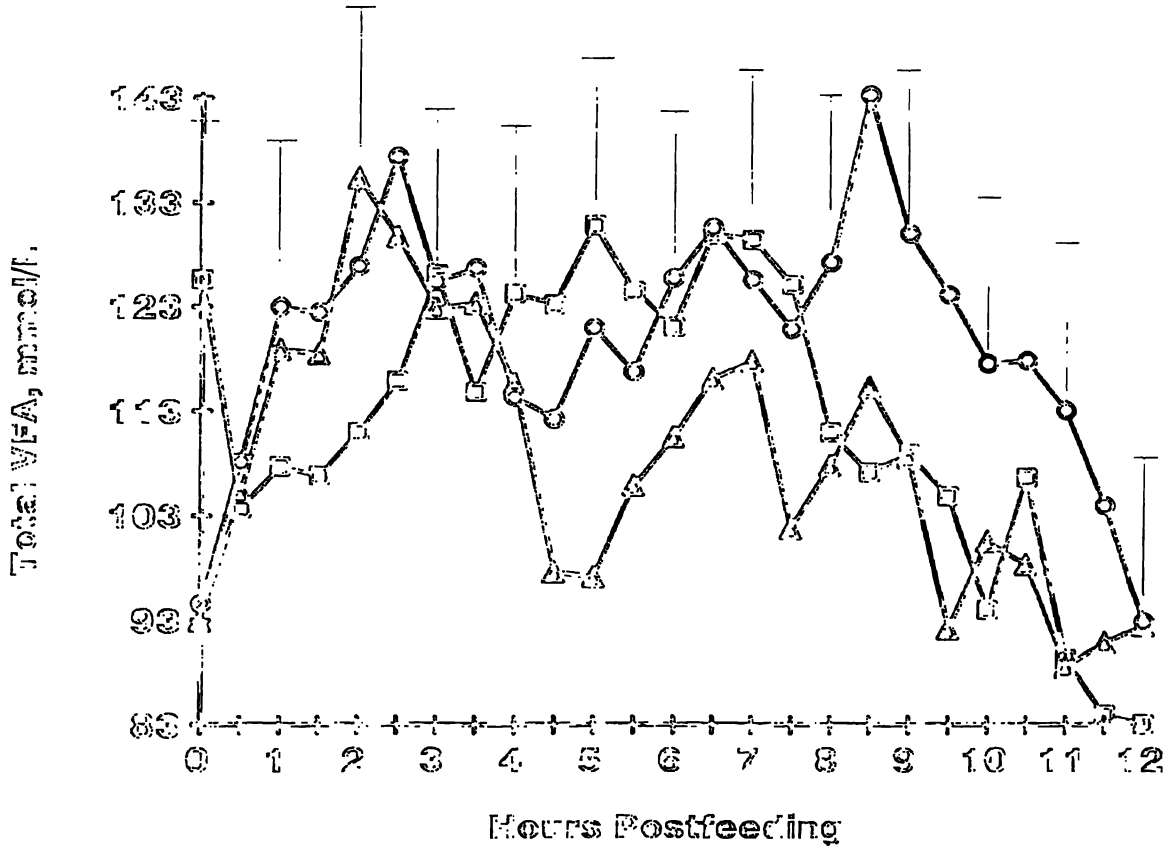

Figure 12. Ruminal Fluid VFA Concentrations for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratio: 50:50, (□); 60:40, (△); 70:30, (○). Vertical Bars Represent Standard Errors.

TABLE 7

ESTIMATES OF TOTAL ACID EQUIVALENTS AND BUFFERING CAPACITY (BC) FROM THE DIET, RUMEN, AND SALIVA

	Acid	BC
	(mol)	
Diet[a]	8	5
Rumen[b]	55	
Salivary $NaHCO_3$[c]		42

[a]Dietary acid calculated from lactic acid content (10%) of sorghum silage; dietary BC calculated from analyzed BC of diets and DMI of test cows.
[b]Ruminal acidity calculated assuming fermentation of 50% of DMI into VFA.
[c]From Erdman (18).

Ruminal fluid volume can be estimated from feed intake as a percentage of BW (9). Total amount of BC in the rumen at any time can be calculated from ruminal volume and ruminal BC. Based on these values (100 to 150 liters) and mean BC (58 meq/L), BC in the rumen at any time is about 7.25 eq compared to the BC provided in the diet of 4 to 6 eq/d. Absorption and outflow of buffers is continuous; thus, the total amount of BC in the rumen during 1 day exceeds the BC present at a given time. Cassida (8) estimated total salivation of 308 L/d and 284 L/d for cows consuming hay crop silage and corn silage based diets, respectively. Erdman (18) estimated total salivary $NaHCO_3$ equivalents to be 3,517 g/d for 50:50 concentrate:forage diets. The contribution of the diet to total buffer in the rumen is approximately 11% of BC in the rumen (Table 7).

According to these estimates, total ruminal and dietary buffering equivalents are inadequate to neutralize total ruminal acid production. Dietary $NaHCO_3$ buffer supplements at the current industry recommendation of 113 to 227 g per cow per day would provide 1.3 to 2.7 equivalents of additional BC. This equals 3 to 6% of estimated ruminal BC. Although this is a minor portion of total BC, dietary $NaHCO_3$ has repeatedly increased ruminal fluid pH (18) and OM intake (59, 60). However, supplementation of corn silage with 4% $NaHCO_3$ (DM basis, 4.2 equivalents) prior to feeding increased OM intake by 1.2 kg/d (60). Neutralizing high silage diets to a pH of 5 to 6 may be optimum. Development of a multi-element buffer to increase pH of acidic diets would be necessary to avoid excessive mineral concentrations in the diet.

Volatile Fatty Acids

Sampling time effects were significant for ruminal fluid concentrations of acetate, propionate, butyrate, valerate, total VFA and the acetate:propionate ratio

(Table 8). Butyrate concentration was highest for the 70:30 diet (Figure 13). Period variation also was significant for butyrate, isovalerate, and total VFA concentrations. The 50:50 diet did not produce the highest acetate concentration (Figure 14), which contrasts with the concept that high fiber diets should yield the highest amount of acetate because of digestion of cellulose. Rapid digestion of starch to propionate occurs several hours after a meal. Propionate concentrations tended to decrease more rapidly with time postfeeding, especially after 6.5 h, for the 50:50 diet than for the 60:40 and 70:30 diets (Figure 14).

Summary

The analyzed BVI of the three TMR were higher than BVI calculated from individual ingredient values. Milk protein yield by cows was 8.8% and 14.4% greater for those fed the 70:30 rather than the 60:40 and 50:50 diets, respectively, due to increased energy content and, presumably, greater propionate production. Milk yield, milk fat content, milk fat production and milk protein content were not affected by the BVI of the diets tested. Ruminal fluid acid-base status was not affected significantly by dietary BVI levels. Dietary buffer equivalents calculated to be only 11% of total buffering equivalents available to the cow and dietary acid equivalents were only 15% of total acid in the rumen. Ruminal fluid pH, H^+, BVI, BC and total VFA were not significantly affected by BVI. Hence, the dietary acid-base status alone is inadequate as a predictor of the need for buffers in the diet of lactating cows fed sorghum silage-based diets.

TABLE 8

MEAN SQUARES FOR RUMINAL FLUID VOLATILE FATTY ACIDS

Source[a]	df	Acetate (A), mmol/L MS[b]	*P*	Propionate (P), mmol/L MS	*P*	A:P Ratio MS	*P*	Isobutyrate, mmol/L MS	*P*
Cow	2	228.1	.651	142.8	.823	.5246	.910	1.043	.746
Period	2	1231.9	.257	2960.0	.183	11.6404	.312	27.135	.101
Diet	2	198.7	.682	342.8	.659	2.1737	.709	.823	.788
Cow by diet	2	425.3		661.7		5.2891		3.055	
Sampling time	24	265.1	<.001	116.5	<.001	.3019	<.001	.381	.108
Diet by sampling time	48	60.9	.913	26.0	.341	.1102	.074	.184	.924
Residual	141	85.6		23.8		.0796		.266	

[a]Main plot variables (cow, period,and diet) were tested by the cow by diet interaction; sub-plot variables were tested by the residual error.

[b]Type I mean squares.

TABLE 8 (Continued)

Source[a]	df	Butyrate, mmol/L MS[b]	*P*	Isovalerate, mmol/L MS	*P*	Valerate, mmol/L MS	*P*	Total VFA, mmol/L MS	*P*
Cow	2	10.02	.261	3.830	.372	2.975	.804	798	.331
Period	2	152.24	.023	54.085	.040	105.576	.104	12,078	.032
Diet	2	77.70	.044	.105	.956	3.041	.801	1640	.194
Cow by diet	2	3.54		2.266		12.233		395	
Sampling time	24	22.83	<.001	.219	.583	1.186	.018	861	<.001
Diet by sampling time	48	3.00	.675	.209	.706	.686	.409	227	.299
Residual	141	3.37		.240		.656		202	

[a]Main plot variables (cow, period and diet) were tested by the cow by diet interaction; sub-plot variables were tested by the residual error.

[b]Type I mean squares.

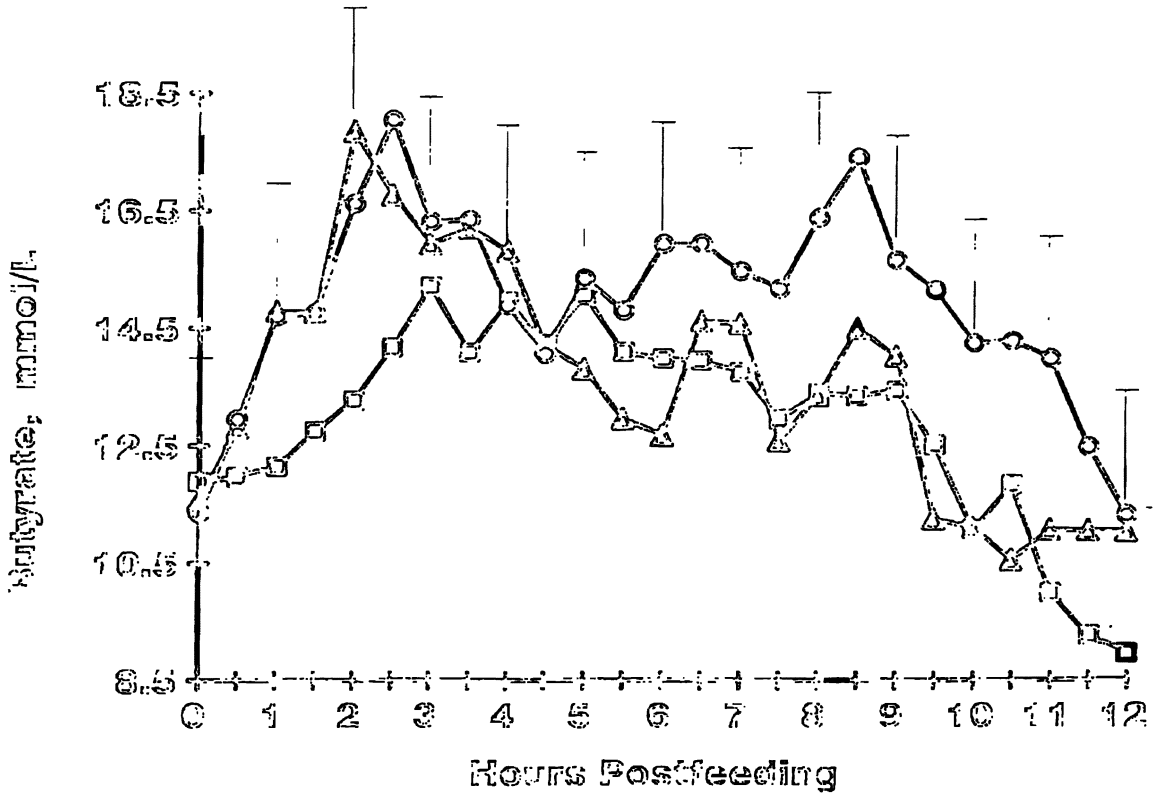

Figure 13. Ruminal Fluid Butyrate Concentrations for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratio: 50:50, (□); 60:40, (Δ); 70:30, (O). Vertical Bars Represent Standard Errors.

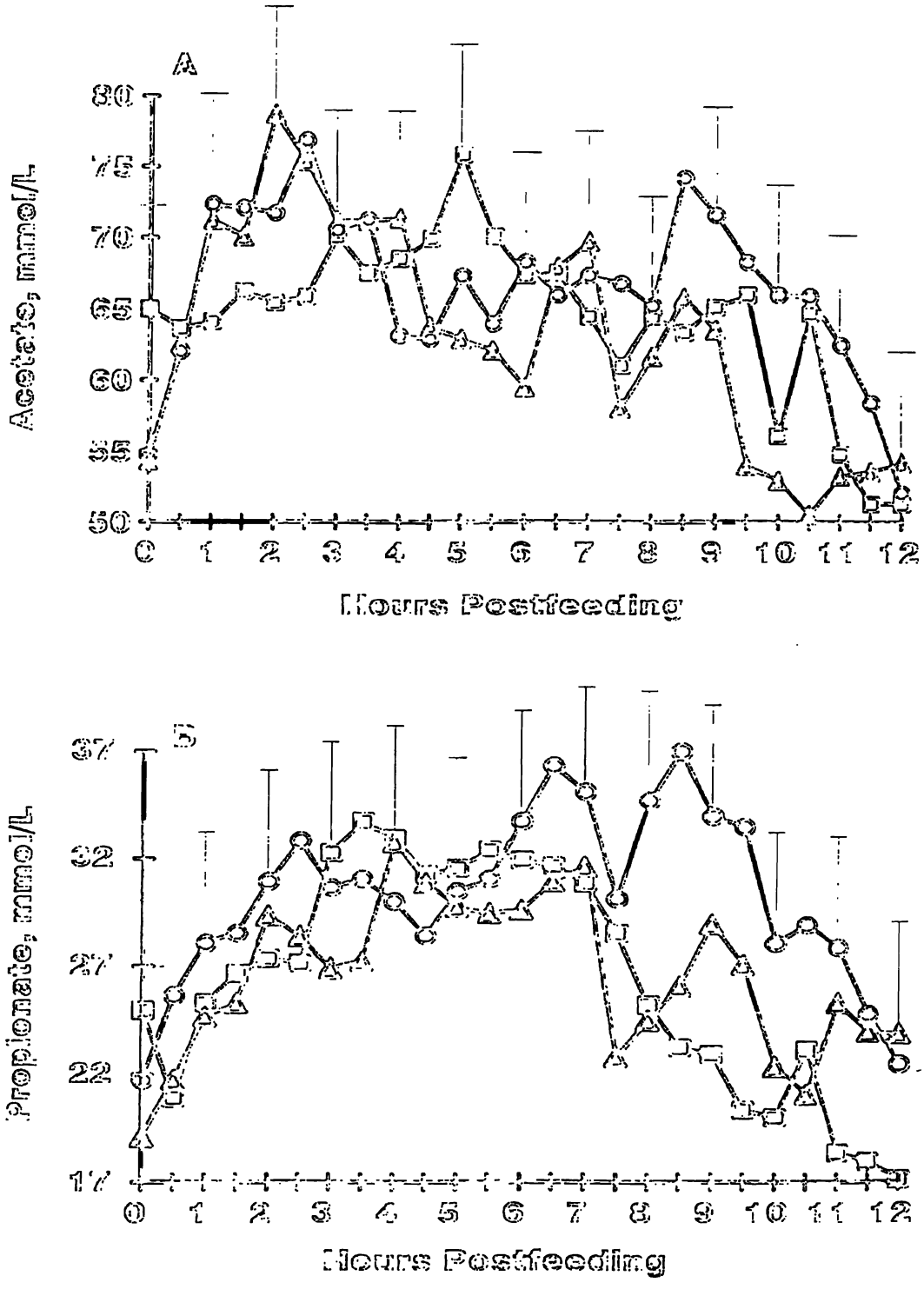

Figure 14. Ruminal Fluid Acetate (A) and Propionate (B) Concentrations for 12 h Postfeeding as Affected by Dietary Concentrate:Forage Ratio: 50:50, (□); 60:40, (△); 70:30, (○). Vertical Bars Represent Standard Errors.

CHAPTER V

EFFECTS OF CALCIUM CHLORIDE ON PREPARTUM UDDER EDEMA AND PLASMA AND URINE ELECTROLYTES IN HOLSTEIN HEIFERS

Abstract

Twenty six nulliparous Holstein heifers, blocked according to pedigree estimate of breeding value, were used to examine the effects of feeding $CaCl_2$ prepartum on udder edema and plasma and urine electrolytes. Heifers were assigned to diets containing either 1.5% $CaCl_2$ (DM basis) or 2.2% limestone. Diets were formulated to be identical, except for the calcium source. Severity of udder edema was evaluated independently by two people on a daily basis throughout the experiment utilizing a 10-point rating system (0 = no edema, 10 = severe edema). Udder edema and body weight were not affected significantly by the addition of $CaCl_2$. Dry matter intake tended to be lower throughout the prepartum period for heifers consuming the $CaCl_2$ diet than for heifers consuming the control diet. Plasma creatinine tended to be higher during the last two weeks prepartum and urine creatinine tended to be lower for heifers consuming $CaCl_2$, possibly indicating dehydration of extracellular fluid. Calcium chloride significantly increased urine calcium and chloride and plasma chloride, while decreasing urine pH and blood pH, HCO_3^-, and PCO_2. This would suggest that feeding $CaCl_2$ at 1.5% of DM may help prevent parturient paresis.

Introduction

Udder edema can be costly to dairy producers. By increasing the strain on the supporting ligaments, edema may lead to pendulous udders and increased incidence of injury and mastitis. This may lead to premature culling of genetically superior animals and increased likelihood of antibiotic residues in the milk from the treatment of mastitis. With the new antibiotic residue laws beginning to be strictly enforced, preventive treatment programs need to be developed.

Feeding calcium chloride has been shown to reduce slightly the severity of edema in nulliparous heifers (41). Because calcium chloride also lowers the dietary cation-anion balance, it also may prove beneficial in decreasing the incidence of milk fever. Lema et al. (41) fed calcium chloride for three weeks prepartum, but they did not measure changes in blood and urine constituents. The objective of the present study was to examine the effect of feeding calcium chloride for three weeks prepartum on udder edema and blood and urine mineral concentrations in nulliparous heifers.

Materials and Methods

Animals, Feeding, and Experimental Design

Twenty six nulliparous Holstein heifers averaging 592 $\pm$ 14 kg BW, blocked according to pedigree estimate of breeding value obtained from DHI records, were assigned to a diet containing either 2.2% limestone (control) or 1.5% $CaCl_2$ (Table 9). All heifers were fed the control diet beginning 28 d before their predicted calving dates. Beginning 21 d prepartum, thirteen heifers were fed the experimental diet. Diets consisted of sorghum silage and grain and were formulated to be identical except

TABLE 9

INGREDIENT AND NUTRIENT COMPOSITION OF DIETS[a]

	Control	$CaCl_2$
Ingredient		
Sorghum silage	61.18	61.61
Shelled corn, ground	25.44	25.61
Soybean meal, 44%	10.72	10.79
Trace mineralized salt[b]	.19	.19
IMC Dynamate[c]	.28	.29
Limestone	2.19	
$CaCl_2$, 78%		1.51
Analyzed nutrient composition		
DM	38.6	36.7
CP	12.5	11.6
NE_L, Mcal/kg[d]	1.52	1.50
ADF	24.8	26.5
NDF	39.1	39.9
Ca	1.05	.77
P	.26	.23
Mg	.34	.33
K	.95	.95
S	.23	.23
Na	.09	.09
Cl	.32	1.29
meq(Na + K) - (Cl + S)/ 100g diet DM	4.9	-22.4
meq(Na + K) - (Cl)/ 100g diet DM	19.3	-8.1

[a]Percent dry matter basis, unless indicated.
[b]Contained 92% NaCl, .250% Mn, .200% Fe, .033% Cu, .007% I, .005% Zn, and .0025% Co.
[c]Double sulfate of potassium and magnesium, Pitman-Moore, Inc., Mundelein, IL.
[d]Estimated.

for the calcium source. However, analysis indicated that the control diet contained 1.05% Ca compared to .77% for the $CaCl_2$ diet (Table 9). Heifers were fed individually at 0200 and 1400 h and orts were recorded daily. Dry matter content of sorghum silage was determined weekly via toluene distillation to maintain a constant ratio of ingredients in the diet DM. Samples of the TMR were obtained weekly and frozen for subsequent nutrient analysis at a commercial laboratory (North East DHIA, Ithaca, NY). Udder edema was scored daily by two people using a ten point rating system (0 = no edema, 10 = severe edema) developed by Tucker et al. (65). The average of the edema scores was used for analysis. Body weights and body condition scores were recorded weekly.

Sample Collection and Analysis

Blood and urine samples were collected weekly at 28, 21, 14, and 7 d prepartum, and within 12 h after calving. Blood (10 ml) was obtained via jugular venipuncture and transferred to two evacuated glass tubes containing Li heparin and placed immediately on crushed ice. One tube was used for analysis of blood pH, pO_2, and pCO_2 (model 1304; Instrumentation Laboratory, Lexington, MA). The other tube was centrifuged and plasma was collected for mineral analysis. Urine, collected in polyethylene vials via manual stimulation of the vulva, was placed on crushed ice and pH was measured within 2 h (model 950 pH-ion analyzer; Fisher Scientific, Pittsburgh, PA). Raw urine was analyzed for P and Cl content, and a 3 ml aliquot was acidified with 90 μl of concentrated HCl. Blood plasma and urine were analyzed for creatinine content (creatinine procedure Number 555-colorimetric; Sigma, St. Louis, MO) via spectrophotometry (Spectronic 21D; Milton Roy, Rochester, NY), Ca and Mg content via atomic absorption spectrophotometry (model 4000; Perkin-Elmer, Norwalk, CT),

Cl via potentiometric titration (Haake-Buchler Instruments, Saddlebrook, NJ), and inorganic P (inorganic P procedure Number 360-UV; Sigma Diagnostics, St. Louis, MO) via spectrophotometry (Response; Gilford Systems, Oberlin, OH).

Statistical analysis

Data were analyzed via SAS General Linear Models (55) with the following model:

$$Y_{gh} = \mu + B_g + D_h + E_{gh},$$

where

Y = dependent variable,

μ = mean,

B = block (g = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13),

D = diet (h = 1, 2), and

E = residual error.

Statistical significance was declared at $P < .05$ unless noted otherwise. Udder edema was averaged by week prepartum, using udder edema score on d 22 prepartum as a covariate. Dry matter intake also was averaged by week prepartum.

Results and Discussion

Performance

Body weight was not significantly affected by the addition of calcium chloride to prepartum diets of Holstein heifers (Table 10). Dry matter intake averaged 7.5%

lower for heifers consuming $CaCl_2$ than for heifers receiving the control diet (Figure 15, Table 10). The addition of $CaCl_2$ may decrease the palatability of the diet, reducing DMI (7, 41). Feeding a negative DCAB diet depressed feed intake by 7% when compared to a positive DCAB diet (64). In another study (41), DMI increased after removal of an acid ingredient. Milk production and energy output during the early postpartum period generally exceeds energy intake because DMI is not maximized. Depressing DMI prepartum by feeding a negative DCAB diet may help increase postpartum DMI, which may increase peak milk yield.

Feeding a negative DCAB may result in metabolic acidosis (75). As blood pH decreases, H^+ concentration increases. Blood HCO_3^- combines with H^+ to buffer the excess acid, decreasing blood HCO_3^- content. Blood HCO_3^- was significantly lower throughout the prepartum period for heifers receiving the $CaCl_2$ diet (Table 11). Tucker et al. (68) reported that blood HCO_3^- decreased as $CaCl_2$ in the diet was increased from 1.0 to 1.5%. The decrease in HCO_3^- due to decreasing DCAB is supported by other studies (64, 67). As HCO_3^- decreases, the respiratory and renal systems attempt to minimize the change in the pCO_2/HCO_3^- ratio (51). Even though respiration rate was not measured in our study, pCO_2 levels should be decreased by increased ventilation. Blood pCO_2 concentrations tended to be lower throughout the entire trial for heifers consuming $CaCl_2$; differences were significant three weeks prepartum and at calving (Table 11). This agrees with Tucker et al. (67, 68).

The addition of $CaCl_2$ to prepartum diets did not significantly reduce the severity of udder edema (Table 10, Figure 16). Development of edema appeared to be retarded during the first week of feeding $CaCl_2$. However, edema scores increased rapidly afterwards. This agrees with Lema et al. (41). Renal excretion of calcium and sodium is very similar; that is, calcium excretion increases urine volume. Plasma volume also should decrease, resulting in a decrease in plasma hydrostatic pressure and net absorption of interstitial fluid. If the effects of $CaCl_2$ last only a few days,

TABLE 10

LEAST SQUARES MEANS FOR BODY WEIGHT, DRY MATTER INTAKE (DMI), AND UDDER EDEMA SCORES FOR 3 WEEKS PREPARTUM AND AT CALVING FOR HEIFERS CONSUMING LIMESTONE (CONTROL) OR $CaCl_2$ DIETS

	Week	Control	$CaCl_2$	SE	*P*
Body weight, kg	-3	593.3	597.3	15.6	.860
	-2	598.6	601.8	14.7	.882
	-1	586.5	603.5	17.4	.503
	0	540.0	565.2	20.3	.398
DMI, kg/d	-3	9.33	9.06	.60	.755
	-2	8.82	7.43	.41	.032
	-1	6.69	6.61	.68	.934
	0	6.10	5.17	.85	.453
Udder edema	-3	3.20	2.60	.45	.379
	-2	4.37	3.98	.40	.500
	-1	5.46	5.06	.31	.383
	0	5.38	5.16	.31	.614

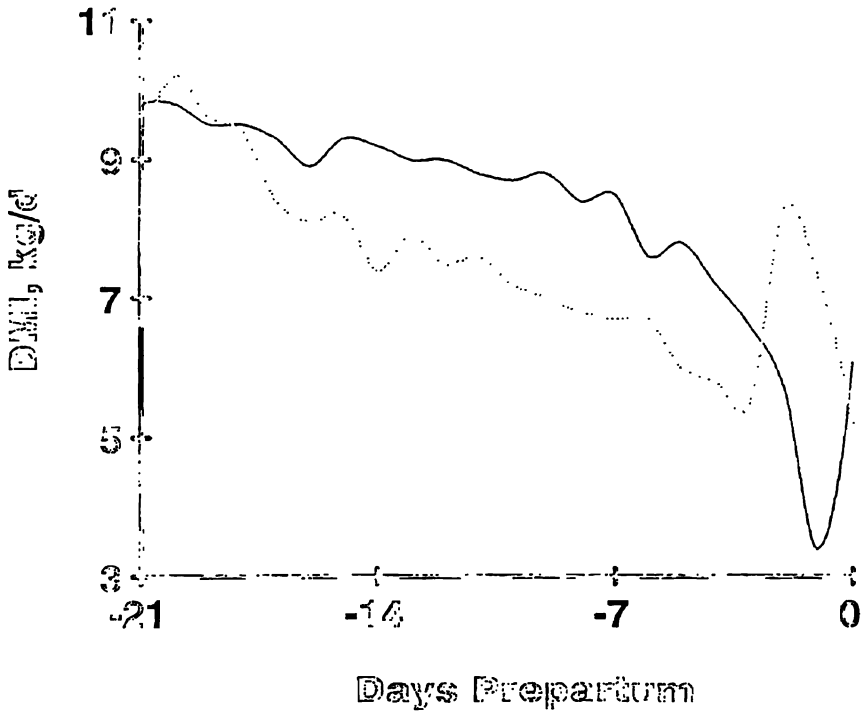

Figure 15. Least Squares Mean Dry Matter Intake (DMI) for Heifers Consuming CaCl2 (...) or Limestone (--).

TABLE 11

LEAST SQUARES MEANS FOR BLOOD AND URINE ACID-BASE STATUS AND ELECTROLYTES FOR 3 WEEKS PREPARTUM AND AT CALVING FOR HEIFERS CONSUMING LIMESTONE (CONTROL) OR $CaCl_2$ DIETS

	Week	Control	$CaCl_2$	SE	*P*
Blood pH	-3	7.420	7.385	.009	.019
	-2	7.435	7.365	.005	<.001
	-1	7.433	7.393	.008	.005
	0	7.449	7.424	.008	.063
Blood HCO_3^-, mm Hg	-3	28.6	24.5	.602	<.001
	-2	27.9	22.5	.614	<.001
	-1	28.1	24.8	.960	.031
	0	30.7	27.1	.781	.007
Blood pCO_2, mm Hg	-3	45.7	42.1	.7	.004
	-2	43.0	40.7	.9	.075
	-1	43.5	41.7	.9	.180
	0	45.6	42.6	.8	.019
Blood pO_2, mm Hg	-3	30.4	30.3	.9	.948
	-2	31.6	33.8	.7	.505
	-1	32.1	33.9	1.8	.505
	0	38.0	34.7	2.0	.263
Urine pH	-3	8.18	6.61	.26	.001
	-2	8.03	5.74	.16	<.001
	-1	7.94	5.81	.15	<.001
	0	7.43	6.27	.19	.082
Plasma creatinine, mg/L	-3	15.26	15.13	.47	.847
	-2	16.04	16.84	.46	.242
	-1	16.21	18.08	.53	.029
	0	16.48	18.65	.65	.036
Urine creatinine, mg/L	-3	1343	1177	154	.464
	-2	1768	1246	128	.014
	-1	2179	1616	190	.058
	0	1293	947	208	.269

TABLE 11 (Continued)

	Week	Control	$CaCl_2$	SE	*P*
Plasma phosphorus, mg/L	-3	61.95	58.00	1.35	.064
	-2	60.83	60.17	1.40	.746
	-1	60.76	65.93	3.39	.302
	0	60.03	59.50	22.61	.893
Urine phosphorus, mg/L[a]	-3	55.53	37.24	18.21	.493
	-2	50.57	14.89	15.11	.121
	-1	47.99	14.91	14.87	.142
	0	188.91	33.51	63.95	.118
Plasma calcium, meq/L	-3	4.93	4.85	.05	.252
	-2	4.88	4.86	.07	.831
	-1	4.75	4.77	.05	.830
	0	4.56	4.46	.07	.221
Urine calcium, meq/L	-3	3.66	18.17	2.577	.002
	-2	3.44	16.79	1.653	<.001
	-1	2.12	8.83	1.002	<.001
	0	3.25	6.56	1.652	.190
Plasma magnesium, meq/L	-3	1.96	1.86	.040	.103
	-2	2.48	1.95	.271	.187
	-1	1.88	1.89	.058	.868
	0	1.95	1.91	.085	.753
Urine magnesium, meq/L	-3	67.0	69.1	7.8	.847
	-2	50.7	41.7	4.3	.171
	-1	35.8	31.1	2.5	.207
	0	84.0	30.6	25.1	.165
Plasma chloride, meq/L	-3	105.8	110.8	.9	.002
	-2	107.4	114.1	.4	<.001
	-1	109.9	113.3	.8	.012
	0	107.5	109.4	1.2	.280
Urine chloride, meq/L	-3	68.3	180.6	20.3	.002
	-2	50.4	153.3	11.8	<.001
	-1	39.0	100.5	13.6	.008
	0	69.9	116.7	21.3	.159

TABLE 11 (Continued)

	Week	Control	$CaCl_2$	SE	*P*
Plasma sodium, meq/L	-3	151.40	141.62	3.5	.077
	-2	153.20	146.59	2.7	.110
	-1	156.56	145.63	4.0	.075
	0	146.27	142.18	2.6	.289
Urine sodium, meq/L	-3	9.29	22.96	4.4	.051
	-2	8.19	17.41	3.0	.048
	-1	11.53	8.93	5.3	.736
	0	35.68	16.01	11.3	.251
Plasma potassium, meq/L	-3	4.74	4.55	.10	.195
	-2	4.77	4.69	.09	.532
	-1	4.96	4.63	.14	.117
	0	4.22	4.40	.15	.403
Urine potassium, meq/L	-3	209.6	222.7	33.2	.785
	-2	148.8	130.6	14.9	.404
	-1	116.2	106.0	18.2	.698
	0	117.2	126.5	18.7	.734
Plasma DCAB, meq/L	-3	50.3	35.3	3.38	.009
	-2	50.5	37.1	2.89	.006
	-1	51.7	37.0	4.28	.032
	0	43.0	37.1	2.83	.169
Urine DCAB, meq/L	-3	150.6	65.0	25.2	.035
	-2	106.6	-5.3	7.75	$<.001$
	-1	88.7	14.4	24.8	.056
	0	81.8	41.0	20.0	.188

[a]Urine mineral concentrations expressed as:
(((urine mineral concentration (meq/L))/(urine creatinine concentration (mg/L)))*1000.

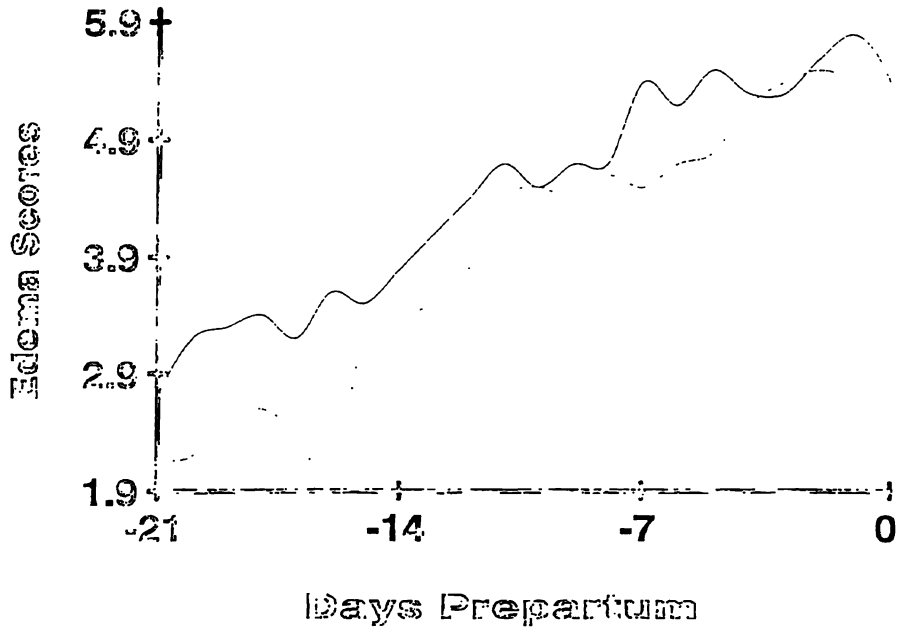

Figure 16. Least Squares Mean Prepartum Edema Scores (0 = No Edema, 10 = Severe Edema) for Heifers Consuming CaCl2 (...) or Limestone (--).

removal for a short period of time and refeeding may prove more useful for controlling udder edema than feeding $CaCl_2$ for an entire three week period.

Mineral Interactions

Addition of $CaCl_2$ significantly reduced urine pH throughout the prepartum period (Table 11). This agrees with Tucker et al. (67, 68). Plasma creatinine concentrations tended to increase as the expected calving date approached for heifers consuming $CaCl_2$. Creatinine concentrations in urine tended to be lower throughout the prepartum period for heifers consuming $CaCl_2$ (Table 11) and were significantly lower at two and one weeks prepartum ($P < .06$). Because the excretion rate of creatinine must equal the production rate of creatinine, the increased plasma creatinine concentrations may indicate a decrease in plasma volume, while the decreased urine creatinine concentrations would indicate an increase in urine output. A decrease in plasma volume would lower blood hydrostatic pressure and increase blood osmotic pressure, which should increase net absorption of extracellular fluid. The decreased urine creatinine concentrations and increased plasma creatinine concentrations suggest that $CaCl_2$ does possess diuretic properties, even though udder edema scores were not significantly affected. Plasma magnesium and phosphorus and urine magnesium and phosphorus concentrations were not affected by supplemental $CaCl_2$ (Table 11). Plasma and urine chloride concentrations were higher ($P < .05$) for heifers consuming $CaCl_2$ (Table 11). Chloride is absorbed from the intestines in association with sodium and also in exchange for HCO_3^- (35). Chloride often is excreted in association with sodium. Plasma sodium tended to be lower for heifers consuming the $CaCl_2$ diet throughout the trial (Table 11). Because calcium and sodium excretion are similar, an increase in urine calcium would increase urine chloride. Urine sodium and calcium

levels decreased as parturition approached; urine chloride excretion aiso decreased during this interval (Table 11). The increased plasma and urine chloride concentrations was the primary reason plasma and urine DCAB was lower for heifers consuming $CaCl_2$. Plasma DCAB was not correlated with udder edema scores, however. Plasma calcium levels were not affected by diet; however, urine calcium levels were significantly increased throughout the prepartum period by the low DCAB. Calcium absorption from the intestine has increased when feeding negative DCAB (43), while the incidence of milk fever has been reduced (7). In response to a negative DCAB diet, calcium is mobilized from bone which should help prevent the occurrence of milk fever (7). Increased excretion of calcium in our study may be in response to increased calcium absorption from the intestines as well as mobilization of calcium from bone.

Summary

Addition of 1.5% $CaCl_2$ did not significantly reduce the development of udder edema. Dry matter intake tended to be lower for heifers consuming the $CaCl_2$ diet. Plasma creatinine tended to be higher and urine creatinine tended to be lower for heifers consuming $CaCl_2$, possibly indicating dehydration of extracellular fluid. Plasma calcium was not affected by treatment. Increased concentrations of calcium in urine may be a result of increased intestinal absorption and mobilization of bone. Hence, feeding $CaCl_2$ at 1.5% of DM may be helpful in preventing parturient paresis.

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