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## CONTROLLED RUMINAL INFUSION OF SODIUM BICARBONATE: EFFECTS OF DIETARY <br> AND INFUSED BUFFER ON RUMINAL MILIEU

Thesis Approved:


Dean of the Graduate College

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## TABLE OF CONTENTS

Chapter page
I. INTRODUCTION ..... 1
II. REVIEW OF THE LITERATURE ..... 5
Buffering sources available to dairy cows ..... 6
Saliva ..... 6
Feed buffering capacity ..... 8
Dietary buffers ..... 9
Sodium bicarbonate as buffering agent ..... 10
Intake ..... 10
Absorption ..... 11
Metabolism ..... 12
Elimination ..... 13
Requirement ..... 13
Effects of Sodium bicarbonate ..... 15
Introduction ..... 15
On rumen pH ..... 16
On rumen VFA ..... 19
On feed intake ..... 23
On milk yield, milk fat test, and FCM ..... 26
On rumen liquid dilution rate ..... 29
On blood and urine acid-base status ..... 31
III. CONTROLLED RUMINAL INFUSION OF SODIUM BICARBONATE: EFFECTS OF DIETARY AND INFUSED BUFFER ON RUMINAL MILIEU ..... 33
Summary ..... 33
Introduction ..... 34
Materials and methods ..... 36
Results and discussion ..... 39
conclusion ..... 44
LITERATURE CITED ..... 58

## LIST OF TABLES

TablePage
3.1. Ingredient and nutrient composition of experimental diet DM ..... 48
3.2. Dry matter intake, milk yield and milk composition of cows receiving ruminal infusion of water from 0 to 2 h or $\mathrm{NaHCO}_{3}$ for 0 to 2,2 to 4 or 4 to 6 h after feeding ..... 49
3.3 Mean alteration in ruminal acid-base status of cows receiving ruminal infusion of water from 0 to 2 h or $\mathrm{NaHCO}_{3}$ for 0 to 2, 2 to 4, or 4 to 6 h after feeding ..... 50
3.4 Ruminal kinetics of cows receiving ruminal infusion of water from 0 to 2 h or $\mathrm{NaHCO}_{3}$ for 0 to 2,2 to 4 , or 4 to 6 h after feeding ..... 52

## TABLE OF FIGURES

Figure Page
1.a. Buffer value index, buffering capacity and rumen pH for 0 to 2 h NaHCO 3 infusion. ..... 53
1.b. Buffer value index, buffering capacity and rumen pH for 2 to 4 h NaHCO 3 infusion ..... 54
l.c. Buffer value index, buffering capacity and rumen pH for 4 to 6 h NaHCO 3 infusion ..... 552. Rumen A:P ratio at 0 to 2,2 to 4 or4 to $6 \mathrm{~h} \mathrm{NaHCO}_{3}$ infusion.56
3. Rumen total VFA, mM at 0 to 2,2 to 4 or 4 to 6 h NaHCO 3 infusion ..... 57

## CHAPTER I

INTRODUCTION

Buffers are widely used in dairy feeding programs to counteract milk fat depression and ruminal acidosis. Chemically, a buffer is the combination of a weak acid and its salt which resists changes in pH . The primary natural source of buffer available to the dairy cow is saliva and feed buffering capacity. Abrupt switching of animals from high roughage prepartum diets to high concentrate postpartum diets often causes failure in regulation of rumen pH , which results in problems like rumen acidosis, milk fat depression and potentially, displaced abomasum. Use of dietary buffers is recommended under these conditions. Absorption of sodium occurs at the luminal membrane by $\mathrm{Na}^{+} / \mathrm{H}^{+}$exchange. The absorption rate of sodium in the rumen is increased by increasing its concentration in the ruminal fluid. Stevens (1970) suggested a model which explains combination of $\mathrm{CO}_{2}$ with water in the ruminal epithelium, ultimately yielding $\mathrm{H}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$. The $\mathrm{H}^{+}$ reacted with the acetate to form acetic acid, which diffused out of the cell into the plasma. The $\mathrm{HCO}_{3}{ }^{-}$ diffused back into the ruminal fluid. The end product of
bicarbonate ion is $\mathrm{CO}_{2}$; excess $\mathrm{CO}_{2}$ is expired from the lungs.

Dietary requirement for sodium bicarbonate in high energy diet in a specific species can not be stated. However, it is well documented that dietary addition of sodium bicarbonate increases the milk fat percent, typically by altering the rumen pH , ruminal volatile fatty acid concentrations and feed intake. Increased feed intake is directly associated with rumen pH , but depends upon the nature of forage, concentration of grains in the diet and the amount of buffer used.

The dietary addition of sodium bicarbonate in alfalfa hay based diets has no effect on rumen pH (Eickelberger et al., 1985; De Peters, 1984; Rogers et al., 1986). Others found an increase in rumen pH with the addition of sodium bicarbonate when diets were based on corn silage (Wiedmeier et al., 1987; Coppock, 1986; Erdman et al., 1980; Rogers et al., 1985). Rumen pH has effects on fiber digestion, fiber-digesting microbial population, and volatile fatty acid production. Slyter et al. (1970) demonstrated a reduction in cellulytic microbial population at pH 5.3 . Low rumen pH facilitates increased molar percentage of propionate; dietary addition of sodium bicarbonate counteracts this phenomenon and increases the acetate to propionate molar ratio (Erdman et al., 1982; Rogers et al., 1980; Snyder et al., 1983) and total volatile fatty acid concentration (Stokes and Bull, 1986). Several scientists
have observed increases in DM intake with the addition of sodium bicarbonate when high concentrate diets were fed (Kilmer et al., 1980; Erdman et al., 1980; Schneider et al., 1986). In some studies, no increase in DM intake was reported with dietary addition of sodium bicarbonate (Hogue et al., 1991; Donker et al., 1980).

Decreased rumen pH results in depression of molar percentage of rumen acetate which leads to decreased milk fat content and reduced milk yield. Dietary addition of sodium bicarbonate in high concentrate diets based on corn silage increases the milk fat, milk yield and FCM (Erdman et al., 1980; Erdman et al., 1982; Rogers et al., 1985), but the response for milk fat content, milk yield and FCM was negligible when diets were based on alfalfa haylage (Donker et al., 1980; Eickelberger et al., 1985).

Another aspect accounting for this increased production is rumen liquid dilution rate. Sodium bicarbonate addition increases the rumen dilution rate, which results in decreases in molar proportion of propionate (Rogers et al., 1979; and Rogers et al., 1982). An increase in ruminal turnover time was observed for 0 to 2 and 4 to 6 h postfeeding intraruminal infusion of sodium bicarbonate (Hogue et al., 1991). The acid-base status of an animal is associated with environmental temperature; however, in some studies addition of sodium bicarbonate has increased urine $\mathrm{HCO}_{3}{ }^{-}$concentration and rate of $\mathrm{HCO}_{3}{ }^{-}$ excretion from urine (Erdman et al., 1982). Others found
alteration in blood $\mathrm{pH}, \mathrm{HCO}_{3}{ }^{-}$, and urine pH when sodium bicarbonate was infused into the rumen (Tucker et al., 1988; Arambel et al., 1988).

## CHAPTER II

## LITERATURE REVIEW

The natural source of ruminal buffering is saliva. It has been estimated that a 700 kg cow consuming hay and grain will produce 190 kg of saliva in a 24 hour period, Bartley (1976). Components of this saliva include 1.1 kg of sodium bicarbonate $\left(\mathrm{NaHCO}_{3}\right)$ and .1 kg of sodium chloride (NaCl). Sodium bicarbonate acts as a buffer in the rumen. A buffer is the salt of a weak acid; when in equilibrium with the weak acid it will resist changes in pH . The pH represents the measure of acidity and alkalinity of a solution on a scale of 0 (acid) to 14 (alkaline). A pH of 7 indicates neutrality, i.e. hydrogen and hydroxyl ions are present in equal concentrations. When early lactating cows are switched to high energy rations a large quantity of buffers are required in order to protect the rumen from pH changes. These rations, which often contain high levels of cereal grains or high moisture silages and low roughages, may cause the following events to occur: 1) decreased rumination, which leads to lower production of saliva and reduces the natural buffer supply to the animal, 2) acidbase imbalance in the rumen (low pH), yielding digestive upset, 3) production of volatile fatty acids in the rumen
is more rapid than normal, and may lead to increased production of lactic acid (lactic acidosis), and 4) changes in the microbial population; reduction in cellulose digesting bacteria and increases in streptococcus and lactobacillus occur most often.

A wide variety of compounds like sodium bicarbonate, potassium bicarbonate, limestone, magnesium oxide, sodium bentonite, sodium hydroxide and calcium hydroxide have been used as feed ingredients to maintain optimal rumen pH . Embry et al. (1969) suggested 28.4 g of sodium bicarbonate per 45 kg of live weight for good results in cattle. Emery et al. (1976) recommended 45 to 136 g of magnesium oxide per day in cattle.

Buffering sources available<br>to dairy cows

Decreasing dietary acid detergent fiber by one percentage unit results in a .0564 unit decline in rumen pH (Erdman, 1988). For neutralizing ruminal acidity the cow has three primary sources of buffers. These include: 1) buffers naturally present in saliva, 2) buffering capacity of ingested feed, and 3) added dietary buffers. Saliva

Saliva plays an important role in digestive physiology of the ruminant. It is required for mastication and swallowing, provides fluid for ruminal fermentation, helps
in buffering the volatile fatty acids produced by the microbial fermentation of feed and also helps passage of ingesta from the rumino-reticulum. It is produced in copious amounts by five paired glands and three unpaired glands, with the parotid glands apparently accounting for 40-50\% of total production. Salivation is under neural control.

Flow of buffers in saliva. Saliva flow is a function of DM intake. Estimates of total flow range from 110 to 308 L/d (Bailey, 1961; Cassida, 1986; Meyer, 1964) with a mean of 171 L/d. This range can be explained by variation in intake, forage to concentrate ratio of the diet, passage rate of ingesta from the rumen and rumen pH . Factors regulating saliva flow include feed intake, feed DM, and particle size (Erdman, 1988). Feed moisture affects saliva flow during eating (Bailey, 1961). Under extremely low flow rate the amount of bicarbonate or phosphate is altered (Bailey, 1961). Mixed saliva is a weak buffer above pH 7.5 and below pH 5.5 and partially neutralizes rumen acids. Saliva is composed of organic and inorganic portions. The organic portion is comprised of mucous and urea while the inorganic portion consists of bicarbonate and phosphate buffers, with the two anions comprising more than $90 \%$ of total anion content. The organic components are an important source of nitrogen for rumen microbes and help promote microbial growth. Saliva is an effective antifoaming agent when it is associated with foaming rumen
digesta in cattle bloating on legumes. It may play a role in grain bloat by transferring antibodies to the rumen. The antibodies are active against some organisms that produce a capsular slime and lead to frothy bloat (Bartley, 1976) .

## Feed Buffering Capacity

Saliva is the primary natural buffering source in the rumen; however, compounds that are found in the feed also influence the buffering capacity of ruminal fluid. The buffering capacity of forage is expressed as milliequivalents of hydrochloric acid needed to decrease the pH of 100 g forage from pH 6.0 to 4.0. The buffering capacity of grasses (orchardgrass, ryegrass, timothy) ranges between 22.0 to 37.0 meq $\mathrm{HCl} / 100 \mathrm{~g}$ of forage, and the buffering capacity of legumes like alfalfa, redclover and white clover ranges between 49.0 to 64.0 meq $\mathrm{HCl} / 100 \mathrm{~g}$ of forage at a pH range of 6.0 to 4.0. There is a great difference between the buffering capacities of grasses and legumes. Organic and inorganic anion and plant protein and fibrous residues accounts for $70-80,10-12$, and $10-20 \%$ buffering capacity, respectively, during fermentation of forage for silage production (Playne and McDonald, 1966). Silages have acid neutralizing capacities (NRC, 1978). Among fresh forages, legumes tend to have higher buffering capacity than grasses and whole corn plant; due to organic acids, silages possess two to three fold increase in
buffering capacity from pH 4 to 6 than unfermented forages. The work of Jasaitis et al. (1987) showed that cereal grains had relatively low buffering capacities but hay and protein sources had three to four fold higher total buffering capacities in the pH range of 4 to 9 . Because rumen pH seldom exceeds the range of 5 to 7 in dairy cows fed diets containing 40 to $90 \%$ forage, buffering capacity measured within that range could be more appropriate than the range of 4 to 9 or 4 to 6 (Playne, 1966).

## Dietary Buffers

Dietary buffers are being advocated to reduce the negative effect of high concentrate diets and sudden diet changes. Dairy cows usually are transferred from high forage diets prepartum to high concentrate diets postpartum in a short time. Problems associated with this change include anorexia, rumen acidosis and displaced abomasum. Energy consumption lags behind energy requirements for up to 8 to 12 wk postpartum. Counotte et al. (1979) observed a decline in rumen pH and bicarbonate concentration several hours before parturition: the decline may have been the result of diminished salivation, because rumination stops several hours before parturition. Several feed buffers in these conditions have been studied in dairy cows. Most of these buffers are sodium and potassium salts of carbonate or bicarbonate ions, i.e. sodium bicarbonate, sodium sesquicarbonate, and potassium bicarbonate. Sodium
sesquicarbonate is a combination of one mole of sodium bicarbonate and one mole of sodium carbonate attached to two moles of water. By definition, buffers must have a defined pKa value; for best buffering the pKa should be close to the pH of the media used. Sodium bicarbonate has a pKa of 6.25 which is within the physiological pH range in the rumen; hence it works well as a buffer in the rumen. Magnesium oxide ( MgO ) is also used in rations of dairy cows; under rumen conditions it acts as a neutralizing agent rather than buffering agent, because it has no defined pKa. The effectiveness of MgO in increasing depressed milk fat content depends upon its particle size because of its low solubility (Thomas et al., 1984; Jesse et al., 1981). In addition to chemical properties and cost, physical properties and palatability would be important considerations for buffer selection by dairy producers and manufacturers.

## Sodium Bicarbonate as Buffering Agent

## Intake

Saliva is the primary source of sodium bicarbonate in ruminants. Several factors that affect salivary quantity and quality also affect the availability of bicarbonate and phosphate in the rumen. These factors include rumination, particle size of the feed consumed, moisture contents of the feed and increased concentrate in the diet. Rumination
is particularly important with forage diets, because it stimulates saliva production. Pelleted and high concentrate diets are not conducive to rumination and result in reduced flow of salivary bicarbonate and phosphate into the rumen (Carter and Grovum, 1988). Osmotic pressure of body fluid, particularly hypertonicity has significant effects on ruminal function and feed intake. Increase in tonicity in body fluid inhibits salivation and feed intake (Carter and Grovum, 1990).

## Absorption

Stevens (1970) hypothesized that the combination of acetate ion with $\mathrm{H}^{+}$occurs in epithelial cells rather than ruminal fluid. The source of $\mathrm{H}^{+}$was dissociation of carbonic acid in the bovine ruminal epithelium. Steven's model has $\mathrm{CO}_{2}$ passing through the ruminal fluid and by the combination of $\mathrm{CO}_{2}$ and $\mathrm{H}_{2} \mathrm{O}$ yield $\mathrm{H}^{+}$and $\mathrm{HCO}_{3}{ }^{-}$. The $\mathrm{H}^{+}$ion reacts with acetate to form acetic acid, and $\mathrm{HCO}_{3}{ }^{-}$diffuses back into the ruminal fluid. Ruminal epithelium contains the $\mathrm{Na}^{+}-\mathrm{K}^{+}$ATP-ase enzyme. There is active absorption of $\mathrm{Na}^{+}$based on cellular arrangement of ruminal epithelium. Carter (1990) in review reported that $\mathrm{Na}^{+}$is pumped by $\mathrm{Na}^{+}{ }^{-}$ $\mathrm{K}^{+}$ATP-ase enzyme from the cell membrane to the blood. This would create a concentration gradient facilitating the diffusion of $\mathrm{Na}^{+}$from the lumen through the cells and tight junctions of strata corneum, granulosum and spinosum. The sodium absorption rate is increased by increasing its
concentration in ruminal fluid (Warner and Stacy, 1972; Martens and Blume, 1978). Increase in $\mathrm{Na}^{+}$absorption occurs provided that water entry into the rumen is minimal. Carter (1990) in a review reported a model which explains a $\mathrm{Na}^{+} / \mathrm{H}^{+}$exchange at the luminal membrane. There is a net flow of $\mathrm{H}^{+}$into the rumen that would neutralize an equivalent amount of $\mathrm{HCO}_{3}{ }^{-}$and this accounts for the disagreement between $\mathrm{HCO}_{3}{ }^{-}$appearance and acetate absorption.

Chloride. Variable results have been reported on absorption of $\mathrm{Na}^{+}$through ruminal epithelium. Marten et al. (1978) reported an increase in ruminal $\mathrm{Cl}^{-}$ concentration with $1.3 \%$ dietary NaCl . Marten and Gabel (1988) proposed a double exchange system involving $\mathrm{Na}^{+} / \mathrm{H}^{+}$ and $\mathrm{Cl}^{-} / \mathrm{HCO}_{3}{ }^{-}$and concluded that mucosal $\mathrm{Na} / \mathrm{H}^{+}$exchange was of major importance in the mechanism for $\mathrm{Na}^{+}$transport across ruminal epithelium.

## Metabolism

Henry's law states that the solubility of a gas in water is proportional to its partial pressure. The pH of bicarbonate is a function of its $\mathrm{CO}_{2}$ partial pressure. If partial pressure of $\mathrm{CO}_{2}$ is increased the pH of a bicarbonate buffer solution will decline, assuming all other variables are constant. The end product of bicarbonate is $\mathrm{CO}_{2}$ in vertebrates. Carbon dioxide has several uses in the body of an animal. It is used in the
process of carboxylation, in which it combines with pyruvate, forming oxaloacetate for gluconeogenesis. This reaction is catalyzed by pyruvate carboxylase in the mitochondria of the cell. Carbon dioxide plays an important role in fatty acid synthesis. Acetyl CoA is a precursor for fatty acid synthesis; in the liver, $\mathrm{CO}_{2}$ combines with acetyl CoA to form acetoacetyl CoA for conversion to a fatty acid. This reaction is supported by liver enzymes and the presence of citrate.

## Elimination

As discussed earlier the end product of bicarbonate is $\mathrm{CO}_{2}$. An excess of $\mathrm{CO}_{2}$ in the cell is eliminated in the bloodstream and finally expired from the lungs. The rate of $\mathrm{CO}_{2}$ production by tissue oxidation and the rate of $\mathrm{CO}_{2}$ loss from the body by expiration affects the $\left[\mathrm{HCO}_{3}\right] /\left[\mathrm{H}_{2} \mathrm{CO}_{3}\right]$ ratio in the blood.

## Requirement

The amount of sodium bicarbonate needed for a specific species cannot be stated unequivocally; several factors influence an animal's need and ability to utilize dietary sodium bicarbonate (Miller et al., 1972). Salivary buffer secretion, buffering capacity of feedstuffs, feed acidity, ADF content of the diet, feed intake, rumen pH , rumen acetate to propionate ratio, stage of production, dietary roughage to concentrate ratio and environment can all
influence sodium bicarbonate requirements in the dairy cow. Thomas et al. (1969), using a $2: 1$ ratio of sodium bicarbonate and magnesium oxide, found a linear increase in the milk fat percentage and decrease in milk production, milk composition and feed intake. Chase et al. (1981) found $.8 \%$ sodium bicarbonate was optimal for maximum milk production when lactating cows were fed a corn silage based diet. De Peters et al. (1984) found no difference in milk production and composition with addition of $1.25 \%$ sodium bicarbonate when the diet was based on alfalfa hay. The results of this experiment suggest that there is no justification for using dietary buffers when the diet is based on alfalfa hay.

Erdman (1988) concluded from the results of several studies that a decrease in dietary ADF of one percent is associated with a . 0564 unit drop in rumen pH. Each . 1 unit pH decrease in rumen pH below 6.3 results in a 3.6 percentage unit decline in ADF digestion. This decrease in ADF digestion may result in depressed feed intake. Digestibility of ADF can be improved by the addition of dietary buffers. Fiber content of the diet is related to salivary buffer secretion. Donker et al. (1985), Emery et al. (1969), Erdman (1988) and others have suggested that fiber content of the diet should be considered as a buffer equivalent. It can be concluded that the need for sodium bicarbonate changes with ADF content of the diet.

Effect of Sodium Bicarbonate

## Introduction

For more than 30 years, scientists have been evaluating the effects of $\mathrm{NaHCO}_{3}$ in dairy rations. Commonly, the addition of $\mathrm{NaHCO}_{3}$ is advocated when high producing dairy cows are switched to a high energy ration, which can cause anorexia, acidosis, displaced abomasum, grain bloat, or low DM intake. All these conditions are related to a decrease in rumen pH . Buffers are added to stabilize the rumen pH which should increase digestion and hence regain the depressed production. Excess of sodium bicarbonate in the animal's body causes metabolic alkalosis. The effects of sodium bicarbonate can be divided into beneficial and adverse.

Beneficial Effects. The major beneficial effect of sodium bicarbonate feeding is to neutralize the acid produced in the rumen so that rumen fluid pH remains above 6.0. Other obvious benefits are:
a. Sodium bicarbonate increases the turnover rate of fluid passing through the rumen. This increases the digestive efficiency of the animal.
b. Animals receiving a high grain diet have an extra exogenous organic acid load on the kidney. Addition of sodium bicarbonate in the diet helps the kidneys by reducing the organic acid load (Chalupa, 1975).
c. Dietary addition of sodium bicarbonate raises the osmolarity of rumen fluid and increases absorption of glucose (Kronfeld, 1979).

Adverse Effects. Dietary addition of excess sodium bicarbonate causes metabolic alkalosis and overhydration. Excess excretion of sodium and carbonate in the urine enhances the incidence of urinary calculi formation by raising urine pH (Kronfeld, 1976).

## Effect of $\mathrm{NaHCO}_{3}$ on Rumen pH

The pKa of bicarbonate in ruminal contents is 6.1 (Kronfeld, 1976), thus its greatest buffering effect would be expected at or close to this pH. Kovacik et al. (1986), using 50\% concentrate:50\% chopped orchardgrass hay diets supplemented with $0,1.5,3.0$, or $4.5 \%$ sodium bicarbonate in lambs, found a quadratic response to dietary level of $\mathrm{NaHCO}_{3}$ for percentage time that ruminal pH was $<6.2$ and $<5.8$ and a linear response for percentage time that ruminal pH was <5.4.

Addition of $2 \%$ sodium bicarbonate did not affect ruminal pH when the diet was based on alfalfa hay and concentrate in a 50:50 ratio (Eickelberger et al., 1985). This agrees with the results of De Peters (1984), who also found no change in rumen pH when diets were based on alfalfa hay. Rumen pH was above 7.0, which is far above the pKa of sodium bicarbonate. Rogers (1986) also found no
change in rumen pH with the addition of $1.5 \%$ sodium bicarbonate when cows consumed an alfalfa hay based diet. These results suggest that there is no justification for using sodium bicarbonate in alfalfa based diets.

Wiedmeier and Arambel (1987) reported that feeding a $50 \%$ concentrate diet with $2 \%$ sodium bicarbonate tended to increase rumen pH in early lactating cows. Increased rumen pH also was observed when $1.5 \%$ sodium bicarbonate was fed with a $55 \%$ concentrate diet. In the same experiment, another group of cows were fed 1.5\% Alkaten with 55\% concentrate diet. The increase in rumen pH tended to be greater for the sodium bicarbonate treatment (Coppock, 1986).

Potassium carbonate $\left(\mathrm{K}_{2} \mathrm{CO}_{3}\right)$ is also a buffer; its pKa value of 10.2 is much higher than sodium bicarbonate. West (1986) reported similar results for maintaining rumen pH when cows were receiving $1.5 \%$ sodium bicarbonate or $1.2 \%$ potassium carbonate, indicating similar buffering capacity. They suggested that $\mathrm{K}_{2} \mathrm{CO}_{3}$ may be a desirable compound for dairy rations but also recommended further investigation for optimal potassium content in the diet.

Erdman (1980) reported no significant increase in rumen pH with the addition of $1.5 \%$ sodium bicarbonate in a diet consisting of $60 \%$ concentrate and $40 \%$ corn silage. By contrast, Rogers (1985) reported a decrease in rumen pH with the addition of $1.2 \%$ sodium bicarbonate when cows were receiving a 60:40 concentrate to roughage ratio. They
suggest that this drop was due to increased feed intake for the sodium bicarbonate treatment. Snyder (1983) reported significant differences in ruminal pH between $0 \% \mathrm{NaHCO}_{3}$ and $1.2 \% \mathrm{NaHCO}_{3}$ at 1 h postfeeding and 3 h postfeeding. The rumen pH was quite stable up to 11 h postfeeding with the addition of $\mathrm{NaHCO}_{3}$. The ratio of concentrate to roughage in this experiment was $75: 25$ or 50:50. Erdman et al. (1982) reported similar findings; cows fed diets with $\mathrm{NaHCO}_{3}$ required 5-7 h for ruminal fluid pH to reach lowest pH vs. 3 to 5 h for the nonsupplemented diet. A more stable ruminal pH postfeeding with added $\mathrm{NaHCO}_{3}$ appears to be a major factor for the increased ADF digestibility. Snyder et al. (1984) with the same concentrate to roughage ratio and 0 or $1.2 \%$ supplemented $\mathrm{NaHCO}_{3}$ claimed that with the addition of sodium bicarbonate in the diet, grain passed more rapidly from the rumen while fiber passed more slowly.

In diets composed of $60 \%$ concentrate and $40 \%$ corn silage, addition of $1 \% \mathrm{NaHCO}_{3}$ significantly increased rumen pH and molar percentage of acetate, while molar percentage of propionate decreased (Erdman et al., 1980). Using a 75:25 concentrate to roughage (corn silage and alfalfa haylage) ratio, Thomas et al. (1984) reported that addition of $1 \% \mathrm{NaHCO}_{3}$ significantly increased rumen pH. From the results of experiments with high concentrate diets we can conclude that addition of sodium bicarbonate increases or maintains rumen pH during 1 to 4 h postfeeding.

Maintaining ruminal fluid pH postfeeding should increase ADF digestion.

Effect of Rumen pH on Fiber Digestion. Low rumen pH is associated with many factors which affect fiber digestion. Mertens (1976) proposed three mechanisms by which rumen pH decreases fiber digestion: (1) pH sensitivity to cellulose activity, (2) alteration of microbial metabolic pathways and (3) selective reduction of fiber-digesting microbial populations. The effect of pH upon cellulose activity in the rumen is unclear. Mertens (1976) concluded from different studies that pH optimum for cellulase is in the range of 6.0 to 6.8 . However, it is generally believed that when pH falls from 7.0 to between 5.0 and 5.5 many ruminal microbes cease growth and might decrease cellulose digestion. More investigation is needed to clarify this mechanism.

Esdale and Satter (1972) concluded that change in ruminal pH without changing the proportion of microbial population, can change their mechanism of fermentation. Snyder et al. (1984) reported that cellulolytic microbial population decreased when pH was below 5.3. However the relationship between rumen pH and cellulolytic microbial population is not clear.

## Effect of NaHCO $_{3}$ on Rumen VFA

Important volatile fatty acids (VFA) in ruminants are acetate, propionate and butyrate. Volatile fatty acids are
produced by specific microbial pathways and continuously absorbed from the rumen. Volatile fatty acids values often are expressed on a molar percentage basis (moles of each VFA/100 moles of total VFA). Despite a great fluctuation in microbial population, differences in feed intake, quality and energy level of diet, ruminal volatile fatty acids proportions are quite stable at molar ratios (moles of acetate:propionate:butyrate) of 65:25:10 with roughage diets and 50:40:10 for concentrate diets. This depends upon ruminal pH . Ruminal pH has a remarkable impact on the production of volatile fatty acids.

Low pH causes pyruvate to be reduced to lactate and propionate. Lactic acid presence increases the problem by depressing pH and $\mathrm{CH}_{4}$ production; it further enhances the concentration of lactate and propionate. So the inactivity of methanogenic bacteria is really responsible for change in VFA production at low pH . Low ruminal pH is usually a result of a high grain diet; typically, less buffers are provided from saliva. The use of dietary buffers in such situations usually decreases the acidity of the rumen making the ruminal atmosphere more feasible for microbial growth.

Several studies where concentrate to roughage ratio was 50:50 failed to show advantage for adding buffer (most commonly sodium bicarbonate) to the diet. Johnson (1988) found no change in rumen acetate to propionate ratio when $1.0 \% \mathrm{NaHCO}_{3}$ was added to the diet. Kilmer et al. (1981)
reported similar responses using 50:50 concentrate to roughage ratio. Dietary addition of $.8 \% \mathrm{NaHCO}_{3}$ did not change the molar percentage of acetate and ruminal volatile fatty acids.

Other worker have shown increased acetate and decreased propionate in the rumen of cows fed $\mathrm{NaHCO}_{3}$. Erdman et al. (1982) found an increase in rumen acetate and decrease in propionate molar ratio with the addition of $1.0 \% \mathrm{NaHCO}_{3}$ and $.8 \% \mathrm{MgO}$ when cows were consuming $40 \%$ corn silage and 60\% concentrate diet. Rogers et al. (1980) reported little effect of $\mathrm{NaHCO}_{3}$ on rate of acetate production, although there is evidence of reduced propionate production. Snyder (1983) reported an increase in rumen acetate to propionate ratio with the addition of $1.2 \%$ sodium bicarbonate either with $50: 50$ or $75: 25$ concentrate to roughage ratio. Sodium bicarbonate supplementation in diets significantly increased the molar proportions of acetate, butyrate and isovalerate and decreased propionate and valerate. Erdman (1988), in a review, reported that sodium bicarbonate supplemented to cows receiving modest to high forage (>30\%) increased ( $\mathrm{P}<.01$ ) rumen acetate to propionate ratio and milk fat percent. Rumen pH tended to increase with $\mathrm{NaHCO}_{3}$ supplementation. Addition of sodium bicarbonate with low forage ( $<30 \%$ ) did not show any change in acetate:propionate ratio. There was significant ( $\mathrm{P}<.05$ ) increase in rumen pH and FCM. Thomas (1984) reported similar results with low
forage diets; they observed no change in rumen acetate:propionate ratio with the addition of $1 \% \mathrm{NaHCO}_{3}$ in a $75 \%$ concentrate diet.

Stokes and Bull (1986) used three diets containing hay crop silage and concentrate ratios of $30: 70,50: 50$, and 70:30; they reported that sodium bicarbonate (.4 or . $7 \%$ of diet DM) increased total volatile fatty acid concentrations with a $30 \%$ concentrate diet, decreased total volatile fatty acid concentrations with a $70 \%$ concentrate diet, and had little effect at $50 \%$ concentrate diet. A linear increase ( $\mathrm{P}<.03$ ) in molar proportion of acetate was found with increasing dietary forage. Greatest effects of buffer were observed with a 50\% concentrate diet. With decreasing concentrate, propionate percentage decreased linearly ( $\mathrm{P}<.03$ ); rumen acetate:propionate ratio increased linearly with increasing forage ratio.

In contrast to these studies, Nicholson et al. (1963) fed steers a high concentrate ration without and with $3 \%$ sodium bicarbonate; they observed that addition of bicarbonate lowered acetate levels in the rumen and increased propionate, butyrate and higher fatty acid levels.

Sodium bicarbonate is effective with ruminants consuming high concentrate diets in preventing the dramatic drop in rumen pH which narrows the acetate to propionate ratio and results in lower milk fat percent.

## Effect of Sodium Bicarbonate on Feed Intake

Switching animals from low energy to high energy diets postpartum often causes sharp decreases in DM intake, basically due to depression in ruminal pH , and often leads to decreased production. Under these conditions, use of buffers is advisable to increase DM intake. Addition of dietary buffer usually increases DM intake when the ration is based on corn silage.

Kilmer et al. (1980) reported that when cows were continued on a low energy prepartum ration until day 4 postpartum, at which time they were switched to a $60 \%$ corn silage: $40 \%$ concentrate ration containing $.72 \% \mathrm{NaHCO}_{3}$, there were trends toward increased DM intake and increased milk production with added $\mathrm{NaHCO}_{3}$. Using a $50 \%$ corn silage and $50 \%$ concentrate diet, Kilmer et al. (1981) reported similar responses; addition of $.8 \%$ sodium bicarbonate increased the DM intake and milk production for 14 days postpartum. Rogers et al. (1985) using a 40\% corn silage diet containing $1.2 \% \mathrm{NaHCO}_{3}$ and $1.4 \%$ limestone, reported a consistent increase in DM intake for cows fed $\mathrm{NaHCO}_{3}$ vs. limestone from wk 1 to 8. Dry matter intake responses as a percentage of body weight did not vary, whereas average milk production, daily fat yield and FCM were enhanced by $\mathrm{NaHCO}_{3}$. Erdman et al. (1980), using 1.5\% sodium bicarbonate or $.8 \%$ magnesium oxide in a $40 \%$ corn silage based diet, demonstrated that cows fed sodium bicarbonate rations had 2.1 kg per day greater ( $\mathrm{P}<.05$ ) intake than
control. There was a tendency for increased DM intake with magnesium oxide supplementation. Schneider et al. (1986) feeding a $38 \%$ corn silage basal diet containing $1 \%$ sodium bicarbonate, showed that feed intake increased $8.5 \%$ when sodium bicarbonate was fed.

Using $15 \%$ whole cottonseed, $30 \%$ corn silage, and $55 \%$ concentrate containing $1.5 \%$ sodium bicarbonate or $1.5 \%$ Alkaten (a naturally occurring mineral containing sodium carbonate and sodium bicarbonate), Coppock et al. (1986) reported that cows fed sodium bicarbonate ate 7 to $10 \%$ more total DM than cows fed control or Alkaten diets. Other measurements, like rumen pH , molar percentage of rumen volatile fatty acid and urine pH means suggested that Alkaten is similar to sodium bicarbonate in buffering action.

Donker et al. (1980) fed corn silage and alfalfa haylage, and showed no increase in DM intake when 1.5\% sodium bicarbonate was added to the concentrate. Control cows ate $93 \%$ of $D M$ offered as compared to $94 \%$ for cows on the experimental diet. With the addition of dietary buffers in hay crop silage based diet no increase in DM intake was reported by Stokes et al. (1985) or Stokes et al. (1986). Addition of dietary buffer (sodium bicarbonate, 1.4 and $2 \%$ ) in alfalfa hay based diets (54:46, 50:50 roughage to concentrate ratios) did not affect feed intake, milk yield, or milk composition (Rogers et al., 1985) .

These experiments indicated that where corn silage was the sole forage fed to early lactating cows, addition of sodium bicarbonate resulted in increased feed intake. When a combination of corn silage and alfalfa haylage or hay crop were the only forages, dietary addition of sodium bicarbonate did not increase feed intake. The mechanism for the increased intake and milk yield in corn silage-based diets with sodium bicarbonate and other buffers is unclear. Changes in rumen pH and rumen acetate:propionate were associated with improved DM, ADF and NDF digestion that might have increased feed intake in corn silage-based diets. Rogers et al. (1979) suggested that increased rumen microbial activity and digestion and corresponding increases in rumen liquid dilution rate might be responsible for increased intake in some types of forages. Effectiveness of buffer addition in improving feed intake may be related to the initial pH of the forage. Corn silage has a lower pH (3.90) than alfalfa haylage and alfalfa hay (4.74 and 6.02, Erdman, 1988). Neutralization of silage acidity before feeding resulted in increased forage intake. Buffers had maximal effects with pH below 4 and minimal effects on forages at pH 4.5 to 5.5 (Shaver et al., 1986).

Effects of Sodium Bicarbonate on Milk Yield, Milk Fat Percent and Fat Corrected Milk

Researchers have investigated the action of "buffers" or alkalinizing agents for dairy cattle consuming high energy, low fiber diets that characteristically cause declines in rumen pH , feed intake, rumen acetate:propionate ratio and ultimately result in milk yield and milk fat depression. Sodium bicarbonate is the common "buffer" fed to dairy cows. Magnesium oxide is another substance fed to improve milk fat test. Studies (Erdman et al., 1980; Erdman et al., 1982; Kilmer et al., 1981; Rogers et al., 1985; Schneider et al., 1986; Snyder et al., 1983; Teh et al., 1985) have reported the effects of sodium bicarbonate on corn silage-based diet in increasing milk yield, milk fat test, and FCM; some of these scientists have evaluated the effects of sodium bicarbonate compared to magnesium oxide or combination of the two. Other studies using corn silage and alfalfa haylage or hay crop silage have reported an increase in milk yield, milk fat test and FCM with the addition of sodium bicarbonate (Donker et al., 1980; Stokes et al., 1986).

Erdman et al. (1980), using a $40 \%$ corn silage: 60\% concentrate diet, found that milk fat test was not affected significantly by the addition of $1.5 \%$ sodium bicarbonate and $.8 \%$ magnesium oxide. There was a slight nonsignificant depression in milk fat percent with $.8 \%$ magnesium oxide addition. Differences in milk yield and fat test
contributed to increased FCM; however, the increase was greater for the combination of sodium bicarbonate and magnesium oxide. Erdman et al. (1982) reported that the addition of $1 \%$ sodium bicarbonate and $.8 \%$ magnesium oxide, to the diet of cows receiving $40 \%$ corn silage and $60 \%$ concentrate, increased milk fat percent ( $\mathrm{P}<.01$ ). The combination of sodium bicarbonate and magnesium oxide was more effective than either sodium bicarbonate or magnesium oxide alone. They suggested that the low ADF of ingested feed might have accounted for the increase in milk fat percent with supplemental buffers. Emery et al. (1965) hypothesized that sodium bicarbonate and magnesium oxide have different modes of action in alleviating milk fat depression. Sodium bicarbonate controls the proportion of rumen propionate via control of ruminal pH . Magnesium oxide affects milk fat by increasing mammary gland uptake of plasma acetate and triglycerides. Magnesium oxide also increases lipoprotein lipase activity of the mammary gland and decreases the linoleic acid content in the mammary gland (unpublished data from Michigan State University). Both of these changes are related to increased milk fat percentage.

Rogers et al. (1985) studied the effects of dietary addition of $1.2 \%$ sodium bicarbonate and $1.4 \%$ limestone, when lactating cows were receiving corn silage and concentrate in a 50:50 ratio. They demonstrated that daily fat yield and FCM were enhanced significantly by sodium
bicarbonate but not by limestone. Milk protein content and milk yield were reduced significantly by limestone inclusion but not by sodium bicarbonate. They concluded that high limestone may have altered the nitrogen utilization and affected milk protein. Schneider et al. (1986) gave evidence of increased milk yield, milk fat percent and FCM when using $1 \%$ sodium bicarbonate with $38 \%$ corn silage and 62\% concentrate in early lactating cows. Snyder et al. (1983), feeding corn silage and grain in a 50:50 or 75:25 ratio, reported higher milk fat percent and increased 4\% FCM production for cows fed diets containing $1.2 \%$ sodium bicarbonate. These results support the conclusion that addition of sodium bicarbonate elevates milk fat production by cows fed corn silage-based ration. Donker et al. (1980), using corn silage and alfalfa haylage as the roughage sources, pointed out that with the addition of $1.5 \%$ sodium bicarbonate, differences in milk fat test between control and sodium bicarbonate treatment were negligible ( $\mathrm{P}>.05$ ) . No difference in milk production, milk fat test, FCM, and DM intake was reported by Eickelberger et al. (1985) when cows were fed an alfalfa hay-based diet (50:50 roughage to concentrate) containing $2 \%$ sodium bicarbonate and $.5 \%$ magnesium oxide.

Stokes and Bull (1986) fed hay crop silage as the only roughage source with roughage to concentrate ratios of 30:70, 50:50, 70:30, and 100:0, to determine effects of dietary addition of sodium bicarbonate equivalent to . 4 and
. $7 \%$ of total ration $D M$. They reported that milk yield, milk fat percentage, $F C M$, milk protein percentage, and efficiency of production of fat corrected milk dropped with decreasing concentrate portion. Milk fat percent was decreased with buffer treatment and milk yield was greater for cows receiving . $4 \%$ sodium bicarbonate than for the cows with . $7 \%$ sodium bicarbonate in their diets.

## Effect of $\mathrm{NaHCO}_{3}$ on Rumen Liquid Dilution Rate

Dietary addition of mineral salts results in alteration of rumen fermentation. Change in rumen fermentation is associated with the amount and nature of the salt as well as composition of the diet. The decomposition of minerals from ingested feed, saliva, or exogenous buffers and production of VFA from microbial fermentation are main components responsible for rise in osmolarity of ruminal fluid. Sodium bicarbonate exerts osmotic properties in the rumen and cause increased dilution rate which leads to increased outflow of soluble nutrients in the fluid phase and increase in production of VFA. Most often, addition of sodium bicarbonate increases the molar proportion of rumen acetate to propionate and results in increased performance of the animal. Rogers et al. (1979), feeding Holstein steers either high concentrate or high roughage diets, concluded that intraruminal infusion of a hypertonic solution of .36 kg or .72 kg $\mathrm{NaHCO}_{3}$ or a hypertonic solution of .5 kg or 1 kg of NaCl
increased the ruminal dilution rate, water consumption and decreased molar proportion of propionate and decreased DM intake with the high concentrate diet. Rogers et al. (1982) reported an increase in liquid dilution rate when feeding a high concentrate diet with the addition of sodium bicarbonate or sodium chloride compared to a high roughage diet. Hodgson and Thomas (1979) suggested that rumen dilution rate has been related to pattern of ruminal fermentation, extent of ruminal digestion (Bull et al., 1979) and efficiency of microbial synthesis of protein (Isaacson et al. 1975).

Stokes et al. (1985) reported no effect on ruminal liquid volume or dilution rate with the supplementation of 58 or 114 g of sodium bicarbonate when cows were receiving hay crop and concentrate (30:70, 50:50, 70:30, 100:0 ratio) ; however, liquid dilution rate was greater during eating than resting and prefeeding. Others (Erdman et al., 1982; Rogers et al., 1982) concluded that up to $2 \%$ exogenous $\mathrm{NaHCO}_{3}$ in DM intake does not increase liquid dilution rate or rumen fluid volume in early lactating cows. Hogue et al. (1991), using sorghum silage and concentrate (35:65 ratio; DM basis), observed the effects of intraruminal infusion of $110 \mathrm{~g} \mathrm{NaHCO}_{3}$ dissolved in 3.8 L of water in lactating cows; they demonstrated an increase in ruminal liquid volume for 0 to 2 and 4 to 6 h NaHCO 3 infusion and an increase in ruminal liquid turnover time for 4 to 6 h NaHCO 3 infusion. For the best results they
recommended that provision of exogenous buffer should be at 2 to 4 h postfeeding.

Effects of Sodium Bicarbonate on Blood and Urine Acid-Base Status

The importance of animal acid-base status on productivity and performance is unclear; however, imbalance in acid-base status leads to poor performance and production. The largest change in blood $\mathrm{pH}, \mathrm{PCO}_{2}$ (partial pressure of $\left.\mathrm{CO}_{2}\right), \mathrm{HCO}_{3}$, and urine pH are related to change in environmental temperature (Erdman, 1988). During cool temperature, blood $\mathrm{pH}, \mathrm{PCO}_{2}$, and $\mathrm{HCO}_{3}$ increases and there is decrease in urine pH (Erdman et al., 1981). The use of buffers and alkalinizing agents has some impact on acidbase metabolism of animals. Erdman et al. (1982) reported blood $\mathrm{pH}, \mathrm{HCO}_{3}{ }^{-}$, and $\mathrm{PCO}_{2}$ tended to be increased with increasing time postfeeding. Ruminant animals secrete alkaline urine. The primary regulator of urine pH is concentration of $\mathrm{HCO}_{3}{ }^{-}$and ammonium ion. Erdman et al. (1982) also reported no significant alteration in blood and urine acid-base measurement with dietary addition of $1 \%$ $\mathrm{NaHCO}_{3}$ or $.8 \% \mathrm{MgO}$ when early lactating cows were consuming 40\% corn silage and 60\% concentrate (DM basis). However, the responses for MgO treatment for blood $\mathrm{pH}, \mathrm{PCO}_{2}$ and $\mathrm{HCO}_{3}{ }^{-}$were slightly higher than for the $\mathrm{NaHCO}_{3}$ treatment. Cows fed $\mathrm{NaHCO}_{3}$ and MgO showed higher urine pH than control treatment. Dietary addition of $\mathrm{NaHCO}_{3}$ and MgO increased
urine $\mathrm{HCO}_{3}{ }^{-}$concentration and rate of $\mathrm{HCO}_{3}{ }^{-}$excretion. Urinary ammonium ion concentration and excretion were not affected significantly by mineral addition in the diet. This agrees with Erdman et al. (1980). Tucker et al. (1988), using 40:60 ratio roughage to concentrate, reported nonsignificant effects on blood $\mathrm{HCO}_{3}{ }^{-}$concentration when . $8 \% \mathrm{NaHCO}_{3}$ was infused intraruminally. Blood and urine pH were not altered by length of infusion time but sampling time and sampling interval had some effects on blood and urine pH. Arambel et al. (1988) concluded that dietary addition of $.8 \% \mathrm{NaHCO}_{3}$ or $.4 \% \mathrm{MgO}$ or a combination of $\mathrm{NaHCO}_{3}$ and MgO had no effect on blood acid-base measurement. In contrast, Kilmer et al. (1981) reported that dietary addition of $.8 \% \mathrm{NaHCO}_{3}$ with $50: 50$ roughage to concentrate ratio increased urine pH . They found a significant reduction in urine pH on day 1 postpartum compared to prepartum period, which is related to abrupt switching of animals to high energy diets.

A study observing the response of dairy cows to $\mathrm{NaHCO}_{3}$
(1.2\%) and limestone (1.4\%) or a combination of sodium bicarbonate and limestone when early lactating cows were receiving a 40:60 roughage to concentrate diet, showed alterations in regulation of blood and urine pH . Concentration of electrolytes in blood and urine and excretion of sodium from urine was higher for $\mathrm{NaHCO}_{3}$ treatment than limestone (Rogers et al., 1985).

## CHAPTER III

CONTROLLED RUMINAL INFUSION OF SODIUM BICARBONATE: EFFECTS OF DIETARY AND INFUSED BUFFER ON RUMINAL MILIEU

## Summary

Four ruminally cannulated, lactating Holstein cows were assigned to a $4 \times 4$ Latin square to monitor the effects of $\mathrm{NaHCO}_{3}$ infusion on ruminal environment of cows receiving dietary sodium bicarbonate. Sodium bicarbonate (110 g) was mixed with 3.8 L water and infused at a constant rate into the rumen from 0 to 2,2 to 4 , or 4 to 6 h postfeeding, twice daily. All cows were fed sorghum silage and concentrate in a $35: 65$ DM ratio for 45 min twice daily. Ruminal fluid was collected at feeding and every 30 min postfeeding for 8 h on the last day of each 1-wk experimental period. Dry matter intake was not affected by $\mathrm{NaHCO}_{3}$ infusion. Yields of milk and its components were reduced with 4 to 6 h NaHCO 3 infusion. At certain isolated times, especially during infusion, $\mathrm{NaHCO}_{3}$ decreased ruminal fluid free proton concentration, and tended to increase
rumen fluid buffering capacity. Concentrations of ruminal fluid total VFA were not affected by $\mathrm{NaHCO}_{3}$ infusions, whereas acetate-to-propionate ratio tended to be reduced. Ruminal liquid volume tended to be increased by 0 to 2 h $\mathrm{NaHCO}_{3}$ infusion, and ruminal outflow rate tended to be reduced by the 2 to 4 h infusion. Potentially because of larger rumen liquid volume and decreased flow of ruminal fluid, VFA concentrations were not affected. Intra-ruminal infusion of $\mathrm{NaHCO}_{3}$ into cows receiving supplemental dietary $\mathrm{NaHCO}_{3}$ altered ruminal acid-base status as typically reported for those receiving dietary $\mathrm{NaHCO}_{3}$; however, these alterations were not accompanied by shifts in ruminal VFA patterns or in milk composition that normally result from such feeding regimens. The effects of $\mathrm{NaHCO}_{3}$ infused directly into the rumen at different intervals postfeeding may be different from those of dietary $\mathrm{NaHCO}_{3}$, possibly being related to the different time of entry into the rumen.

## Introduction

Wiedmeier et al. (1987) reported that feeding high concentrate diets to ruminants increased the occurrence of grain bloat, liver abcesses and lactic acidosis; these disorders were described as resulting from alterations in the ruminal milieu accompanying fermentation of soluble carbohydrates, including increased ruminal acidity and lactate production, and reduced degradation of cellulose.

Dietary buffers reduce ruminal acidity and increase cellulose digestion (Donker and Marx, 1980); hence, they may prevent metabolic disorders associated with high concentrate diets. Sodium bicarbonate is often included in the diet of lactating dairy cattle to prevent reductions in ruminal pH after feeding (Boisclair et al., 1987; Coppock et al., 1988; Donker and Marx, 1985; Erdman, 1988; Haaland et al., 1982; Rogers et al., 1979). When high-concentrate diets are fed twice per day, ruminal pH is dramatically lower from 4 to 6 h postfeeding (Erdman, 1988; Harrison et al., 1986; Snyder et al., 1983). Therefore, regimens developed to maintain a stable ruminal environment should include the provision of buffering capacity several hours postfeeding.

Hogue et al. (1991) observed that intraruminal infusion of $\mathrm{NaHCO}_{3}$ increased ruminal pH and buffering capacity; however, they also reported that these effects were transient, diminishing soon after cessation of infusion. Consumption of fermented diets presents an immediate acid challenge to the rumen; fermentation of starch and other carbohydrates presents another acid challenge which is most severe at 4 to 6 h postfeeding. Hence, given the transient effects of dietary buffers, maintenance of a stable ruminal environment requires the release of buffers in the rumen at two, separate intervals postfeeding. This might be achieved via feeding a combination of unprotected and controlled-release buffers,
which would provide buffering capacity to the rumen during each of these critical intervals. The objective of our study was to evaluate this thesis by examining the effect of a combination of dietary $\mathrm{NaHCO}_{3}$ and several different intraruminal $\mathrm{NaHCO}_{3}$ infusion intervals on temporal changes in buffering capacity and ruminal free proton concentration ([ $\left.\mathrm{H}^{+}\right]$).

## Materials and Methods

One primiparous and 3 multiparous ruminally fistulated Holstein cows were arranged in a $4 \times 4$ Latin square design with 1-wk experimental periods. A total mixed diet containing . $5 \% \mathrm{NaHCO}_{3}$ (DM basis; Table 1) was offered at 12-h intervals in an amount that would be consumed within 45 min ; the diet consisted of sorghum silage and concentrate in a 35:65 ratio (DM basis). Samples of the total mixed diet were collected weekly and composited at the end of the trial for subsequent nutrient analysis at a commercial laboratory (NEDHIA, Ithaca, NY). Dry matter composition of the sorghum silage was determined weekly and utilized to maintain a consistent ratio of ingredients in diet DM.

Treatments consisted of infusion of water from 0 to 2 h (Ctrl) or $\mathrm{NaHCO}_{3}$ for 2 h beginning either at 0 (0-2Bic), 2 (2-4Bic) or 4 h (4-6Bic) postfeeding. Sodium bicarbonate solution was prepared for infusion at each feeding by dissolving . $11 \mathrm{~kg} \mathrm{NaHCO}_{3}$ in 3.8 L of water and was allowed
to flow by gravity at a controlled rate into the rumen. Although intervals postfeeding at which infusion was initiated varied among treatments, the amount of $\mathrm{NaHCO}_{3}$ (.11 kg) per infusion was constant across all treatments.

On the morning of the last day of each experimental period, immediately before feeding, cows were dosed intraruminally with 550 mg Cr as Cr-EDTA; thereafter, ruminal fluid samples were collected every 30 min from 0 to 8 h postfeeding. Samples were collected via application of vacuum to a perforated tube positioned in the ventral sac of the rumen. Ruminal fluid pH was analyzed each half hour (Fisher Model 950 pH/ion analyzer, Fisher Scientific, Pittsburgh, PA); ruminal fluid was strained through four layers of cheesecloth, and a 100-ml aliquot was collected in a polyethylene vial, tightly capped and frozen for subsequent buffering capacity analysis. An additional 100ml aliquot was collected into a polyethylene vial, acidified with 1 g of crystalline metaphosphoric acid, tightly capped, and frozen for VFA and Cr analyses. Upon thawing, ruminal fluid was centrifuged at $10,000 \mathrm{x}$ g for 10 min; the supernatant was analyzed for VFA via GLC (Tracor 550, Tracor Instruments, Austin, TX) and Cr via atomic absorption spectrophotometry (Perkin-Elmer, Model 4000, Norwalk, CT).

Buffering capacity was determined by titrating a $30-\mathrm{ml}$ aliquot of ruminal fluid with continuous stirring from its initial pH to pH 5 with $\mathrm{HCl}(.5$ or 1.0 N ), and by titrating
an additional 30-ml aliquot from its initial pH to pH 7 with $\mathrm{NaOH}(.1$ or 1.0 N$)$. All pH measurements were recorded following 30 s of equilibration. Total volume of base or acid added to each aliquot was summed and utilized to calculate ruminal fluid buffering capacity. Buffering capacity was expressed as total milliequivalents of free protons required to change 1 L ruminal fluid from pH 5 to pH 7.

In an attempt to more accurately characterize alterations in ruminal acid-base status effected by dietary buffers, we utilized a buffer value index (BVI) developed previously (Hogue et al., 1991), which accounts for alterations in both ruminal $\left[\mathrm{H}^{+}\right]$and ruminal fluid buffering capacity. With decrease in the $\left[\mathrm{H}^{+}\right.$] in the rumen there is increase in the buffering capacity and increase in buffering value index. To calculate BVI, a standard pH (STPH) of 6 and standard buffering capacity (STBC) of 50 meq/L were utilized with the ruminal fluid sample pH (SAPH) and buffering capacity (SABC; meq/L) in the following formula:

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BVI = (((antilog}10(-STPH)) - (antilog 10 (-SAPH))) /
antilog}10(-STPH))+((SABC - STBC) / STBC) + 100
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Milk was sampled weekly during consecutive p.m. and a.m. milkings and analyzed for fat, protein, lactose and SNF composition via infrared spectrophotometry (Multispec

2, Multispec Limited, Wheldrake, York, England) and for SCC via electronic counting (Coulter Milk Cell Counter, Coulter Electronics, Inc., Opalocka, FL).

Statistical analysis consisted of a linear model ANOVA for each sampling time for ruminal variables; DM intake, milk yield and milk composition were analyzed as weekly means. In each instance, cow, period, treatment and residual error were included in the model. Contrasts were employed to compare Ctrl to 0-2Bic, Ctrl to 2-4Bic, and Ctrl to 4-6Bic. Statistical significance was established at $\mathrm{P}<.10$.

Results and Discussion

## Ruminal Acid-Base Status

Free proton concentration tended to be lower for 02Bic than Ctrl at 1.5 and 3.5 h postfeeding (Figure 1A). The difference at 1.5 h was during the period of supplemental $\mathrm{NaHCO}_{3}$ infusion into the rumen. The $\left[\mathrm{H}^{+}\right]$for 2-4Bic also tended to be lower than for Ctrl during $\mathrm{NaHCO}_{3}$ infusion ( 4 h ) and was significantly lower at 8 h postfeeding. The 4-6Bic infusion resulted in a higher [ $\mathrm{H}^{+}$] than for Ctrl at 0 and 2 h , but reduced $\left[\mathrm{H}^{+}\right.$] after $\mathrm{NaHCO}_{3}$ infusion began ( 5.5 h ) and again at 7.5 h postfeeding. Hogue et al. (1991) also reported that the effects of intra-ruminal infusion of $\mathrm{NaHCO}_{3}$ on $\left[\mathrm{H}^{+}\right.$] were most dramatic during the infusion interval. As compared to Ctrl, mean
ruminal $\left[\mathrm{H}^{+}\right]$from 0 to 8 h postfeeding was lowered by $2-$ 4Bic and 4-6Bic, but not affected by 0-2Bic (Table 3). The rapid rise in $\left[\mathrm{H}^{+}\right.$] during hour 2 to 4 postfeeding (Table 3) for Ctrl and 0-2Bic indicated that, whether resulting from dietary or exogenous buffer, ruminal effects dissipated rapidly.

Infusion of $\mathrm{NaHCO}_{3}$ from 0-2 h postfeeding tended to increase buffering capacity of the rumen fluid (Figure 1A, Table 3); buffering capacity was higher for this treatment than for the Ctrl from 0 to 6 h postfeeding and clearly was increased at 2.5 and 3.5 h after feeding. Buffering capacity for 2-4Bic was similar to that of Ctrl throughout the 8 -h postfeeding interval, whereas 4-6Bic increased buffering capacity above Ctrl at $2.5,3.5,5.5$ and 7.5 h postfeeding.

Overall, ruminal infusion of $\mathrm{NaHCO}_{3}$ tended to increase buffering capacity above that for the water infusion throughout the postfeeding interval (Table 3), but the increases did not always correspond temporally to $\mathrm{NaHCO}_{3}$ infusion. For example, 4-6Bic was higher than Ctrl at 2.5 and 3.5 h postfeeding, before $\mathrm{NaHCO}_{3}$ infusion was initiated. In contrast to the present study, Hogue et al. (1991) observed increased ruminal fluid buffering capacity during $\mathrm{NaHCO}_{3}$ infusion. In our study, $\mathrm{NaHCO}_{3}$ was included in the diet in addition to the intra-ruminal infusions; hence, the $\mathrm{NaHCO}_{3}$ infusion treatments were compared to a Ctrl cow receiving some $\mathrm{NaHCO}_{3}$ via the diet in order to
evaluate the effects of combination of unprotected and controlled released buffer on ruminal acid-base status. This presence of buffer in the diet may explain the lack of buffering capacity response to the $\mathrm{NaHCO}_{3}$ infusions.

The BVI (Figure 1) increased with $\mathrm{NaHCO}_{3}$ infusion; it was higher for 0 -2Bic than for Ctrl from 1.5 to 2.5 h , for $2-4$ Bic at 3.5 and 4.5 h , and for $4-6 \mathrm{Bic}$ at 5.5 and 7.5 h postfeeding. Mean BVI from 0 to 8 h postfeeding was higher for each of the $\mathrm{NaHCO}_{3}$ infusion treatments than for Ctrl (Table 3); however, these differences were not detected with each $\mathrm{NaHCO}_{3}$ infusion treatment for buffering capacity and ruminal $\left[\mathrm{H}^{+}\right]$during this interval. Buffer value index is an indicator of alterations in both ruminal [ $\mathrm{H}^{+}$] and rumen buffering capacity. As such, it may be useful to identify changes in ruminal acid-base status that are not apparent when examining $\left[\mathrm{H}^{+}\right.$] or buffering capacity separately.

## Ruminal Volatile Fatty Acids

Total ruminal fluid VFA concentrations (Figure 2) generally were unaffected, whereas the ruminal acetate-topropionate ratio (A:P) (Fig 3) tended to be reduced by $\mathrm{NaHCO}_{3}$ infusion. Total ruminal fluid VFA concentrations may be related to DM intake, Kovacik et al. (1986); in our study, DM intake was not altered by treatments, possibly explaining the consistent total VFA concentrations. The reduction in $A: P$ with $\mathrm{NaHCO}_{3}$ infusion (Figure 3) was
observed for each infusion interval, but contrasts with the results of Rogers et al. (1985), and Rogers and Davis (1982), who reported that dietary $\mathrm{NaHCO}_{3}$ supplementation increased A:P. Hogue et al. (1991) also observed an increase in $A: P$ with infusion of $\mathrm{NaHCO}_{3}$ from 0 to 2 h postfeeding. The reason for this discrepancy is unclear.

## Ruminal Kinetics

The natural logarithm of concentrations of ruminal fluid $C r$ was regressed on time postfeeding to allow calculation of ruminal kinetics. Mean $r^{2}$ for linearity of the regression was $.695 \pm .239$, $\mathrm{n}=16$. Ruminal liquid volume (Table 4) was higher for 0-2Bic than for Ctrl. This agrees with Hogue et al. (1991), who reported that ruminal liquid volume was increased by infusing $\mathrm{NaHCO}_{3}$ from 0 to 2 h, and from 4 to 6 h postfeeding. Others (Kovacik et al., 1986; Erdman, 1980) have reported that feeding $\mathrm{NaHCO}_{3}$ at as much as $2 \%$ of diet $D M$ had no effect on ruminal liquid volume. In our study, liquid flow rate was lower for 24Bic than for Ctrl and tended to be lower for 2-4Bic than for other $\mathrm{NaHCO}_{3}$ infusion intervals. This contrasts with Rogers et al. (1985), who observed that supplementation of $1.4 \% \mathrm{NaHCO}_{3}$ increased total daily rumen fluid outflow in cows receiving either long-stemmed or chopped alfalfa hays. Neither ruminal liquid dilution rate nor liquid turnover time were affected by ruminal infusion of $\mathrm{NaHCO}_{3}$.

The diet we used was similar to that used by Hogue et al. (1991) with the exception that their diet contained no $\mathrm{NaHCO}_{3}$; they reported that, compared with the water-infused control, infusing $\mathrm{NaHCO}_{3}$ from 2 to 4 h postfeeding reduced DM intake. In our study, DM intake (Table 2) was not affected by any $\mathrm{NaHCO}_{3}$ infusion interval. The reduction in intake reported by some researchers when $\mathrm{NaHCO}_{3}$ was supplemented to the diet of lactating cows probably can be attributed to poor palatability; gradual introduction of the buffer into the diet should eliminate this problem (Donker and Marx, 1980). Others (Coppock et al., 1986; Haaland et al., 1982) have observed that DM intake increased with dietary $\mathrm{NaHCO}_{3}$ supplementation.

In contrast with Hogue et al. (1991), milk yield was higher for Ctrl than for 4-6Bic, potentially a result of the tendency for lower DM intake with 4-6Bic. Cows were released from the stanchions immediately following termination of their infusion interval; therefore, 4-6Bic cows were confined for longer intervals than the controled cows. This added stress may have contributed to reduced milk yield. Despite the high concentrate-to-forage ratio of the test diet, milk fat percentage, fat yield and protein yield were not affected by treatment (P>.10). In a recent review, Erdman (1980) reported that $\mathrm{NaHCO}_{3}$ supplementation had no effect on milk fat percentage when cows were consuming grass silages. In our study, milk
protein yield, lactose yield, percentage SNF and SNF yield all were reduced for 4-6Bic vs. Ctrl, potentially a result of reduced intake or increased stress for that treatment. Milk lactose percentage, although typically quite constant, was lower in our study for 0-2Bic than Ctrl; the reason for this response is unclear. Milk SCC were not affected by treatment and remained within the range typical for wellmanaged dairy herds. The short adaptation time for infusions (1 wk) may not have permitted an accurate evaluation of infusion-effected alterations in milk yield and milk composition.

Conclusions

Addition of $\mathrm{NaHCO}_{3}$ to the diets of cows receiving intra-ruminal $\mathrm{NaHCO}_{3}$ infusions attenuated the alterations in ruminal acid-base status observed previously for infusion without dietary $\mathrm{NaHCO}_{3}$ (Hogue et al., 1991). Alterations were evident; however, they were observed primarily during the infusion intervals and dissipated rapidly when infusion ceased. Infusion-effected changes in ruminal acid-base status were not accompanied by the expected alterations in ruminal VFA concentrations or in ruminal liquid kinetics. Therefore, we suggest that the influence of $\mathrm{NaHCO}_{3}$ infused directly into the rumen at different intervals postfeeding may differ from that
previously established (Rogers et al., 1985; Rogers and Davis, 1982) for dietary $\mathrm{NaHCO}_{3}$.

## Figure Legends

Figure 1. Ruminal buffer value index, buffering capacity (meq/L) and free proton concentration (neq/L) for Ctrl vs. 0 to $2 \mathrm{~h} \mathrm{NaHCO}_{3}$ infusion, panel A; Ctrl vs. 2 to 4 $\mathrm{h} \mathrm{NaHCO}_{3}$ infusion, panel B; and Ctrl vs. 4 to 6 h $\mathrm{NaHCO}_{3}$ infusion, panel C. Ctrl $=(\cdots) ; \mathrm{NaHCO}_{3}$ infusion $=(----)$. Free proton concentration of 1,000 neq $/ L=\mathrm{a} \mathrm{pH}$ of 6; $316 \mathrm{neq} / \mathrm{L}=\mathrm{a} \mathrm{pH}$ of 6.5; and 100 neq $/ L=a \mathrm{pH}$ of 7 . Vertical, dashed lines represent beginning and end of $\mathrm{NaHCO}_{3}$ infusion. * = probability that treatment means are not different ( $\mathrm{P}<.10$ ).

Figure 2. Total ruminal volatile fatty acids (mM) for Ctrl vs. 0 to $2 \mathrm{~h} \mathrm{NaHCO}_{3}$ infusion, bottom panel; Ctrl vs. 2 to $4 \mathrm{~h} \mathrm{NaHCO}_{3}$ infusion, middle panel; and Ctrl vs. 4 to 6 h NaHCO 3 infusion, top panel. Ctrl $=(\cdots) ; \mathrm{NaHCO}_{3}$ infusion $=$ (----). $*=$ probability that treatment means are not different ( $\mathrm{P}<.10$ ) .

Figure 3. Ruminal acetate to propionate ratio (A:P) for Ctrl vs. 0 to 2 h NaHCO 3 infusion, bottom panel; Ctrl vs. 2 to $4 \mathrm{~h} \mathrm{NaHCO}_{3}$ infusion, middle panel; and Ctrl vs. 4 to 6 h NaHCO 3 infusion, top panel. Ctrl = (•••); $\mathrm{NaHCO}_{3}$ infusion = (----). $\quad$ * = probability that treatment means are not different ( $\mathrm{P}<.10$ ).

Alphabetical Key to Author Abbreviations

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A:P = acetate to propionate ratio
0-2Bic = intra-ruminal infusion of NaHCO
    postfeeding
2-4Bic = intra-ruminal infusion of NaHCO}3\mathrm{ from 2 to 4 h
    postfeeding
4-6Bic = intra-ruminal infusion of NaHCO}3\mathrm{ from 4 to 6 h
    postfeeding
BVI = buffer value index
Ctrl = intra-ruminal infusion of water from 0 to 2 h
    postfeeding
[H+}]=\mathrm{ free proton concentration
SABC = sample buffering capacity
SAPH = sample pH
STBC = standard buffering capacity
STPH = standard pH
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Table 1
INGREDIENTS AND NUTRIENT COMPOSITION OF EXPERIMENTAL DIET DM.
Ingredient: ..... \%
Sorghum silage ..... 35.01
Ground shelled corn ..... 40.57
Soybean meal, 44\% CP ..... 21.64
Limestone ..... 92
Dicalciuq phosphate ..... 54
Dynamate ..... 36
Trace mineralized salt ${ }^{2}$ ..... 46
Sodium bicarbonate ..... 50
Nutrient ${ }^{3}$ :
DM (as fed) ..... 43.3
Crude protein ..... 17.3
ADF ..... 20.3
NDF ..... 32.4
$\mathrm{NE}_{1}, \mathrm{Mcal} / \mathrm{kg}^{4}$ ..... 1.59
Ca .....  57
P ..... 48
Mg ..... 33
K ..... 1.39
Na ..... 39
S ..... 31
Cl ..... 42
${ }^{1}$ Double sulfate of K and Mg .
${ }^{2}$ Contained $92 \% \mathrm{NaCl}, .250 \% \mathrm{Mn}, .200 \% \mathrm{Fe}, .033 \%$ $\mathrm{Cu}, .007 \% \mathrm{I}, .005 \% \mathrm{Zn}$, and .0025\% Co.
${ }^{3}$ Composition from laboratory analyses.
${ }^{4}$ Calculated from ADF.

Table 2.
DRY MATTER INTAKE, MILK YIELD AND MILK COMPOSITION OF COWS RECEIVING RUMINAL INFUSION OF WATER FROM 0 TO 2 H , OR NaHCO 3 FOR 0 TO-2, 2 TO 4, OR 4 TO 6 H AFTER FEEDING.

| Infusion interval |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{gathered} 1 \\ 0-2 \mathrm{~h} \\ \text { Water } \end{gathered}$ | $\begin{array}{r} 2 \\ 0-2 \mathrm{~h} \\ \mathrm{NaHCO}_{3} \end{array}$ | $\begin{gathered} 3 \\ 2-4 \mathrm{~h} \\ \mathrm{NaHCO}_{3} \end{gathered}$ | $\begin{gathered} 4 \\ 4-6 \mathrm{~h} \\ \mathrm{NaHCO}_{3} \end{gathered}$ | SE |  | Effect | P.Value |
| DM intake, kg/d Milk: | 14.8 | 15.2 | 14.5 | 14.2 | . 5 |  | NS ${ }^{1}$ |  |
| Yield, kg/d | 21.2 | 20.0 | 21.2 | 19.1 | . 7 | 1 | vs 4 | . 073 |
| Fat, \% | 3.39 | 3.54 | 3.40 | 3.45 | . 18 |  | NS |  |
| Fat: |  |  |  |  |  |  |  |  |
| Yield, kg/d | 0.71 | . 72 | . 71 | . 65 | . 04 |  | NS |  |
| Protein, \% | 3.00 | 3.03 | 2.97 | 2.98 | . 03 |  | NS |  |
| Protein: |  |  |  |  |  |  |  |  |
| Yield, kg/d | . 63 | . 61 | . 62 | . 56 | . 02 |  | vs 4 | . 063 |
| Lactose, \% | 5.05 | 5.00 | 5.06 | 5.01 | . 02 | 1 | vs 2 | . 071 |
| Lactose: |  |  |  |  |  |  |  |  |
| Yield, kg/d | 1.07 | 1.00 | 1.07 | 0.96 | . 03 |  | vs 4 | . 052 |
| SNF, \% | 8.68 | 8.66 | 8.65 | 8.62 | . 02 |  | vs 4 | . 038 |
| SNF: |  |  |  |  |  |  |  |  |
| Yield, kg/d | 1.84 | 1.73 | 1.83 | 1.64 | . 06 | 1 | vs 4 | . 054 |
| SCC (1,000) | 130 | 161 | 127 | 132 | 24 |  | NS |  |

[^0]Table 3.
MEAN ALTERATIONS IN RUMINAL ACID-BASE STATUS OF COWS RECEIVING RUMINAL INFUSION OF WATER FROM 0 TO 2 H , OR NaHCO 3 FOR

0 TO 2,2 TO 4, OR 4 TO 6 H AFTER FEEDING.

| Infusion interval |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | 1 | 2 | 3 | 4 |  |  |  |
|  | 0-2h | 0-2h | $2-4 \mathrm{~h}$ | 4-6h |  |  |  |
|  | Water | $\mathrm{NaHCO}_{3}$ | $\mathrm{NaHCO}_{3}$ | $\mathrm{NaHCO}_{3}$ | SE | Effect | P Value |
| $\left[\mathrm{H}^{+}\right], \mathrm{neq} / \mathrm{L}$ |  |  |  |  |  |  |  |
|  | 582 | 405 | 519 | 639 | 82 | NS ${ }^{1}$ |  |
| 2 to 4 h | 618 | 684 | 454 | 761 | 101 | NS |  |
| 4 to 6 h | 859 | 939 | 472 | 426 | 220 | NS |  |
| 6 to 8 h | 563 | 588 | 422 | 306 | 147 | NS |  |
| 0 to 8 h | 657 | 645 | 464 | 521 | 46 | 1 Vs 3 | . 003 |
|  |  |  |  |  |  | 1 Vs 4 | . 038 |
| Mean rumen pH |  |  |  |  |  |  |  |
| 0 to 2 h | 6.35 | 6.54 | 6.37 | 6.27 |  | 1 Vs 2 | . 118 |
| 2 to 4 h | 6.25 | 6.36 | 6.43 | 6.18 |  | 1 vs 3 | . 129 |
| 4 to 6 h | 6.14 | 6.21 | 6.43 | 6.45 |  | NS |  |
| 6 to 8 h | 6.30 | 6.46 | 6.46 | 6.58 |  | NS |  |
| 0 to 8 h | 6.26 | 6.40 | 6.43 | 6.38 |  | 1 vs 2 | . 011 |
|  |  |  |  |  |  | 1 vs 3 | . 001 |
|  |  |  |  |  |  | 1 vs 4 | . 024 |

Table 3. (Continued)

|  | $\begin{aligned} & 0-2 h \\ & \text { Water } \end{aligned}$ | $\begin{aligned} & 0-2 \mathrm{~h} \\ & \mathrm{NaHCO}_{3} \end{aligned}$ | $\begin{aligned} & 2-4 \mathrm{~h} \\ & \mathrm{NaHCO}_{3} \end{aligned}$ | $\begin{aligned} & 4-6 \mathrm{~h} \\ & \mathrm{NaHCO}_{3} \end{aligned}$ | SE | Effect | P.Value |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Mean buffering capacity, meq/L |  |  |  |  |  |  |  |
| 0 to 2 h | 55.5 | 65.1 | 56.6 | 55.4 | 5.4 | NS |  |
| 2 to 4 h | 50.0 | 59.3 | 54.2 | 55.3 | 2.3 | 1 vs 2 | . 028 |
| 4 to 6 h | 54.9 | 59.2 | 57.3 | 59.9 | 1.7 | 1 vs 2 | . 129 |
|  |  |  |  |  |  | 1 vs 4 | . 088 |
| 6 to 8 h | 54.6 | 55.9 | 56.2 | 60.8 | 2.2 | 1 vs 4 | . 093 |
| 0 to 8 h | 54.2 | 59.8 | 56.3 | 58.2 | 1.1 | 1 vs 2 | . 005 |
|  |  |  |  |  |  | 1 vs 4 | . 012 |
| Mean buffer value index |  |  |  |  |  |  |  |
| 0 to 2 h | 100.54 | 100.90 | 100.60 | 100.47 | . 16 | NS |  |
| 2 to 4 h | 100.38 | 100.50 | 100.63 | 100.34 | . 13 | NS |  |
| 4 to 6 h | 100.24 | 100.24 | 100.67 | 100.77 | . 23 | NS |  |
| 6 to 8 h | 101.53 | 100.53 | 100.70 | 100.91 | . 18 | NS |  |
| 0 to 8 h | 100.43 | 100.55 | 100.67 | 100.64 | . 05 | 1 vs 2 | . 117 |
|  |  |  |  |  |  | 1 vs 3 | . 002 |
|  |  |  |  |  |  | 1 vs 4 | . 006 |

[^1]Table 4.
RUMEN KINETICS OF COWS RECEIVING RUMINAL INFUSION OF WATER FROM 0 TO $2 \mathrm{H}, \mathrm{OR} \mathrm{NaHCO}_{3}$ FOR 0 TO 2, 2 TO 4, OR 4 TO 6 H AFTER FEEDING.

Infusion interval

| 1 | 2 | 3 | 4 |
| :---: | :---: | :---: | :---: |
| $0-2$ | h | $0-2 \mathrm{~h}$ | $2-4 \mathrm{~h}$ |
| O | $4-6 \mathrm{~h}$ |  |  |
| Water | $\mathrm{NaHCO}_{3}$ | $\mathrm{NaHCO}_{3}$ |  |
| $\mathrm{NaHCO}_{3}$ |  |  |  | $\mathrm{SE}^{2} \quad$ Effect $\quad$ P.Value

Rumen:
$\left.\begin{array}{lcccccccc}\text { Liquid Volume, L } 54.1 & 62.0 & 52.2 & 51.1 & 3.2 & 1 & \text { vs } 2 & .129 \\ \text { Flow rate, L/h } & 5.97 & 4.37 & 3.62 & 5.44 & 1.00 & 1 & \text { vs } & 3\end{array}\right) .148$

[^2]

A


FIGURE 1. A



FIGURE 1. B



FIGURE 1. C



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[^0]:    ${ }^{1}$ Nonsignificant; $\mathrm{P}>.10$.

[^1]:    $1_{\text {Nonsignificant; }} \mathrm{P}>.10$.

[^2]:    ${ }^{1}$ Nonsignificant; $\mathrm{P}>.10$.

