This is to certify that the
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REGULATION OF THE RUMINAL ENVIRONMENT
BY LACTATING DAIRY COWS

presented by

CHARLES STEVEN MOONEY

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Michael S. Allen
Major Professor's Signature

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REGULATION OF THE RUMINAL ENVIRONMENT BY LACTATING DAIRY COWS

By

Charles Steven Mooney

A DISSERTATION

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2006

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ABSTRACT

REGULATION OF THE RUMINAL ENVIRONMENT
BY LACTATING DAIRY COWS

By

Charles Steven Mooney

The ruminal environment must be regulated for the health and productivity of ruminants. Sodium is the most abundant cation in the ruminal solution and is the most likely candidate as the regulated ion. Three experiments were conducted to evaluate the role of strong ions in the ruminal environment. If sodium compounds are infused into the ruminal solution, rumination time is reduced markedly, however, these infusions may generate ruminal conditions that are not representative of normal physiological or nutritional conditions. In the first experiment, we hypothesized that additional dietary sodium at normal concentrations would reduce rumination time of dairy cows. Additional dietary sodium decreased rumination time as did additional dietary potassium indicating that the general decrease in rumination was caused by a tonic increase in ruminal osmolality. Sodium is often added to lactating dairy cow diets in the form of sodium bicarbonate. The benefits of sodium bicarbonate addition are well documented but the mechanism of its action has not been defined. In experiment two, addition of dietary sodium increased total tract neutral detergent fiber digestibility probably by an expansion of ruminal contents and slowing of passage of digesta from the rumen. These effects are likely only a component of the mechanism of sodium bicarbonate action in lactating dairy cows. In this experiment, the addition of dietary sodium bicarbonate did not affect ruminal pH or alter the site of starch digestion. Sodium is a strong ion and

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strong ions are one of the determinants of the pH of a solution. In experiment three, we hypothesized that strong ion concentrations in the ruminal solution would be related to ruminal pH. Ruminal pH was correlated positively with ruminal sodium concentration, the sum of ruminal sodium and potassium, and ruminal strong ion difference, and was correlated negatively with total volatile fatty acid concentration, ruminal ammonium, and the sum of ruminal ammonium plus potassium. Also, ruminal sodium concentration was negatively related to the sum of ruminal potassium plus ammonium concentrations. Therefore, the total concentration of cations is controlled, balancing ruminal acidity and limiting ruminal osmolality. A uniform, alkalizing strong ion difference was maintained in the ruminal solution across animals and dietary treatments and this plus the charge balance in the rumen are likely regulated by modifying sodium flux across the ruminal epithelium. Sodium, as well as bicarbonate, are likely key in the whole body regulation of the volatile fatty acid load. These experiments suggest that lactating dairy cows actively regulate the ruminal environment especially sodium in the ruminal solution.

DEDICATION

To SG and BW and LL, without whom this would not be.

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To Dr. A

To my G

for their patience

To Drs. A

Burton, Benson, D

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Gordon, Kevin,

Mike, Joy, Sher

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Heather, Tina,

Jessica, for al

To my

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02CSM2	
99MO01	
ADF	
AOAC	
BCS	b
BHBA	β
BW	bo
CP	cr
DCAD	die
DCAD3	die
DCAD4	die
DHIA	Dai
DIM	days
DM	dry
DMI	dry
ECF	extr
EDTA	ethy
FCM	fat-c
HPLC	high
ICF	intra

KEY TO ABBREVIATIONS

01CSM1	Experiment: 40 cows, 5 x 5 Latin square (n = 8), 14 d periods (Chapter 2)
02CSM2	Experiment: 6 cows, 3 x 3 Latin square (n = 2), 28 d periods (Chapter 3)
99MO01	Experiment: 8 cows, 4 x 4 Latin square (n = 2), 21 d periods (Chapter 4)
ADF	acid detergent fiber
AOAC	Association of Official Analytical Chemists International
BCS	body condition score
BHBA	β-hydroxybutyrate
BW	body weight
CP	crude protein
DCAD	dietary cation anion difference
DCAD3	dietary cation anion difference based on Na, K and Cl
DCAD4	dietary cation anion difference based on Na, K, Cl, and S
DHIA	Dairy Herd Improvement Association
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECF	extracellular fluid
EDTA	ethylenediaminetetraacetate
FCM	fat-corrected milk
HPLC	high performance (pressure) liquid chromatography
ICF	intracellular fluid

iNDF	
MN	
MUN	
n	n
NAN	n
NDF	n
NEFA	n
NE_L	n
NFC	no
NANMN	no
NRC	Na
OM	org
OMI	org
pdNDF	pot
r	cor
R^2	coe
ROO	reti
RR	reti
SARA	sub
SAS	Sta
SCC	som

KEY TO ABBREVIATIONS (continued)

iNDF	indigestible NDF
MN	microbial nitrogen
MUN	milk urea nitrogen
n	number of samples
NAN	nonammonia nitrogen
NDF	neutral detergent fiber
NEFA	nonesterified fatty acids
NE_L	net energy for lactation
NFC	nonfiber carbohydrate
NANMN	non-ammonia non-microbial N
NRC	National Research Council
OM	organic matter
OMI	organic matter intake
pdNDF	potentially digestible NDF
r	correlation coefficient
R^2	coefficient of determination
ROO	reticular-omasal orifice
RR	reticulo-rumen or reticulorumen
SARA	subacute ruminal acidosis
SAS	Statistical Analysis System
SCC	somatic cell count

SCM	soli
SCS	sor
SD	star
SE	star
SID	str
SNF	soli
TRDOM	trul
TMR	tota
VFA	vol.

KEY TO ABBREVIATIONS (continued)

SCM	solids-corrected milk
SCS	somatic cell score
SD	standard deviation
SE	standard error
SID	strong ion difference
SNF	solids-not-fat
TRDOM	truly ruminally degraded organic matter
TMR	total mixed ration(s)
VFA	volatile fatty acid(s)

CHAPTER 1:

Overview

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Ruminants

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CHAPTER 1: Literature Review

THE LACTATING DAIRY COW AS A RUMINANT

Overview

Lactating dairy cows are unique among domestic ruminants for their ability to consume feed, commonly consuming 4% of their BW on a daily basis (NRC, 2001). This consumed feed ferments in the forestomach and leads to a significant acid load which cows must regulate to maintain homeostasis. In this regulation, the production of copious sodium bicarbonate by the salivary glands is a key control. Total salivary sodium bicarbonate is proportional to total saliva flow which is influenced by many factors.

Ruminants

Ruminants have a symbiotic relationship with the microbes of their foregut (Russell and Rychlik, 2001). The ruminant provides water, warmth, substrate, and endproduct removal to a dense, diverse, and interacting collection of suitable bacteria ($>10^{10}$ cells per gram of contents), protozoa ($=10^{6}$ cells per gram of contents), and fungi (Russell and Rychlik, 2001). In return, the ruminant obtains nutrients (energy from VFA, microbial protein) from plant fiber (cellulose etc.) unavailable by mammalian digestion (Russell and Rychlik, 2001).

Ruminants are characterized by a fermentation of feed in a highly specialized four-chambered stomach (Van Soest, 1994). Feed is ingested and fermented to volatile fatty acids (VFA) by microbes in the forestomach (Hofmann, 1988). Consumed sugars,

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starches, cellulose, hemicellulose and pectin are fermented to VFA and gases (Stevens and Hume, 1995). In the forestomach, acetic, propionic, and butyric acids usually account for 95% of the VFA in solution and, of the gases, carbon dioxide predominates with half as much methane and much smaller amounts of hydrogen and hydrogen sulfide (Leek, 2004). Proportions of VFA and gases are dependent on substrate and microbes (Stevens and Hume, 1995).

Microbial fermentation occurs in the rumen and reticulum, the first two of the three forestomach chambers (Hofmann, 1988). As the rumen and the reticulum are only partially separated by the reticuloruminal fold, these two organs can be considered together as the reticulorumen (RR; Van Soest, 1994). The RR is major site of absorption, absorbing water, VFA, and ions (Van Soest, 1994) and, with its contents, can represent 15% of the body weight but the percentage is highly variable (Stevens and Hume, 1995). The omasum, the third chamber of the forestomach, controls the flow of the water and particles from the RR and absorbs water and VFA from the passed digesta (Hofmann, 1988). Cows have a more prominent omasum having twice the relative surface area when compared to sheep and goats (Engelhardt and Hauffe, 1975). The papillated lamellae of the omasum can constitute one-third of the surface area of the entire forestomach in domestic cattle (Stevens and Hume, 1995). This surface area absorbs water, VFA, sodium and potassium and can start the gastric secretion of chloride (Engelhardt and Hauffe, 1975). This absorption reduces the digesta volume and changes solute concentrations in preparation of HCl digestion in the abomasum, the fourth chamber and true stomach (Stevens and Hume, 1995).

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The forestomach chambers are lined on the luminal side with nonglandular stratified squamous epithelium with slight keratinization (Stevens and Hume, 1995) and are not secretory tissues (Leek, 2004). The forestomach's three compartments have structures that increase the surface area. The rumen has papillae, the reticulum has a "honeycomb" network of low ridges and the omasum has papillated lamellae (Leek, 2004).

The four-chambered stomach of dairy cows is well vascularized and innervated. The stomach receives blood from the branches off the abdominal aorta and is drained by the hepatic vein (Leek, 2004). The stomach is innervated by parasympathetic and sympathetic pathways (Leek, 2004). The parasympathetic pathways along the vagus nerve collect sensory input from the forestomach (monitoring tension, mechanical and chemical stimulation) which is integrated in the gastric centers of the brain (Leek, 2004). Motor signals return from the brain and are essential for the primary and secondary contraction cycles of the forestomach and also rumination and eructation (Leek, 2004). The sympathetic pathways along the splanchnic nerve can inhibit gastric motility (Leek, 2004).

Tension receptors and epithelial receptors have been found in the RR (Iggo and Leek, 1970; Leek and Harding, 1975; Leek, 1984). Tension receptors are located in the smooth muscle layer of the RR (Iggo and Leek, 1970; Leek and Harding, 1975; Leek, 1984) and appear to monitor the tension the RR wall (Leek, 2004). They are most apparent in the medial walls of reticulum, cranial ruminal sac, ruminorecticular fold, in the cranial pillar, outside of the lips of the reticular fold, and around the cardia and the reticulo-omasal orifice (Leek, 1984; Leek, 2004). These receptors are excited by passive

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distention caused by luminal contents and by active contraction of smooth muscle (Leek, 1984; Leek, 2004). Low to moderate excitation of these receptors increases the rate and amplitude of primary and secondary contractions and an increase in flow rate of saliva also follows (Leek, 1984; Leek, 2004). High excitation has the opposite effects (Leek, 1984; Leek, 2004).

The epithelial receptors in the RR are located near basement membrane of luminal epithelium (Iggo and Leek, 1970; Leek and Harding, 1975; Leek, 1984; Leek, 2004). These receptors respond to both mechanical and chemical stimulation (Leek and Harding, 1975; Leek, 1984; Leek, 2004). Mechanically, these receptors are excited by lightly moving tactile stimuli (i.e. "rapid light brushing") with a very low threshold (Leek, 1984; Leek, 2004). This excitation stimulates rumination which is likely the primary function of these receptors (Leek, 1984; Leek, 2004). Chemically, these receptors respond to a range of chemical stimuli (Leek and Harding, 1975). These receptors are stimulated by increases in "tritratable acidity" and this excitement inhibits the primary contraction cycle (Leek and Harding, 1975). In the extreme, the high acidity of acidosis will lead to ruminal stasis (Leek and Harding, 1975). In addition, a pH below 6 seems required for "significant epithelial receptor excitation" (Crichlow and Leek, 1981). Lower molecular weight acids evoke a quicker response and high molecular weight acids are ineffective (Leek and Harding, 1975). Of the VFA, butyric acid is particularly potent (Leek and Harding, 1975). Hypertonic and alkali solutions will also excite these receptors but at concentrations tested were outside the normal physiological range (Leek and Harding, 1975). Water and hypotonic solutions also excite these receptors (Leek and Harding, 1975).

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Rumination

Rumination is one of the defining behaviors of a ruminant. It aids in the regulation of ions and pH in the forestomach and is defined as the post prandial regurgitation of ingesta (Van Soest, 1994) and is a highly coordinated, highly rhythmic, cyclical process characterized by regurgitation, remastication, and reswallowing (Welch and Hooper, 1988). To ruminate, the central nervous and enteric nervous systems integrate ruminal and reticular stimuli and coordinate the diaphragm, rumen, reticulum, esophagus, and mouth (Van Soest, 1994). Rumination begins with the regurgitation of a bolus of ingesta from the reticulum to mouth (Beauchemin, 1991). Excess liquid is reswallowed and the remaining bolus is rechewed for about 60 seconds while salvia is added (Van Soest, 1994). The bolus is reswallowed to the RR and another will be regurgitated after a short pause (Beauchemin, 1991).

Dairy cows will normally spend more time ruminating than eating during a day (Van Soest, 1994). They will spend 5 to 9 h per d ruminating and 4 to 7 h per d eating (Beauchemin, 1991). Actual amount of time depends on physical form and composition of diet and the total amount ruminating time per d seems to have an upper limit of about 10 h per d (Welch, 1982). Cows will spend 10 to 20 periods per d ruminating (Beauchemin, 1991). The 30,000 to 50,000 chews per d occur with a lateral motion on the jaw that crushes not cuts the ingesta with the molars exposing the plant flesh to microbial attack (Beauchemin, 1991).

Rumination behavior is influenced by many factors. Rumination is inhibited by low pH, anorexia, high ruminal osmolality, and high VFA concentration (Welch, 1982; Welch and Hooper, 1988; Beauchemin, 1991; Van Soest, 1994; Leek, 2004). Rumination

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can be completely stopped by osmolality above a threshold (suggested as 350 mOsm in sheep; Welch, 1982). Rumination is stimulated by increased particle size, dietary fiber and feed intake, and decreased forage quality (Welch and Smith, 1969; Sudweeks et al., 1980; Welch and Hooper, 1988; Beauchemin, 1991; Van Soest, 1994). These stimulatory factors are associated with greater mechanical stimulation of the rumen wall (Baumont et al, 1990) and, therefore, excitation of tension and epithelial receptors of RR (Leek, 2004).

Salivation

The increase in saliva production is one of the important aspects of rumination. Compared to other species, ruminants secrete large quantities of saliva with enhanced quantities of buffer (Herdt, 2002). Ruminant saliva is more basic and contains more sodium and bicarbonate than other species (McDougall, 1948; Table 1.1) This saliva functions to aid in lubrication of ingested feed, in taste, in forming and swallowing a bolus of food, to provide some nutrients to rumen microorganisms (urea, minerals), to add fluid for proper microbial actions in RR, and to supply bicarbonate and phosphate buffers to the rumen (Bartley, 1976; Church, 1988a; Beauchemin, 1991; Ruckebusch et al., 1991).

Saliva is secreted from several glands. Ruminants have five bilateral and 3 unpaired glands(Kay, 1960). The five bilateral glands are the parotid, submaxillary, inferior molar, sublingual, and buccal (Kay, 1960). The three unpaired are the labial, pharyngeal, and palatine (Kay, 1960). These glands fall into 3 histological groups: serous, mucous, and mixed (Kay, 1960). The parotids secrete 40-50% of the daily saliva (Kay, 1960). The total flow from all glands is referred to as the "mixed" flow (Stevens and Hume, 1995).

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Saliva Composition

Ruminant saliva has an alkaline pH of near 8.2 (McDougall, 1948) and buffers well between pH 6 and 7 (Turner and Hodgetts, 1955b) but not well above pH 7.5 or below pH 5.5 (Bartley, 1976). Mixed saliva contains sodium, potassium, chloride and phosphate from the blood and bicarbonate from the salivary cell (Stevens and Hume, 1995) and is near isotonic with serum at standard ranges of secretion (Kay, 1960). Ruminant saliva contains higher concentrations of bicarbonate, phosphate and sodium and a lower concentration of chloride when compared to serum (Herdt, 2002). It also has some N containing compounds, mostly in the form of urea (Bartley, 1976). Saliva also contains an antifoaming agent to prevent bloat and an limited amount of the digestive enzymes lipase and amylase (Church, 1988a). Overall, the DM of saliva is very low at 1 to 1.5% (McDougall, 1948; Bailey and Balch, 1961b).

Saliva composition is relatively constant at higher rates of secretion under normal conditions (Bailey and Balch, 1961b) and the sum of cations (mEq/L) will equal the sum of anions (mEq/L) (McDougall, 1948; Bailey and Balch, 1961b). At resting flows, mixed saliva composition is about equal to parotid saliva composition (Bailey and Balch, 1961b). However, at mixed saliva flow rates below 30 ml/min, bicarbonate concentration decreases and phosphate concentration increases (Bailey and Balch, 1961b). Saliva composition has been measured in several experiments (Table 1.2).

The primary cation of mixed saliva of the ruminant is sodium (Kay, 1960). Sodium is usually present at more than ten times the potassium concentration (Kay, 1960). When the body is depleted of sodium (either by prolonged dietary sodium deficiency or by an artificial sodium draw), potassium can replace sodium in mixed saliva

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so that the sum of sodium and potassium concentrations remains constant (Kay, 1960; Bailey and Balch, 1961a; Bailey and Balch, 1961b; Hawkins et al., 1965). The parotid, inferior molar, and submaxillary glands affect this change (Kay, 1960). The sum of Na and K in cows saliva is likely constant (166 mEq/L; Bailey and Balch, 1961b). Saliva concentrations of sodium, potassium and urea can be influenced by intake (either dietary or infusion) with the greater ingestion leading to higher saliva concentrations (Warner and Stacy, 1977). Sodium concentration of saliva appears constant over a range of flow rates (Carter and Grovum, 1990a; Table 1.2 for more detail.)

The primary anion of mixed saliva of the ruminant is bicarbonate with phosphate then chloride following in concentration (Kay, 1960). Saliva bicarbonate concentration is about four times, saliva phosphate is about fifteen times, and saliva chloride is about 1/6 of concentrations found in serum (McDougall, 1948). These anions of mixed saliva tend to remain proportional (Bailey and Balch, 1961a) and are not affected strongly by the animal, diet or experimental treatment (Bailey and Balch, 1961b). (Table 1.2 for more detail.)

Total flow of bicarbonate and phosphate into the RR will be proportional to the total salivary flow (Erdman, 1988a) and can be predicted to provide buffering of 19.0 and 6.6 Eq/d, respectively, for a typical lactating dairy cow on a typical diet (Allen, 1997).

Saliva Flow

Cows secrete saliva continuously with increases in flow rate during eating and ruminating (Church, 1988a). Several experiments have measured saliva flow at rest and during eating. (Table 1.3) Earlier work determined that saliva flow rate during eating was two to four times the flow rate at rest (Bailey, 1961a). More recent work with

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lactating dairy cows has shown a smaller difference between resting flow and eating flow. Cassida and Stokes (1986), using cardial collection in lactating cows, found mixed saliva flows were 151 ml/min during resting and 171 ml/min during eating. Maekawa et al. (2002b), using cardial collection in lactating cows, mixed saliva flows were 101 ml/min during resting and 225 ml/min during eating. (Table 1.3)

Parotid cannulation has been used to calculate mixed saliva flow during rumination. An early measure of flow from a single cannulated parotid gland of a dry cow was 10 ml/min for resting, 20 ml/min during eating, and 25 ml/min during rumination (Bailey and Balch, 1961a). Based on this, mixed saliva flow during rumination was estimated at 2.5 times the resting flow or 100 ml/min (Bailey and Balch, 1961a). A later review summarized 19 published parotid cannulations during rumination and calculated a mean for flow during rumination of 1.7 times resting and showed that less than a third of the studies over 2.0 times (Cassida and Stokes, 1986). The authors concluded that 2.5 times was "not warranted" and a ruminating flow rate of 272 ml/min or 1.8 times the resting rate for lactating dairy cows on "high concentrate diets" was recommended.

In ruminants, flow of saliva can be influenced by a number of factors. Salivary glands under the control of parasympathetic nervous system (Herdt, 2002). The parotids and inferior molar (and thus over half of the flow) are under tonic nervous inhibition and submaxillary is under tonic stimulation (Kay, 1960). Flow from the parotids, inferior molar, palatine, buccal, and pharyngeal is increased with the stimulation of the mouth, oesophagus, and RR (Kay, 1960).

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Flow of saliva can be influenced by feed, feed characteristics, and intake. Resting flow is greatest on grass and lowest on silage with hay diets being intermediate, however, these flow differences do not produce ruminal differences (Bailey and Balch, 1961b). Flows during resting and rumination are reduced by pelleting and grinding of feeds (Putnam et al., 1966). Meal length is probably more important in determining the saliva produced (Bailey, 1961a) with feeds most difficult to process through the mouth producing longer eating times and therefore more saliva. Flow is influenced by meal size with higher flow after small meal and lower flow after big meal (Bailey and Balch, 1961b) and by time relative to a meal with slowest flow after feeding and a gradual increase to the highest rate before the next feeding (Bailey and Balch, 1961b; Wilson, 1963; Bartley, 1976). Distention caused by food, water and saliva entering the RR during a meal can generate an inhibition of saliva flow that gradually subsides as the volume of the RR diminishes between meals (Bartley, 1976). Resting flow can also show a diurnal variation (Meyer et al., 1964).

Flow is influenced by the ruminal contents and blood composition. Flow from the parotids can be increased by stretching near the oesophagus, cardia, reticulo-rumen fold or reticulo-omasal orifice (Ash and Kay, 1959). Flow is not directly influenced by light tactical stimulation in the RR but this stimulation can initiate rumination which will be accompanied by increased flow of saliva (Ash and Kay, 1959). Flow of saliva (and motility) are inhibited by distension of RR (Ash and Kay, 1959).

Flow is reduced by the ruminal infusion of artificial saliva or 1% sodium chloride (Wilson and Tribe, 1963). Whether flow is affected by ruminal changes depends on effects on plasma (Warner and Stacy, 1977) suggesting the increased ruminal osmolality

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leads to increased plasma osmolality. Flow is reduced by the intravenous perfusion or intraruminal infusion of mixed VFA with reduction via intravenous effects being faster, stronger and longer (Focant et al., 1979). Saliva flow is decreased by increased osmotic pressure in the plasma and vice versa (Warner and Stacy, 1977). The +10 mOsm increase in plasma osmolality associated with the end of a large meal leads to inhibited parotid saliva flow due to reduced parasympathetic stimulation (Carter and Grovum, 1990a).

Flow of saliva may be influenced by stage of lactation and parity. Resting flow of saliva has been reported as lower in early lactation than at peak (Cassida and Stokes, 1986). While having similar eating salivation rates, multiparous cows have greater resting salivation rates and spend more time ruminating per d than primiparous (Maekawa et al., 2002a). The proportion of these effects that is attributed to differences in intake and to differences among the animals remains to be determined.

Saliva flow does not appear to be influenced by water intake or percent moisture of feeds (Wilson and Tribe, 1963). Total flow usually proportional to feed intake (Poutiainen, 1966). Feed intake has a greater influence of flow than any other dietary factors except particle size (Wilson and Tribe, 1963).

Total daily flow of saliva reports vary for cattle. Early reports estimated total daily flow as 56 L/d for oxen and 50 L/d for cattle (Colin, 1886; Markoff, 1913, respectfully as reported in McDougall, 1948). Research in the 1960s reported average daily production near 140 L/d with a range of plus or minus 50 L/d (Bailey, 1961a; Meyer et al., 1964; Poutiainen, 1966). More recent studies of lactating dairy cows show they produce even more saliva (Cassida and Stokes, 1986; Maekawa et al., 2002a;

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Maekawa et al., 2002b). Rumination times in most of these studies were not measured and are estimated based on previously reported time relationships between eating and rumination (Bailey, 1961a; Meyer et al., 1964; Poutiainen, 1966; Cassida and Stokes, 1986; Table 1.4)

Where rumination behavior was monitored, lactating cows produced 239 L/d of saliva regardless of treatment diet (Maekawa et al., 2002b). Lack of diet difference was attributed to continuous flow of saliva in the lactating dairy cow (Maekawa et al., 2002b). This study reported a wider gap between resting flow (101 ml/min) and eating flow (225 ml/min) and that rate of salivary secretion during eating was 2.2 times higher than during resting (Maekawa et al., 2002b). They assumed the flow of saliva during rumination was the same as during eating when calculating total salivary flow per d (Maekawa et al., 2002b).

The study of flow rates of saliva is an area of research that is still in a descriptive phase and its many assumptions need testing and validation before a working model can be put into practice. A stronger, quantitative understanding of the variations of flows within a day and across diets awaits further study with methods and ideas yet to be discovered.

Even with differences in chewing behavior, differences in ruminal digesta weight, ruminal liquid volume and ruminal liquid turnover rate were not found (Cassida and Stokes, 1986; Maekawa et al., 2002b). Increased physically effective fiber (peNDF) resulted in increased ruminating and total chewing, but ruminal pH expressed as mean, area or time under the curve was not affected (Yang and Beauchemin, 2006).

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In summary, saliva of lactating dairy cows is relatively constant in composition and flows continuously. Rate of saliva flow is modulated by several factors and is increased markedly with chewing. For lactating dairy cows, daily saliva flow is likely ~260 L/d.

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THE RUMINAL ENVIRONMENT

Overview

The forestomach of lactating dairy cows contains an active microbial fermentation and a mixture of substrates and products. A complete understanding of the diverse processes occurring is essential.

The Ruminal Solution

Ruminal pH or acidity in the rumen of a lactating dairy has a normal range of 5.5 to 7.0 (Stevens and Hume, 1995). Ruminal pH is function of microbial activity, fermentation stoichiometry, intake of the different carbohydrate fractions in feeds, net VFA and lactate concentrations, and saliva production (Pitt et al., 1996). Factors that contribute to ruminal acidity include the adaptation of rumen to a new diet, intake, diet characteristics, and the variability of the diet (Nordlund, 2003).

Ruminal fluid resists pH change to the addition of acid (Turner and Hodgetts, 1955b). But this varies with interval after feeding, nature of the diet, and consumption of water which determine VFA, bicarbonate and hydrogen phosphate concentrations in solution (Turner and Hodgetts, 1955b). The ruminal solution's key buffers are bicarbonate and VFA (Counotte et al., 1979).

The ruminal solution has tremendous buffering capacity from pH 4 to 5 (Van Soest, 1994). The VFA pool has an average or aggregate pK_a of approximately 4.8. As pH drops, the dominate VFA anion combines with a proton and buffers the pH drops below pH 6 (Van Campen, 1976). The buffering by feeds is more important as pH

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declines below 5 (McBurney et al., 1983; Wohlt et al., 1987). Overall, the ruminal solution is very complex and continually modified by the processes within and by the physiology of the encapsulating animal.

Several predictable changes occur in the ruminal solution when a ruminant consumes a meal. After consumption, feed is fermented to VFA. VFA concentrations, osmolality and distension increase during feeding (Forbes and Barrio, 1992). The VFA accumulates, protons separate from anions and the pH of ruminal solution decreases. In a ruminant fed a single meal per d, ruminal VFA production and pH vary in a regular daily pattern with the VFA concentration negatively related to ruminal pH (Phillipson, 1942; Briggs et al., 1957; Emmanuel et al., 1969; Sutton et al., 1986). However, across cows and diets, ruminal VFA concentration is not predictive of ruminal pH (Allen, 1997). Ruminal pH decreases with an increasing rate of decline with increased meal size and decreased dietary NDF concentration (Dado and Allen, 1993b). Higher proportion of dietary concentrates leads to more VFA produced and a higher proportion of propionate (Sutton et al., 1986; Lana et al., 1998). Ruminal pH and bicarbonate are lowest a few hours after feeding with the timing of nadir dependent on many factors (Phillipson, 1942; Turner and Hodgetts, 1955b; Emmanuel et al., 1969, Fernandez et al., 2000). Osmolality (Phillipson, 1942) and carbon dioxide production (Emmanuel et al., 1969) is also greatest during this time.

The homeostasis of lactating dairy cows is challenged by the endproducts of fermentation in the RR - VFA, gases and heat (Van Soest, 1994). A theoretical ruminal fermentation of 57.5 moles of glucose equivalent (approximately 10 kg at 180g/mole) produces 65 moles of acetic acid, 20 moles of propionic acid, 15 moles of butyric acid,

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60 moles of carbon dioxide, 35 moles of methane, and 25 moles of water (Wolin, 1960). The ruminal contents average 15% of the body weight in lactating dairy cows (NRC, 2001). The ruminal environment, while technically outside the body, is surrounded by the rest of the cow and is maintained within certain rough boundaries.

Gas Production And Removal

An active ruminal fermentation will generate more than one liter of mixed gases per min (Steven and Hume, 1995). In a theoretical ruminal fermentation, 57.5 moles of glucose equivalent will yield 60 moles of carbon dioxide and 35 moles of methane (Wolin, 1960) or 2128 L of gases at standard temperature and pressure. However, the actual gas mixture is variable and dependent on the ruminal ecology, the fermentation balance, and intake (Van Soest, 1994). These gases collect in the dorsal rumen and must be eructated (Van Soest, 1994). Eructation is the removal of gases from fermentation from the RR and is stimulated by gas pressure in RR (Stevens and Hume, 1995). Eructation by dairy cows is necessary because at the height of fermentation the gas production exceeds absorption (Stevens and Hume, 1995). Ruminal gas pressure does not greatly exceed one atmosphere (+10 to 20 mm Hg) and, when it does, only for transient periods (Stevens and Sellers, 1960). These pressure differentials are great enough to move gas out of the RR. To eructate, the reticulum is contracted to remove ingesta. Gases are moved to the reticulum, pushed up the esophagus, and expelled with the normal rhythms of breathing (Ruckebusch, 1988).

Carbon dioxide, methane, and nitrogen are the dominant gases in the RR. Carbon dioxide, present usually in the greatest percentage in the RR, is generated as a byproduct of VFA production, from the decomposition of carbonic acid formed from bicarbonate

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and a proton, and from the hydrolysis of urea (Hoernicke et al., 1965; Stevens and Hume, 1995; Leek, 2004). Carbon dioxide is removed from the RR primarily by accumulation in the dorsal rumen and eructation, and by absorption to the blood stream and exhalation (Stevens and Hume, 1995). Methane is generated from the reduction of carbon dioxide with formate, succinate and hydrogen (Stevens and Hume, 1995) and can be a hydrogen sink for surplus reducing equivalents (Leek, 2004). Methane is removed from the RR by eructation and by absorption and exhalation (Stevens and Hume, 1995) though methane is not as soluble across the RR wall as carbon dioxide (Hoernicke et al, 1965). Nitrogen enters the RR when it is swallowed or when it diffuses down its concentration gradient from the blood (Stevens and Hume, 1995).

Carbon dioxide usually accounts for 60% of the mixed gas and methane 30 to 40% (Leek, 2004) but ruminal gas composition is quite variable within day (Washburn and Brody, 1937; Hoernicke et al, 1965; Barry et al., 1967; Emmanuel et al., 1969). (Table 1.5) With a meal, substrate is added to the fermentation and carbon dioxide and methane production increases. These fermentation gases displace atmospheric gases and, therefore, nitrogen and oxygen concentrations varying inversely to carbon dioxide (Washburn and Brody, 1937). Carbon dioxide is the most variable gas with a range in portion of total gas of up to 50 percentage points within a day (Washburn and Brody, 1937; Turner and Hodgetts, 1955a; Hoernicke et al, 1965; Barry et al., 1967; Emmanuel et al., 1969). Carbon dioxide is higher and more variable on diets with concentrates than with pure forage diets (Barry et al., 1967). The proportion of carbon dioxide is equal or greater (up to 3X) than methane with concentrate diets increasing this difference (Washburn and Brody, 1937; Hoernicke et al, 1965; Barry et al., 1967). With the ad

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libitum feeding of lactating dairy cows, the fermentation in the RR is likely more or less continuous. Given previously reported work on cattle fed single meals (Table 1.5), carbon dioxide proportion of the ruminal gas in these cows is likely 50% or greater at all times, however, this approximation needs to be verified experimentally.

Oxygen and other gases are also present in the RR but a lesser extent. Like nitrogen, oxygen enters the RR when swallowed and by diffusion from the blood. Oxygen that does enter the RR is quickly utilized by the microbes and thus remains at low concentration (Stevens and Hume, 1995). Hydrogen in small quantities is nearly always present (Washburn and Brody, 1937) and hydrogen sulfide is also present in trace amounts (Leek, 2004). Water vapor is also present in the collected gas with a vapor pressure of 50.5 mm Hg at 38.5°C (Weast, 1978).

Ruminal gas composition differs from the atmosphere and from blood. The Earth's atmosphere is much higher in nitrogen and oxygen and almost devoid of carbon dioxide and methane (Weast, 1978; Table 1.6). Blood is also higher in nitrogen and oxygen than ruminal gas and has significant carbon dioxide (Rhoades and Tanner, 1995) These gradients are important as the ruminal gases are always moving toward an equilibrium with surrounding environments. Generally, the RR can be characterized as a mixture of atmospheric and fermentation gases. An active fermentation displaces the atmospheric gases and carbon dioxide and methane dominate. As fermentation in the RR subsides, atmospheric gases return.

VFA Production And Removal

The VFA generated during the ruminal fermentation are used as an energy source by the cows (Stevens and Hume, 1995). The dominant VFA produced are acetic acid

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(two carbon), propionic acid (three carbon), and butyric acid (four carbon) (Stevens and Hume, 1995). The molar ratios of acetic acid to propionic acid to butyric acid are variable and can range from 75:15:10 to 40:40:20 (Bergman, 1990). The actual pattern of fermentation is dependent on meal size and frequency (Van Soest, 1994). Profile of ruminal VFA can also be influenced by pH (Esdale and Satter, 1972). A theoretical ruminal fermentation of 57.5 moles of glucose equivalent will generate 100 moles of VFA (Wolin, 1960) and each mole of acid has a mole of protons with the potential to disassociate. The daily VFA load that must be managed by cows is a function of organic matter intake and the fermentibility of that intake and the ruminal fermentation in lactating dairy cows may generate more than 100 Eq of protons each day (Allen, 1997). Daily intake is function of meal size and the number of meals each day. Fermentibility of the intake is determined by the character and composition of the substrate and population of microbes present in the RR.

The first determinant of VFA production is feed intake and feed intake in lactating dairy cows is a determined by the integration of many factors. Factors important in lactating dairy cows are physical fill of the RR which is determined primarily by diet forage NDF and its digestibility (Allen, 1996), the actions of absorbed fuels (Allen, 2000), ability of the tissues to metabolize nutrients (Forbes, 1996), oxygen consumption (NRC, 2001), ruminal acidity and(or) osmolality (Forbes, 1996), and psychological and sensory ability of animals (NRC, 2001). Daily DMI can be influenced by environmental temperature, genetics, physiological state, water intake, behavior, management, and diet (NRC, 2001). In lactating cows, DMI is correlated positively with milk production (Dado and Allen, 1994).

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The other determinant of VFA production is the composition of the diet ingested. More VFA is produced as fermentibility of the diet increases. Fermentibility of the diet is determined primarily by the carbohydrate proportions of NFC (nonfiber carbohydrate) and NDF with an increasing NFC proportion increasing the fermentibility and, therefore, the total VFA production. Fermentibility of the NFC in the RR is increased by increased moisture, by more fermentable sources, by decreased particle size, by increased processing (physical and chemical) and by decreased vitriousness of the starch (Nocek and Tamminga, 1991). The amount of NDF fermented in the RR is determined by the rate of fiber fermentation and by ruminal retention time (Allen and Mertens, 1988). Rate of fiber fermentation is a function of the intrinsic characteristic of the feed (Allen and Mertens, 1988) and ruminal pH over time, with pH <6.0 (Hoover, 1986; Grant and Mertens, 1992; Krajcarski-Hunt et al., 2002) and increased variability of pH (Wales et al., 2004) decreasing fiber fermentibility. Ruminal retention time is a function of particle size and buoyancy (Allen and Mertens, 1988). Increased intake can decrease fermentibility of the diet by decrease ruminal retention time (NRC 2001). The resulting range of ruminally degraded organic matter across diets is wide ranging from 29% to 67% with a mean of 50% (Allen, 1997).

Once produced, VFA are absorbed across the ruminant forestomach epithelium in undisassociated (or free acid) and disassociated (or anion) forms (Danielli et al., 1945; Dijkstra et al., 1993; Kramer et al. 1996; Gaebel and Sehested, 1997; Figure 1.1). VFA are transported transcellularly and not paracellularly (Sehested et al., 1999a).

Undisassociated VFA are lipid soluble and can diffuse across the lipid bilayers of the epithelium cell membrane (Stevens and Hume, 1995). The concentration of

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undisassociated VFA for absorption is increased by lower ruminal pH and higher concentrations of VFA (Van Soest, 1994). The mucus on the lining of the lower gastrointestinal tract can divide the lumen effectively into two compartments and hold excreted protons close to the epithelium (Stevens and Hume, 1995). However, this mucus layer is not present in the RR (Leek, 2004).

With a collective pKa of 4.8, most VFA (>90%) in the forestomach of the ruminant are in disassociated or anion form (Bugaut, 1978; Bergman, 1990). Disassociated VFA can be absorbed by non-selective, electroneutral anion exchangers (Gaebel and Sehested, 1997). Results from early work in emptied, washed, and isolated rumens, in retrospect, support these mechanisms (Masson and Phillipson, 1951; Dobson, 1959; Ash and Dobson, 1963) but more recent work (isolated rumen and in vitro) has been more conclusive (Rechkemmer et al., 1995; Kramer et al., 1996, Gaebel and Sehested, 1997; Sehested et al., 1999a; Sehested et al., 1999b). VFA anions are exchanged for bicarbonate anion across the ruminal epithelium (Gaebel and Sehested, 1997; Sehested et al., 1999b). This absorption in promoted by carbon dioxide and is linked to carbon dioxide inside the cell (Gaebel and Sehested, 1997) and is abolished with removal of bicarbonate from solution (Sehested et al., 1999b). Increased ruminal chloride can inhibit VFA uptake (Kramer et al., 1996) suggesting a competition but not a direct link (Gaebel and Sehested, 1997). VFA absorption is connected positively to sodium absorption via sodium/proton exchanger (Gaebel and Sehested, 1997; Sehested, 1999b). The relative amounts of VFA absorbed as undisassociated and disassociated remain to be quantified in dairy cows in vivo but a recent estimate is 50:50 (Leek, 2004).

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The energy required for absorption of undisassociated and disassociated VFA remains to be determined and awaits a more complete description of transport systems.

Whether in undisassociated or disassociated forms, VFA absorption can be increased with increased surface area, increased concentration gradient, and decreased concentration gradient distance. The surface area of the ruminant forestomach is increased by the papillae of the rumen, the reticular ridges of the reticulum, and papillated lamellae of the omasum (Leek, 2004). Ruminal papillae can increased in length, width, and surface area with more fermentable diets (Dirksen et al., 1985; Xu and Allen, 1999). Increasing absorptive surface area of the RR leads to increased VFA rates of absorption (Dirksen et al., 1985; Xu and Allen, 1999). VFA concentration in the RR is usually much more than the concentrations in the blood (Bugaut, 1978). Metabolism and clearance can increase the difference between RR and blood concentrations (Bergman, 1990). The distance of the concentration gradient from active fermentation in the particles in the RR to the blood is function of the homogeneity of the rumen. The homogeneity of the RR is determined by forestomach contractions and the viscosity of the contents (Van Soest, 1994). A greater volume of the ruminal solution can increase the distance of the concentration gradient by decreasing the surface to volume ratio (Dijkstra et al., 1993).

In isolated and washed rumens, lower ruminal pH can lead to increased VFA absorption rates from bathing solutions (Danielli et al., 1945; Thorlacius and Lodge, 1973; Dijkstra et al., 1993). In vivo, a decrease in ruminal pH may lead to a decrease in absorption possibly from a decrease in ruminal motility (Allen, 2004). Over a range of

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experimental conditions in a washed rumen, butyrate has the greatest fractional absorption rate followed by propionate then acetate (Dijkstra et al., 1993).

An increase in ruminal fermentable organic matter intake (which can cause increased VFA concentration and lower pH) leads to greater VFA and ion absorption (Gaebel et al., 1987a; Gaebel et al., 1987b; Thorlacius and Lodge, 1973; Uppal and Martens, 2002; Doreau et al., 1997). These increases taken together suggest transporter adaptation and molecular interactions.

In dairy cows, an estimated 76% of VFA of the ruminal fermentation are absorbed in the RR, 19% in the omasum and abomasum and 5% in the small intestine (Ruckebusch et al., 1991) but proportions vary depending animal and diet description (Rupp et al., 1994; Bergman, 1990; Edrise and Smith, 1977; Peters et al., 1990a; Peters et al., 1990b, Peters et al., 1991). However, this estimate of ruminal disappearance may be high. A range of 65 to 80% of the VFA absorbed in RR has been proposed for dairy cattle (Dijkstra et al., 1993) but these measurement were done with washed rumen containing solutions with less volume than in vivo and this can lead to artificially high rates of VFA flux. A prediction of 53% of ruminal protons absorbed in the rumen has been calculated based on established rates of absorption and liquid passage, ruminal volumes, and ruminal VFA concentrations (Allen, 1997). Regardless of specific proportions, high clearance of VFA and the associated buffering potential by the forestomach is necessary for proper acidification in the abomasum.

Greater than 80% of butyrate is utilized by the visceral tissue; propionate and acetate to lesser degree (approximately 50% and 30% respectively; Bugaut, 1978). Of the VFA entering the blood, acetate is primarily utilized by the peripheral tissue as either

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fuel or for lipid synthesis, propionate is largely cleared by the liver and is used to make glucose, and butyrate is mostly cleared by the liver (Bergman, 1975). These processes lead to acetate being the primary circulating VFA (Bergman, 1990).

Most of the VFA from ruminal fermentation disassociate and donate protons to the ruminal solution. (Stevens and Hume, 1995). These protons can be removed from the RR by passage of ruminal materials, absorption across the RR wall, neutralization by the bicarbonate and phosphate in saliva, cation exchange in fiber, oxidation, and microbial efficiency (Van Soest, 1994). Passage from the RR takes protons associated with digesta, phosphate, VFA, and ammonium (Allen, 1997). Protons can be adsorbed to feedstuffs entering the RR with a process called cation exchange (McBurney et al., 1983) - particularly if the feed is a high protein feed or a legume forage (Jasaitis et al., 1987).

The relative importance of each process in proton removal in the rumen has been modeled (Allen, 1997; Allen, 2004; Table 1.7). Here, the majority of protons (>50%) are removed during the absorption of VFA across the ruminal wall. The other process of significance is the sodium bicarbonate produced with the saliva which accounts for the removal of approximately a third of the protons produced. Together, these two processes are predicted to remove the majority of the protons produced each day.

In summary, VFA are produced by the microbial fermentation and are >90% disassociated (anion form) in the ruminal solution. VFA are absorbed across the RR wall in undisassociated and disassociated forms by controlled processes.

Excess VFA

With excess VFA, lactating dairy cows become susceptible to total VFA acidosis (NRC, 2001). Acidosis in dairy cows is defined as either acute or subacute (Nocek, 1997;

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Owens et al., 1998). Acute acidosis is defined as a decrease in ruminal pH below the threshold of 5 (Nocek, 1997). Excess ruminal fermentable organic matter intake starts a fermentation that overwhelms VFA removal mechanisms and leads to excess ruminal VFA and osmolality (Owens et al., 1998). The resulting ruminal acidosis leads to problems that include damage to RR lining, decreased forestomach motility and abnormal fermentation from an abnormal microbial population (Dougherty, 1976; Huber, 1976A; Huber, 1976B; Slyter, 1976; Nocek, 1997; Owens et al., 1998). Ruminal acidosis leads to metabolic acidosis (NRC, 2001), decreased blood pH and systematic acidosis, dehydration, cardiovascular and respiratory failure, and often death (Dougherty, 1976; Huber, 1976A; Huber, 1976B; Slyter, 1976; Owens et al., 1998; Brown et al., 2000).

Subclinical or subacute or chronic acidosis is more common in dairy cattle (NRC, 2001) and is commonly referred to as subacute ruminal acidosis (SARA; Nocek, 1997; Keunen et al, 2002; Oetzel, 2003). It is defined as a drop (or repeated drops) below a critical pH of 5.5 (Nocek, 1997; Keunen et al, 2002; Oetzel, 2003), however, critical pH is should be adjusted for the method of ruminal fluid collection (Oetzel, 2005; $\leq$5.6 for indwelling pH probe and $\leq$6.0 for oral collection tube). When using rumenocentesis to diagnosis lactating herd SARA, sampling should focus on the high risk groups: early lactation (3 to 20 DIM) and peak lactation (Nordlund, 2003) or 5 to 250 DIM (Oetzel, 2004). For accurate herd diagnosis, samples should be taken from 12 or more cows from these groups and timing of sampling should be near the projected nadir of ruminal pH (Oetzel, 2004) suggested at 2 to 4 hours after feeding. SARA can be considered a herd problem if 25% of cows test below pH 5.5 (Pereira et al., 1999; Garrett et al., 1999; Oetzel, 2004).

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Factors that contribute to SARA include lack of adaptation to diet, high DMI, rapid changes in intake, component feeding and certain diets (Nordlund, 2003). SARA is characterized by a reduced and(or) inconsistent feed intake (Huber, 1976A; Slyter, 1976; Nocek, 1997; Owens et al., 1998). Other symptoms of SARA in cattle and dairy operations include decrease production efficiency, reduced milk fat test, poor body condition, high culling rates, unexplained diarrhea and laminitis (Nocek, 1997). Again, increases in acidity and osmolality are characteristic but not to the extent of acute acidosis and the extent of problems are also proportional (Nocek, 1997; Owens et al., 1998; NRC, 2001). Laminitis and liver abscesses are associated with both conditions (Nocek, 1997; Owens et al., 1998; NRC, 2001).

The incidence of SARA in individual and in herds is variable and is dependent on many factors (NRC, 2001). SARA can be described as a consequence of an attempt to maximize energy intake and is often found in "well-managed, high producing herds" (Nocek, 1997). Prevention of acidosis has been attempted with feed additives, direct-fed microbials and dietary roughage increases (Owens et al., 1998) with specific choice dependent on economics and goals. However, feed management may be more critical than the actual diet. "Cycles of feed deprivation and re-feeding are more important risk factors for SARA than is diet formulation itself." (Oetzel, 2003). Experimentally, SARA can be induced by restricting feed (e.g. 50% of previous intake) for one day followed by refeeding of a diet of increased fermentibility (Krause and Oetzel, 2005).

A highly ruminally fermentable diet leads to changes in the ruminal fermentation. With a high grain, low forage diet, ruminal acetate to propionate ratio is decreased (Grummer et al., 1987; Owens et al., 1998). Total VFA production per d is also

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increased (Allen, 1997; Owens et al., 1998). With lactating dairy cows, milk fat is depressed (Grummer et al., 1987; NRC, 2001). Total chewing time can also be decreased (Grummer et al., 1987; Allen, 1997; NRC, 2001). SARA is also associated with a concurrent increase in water consumption (Cottee et al., 2004) possibly due to a short term osmotic effect.

Acidosis can lead to acute and chronic damage to the RR lining. The acute damage involves sloughing the lining of the RR (Hinders and Owens, 1965; Owens et al., 1998) and chronic is a swelling and keratinization of the lining called parakerotosis (Hinders and Owens, 1965). This damage as decreased absorptive surface area, physical barriers of scars and keratinization, reduced blood flow and(or) disruption of the transport mechanisms leads to decreased absorptive capacity (Hinders and Owens, 1965).

Often, acidosis involves a significant increase in ruminal osmolality (Owens et al., 1998). If ruminal osmolality exceeds blood osmolality, water can be drawn into the RR and, with enough time and a sufficient gradient, this movement can compromise the integrity of the ruminal epithelium (Engelhardt, 1970; Gemmell and Stacy, 1973; Owens et al., 1998) where a separation in the epithelium can lead to blistering and sloughing of the epithelium. Conversely, blood osmolality greater than ruminal can be handled without damage to the ruminal epithelium for extended periods of time (Engelhardt, 1970).

Damage to the ruminal epithelium integrity leads to two situations. Sloughing can leave the patches in the RR wall freely permeable to water and ions (Gemmell and Stacy, 1973) and, with sufficient damage, larger materials (Nocek, 1997). Damage can also lead to scarring and keritinazation that cause impermeability (Hinders and Owens,

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1965; Owens et al., 1998). This impermeability can lead to long term or permanent reductions in ruminal VFA absorption (Krehbiel et al., 1995; Owens et al., 1998).

Strong Ions

Strong ions are another component of the ruminal solution that are regulated. Strong ions are defined as ions completely disassociated in solutions (Stewart, 1983). In the ruminal solution, sodium, potassium, and chloride are the primary strong ions, each having a single valence (Tables 1.8, 1.9, and 1.10). These ions enter the RR by controlled (i.e. in the saliva) and less controlled (i.e. in the diet) ways. Within RR, these strong ions appear to be regulated primarily by absorption.

Sodium is usually the most abundant cation in the RR (Bennick et al., 1978) and a typical ruminal concentration in cattle is 120 mEq/L (Tables 1.9 and 1.10). Sodium enters the rumen in the diet or in the saliva and can leave the ruminal lumen by absorption across the ruminal epithelium or passage to lower tract (Stevens and Hume, 1995). The concentration of sodium in the RR is proportional but lower than the concentration of sodium in the saliva (Bailey, 1961b). Meals do not appear to influence ruminal sodium concentration (Tucker et al., 1993). Sodium chloride can be included in cattle diets at quite high levels ($\geq$5%) without apparent problems (Cardon, 1953; Merchen, 1988) assuming adequate water is provided.

Sodium is absorbed into the ruminal epithelium by sodium-hydrogen exchange and by the electrogenic diffusion of sodium through ion channels (Martens and Gaebel, 1988). The exchange of an absorbed sodium ion and excretion of a proton across the apical membrane of ruminal epithelial cell is electrically silent and accounts for the majority of the sodium translocation (Martens and Gaebel, 1988). The reticulum (Gaebel

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et al., 1993) and omasum (Martens and Gaebel, 1988) appear to have the same sodium absorption mechanism as the rumen. This sodium absorption is inhibited by decreases in luminal pH (Gaebel et al., 1987a; Gaebel et al., 1987b) and enhanced with increases in VFA (Gaebel et al., 1991; Rechkemmer et al., 1995; Sehested et al., 1999b; Uppal et al., 2003) and pCO2 (Gaebel et al., 1991; Gaebel and Sehested, 1997). Increased VFA and pCO2 are speculated to increase intracellular hydrogen ion concentration to promote the sodium-hydrogen exchange (Gaebel et al., 1991; Gaebel and Sehested, 1997). Sodium absorption has been linked positively to ATP supply (Harrison et al., 1975b; Gaebel et al., 1999). The ruminal epithelium has shown the reversible ability to adapt sodium absorption by increasing with increased luminal VFA (Gaebel et al., 1987a; Uppal and Martens, 2002; Uppal et al., 2003). Increased lactate (Gaebel et al., 1987b) and hypertonicity (Gaebel et al., 1987a; Gaebel et al., 1987b) does not appear to influence sodium absorption. (Figure 1.2)

Potassium enters the rumen from the diet and, to a lesser extent, in the saliva and will leave the ruminal lumen by paracellular absorption through the ruminal epithelium diffusing down the concentration gradient from lumen to the blood and by passage from the RR to the lower tract (Stevens and Hume, 1995; Figure 1.2). In the ruminal solution, potassium is usually a fraction of sodium at concentrations of 25-40 meq/L (Tables 1.9 and 1.10). The concentration of potassium in the ruminal solution is usually higher than that found in saliva (Bailey, 1961b) and primarily a function of diet concentrations (Bailey, 1961b; Bennick et al., 1978; Tucker et al., 1993).

Chloride is readily soluble in solution and is absorbed throughout the gastrointestinal tract and is excreted in urine and feces (NRC, 2001). Chloride enters the

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RR from the diet and saliva and leaves by absorption and passage (Stevens and Hume, 1995). It is absorbed in the RR by the exchange of chloride anion for bicarbonate anion (Gaebel et al., 1991; Martens et al., 1991; Gaebel et al., 1993) across the apical membrane of ruminal epithelial cell. The reticulum (Gaebel et al., 1993) and omasum (Martens and Gaebel, 1988) appear to have the same chloride absorption mechanism as the rumen. (Figure 1.2) Chloride absorption is inhibited by lower ruminal pH but not as greatly as sodium (Gaebel et al., 1987b, Gaebel et al., 1987a). Chloride absorption has been linked positively to the ATP supply but more weakly than sodium (Harrison et al., 1975b; Gaebel et al., 1999).

Absorptions of sodium, chloride and potassium from the RR interact. Sodium and chloride have been shown to be absorbed proportionally but without a direct link (Trenkle, 1979; Gaebel et al., 1987b; Gaebel et al., 1987a; Martens and Blume, 1987; Gaebel et al., 1991; Diernaes et al., 1994; Rechkemmer et al., 1995). Intracellular pH or perhaps intracellular carbonic acid is the proposed indirect link and the mechanism is the sodium-hydrogen exchange working in parallel with a chloride-bicarbonate exchange (Martens et al., 1991). Sodium and chloride co-transport systems in the RR have been ruled out (Martens and Gaebel, 1988).

Sodium and potassium concentrations in the RR have a reciprocal relationship in sheep (Sellers and Dobson, 1960; Scott, 1966; Stacy and Warner, 1966; Scott, 1967; Ternouth, 1967; Warner and Stacy, 1972a) and cattle (Emery et al., 1960; Bailey, 1961b; Bennick et al., 1978; Tucker et al., 1988a; Tucker et al., 1993). In some experiments, the relationship is reported as peripheral observation (Emery et al., 1960; Bennick et al.,

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1978; Tucker et al., 1993) and, in others, more intensively quantified (Sellers and Dobson, 1960; Bailey, 1961b; Scott, 1966; Stacy and Warner, 1966; Scott, 1967).

Evidence exists to suggest that the ruminal solution is regulated to a "constant" total mEq of sodium plus potassium (162 mEq/L in cattle (Bailey, 1961b) and 140 mEq/L in sheep (Lang and Martens, 1999). This constancy is likely accomplished by modulation of sodium absorption (Scott, 1967; Warner and Stacy, 1972a, and Lang and Martens, 1999). A mechanism for the modulation of sodium absorption has been proposed:

> "an increase in ruminal K concentration depolarizes the apical membrane and increases or induces a PD-dependent cation conductance, which enhances Na uptake (despite a reduced electrical driving force) and finally increases transepithelial Na transport via the basolateral Na-K-ATPase" (Lang and Martens, 1999).

This mechanism of absorption has not been described previously and is believed to be unique to the rumen (Lang and Martens, 1999).

The high potassium enhancement of sodium absorption is an effective mechanism for the management of charge and osmolality in the RR (Lang and Martens, 1999). Sodium and potassium are osmotic and charge equals but are managed in opposition in the body (Rhoades and Tanner, 1995). The inverse relationship within the RR space (outside the body) is an effective way to manage the variation in diets and suggests that, within the RR, charge and osmotic character are more important than the specific element. This relationship is likely be part of the adjustment to excess dietary potassium (Warner and Stacy, 1972a) or sodium depletion (Scott, 1966).

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Sodium may also have a reciprocal, diet-dependent, relationship with ammonia in the RR (Abdoun et al., 2003). Urea diffuses from the blood to the saliva and across the ruminal epithelium and can be hydrolyzed to ammonia by urease in the ruminal epithelium or by bacteria near the RR wall (Houpt, 1970). Ammonia is protonated to ammonium at ruminal pH (Hogan, 1961) and, therefore, has a charge of +1. Ammonia absorption can be increased by increased VFA, more so with higher pH (6.5 vs. 4.5; Hogan, 1961). Added ammonia to the solution can inhibit sodium uptake by ruminal epithelium adapted to hay only diets but promote sodium uptake of ruminal epithelium adapted to diets containing concentrate or urea in sheep epithelium in vitro (Abdoun et al., 2003). The addition of urea to the RR can promote sodium absorption in vivo (Stacy and Warner, 1966). In general, an inverse relationship appears to exist between ruminal ammonia and sodium concentrations and this interaction may play a role in the management of total cation concentration in the RR.

In summary, sodium, potassium, and chloride absorption is regulated and these ions interact. Sodium and chloride are coupled through the ruminal epithelial bicarbonate system. With respect to the ruminal solution, the charge and osmotic effects of the strong ions are most important.

Dietary Cation-Anion Difference

Minerals in the diet need to not only meet requirements but also must be in balance with each other for optimal performance (Mongin, 1960). Interrelationships among monovalent macromineral elements of sodium, potassium, and chloride within the body are recognized (NRC, 2001) and one expression of these relationships is dietary cation-anion difference (DCAD; NRC, 2001). DCAD is used in management of dairy

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cattle diets (Sanchez and Beede, 2005). Low DCAD diets are provided to dry cows to induce a mild metabolic acidosis which enhances calcium homeostasis at calving (NRC, 2001). Positive DCAD diets are considered for lactating cows as a management tool to help counter or neutralize the "accelerated or greater" acid load associated with more fermentable diets (Sanchez and Beede, 2005). DCAD is commonly expressed two ways:

Equation 1.1. DCAD3 = K + Na - Cl in mEq/100gDM

(Beede, 2003; Sanchez and Beede, 2005)

Equation 1.2. DCAD4 = K + Na - Cl - S in mEq/100gDM

(Beede, 2003; Sanchez and Beede, 2005)

These equations are commonly used in diet formulation and DCAD3 is recommended for nonruminants and DCAD4 is recommended for ruminants (Tucker et al., 1991; Sanchez and Beede, 2005).

In addition to sodium, potassium, chloride and sulfur, the elements of calcium, magnesium and phosphorus are important in body acid-base but to a lesser extent (Goff et al., 2004; Sanchez and Beede, 2005). A full expression of major cations and anions would be mEq (Na + K + Ca + Mg) - (Cl + S + P)/100g dietary DM (Equation 1.3.; Goff et al., 2004; Sanchez and Beede, 2005). Source of cations and anions in diet is also important as source may affect availability or potency (Goff and Horst, 1998; Goff et al., 2004).

Research with lactating cows shows a positive response in DMI and milk yield and increases in blood pH and bicarbonate to increasing DCAD (Tucker et al., 1988a; Apper-Bossard and Peyraud, 2004) but an upper limit to benefit (Roche et al., 2003). Small increases in ruminal pH and other small ruminal effects have been reported as well

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as overall benefits to acid-base status (Tucker et al., 1988a; Apper-Bossard and Peyraud, 2004). Meta-analysis has suggested an optimum DCAD4 for lactating cows might fall between +20 to +50 mEq/100g DM (Sanchez et al., 1994a, Sanchez et al., 1994b, Sanchez et al., 1994c; Sanchez and Beede, 1996; Block and Sanchez, 2000; Hu and Murphy, 2004, Sanchez and Beede, 2005) but more research is needed (Sanchez et al., 1994a, Hu and Murphy, 2004, Sanchez and Beede, 2005). DCAD of a balanced ration for lactating cows rarely falls outside this optimum rage (Sanchez and Beede, 1996) but diet minerals should be monitored with wet chemistry analysis to guard against anomalies (Sanchez and Beede, 1996; Beede, 2003; Sanchez and Beede, 2005).

Water

The VFA and strong ions are all contained in an aqueous solution. The water of the RR needs to be understood in the context of the total body water of lactating dairy cows. Lactating dairy cows are approximately 65% water but the exact proportion is dependent on physiological state and body composition (Andrew et al., 1995; Tables 1.11 and 1.12). The water in lactating cows is found in three pools: intracellular fluid (ICF), extracellular fluid (ECF), and gastrointestinal fluids (English, 1966). ICF is the fluid found within cells and its volume is determined primarily by potassium content (Carlson, 1997) and accounts for about two-thirds of the water in the body (NRC, 2001). ECF includes plasma, interstitial fluids, and lymph and its volume is determined primarily by sodium content (Carlson, 1997) because cations in ECF are regulated and bicarbonate and chloride follow to balance charge (Houpt, 2004). The water in the gastrointestinal tract of lactating dairy cows is approximately 13% of the live weight or 20% of the total body water (Andrew et al., 1995; Tables 1.11 and 1.12). Total body water is controlled by

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antidiuretic hormone (ADH; regulates the osmolality of the body fluids), the renin-angiotensin system (maintains effective circulating fluid volume), aldosterone (maintains effective circulating fluid volume and potassium balance), and atrial natriuretic factor (ANF; response to increased central venous pressure and stretching of the atrial wall; Carlson, 1997). Concentrations and totals for bovine blood have been reported (Table 1.13) and these concentrations are similar to other species (Carlson, 1997).

The water in the RR of lactating dairy cows is approximately 8.1% of the live weight or 12.4% of the total body water (Andrew et al., 1995; Tables 1.11 and 1.12). However, the RR volume and liquid pool size are not constant (Van Soest, 1994). Water enters the RR by diffusion, in saliva, and by intake of water and feed (Murphy, 1992) and leaves by diffusion across RR and passage to lower tract (Murphy, 1992). In lactating cows, most of the water entering the RR is from saliva (Allen, 1997). Feeding and drinking both expand RR volume and also increase outflow (Warner and Stacy, 1968b). Water and feed entering the RR causes a nonsteady-state dilution (Warner and Stacy, 1968a; Warner and Stacy, 1968b). Not all water consumed completely mixes in the RR. As much as 18% of a drink bypasses RR to the abomasum (Woodford et al., 1984) and mixing of the proportion of the drink retained can be incomplete (Warner and Stacy, 1968b). Some of the saliva can be expected to bypass the RR as well (Allen, 1997).

Water Movement Across The Ruminal Wall

In the absence of a barrier, water follows solute (Stevens and Hume, 1995). However, the RR appears to have "an appreciable barrier to the net flux of water due to osmotic gradients normally present between rumen contents and blood" (Engelhart, 1970). Except during a very active fermentation, osmolality of the blood is generally

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higher than the contents of the RR (Van Soest, 1994). Little net water is believed to diffuse from the blood into the RR (Warner and Stacy, 1968b; Engelhart, 1970; Dobson, 1970) and most of the transepithelial flux is thought be from the RR to the blood (Engelhart, 1970). Across the day, the absorption of water from the RR to the blood, though slow, is significant and, in sheep, may equal the amount of water consumed within the day (Warner and Stacy, 1968b). Most of the absorption from the RR to blood likely occurs at lower ruminal osmolalities when osmotic pressure across the ruminal wall is greater. In sheep, little to no net transepithelial water movement occurred at ruminal osmolarities of 260 to 340 mOsm/L (Engelhart, 1970). The net flux of water is "prevented or intensely inhibited by a zone of high osmotic pressure in the deeper layers of the epithelium" (Engelhardt, 1970).

The ruminal epithelium appears resistant to potential damage of higher osmotic pressure normally present in the ruminal solution (Engelhart, 1970). In contrast, the ruminal osmolality needed to drawn water into the rumen appears, with time, to damage to the ruminal epithelium by forming spaces in the tissue (Engelhardt, 1970; Gemmell and Stacy, 1973). If the spaces formed in the epithelium connect and form a breach, the flux of water (Engelhart, 1970) and ions (Gemmell and Stacy, 1973) across the ruminal epithelium greatly increases.

Evidence for the ruminal epithelium is not being freely permeable to water include that higher osmotic pressures in the RR do not equalize and that the reverse osmotic pressures, with time, cause damage. The barrier to water flux probably is by design. Free water flux across the RR epithelium should lead to large shifts in the water of blood pools. As an example, a 650 kg cow would be expected to have 53 kg of water

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in rumen and 34 kg of water in blood (Table 1.12). To bring the ruminal solution at 350 mOsm down to 300 mOsm would require the addition of almost 12 kg of water which would significantly expand the RR volume and draw heavily on the ECF, particularly the blood. This calculation assumes the active defense of blood osmolality at 300 mOsm. So, the restriction of water movement is beneficial to ruminants.

Osmolality Of The Ruminal Solution

The osmolality of the ruminal solution is variable. Ruminal osmolality increases with a meal primarily because of the production of VFA (Scott, 1975; Bennick et al., 1978; Trenkle, 1979; Carter and Grovum, 1990a). Diet components entering ruminal solution can also contribute to ruminal osmolality (Bailey, 1961, Scott, 1975, Bennick et al., 1978). Osmolality is decreased by absorption and by saliva and water entering the RR (Scott, 1975). Inflow of saliva appears to be essential to the reduction of ruminal osmolality because removal of saliva flow to the RR leads to a very slow return to normal osmolality (Warner and Stacy, 1972b).

Increased osmolality of the ruminal solution can affect animal behavior. It can influence intake (Forbes and Barrio, 1992) or decrease intake proportionally (Ternouth and Beattie, 1971). Ruminal infusions of solutions containing sodium chloride and sodium salts of VFA at the start of spontaneous meals reduced meal size and total intake respectively (Choi and Allen, 2000). Ruminal osmolality artificially increased to greater than 400 mOsm can shut down a meal and markedly decrease overall intake (Bergen, 1972). The administration of a local anaesthetic to the RR stops this effect (Bergen, 1972). The signal to cease eating is sensed in the wall of the RR and not the abomasum or via the plasma (Carter and Grovum, 1990a; Carter and Grovum, 1990b). Neuronal

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receptors in the RR appear to be able to detect osmotic pressures within the physiological range (Carter and Grovum, 1990b) but these nerves and(or) receptors have not been specifically identified (Leek and Harding, 1975).

Normal increases in osmolality within the RR may not inhibit mixing contractions but can delay the time to rumination following a meal (Carter and Grovum, 1990a). High osmotic concentration in the RR can completely inhibit rumination, acute infusion of 1.2 moles of sodium or potassium bicarbonate inhibited rumination in rams until the ruminal solution returned to a threshold of approximately 350 mOsm, sometimes for more than 12 hours (Welch, 1982).

Two systems that are well defined for the blood are the bicarbonate and strong ion difference systems. The application of these two systems to the ruminal solution could be useful.

The Bicarbonate System

Bicarbonate is formed when carbon dioxide and water combine to form carbonic acid then decompose to a proton and a bicarbonate ion.

Equation 1.4. $CO_2 + H_20 <-> H_2CO_3 <-> H^+ + HCO_3^-$

In the body, the formation of carbonic acid from carbon dioxide and water is catalyzed by the enzyme carbonic anhydrase (Rose and Post, 2001). Carbonic anhydrase is plentiful in the RBC and the renal tubular epithelium (Rose and Post, 2001). Carbon dioxide and bicarbonate are dominant in solution as carbonic acid is an unstable intermediate (Segel, 1976). The disassociation of carbonic acid to bicarbonate and a proton and back is spontaneous (Rose and Post, 2001). This reaction is reversible and, as with all equilibrium reactions, responsive to mass action (Segel, 1976).

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Bicarbonate and its relationship to blood pH is well defined and described in the following equation:

Equation 1.5. $pH = 6.1 + \log [HCO_3^-]/[CO_2]$ (Segel, 1976)

Bicarbonate In The Ruminal Solution

Compared to the blood, the ruminal bicarbonate system less quantified but still has the same reactive species of carbon dioxide and carbonic acid (Equation 1.4). The carbon dioxide proportion of the gas phase above the ruminal solution is high relative to the atmosphere and the blood and is quite variable within a day (ranging from 0.20 to 0.75 atm; Table 1.5). The ruminal gas system with its active and passive gas removal is considered open with RR gas pressures rarely and only marginally exceeds atmospheric pressure (Stevens and Sellers, 1960). If the ruminal solution is experimentally removed and equilibrated in the carbon dioxide poor atmosphere, the pH will raise (Turner and Hodgetts, 1955a). Bicarbonate combined with a proton and, with equilibration, carbon dioxide is lost and pH is elevated (Turner and Hodgetts, 1955a). The reaction is reversible assuming no bacterial lysis (Turner and Hodgetts, 1955a).

The formation of carbonic acid from carbon dioxide and water is catalyzed by the enzyme carbonic anhydrase in the ruminal epithelium (Bergman, 1990) and, under the partial pressure of carbon dioxide of the RR, is spontaneous (Van Soest, 1994). Bicarbonate ion is osmotically active (Weast, 1978) and, in the ruminal solution, is usually an alkalizer.

The pK_a of carbonic acid is reported as a range from 6.0 to 6.8 (Table 1.14). The reason for this range is probably due to the conditions of the determination (concentration, temperature and whether carbon dioxide is allowed to escape), character

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of the solution (pure, blood or ruminal system) and methods (theoretical or experimental). The pK_a of ruminal fluid titrated under "closed" conditions has been measured as 6.25 (Turner and Hodgetts, 1955a; Fernandez et al., 2000). In the earlier case (Turner and Hodgetts, 1955a), the system for determination was done as a closed system at 25 C.

The primary gas produced by the ruminal fermentation is carbon dioxide and, while this high carbon dioxide atmosphere over the ruminal contents does not contribute to buffering directly (as it contributes both a proton and a bicarbonate), it does maintain a reserve of bicarbonate in the ruminal solution by mass action. The ruminal fermentation also supplies carbon dioxide to the ruminal epithelium.

Strong Ion Difference Theory

The carbonic acid equation (Equation 1.4) is the basis for the Henderson-Hasselbalch equation:

Equation 1.6: $pH = 6.10 + \log ([HCO_3^-]/(0.03)(pCO_2))$ (Rose and Post, 2001)

The Henderson-Hasselbach equation is very simple and clinically useful (Rose and Post, 2001), however, it is more descriptive than mechanistic and does not separate independent and dependent variables for the determination of pH (Constable, 1999). The hydrogen ion concentration or pH is a dependent variable, a result or net function of the influence and actions of independent variables (Stewart, 1983). Blood acid-base or pH is determined by more than carbon dioxide and a more complete model would consider plasma cations, anions, and plasma protein (Singer and Hastings, 1948).

Alternatives to the Henderson-Hasselbalch model are the strong ion models for pH determination. In addition to the partial pressure of carbon dioxide, plasma strong ions and weak acids are considered determinants of plasma pH (Stewart, 1983;

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Constable, 2000; Heisey and Adams, 2002). Strong ions are ions that stay disassociated in solution and those of primary importance in the blood or plasma are sodium, potassium, and chloride (Stewart, 1983). Strong ion difference (SID) is aggregate term used to describe strong ions in solution and is defined as the sum of strong cations (mEq/L) minus sum of strong anions expressed as mEq/L (Stewart, 1983).

In a simple solution of only water and strong ions, the balance of strong ions or SID determines the pH of the solution. If the concentration of strong cations exceeds strong anion (also called a positive SID), then the charge is balanced with a hydroxyl and the resulting solution is basic. If the concentration of strong anions exceeds strong cations (also called a negative SID), then the charge balanced with protons results in an acidic solution. If the strong cations equal the strong anions, the pH of the solution is neutral or 6.67 at 37°C (Stewart, 1983).

A quantitative strong ion model of blood pH has been proposed and states that hydrogen ion concentration is a function of eight independent factors (Stewart, 1983). These factors are the partial pressure of carbon dioxide, the strong ion difference, the total weak acid, the solubility of carbon dioxide in plasma, and four chemical constants (Stewart, 1983). In this model, the only strong ions considered are sodium, potassium, and chloride and the weak acids are defined as the plasma proteins (Stewart, 1983). These eight factors combine to determine six dependent variables which are the concentrations of bicarbonate, carbonate, weak acid, weak anion, hydroxide and protons (Stewart, 1983). Also, the conditions required include electrical neutrality (positive charges must be equal to negative charges) and that normal arterial pH is 7.4 and bicarbonate is 24 mEq/L (Stewart, 1983).

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Accuracy of pH calculated based on SID is dependent on which ions are measured and(or) included in calculations and can be dependent of methods of analysis (Constable, 1997; Constable, 2000; Constable, 2001). Measurement of total weak acid is essential and should not be assumed because its measurement will define bicarbonate concentration and, thus, differentiate between metabolic alkalosis and metabolic acidosis (Rossing et al., 1986). Species specific plasma protein concentrations also make plasma protein assumptions inappropriate (Constable, 1997; Constable, 2002)

The Stewart strong ion difference model of plasma pH provides insight by being able to discern multiple types of nonrespiratory acidosis and alkalosis (Constable, 1999). While theoretically useful, the model is, however, not as useful in a clinical or diagnostic setting because of the difficulties of rapid and quantitative measurements of SID and total weak acid, algebraic complexity and questions of appropriateness of constants (Constable 2000; Constable, 2001).

Because of the analytical, chemical and mathematical difficulties, more simplified models of blood pH have been proposed (Constable, 2000; Heisey and Adams, 2002). One model proposed that plasma pH is function of partial pressure of carbon dioxide, SID as sodium plus potassium minus chloride and lactate and total weak acid as the sum of albumin, globulin and phosphate (Constable, 2000). Sensitivity analysis was used to eliminate the constants for the apparent equilibrium disassociation constant for bicarbonate and ion product of water from the equations and the contribution of minor cations and anions (calcium, magnesium, ammonium, sulfate, NEFA, urate, succinate, ketone bodies, pyruvate) to SID were assumed equal and discarded from the calculations.

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Another model proposed plasma pH as a function of partial pressure of carbon dioxide, SID, and the concentrations of albumin and inorganic phosphate (Heisey and Adams, 2002). An extensive and critical review of the literature was used to generate the parameters of this model.

Most strong ion difference models have focused on blood or plasma pH determination (Stewart, 1983; Constable, 2000; Heisey and Adams, 2002). However, strong ion difference could be applied to other bodily solutions. The ruminal milieu seems a likely candidate of application of SID. The pH of the ruminal solution has proposed to be a function of three conditions: SID, VFA concentration, and partial pressure of carbon dioxide (Kohn, 2000). In this model, SID would include ruminal sodium, potassium, and chloride as well as other ions yet to determined. Disassociated VFA would be the major anion and the partial pressure of carbon dioxide in the ruminal gas would be variable (Table 1.5) and dependent of the gas production of the fermentation.

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EXOGENOUS SODIUM BICARBONATE

Background

Because of the important role endogenous sodium bicarbonate plays in the regulation of the RR, exogenous sodium bicarbonate is commonly added to the diets of lactating dairy cows. The purpose of this addition is to aid in the buffering of the ruminal solution (Erdman, 1988a). The use of sodium bicarbonate and other dietary buffers are recommended when "buffer flow from saliva is inadequate" (Erdman, 1988a), when ruminal pH is low (Kronfeld, 1976) or when herds exhibiting low milk fat test and low or irregular DMI (NRC, 2001). Specific cases where added buffer such as sodium bicarbonate is recommended include high corn silage diets, high ruminal fermentable OM consumption (proportion or amount), low fiber diets, and component feeding system (Hutjens, 1991; NRC, 2001). Sodium bicarbonate is fed free-choice, in the grain mix, or in the total diet (TMR). Recommendation for sodium bicarbonate inclusion commonly appears as 0.75% of the total diet DM but ranges from approximately 0.5% to 1.0% (Table 1.15).

The addition of sodium bicarbonate relieves milk fat depression (defined as milk fat percentage of less than 3.0%), increasing milk fat percentage and yield (Emery, 1976). Milk fat depression is known to occur when lactating cows are fed high levels of concentrates, finely chopped forages, and high amounts of polyunsaturated fatty acids (NRC, 2001). Often, milk fat percentage has been used as an indirect measure of ruminal acidity (NRC, 2001) even though the relationship is very poor (Erdman, 1988a).

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In solution, sodium bicarbonate disassociates into two components: sodium, a strong ion, and bicarbonate, a weak acid anion. In ruminal conditions, sodium bicarbonate can be expected to have an osmotic index of more than 1.80 times its molar concentration (Table 1.16). Sodium is a single valence cation, an alkalizer, and osmotically active. In ruminal conditions, bicarbonate is an alkalizer, osmotically active, and perhaps a buffer depending on ruminal pH. This bicarbonate ion can combine with a proton and decompose to form water and carbon dioxide.

Sodium Bicarbonate As A Ruminal Infusate

The daily infusion of sodium bicarbonate solutions into the RR can, depending on amounts, increase ruminal pH, increase liquid dilution rate, alter fermentation and(or) possibly change efficiency of microbial protein synthesis in sheep (Harrison et al., 1975a; Harrison et al., 1976) and in cattle (Roger et al., 1979; Rogers and Davis, 1982a; Newbold et al., 1988). These effects are generally dose dependent with stronger effects resulting from greater rates of sodium bicarbonate inclusion. All of these infusion experiments show some ruminal effects but these effects must be kept in context. With sheep, 4 L of artificial saliva ruminally infused per d is equivalent to 25 to 50% of natural saliva flow of a sheep (Kay, 1960) and should be viewed as artificial situation. With the experiments using cattle, sodium bicarbonate that was infused was equal to 3% or more of the daily DMI and is not representative of the current diets of lactating cows. A fuller accounting of water and ruminal dynamics in these artificial situations seems warranted.

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Sodium Bicarbonate As A Feed Additive

Early work with sodium bicarbonate in lactating dairy cows explored restricted forage diets (Emery and Brown, 1961; Emery et al., 1964; Emery et al., 1965; Muller and Kilmer, 1979). The daily diets were generally 454 g sodium bicarbonate, forage restricted to less than 10% of intake (less than 2.4 kg forage), and ad libitum concentrate. In these short term experiments (2 to 8 weeks), the inclusion of sodium bicarbonate increased milk and 4.0% FCM, prevented milk fat depression, increased ruminal A:P, and increased ruminal pH (Muller and Kilmer, 1979). Effects on BW were variable (Muller and Kilmer, 1979). However, these diets and lactating cows (<20 kg DMI) are no longer representative of the dairy industry.

A summary of sodium bicarbonate studies with lactating dairy cows from 1960 to 1988 (Erdman, 1988a) showed that, on average, 205 g/d (equal to 1.1% of diet DM) increased milk fat percentage (3.54 to 3.64) and increased ruminal A:P (2.45 to 2.65). Inclusion of sodium bicarbonate had no affect on DMI (either kg/d or as percentage of BW), milk produced (kg/d), milk protein (%), FCM (kg/d), ruminal pH, or total VFA (mEq/L). (Table 1.17). Responses of sodium bicarbonate inclusion in the diet were forage dependent. Corn silage based diets with an average intake of sodium bicarbonate of 207 g/d increased DMI (19.1 to 19.6 kg/d), milk fat percentage (3.49 to 3.65), FCM (27.5 to 28.7 kg/d), ruminal A:P (2.16 to 2.46), apparent total tract DM digestibility (70.3 to 71.9), increased apparent total tract NDF digestibility (51.2 to 54.7). Sodium bicarbonate inclusion had not affect on DMI as a percentage of BW, milk produced (kg/d), milk protein (%), ruminal pH, or total VFA (mEq/L) in corn silage based diets. Sodium bicarbonate inclusion did not affect measurements on diets with forage bases of

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corn silage with alfalfa haylage or grass silage, alfalfa haylage, grass silage, or alfalfa hay. "A direct relationship between blood pH, HCO_3, pCO_2 and milk production has not been shown."

Another summary of sodium bicarbonate inclusion focused on studies with lactating cows from 1980 to 1989 (Staples and Lough, 1989). This decade contained 41 published experiments that were more representative of current diets having more corn silage and more total forage in diet. (Table 1.18) The average diet contained 57% concentrate and 1.1% sodium bicarbonate with maximums for most studies of sodium bicarbonate of 1.5% of diet DM and corn silage as 60% of forage base. In diets where corn silage was the main forage and sodium bicarbonate was included at an average of 1.1% of diet DM, sodium bicarbonate inclusion increased milk produced (0.8 kg/d), milk fat percentage (0.22%), and increased 4% FCM (1.6 kg/d). Mid lactation cows had a slightly greater response in these variables than early lactation cows. Results in other forage bases were not consistent. Sodium bicarbonate inclusion increased ruminal pH, increased ADF digestibility (9 of 12 studies but only 4 of 12 were statistically significant), increased molar percentage of ruminal acetate and decreased molar percentage of ruminal propionate. The inclusion of sodium bicarbonate never increased ruminal liquid dilution rates nor did it change blood pH, pCO_2, pO_2, or bicarbonate. Sodium bicarbonate inclusion increased BW loss in early lactation (0.16 vs. 1.03 kg/cow/week) and increased gain in mid lactation (0.76 vs. 2.53 kg/cow/week). The optimum inclusion rate was concluded to be 0.6 to 0.8% of diet DM.

Since these two reviews, several other experiments have investigated sodium bicarbonate inclusion in the diet. Inclusion of sodium bicarbonate in daily intake can

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increase ruminal pH (Kovacik et al., 1986; Ghorbani et al., 1989; Clayton et al., 1999) but does not always (Solorzano et al., 1989; McKinnon et al., 1990; Hadjipanayiotou et al., 1992). Inclusion of sodium bicarbonate had inconsistent effects on ruminal VFA- both total and proportions. Ruminal VFA (mEq/L) was increased (Kennelly et al., 1999; Khorasani and Kennelly, 2001) or not changed or decreased (Solorzano et al., 1989; Ghorbani et al., 1989; McKinnon et al., 1990; Hadjipanayiotou et al., 1992; Clayton et al., 1999). Ruminal acetate concentration was increased and propionate was decreased (Hadjipanayiotou et al., 1992; Clayton et al., 1999; Kennelly et al., 1999; Khorasani and Kennelly, 2001) or remained unchanged (Solorzano et al., 1989; Ghorbani et al., 1989). Sodium bicarbonate inclusion can increase ruminal liquid dilution rate and(or) rumen dilution outflow (Okeke et al., 1983a; Stokes, 1983) but these increases are associated with sodium bicarbonate inclusions of $\geq$2.5% of dietary DM. Sodium bicarbonate inclusion at 1.5% or less does not appear to influence ruminal liquid dilution (Okeke et al., 1983a; Stokes, 1983; Stokes et al., 1985; Staples and Lough, 1989). Sodium bicarbonate inclusion can increase total tract digestibility a few percent usually through increased fiber digestibility (Solorzano et al., 1989; Ghorbani et al., 1989) but not always (McKinnon et al., 1990; Kennelly et al., 1999; Khorasani and Kennelly, 2001). Sodium bicarbonate inclusion does not always affect DMI (Ghorbani et al., 1989; McKinnon et al., 1990; Kennelly et al., 1999; Khorasani and Kennelly, 2001) and when it does usually a small increase (<10%; Schnedier et al., 1986; Solorzano et al., 1989; Hadjipanayiotou et al., 1992). Inclusion of sodium bicarbonate has little to no affect on blood acid-base measures (Schnedier et al., 1986; Ghorbani et al., 1989; McKinnon et al., 1990) or hormone or metabolites measures (Boisclair et al., 1987; Vicini et al., 1988). Oral dosing

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with sodium bicarbonate or sodium propionate are equally effective in correcting acute metabolic acidosis (Bigner et al., 1997). Inclusion of sodium bicarbonate can increase milk, FCM, milk fat percentage (Schnedier et al., 1986; Hadjipanayiotou et al., 1992; Kennelly et al., 1999; Khorasani and Kennelly, 2001), milk components (Hadjipanayiotou et al., 1992; Kennelly et al., 1999; Khorasani and Kennelly, 2001) but not always (Solorzano et al., 1989; Ghorbani et al., 1989; Clayton et al., 1999). Sodium bicarbonate effect on components is found to be more pronounced during late lactation (Tucker et al., 1994). Production responses to sodium bicarbonate inclusion in early lactation may be dependent on diet base forage (Canale and Stokes, 1988). Sodium bicarbonate inclusion alters fatty acid profile of milk fat (Thivierge et al., 1998; Kennelly et al., 1999; Khorasani and Kennelly, 2001) by increasing saturated and decreasing monounsaturated (Kennelly et al., 1999; Khorasani and Kennelly, 2001). Lactating cows with SARA do not have conclusive preference to consume sodium bicarbonate (Cumby et al., 2001; Keunen et al., 2003) or drink water containing sodium bicarbonate (Cottee et al., 2004).

Including sodium bicarbonate in diets can increase ruminal liquid dilution rate. Sodium bicarbonate at 2% of diet DM of dry Holstein increased ruminal liquid dilution rate from 10.3 to 12.2%/h (Rogers et al., 1982). Sodium bicarbonate at 5% of the diet of steers (145 kg) increased feed intake (5.2 vs. 5.6 kg/d), water intake (16.2 vs. 23.2 kg/d), ruminal pH (6.44 vs. 6.68), ruminal osmolality (273 vs. 288 mOsm), ruminal liquid dilution rate (10.6 vs. 11.3 %/h), and ruminal volume (17.3 vs. 19.7 L) and decreased both molar proportion and production of propionate (Rogers and Davis, 1982b). In both

cases, sodium bicarbonate inclusion was higher than normal for lactating dairy cows and effects should extrapolated with caution.

The sudden introduction of 1.5% sodium bicarbonate in the concentrate portion of a component feeding system decreased intake (0.8 kg/d of concentrate) but this drop was avoided with gradual introduction (Erdman et al., 1982a). This effect is attributed to palatability but ruminal osmolality may also play a role.

Ruminants with multi-cannulated gastrointestinal tracts are used to compartmentalize effects along the gastrointestinal tract. Work with dietary sodium bicarbonate as either a treatment or positive control has been limited and no studies have been found reporting the study of fed sodium bicarbonate mechanism as the primary objective. A review of the literature reveals two lactating cow studies (Kalscheur et al., 1997; Qiu et al., 2004), two steer studies (T e h et al., 1985; Boerner et al., 1987) and three sheep studies (Mees et al., 1985; Wedekind et al., 1986; Hsu et al., 1991).

The two lactating cow studies investigated the effect of different diets on long-chain fatty acid flow to the duodenum. In the first study (Kalscheur et al., 1997), sodium bicarbonate at 1.5% of the diet DM with MgO at 0.5% of diet DM increased average ruminal pH, partially corrected the milk fat depression of the low fiber diet, and decreased flow of the trans-C18:1 fatty acids to duodenum. In the second study (Qiu et al., 2004), sodium bicarbonate at 0.8% of DM numerically but not significantly increased ruminal pH and did not affect flow of trans-C18:1 and conjugated linoleic acid to duodenum.

In the two steer studies, fed sodium bicarbonate was used as a positive control when investigating other buffers. In the first study, 1% fed sodium bicarbonate in a 60%

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concentrate diet increased ruminal pH (6.46 vs. 6.66) and lowered ruminal osmolality (265 vs. 234 mOsm) relative to control and did not affect ruminal liquid dilution rate, blood measures, or ruminal VFA (T e h et al., 1985). In the second study, 1% fed sodium bicarbonate in diet with cottonseed hulls as the fiber source "enhanced" diet digestibility relative to control (Boerner et al., 1987).

In the three lamb studies, higher rates of fed sodium bicarbonate were part of factorial designs. In the first study, 3.5% fed sodium bicarbonate in a 75% concentrate diet increased ruminal pH 2 h postfeeding, increased particulate dilution rate (3.8 to 4.8%), increased microbial nitrogen to small intestine (14.3 to 15.3 g/d), and microbial crude protein efficiency (16.0 to 17.1) g N/1000g OM), but had no affect on total VFA, VFA profile, fluid dilution rate or ruminal volume (Mees et al., 1985). In the second study, 7.5% fed sodium bicarbonate in semi-purified, ground diets increased ruminal fiber digestion but had no effect on total tract digestion (Wedekind et al., 1986). In the third study, 2% fed sodium bicarbonate in 45% bromegrass and 17% soybean hull diets increased ruminal fluid pH (6.2 to 6.4), total tract ADF digestibility (54.2 to 57.6%) and ruminal NDF digestibility (28.5 to 41.6%; Hsu et al., 1991).

Across these studies, an increase in ruminal pH was usually observed with sodium bicarbonate in the diet. Sodium bicarbonate was less likely to produce ruminal effects and increased fiber digestion.

Simulation Of Time-Release Sodium Bicarbonate

In a series of experiments, ruminal infusion of sodium bicarbonate was used to simulate a time-release sodium bicarbonate (Tucker et al., 1988c; Tucker et al., 1988b; Hogue et al., 1991; Aslam et al., 1991; Tucker et al., 1992; Tucker et al., 1993). In these

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experiments, lactating cows were trained to eat during two meals per d. Cows were offered a TMR that was 65 to 75% concentrate for 45 minutes at 12 h intervals. Observed intake was estimated to be 95% of ad libitum. As a consequence, 8 to 10 kg DM was consumed at each meal for a daily intake total of approximately 19 kg of DM. Milk produced ranged from 20 to 25 kg/d. Ruminal fluid, blood, and urine were usually collected every 30 minutes and measures were averaged by timepoint across cows. Given the differences in saliva flow due to chewing behavior or water consumption pattern of individual cows, averages of ruminal sodium, bicarbonate, and pH across cows may not be appropriate.

Several dietary buffer treatments were applied to this experimental model. Inclusion of buffers as 1.4% diet after 2 week adaptation did not change plasma mineral concentration (Tucker et al., 1988c). Twice daily ruminal infusion of sodium bicarbonate at an equivalent of 0.8% of diet DM (Tucker et al., 1988b) and 1.5% of diet DM (Hogue et al., 1991) at different intervals postfeeding (to simulate a time release sodium bicarbonate) had no effects on all ruminal liquid measures at lower rate and only transient buffer effects near the infusion window on the higher rate. Neither infusion scheme affected milk production or components. The higher infusion rate decreased intake (18.1 vs. 17.2 kg) when in the 2 to 4 h postfeeding interval only. No speculation was provided for this decreased intake only associated with this postfeeding interval. Two to four hours after the increased ruminal pH caused by the twice daily infusion of sodium bicarbonate, there was a significant decrease in ruminal pH (Hogue et al., 1991). The authors did not speculate on cause. This rebound in ruminal pH was perhaps associated with a decrease in rumination caused by sodium bicarbonate infusion but rumination behavior and

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ruminal sodium concentrations were not reported. Inclusion of 0.5% diet DM as sodium bicarbonate coupled with twice daily ruminal infusions approximately equal to an additional 1.5% of diet DM showed no effect of treatment on DMI, rumen liquid volume (L), liquid dilution rate (%/h), or liquid turnover (Aslam et al., 1991). Similar ruminal water effects were reported by Tucker et al. (1992). In a 0.5% sodium bicarbonate diet with an increasing rate of twice daily sodium bicarbonate infusion in 2 to 4 h postfeeding window (approximately +0, +1.5%, +3.0%, +4.5% of diet), infusion did not affect DMI (18.4 kg/d), FCM (23.2 kg/d), blood measures, or systematic acid-base status. Ruminal potassium, chloride, calcium and magnesium concentrations were related to intake and sodium was not (Tucker et al., 1993).

Overall, results from these experiment tended to be dose dependent and effects were most prominent near the time of infusion. Higher ruminal pH was recorded usually during infusion. Changes in ruminal liquid measures were associated with higher rates of sodium bicarbonate infusion. No strong intake or production responses were reported.

The Mechanism Of Sodium Bicarbonate Action In The Diets Of Lactating Cows

The beneficial effects of sodium bicarbonate feeding are well documented but, the actions of sodium bicarbonate are probably more complex than the simple elevation of ruminal pH. Many have speculated on the mechanism of sodium bicarbonate action but the actual mechanism has never been documented in the scientific literature.

Sodium bicarbonate inclusion in the diets of lactating dairy cows generally increases milk fat percentage and possibly FCM yield without changing DMI (Erdman, 1988a; Staples and Lough, 1989). The increase in efficiency can be from small increase in apparent digestibility of the DM (Erdman, 1988a) or, more specifically, increases in

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ADF digestibility-usually in the RR (Staples and Lough, 1989). This increase in ruminal fiber digestion may be associated with an increase in ruminal pH and an increase in the A:P ratios (Erdman, 1988a, Staples and Lough, 1989). However, results seem to be diet dependent (Erdman, 1988a) and a specific mechanism has not been documented.

Another theory of the mechanism of sodium bicarbonate action is an altering of the liquid dynamics in the RR (Russell and Chow, 1993). An increase in efficiency of diet utilization could be achieved by an increase in water intake due to sodium bicarbonate inclusion. This could lead to an increased flow of liquid through the ROO and increased the flow of other components leaving the rumen such as particulate matter, rumen microbes and VFA. An increase in particulate matter escape should increase the flow of starch to the small intestine thus reducing starch fermented to VFA and increase starch digested and absorbed as glucose from the small intestine. (Figure 1.3)

Alternatively, milk fat depression may also be caused by a postabsorptive effect caused by altered biohydrogenation of FA in the RR (Bauman and Griinari, 2001). Altering the biohydrogenation in the RR will change the profile of unsaturated FA leaving the RR (Bauman and Griinari, 2001; AbuGhazaleh et al., 2005) which may alter milk fat synthesis (Bauman and Griinari, 2001). Perhaps sodium bicarbonate inclusion is involved in a change in biohydrogenation in the RR.

The increases in FCM or milk fat percentage associated with sodium bicarbonate inclusion may be due to increased efficiency of diet utilization, either by increase fiber digestion (Erdman, 1988a; Staples and Lough, 1989) or altering the site of starch digestion (Russell and Chow, 1993) or due to the altering of lipid metabolism (Bauman

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and Griinari, 2001). More work is needed before the mechanism of sodium bicarbonate is firmly concluded.

SUMMARY

Lactating dairy cows have the ability to generate and regulate a large VFA load in their RR. The RR contains the ruminal fermentation, a heterogeneous mix of gas, liquid, and solids. The ruminal solution contains ions, VFA, and other metabolites. During ruminal fermentation, the microbial mass produces endproducts and releases cell contents which the regulatory systems of cows remove.

The RR absorbs gases, VFA, ions, and water. Ruminal gases move passively down gradients. VFA and ions have transporters in the ruminal epithelium that require energy and the movements of ions and water are restricted and regulated. Overall, the forestomach of lactating dairy cows limits diffusion of water while removing osmotically active particles from the ruminal solution. In a manner analogous to the kidney, the RR actively removes ions from the ruminal solution with a recycling bicarbonate system.

The sodium bicarbonate secreted in the saliva is the key to the removal of ruminal VFA and osmolality from the ruminal solution. Lactating dairy cows are capable of producing large amounts sodium bicarbonate in their saliva each day. With sodium bicarbonate in solution, sodium provides a positive charge and bicarbonate provides a negative charge and both ions are osmotically active. In contrast to sodium, bicarbonate is ephemeral and this provides a mechanism for the removal of a proton.

In the RR, the fermentation generates VFA which, at ruminal pH, mostly disassociate to generate anions and protons. The VFA anion can be absorbed across the ruminal epithelium either by recombining with a proton and diffusing (Figure 1.4) or by an exchange with a bicarbonate (Figure 1.5). In both cases, the cytoplasm of the ruminal

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epithelial cell gains a VFA anion and a proton. This proton must be removed to maintain intracellular pH.

The proton can be exchanged across the epithelial membrane for a luminal sodium and the proton then combines with a ruminal bicarbonate to eventually form carbon dioxide and water. This exchange of cations across the apical membrane maintains the balance of charge while removing the more reactive cation, the proton. Or the intracellular proton can be combined with a VFA anion as cellular metabolism respires the set to carbon dioxide and water. In both cases, the proton is stored in a water molecule and thus, neutralized.

Without intracellular metabolism of the VFA, the cytoplasm contains a sodium and VFA anion which are transported to the blood as a charge-balanced pair. With metabolism or transformation of the VFA in the body, the proton is removed from water and the sodium bicarbonate is regenerated, becoming available for resecretion from the salivary glands.

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The overall net result of this scheme is that osmotic particles are removed from ruminal solution with a balance of charge. Within this scheme, sodium is just as important as bicarbonate in the regulation of acids produced by the microbial mass in the RR. A common tendency is to consider independently the four moieties of VFA and sodium bicarbonate in the ruminal solution (i.e. to focus on pH, the acid/anion, sodium, or bicarbonate independently of the others). However, to truly understand lactating dairy cows' regulation of their VFA load, the interaction of these four moieties must be considered. (Figure 1.6)

The inclusion of sodium bicarbonate in the diets of lactating dairy cows will interact with this four moiety system. Overall, the addition of sodium bicarbonate to lactating dairy cow diets usually increases ruminal pH, can alter VFA profile in RR, does not affect DMI, increases milk fat percentage and FCM, can increase other milk components, and may increase fiber digestion in the gastrointestinal tract. At higher inclusion rates ($\geq$2.5%), water dynamics in the RR may be altered. The effects of sodium bicarbonate inclusion are generally dose and diet dependent. The definitive mechanism of fed sodium bicarbonate action in lactating dairy cows remains to be documented.

Sodium as an osmotically active particle in the rumen could have an effect on rumination but the literature contains limited documentation of the relationship between ruminal sodium and behavior such as rumination. Acute, supraphysiological infusions of sodium into the rumen have decreased ruminating behavior. In these infusions, sodium was introduced in solutions of either sodium salts of VFA (Choi and Allen, 1999) or sodium bicarbonate (Welch, 1982). In both cases, rumination was reduced in the hours

following infusion. In contrast, the effects of additional sodium on rumination has not been reported for typical dairy production situations.

Using sodium bicarbonate added at recommended rates to the diets of lactating dairy cows, sodium's effect on rumination was investigated. The null hypothesis for this study was:

H_o: Inclusion of additional sodium in standard lactating dairy cow diets does not affect ruminating behavior.

The results of this experiment should lead to a better understanding of the interaction of diet (esp. strong ions) and the behavior of lactating dairy cows which will improve ration formulation.

The use of sodium bicarbonate as an additive in the diets of lactating dairy cows changed over the decades. Inclusion rates have decreased as the objective of inclusion has changed and this has lead to the current recommendations averaging approximately 0.75% of dietary DM. The benefits of sodium bicarbonate addition are well documented but the mechanisms of action have only been theorized. Given this lack and the changing objectives, an intensive investigation into actions of sodium bicarbonate addition in the diets of lactating dairy cows is warranted. The null hypothesis for this study was:

H_o: Adding sodium bicarbonate at a recommended rate to diets of lactating dairy cows does not affect milk production, diet digestibility, MN production, ruminal liquid turnover, or chewing behavior.

The parameters of the theories of the mechanism of sodium bicarbonate action in lactating dairy cows will be incorporated into an extensive and comprehensive experimental design. The results should give new insight into the mechanism of action.

More generally, strong ions such as sodium are a determinant of pH of a solution (Stewart, 1983). Sodium is a cation and therefore alkalogenic in solution (Stewart, 1983) and is the most abundant in the ruminal solution (Table 1.10). Ruminal sodium should be related to ruminal pH but investigations of this relationship are limited in the literature. The null hypothesis for this experiment was:

H_o: Sodium and other strong ions in the ruminal solution are not related to ruminal pH or each other.

The results of this descriptive study will yield an increased understanding of strong ions in the ruminal solution.

Overall, this dissertation investigates the role of strong ions (particularly sodium) in the relationships and solutions beyond the empirical measurement of requirement. A more mechanistic and comprehensive view of strong ion will lead to improved diet formulation for lactating dairy cows.

Table 1.1. Comparison of mixed saliva composition of human and ruminant (Adapted from McDougall, 1948).

Table 1.1. Comparison of mixed saliva composition of human and ruminant (Adapted from McDougall, 1948).

	Human	**Ruminant**
Na, mEq/L	11	181
K, mEq/L	17	8
Cl, mEq/L	12	10
HCO_3, mEq/L	13	69
pH	6.6	8.2

Table 1.2. Measured mean saliva compositions.

Table 1.2. Measured mean saliva compositions.

Citation	Animal	Saliva Source	Na, mEq/L	K, mEq/L	HCO_3, mEq/L	H_2PO_4, mEq/L	Cl, mEq/L
McDougall, 1948	Sheep	Mixed	181	8	69	-	10
McDougall, 1948	Sheep	Parotid	177	8	104	52	17
Phillipson and Mangan, 1959	Calves	Mixed	133	16	117	24	8
Phillipson and Mangan, 1959	Calves	Parotid	137	14	108	21	15
Emery et al., 1960	Cows	Mixed	133	14	-	-	-
Kay, 1960	Sheep	Parotid	186	5	95	75	13
Bailey and Balch, 1961a	Cows	Parotid	157	7	127	23	7
Bailey and Balch, 1961b	Cows	Mixed	161	6	126	26	7
Poutiainen, 1966	Cows	Mixed	155	8	-	-	-

Table 1.3. Measured saliva flows during resting and eating in cattle.

Table 1.3. Measured saliva flows during resting and eating in cattle.

Citation	Cattle	Resting Flow, ml/min	Eating Flow, ml/min
Emery et al., 1960	Unspecified	201[1]	-
Bailey, 1961	Dry	-	229
Bailey and Balch, 1961b	Dry	58	-
Meyer et al., 1964	Unspecified	81	-
Cassida and Stokes, 1986	Lactating	151	177
Maekawa et al., 2002a And Maekawa et al., 2002b	Lactating	101	225

[1] High rate attributed to stimulation of the cardia by the collection method.

4. *Estimated daily flow of saliva in cattle.*

Citation	Cows	Estimates in L/d Mean	Low	High	Treatment effects

Table 1.4. Estimated daily flow of saliva in cattle.

Citation	Cows	Estimates in L/d			Treatment effects
		Mean	Low	High	
McDougall, 1948	Not specified	53	50	56	not applicable
Bailey, 1961	Dry	139	98	190	yes, feed character response
Meyer et al., 1964	Not specified	-	117	183	not applicable
Poutiainen, 1966	Not specified	-	70	172	Yes, intake response
Cassida and Stokes, 1986	Lactating	-	285	308	yes, feed character response
Maekawa et al., 2002b	Lactating	239	223	255	no

Table 1.5. Composition of mixed gases in the reticulorumen.

Citation	Species	Carbon dioxide	Methane	Nitrogen	Comments
Wenham and Hoch, 1957	Cattle	20 to 60%	16 to 44%	0 to 35%	

Table 1.5. Composition of mixed gases in the reticulorumen.

Citation	Species	Carbon dioxide	Methane	Nitrogen	Comments
Washburn and Brody, 1937	cattle	20 to 69%	16 to 41%	0 to 35%	
Turner and Hodgetts, 1955a	Sheep	28.3 to 72.8% with a mean of "about 50%)	-	-	
Hoernicke et al., 1965	cattle	10 to 50%	1x to 1/3 of carbon dioxide	-	gases were highly variable
Emmanuel et al., 1969	sheep	35 to 65%	-	-	diet was 45% ground barley straw
Barry et al., 1977	sheep	modal percentage was about 50%; range was about 25 to 55%	modal percentage was about 30%; range was about 12 to 35%	modal percentage was about 10%; range was about 9 to 52%	diet was 100% hay.
Barry et al., 1977	sheep	modal percentage was about 65%; range was about 35 to 75%	modal percentage was about 25%; range was about 16 to 25%	modal percentage was about <5%; range was about 3 to 37%	diet was 20% hay

Table 1.6. Dry atmospheric composition at sea level[1] (Weast, 1978).

Gas	Percentage
	78 (99)

Table 1.6. Dry atmospheric composition at sea level[1] (Weast, 1978).

Gas	Percentage
Nitrogen	78.09%
Oxygen	20.95%
Argon	0.93%
Carbon dioxide	0.03%
Neon	trace
Helium	trace
Krypton	trace
Hydrogen	trace
Xenon	trace
Ozone	trace
Radon	trace

[1] 760 mm Hg

Table 1.7. Predicted
2004).

ved from the RR solution by processes a

Table 1.7. Predicted percentage of protons produced daily removed from the RR solution by processes as modeled by Allen (1997, 2004).

Process	Approximate percentage removed
Absorption of VFA across RR wall	>50
Combined with bicarbonate	28 to 35
Associated and passed with hydrogen phosphate	9
Associated and passed with undisassociated VFA	3
Passage adsorbed to digesta	1
Associated and passed with ammonia	2
Passed as hydronium ion	0

Table 1.8. Typical

Table 1.8. Typical concentrations in lactating dairy cows (NRC, 2001).

	In mEq/L				
Ion	**Blood**	**Saliva**	**Rumen**	**Milk**	**Urine**
Sodium	150	170	Not reported	28	Variable
Potassium	8	<10	70	38	Variable
Chloride	95	Not reported	20	23	Variable

Table 1.9. Estimated typical concentrations of sodium, potassium, chloride, and bicarbonate for spaces related to dairy cows.

	Saliva	RR,	Extracellular,	Intracellular,	Urine

Table 1.9. Estimated typical concentrations of sodium, potassium, chloride, and bicarbonate for spaces related to dairy cows.

Ion	Diet, mEq/100g DM[1]	Saliva, mEq/L[2]	RR, mEq/L[3]	Extracellular, mEq/L[4]	Intracellular, mEq/L[5]	Urine (mEq/L)
Sodium	9.6	161	120	142	12	varies
Potassium	27.1	6	25	5	150	varies
Chloride	8.0	7	11	104	4	varies
Bicarbonate	0	126	30[6]	23	8	varies
$Na + K - Cl$[7]	+28.7	+160	+134	+43	+158	-
$(Na + K) - (Cl + HCO_3)$[8]	+28.7	+34	+104	+20	+150	-

[1] Source: NRC, 2001, p 266. Balanced for mature, 680 kg BW, BCS 3.0, 45 kg milk, 3.5 milk fat %, 3.0 true protein %.
[2] Source: Bailey, 1961a.
[3] Source: Tucker et al., 1993.
[4] Source: Carlson, 2002.
[5] Source: Rhoades and Tanner, 1995.
[6] Source: Dobson, 1970.
[7] Na + K – Cl in mEq/L is SID (Stewart, 1983).
[8] Sodium plus potassium minus chloride minus bicarbonate in mEq/L is anion gap (Carlson, 1997).

Table 1.10. Measured ruminal strong ion concentrations in vivo.

Animal	Sodium,	Potassium,	Chloride,	Comments

Table 1.10. Measured ruminal strong ion concentrations in vivo.

Citation	Animal	Sodium, meq/L	Potassium, meq/L	Chloride, meq/L	Comments
Emery et al., 1960	Unspecified cows	102	37	-	Varied forage to concentrate ratio
Sellers and Dobson, 1960	Unspecified sheep	~84	~46	-	Varied forage to concentrate ratio
Bailey, 1961b	Nonlactating cows	120±23	42±19	16±6	Range of diets and times relative to feeding
Stacy and Warner, 1966	Adult sheep	112-136	16-65	-	Control feeding period
Scott, 1967	Adult sheep	35-95	35-110	-	Testing the effect of potassium supplementation
Ternouth, 1967	Adult sheep	~105	~45	-	Hay diets
Warner and Stacy, 1972a	Adult sheep	119	24	-	Control period
Bennick et al., 1978	Unspecified cows	121±30	50±19	14±7	Range of diets and times relative to feeding
Tucker et al., 1988	Lactating cows	~164	~28	~11	DCAD treatments
Tucker et al., 1993	Lactating cows	~120 (90 to 135)	~25 (15 to 35)	~11 (6 to 17)	Control diet only

1. Distribution of water in the body of dairy cows adapted from Andrew et al. (1995).

Physiological State

Table 1.11. Distribution of water in the body of dairy cows adapted from Andrew et al. (1995).

	Physiological State			
Measurement	**prepartum**	**early lactation**	**late lactation**	**mean lactating**
DIM	-7	63	269	
Live BW, kg	584	555	556	556
Total body water, kg[1]	378	383	347	365
Total body water, % of live wt.	64.7	69.0	62.4	65.7
Total GI water, kg	61	84	59	72
Total GI water, % of live wt.	10.4	15.1	10.6	12.9
Total GI water, % of total body water	16.1	21.9	17.0	19.5
Total RR water, kg	40	52	36	44
Total RR water, % of live wt.	6.8	9.4	6.5	7.9
Total RR water, % of total body water	10.6	13.6	10.4	12.0
Total non-RR, non GI water, kg[4]	21	32	23	28
Total non-RR, non GI water, % of live wt.	3.6	5.8	4.1	5.0
Total non-RR, non GI water, % of total body water	5.6	8.4	6.6	7.5
Proportion GI water in RR	0.65	0.62	0.61	0.62

[1] Includes empty-body water, gastrointestinal water, and water associated with conceptus.
[2] GI is gastrointestinal.
[3] RR is reticulorumen.
[4] Includes water in contents of omasum, abomasum, small intestine, cecum, and large intestine.

1.12. Estimated water pools in lactating dairy cows[1].

Water pool	% of total body water	% of live weight[2]

Table 1.12. Estimated water pools in lactating dairy cows[1].

Water pool	% of total body water	% of live weight[2]
Intracellular	65	42.2
Gastrointestinal	20	13.0
RR[3]	12.4	8.1
Non RR[3]	7.6	4.9
Plasma	8	5.2
Other extracellular fluid	7	4.6
Total	**100**	**65**

[1] Based on Andrew et al., 1995; NRC, 2001; Reece and Swanson, 2004; and difference.
[2] Assumes 65% of live weight as water.
[3] RR is reticulorumen.

Table 1.13a. Typical blood measures of bovines.

Item	Blood volume, % of BW	Hemocrit, %

Table 1.13a. Typical blood measures of bovines.

Item	Blood volume, % of BW	Hemocrit, %
Putnam, 1991	5 or 7.7	
Ruckebusch, et al., 1991	6	
Frandson and Spurgeon, 1992	7.7	40
Reece, 1997	5 or 6	35
Reece and Swenson, 2004	5.2-6.0	24-46

Table 1.13b. Typical blood measures of bovines.

Citation

Table 1.13b. Typical blood measures of bovines.

	Citation		
Item	**Carlson, 1997**	**Carlson, 2002**	**Reece and Swenson, 2004**
Arterial blood pH		7.38	7.38 (7.27-7.49)
Venous blood pH	7.35-7.50	7.31-7.53	
Venous pCO2, mm Hg	35-44	35-44	
Venous bicarbonate	20-30	17-29	
Venous TCO2		21-32	
Sodium, mEq/L	132-152	132-152	132-152
Potassium, mEq/L	3.9-5.8	3.9-5.8	3.9-5.8
Chloride, mEq/L	97-111	97-111	97-111
Calcium, mEq/L			4.5-6.0
Calcium, mg/dl	9.7-12.4	9.7-12.4	
Phosphorus, mEq/L			2.7
Phosphorus, mg/dl	5.6-6.5	5.6-6.5	
Magnesium, mEq/L			1.5-2.5
Magnesium, mg/dl	1.8-2.33	1.8-2.3	
Osmolality, mOsm	270-300	270-300	
Anion Gap, mEq/L	14-20	14-20	

Table 1.14. Reported effective pKa of carbonic acid.

Citation	effective pK_a	solution?	measured?	comments

Table 1.14. Reported effective pKa of carbonic acid.

Citation	effective pK_a	solution?	measured?	comments
McDougall, 1948	6.1	saliva	Yes	at 38.5°C
Turner and Hodgetts, 1955a	6.25	Rumen	Yes	@ 25°C
Kronfeld, 1976	6.1	Body		
Segel, 1976	6.1, 10.25	Blood		1st and 2nd H+
Weast, 1978	6.37, 10.25	Pure		1st and 2nd H+ at 25°C
Trenkle, 1979	6.35	Rumen		
Nova Manual, 1990	6.1	Blood		37°C
Van Soest, 1994	6.4	Rumen		
Pitt et al., 1996	6.4	Rumen		
Allen, 1997	6.1-6.8	Rumen		
Atkins, 1997	6.37, 10.25	Pure		1st and 2nd H+
Constable, 1997	6.0-6.4	Blood		
Erdman, 1988a	6.25	Rumen		
Hutjens, 1991	6.2	Rumen		
Kohn and Dunlop, 1998	6.20	Rumen	Yes	at 37°C
Fernandez et al., 2000	6.25, 10.25	Rumen	Yes	1st and 2nd H+
Russell, 2002	6.7	Rumen		
Leek, 2004	6.1	Rumen		

Table 1.1 S

Table 1.15. Recommendation for sodium bicarbonate inclusion in lactating dairy cow diets.

Citation	Recommendation
Erdman, 1988a	0.75 to 1.0% of diet DM
Staples and Lough, 1989	0.6 to 0.8% of diet DM
Hutjens, 1991	110 to 225 g/cow/d
Church & Dwight Co., Inc., 2001	5 g/kg of milk produced
Church & Dwight Co., Inc., 2001	1.5% of concentrate mixture
Church & Dwight Co., Inc., 2001	0.75% of diet DM
NRC, 2001	1.2 to 1.6% of concentrate mixture
NRC, 2001	0.6 to 0.8% of diet DM

Table 1.16. Sodium bicarbonate solution molality and osmolality as adapted from Weast (1978)[1].

Anhydrous g /100g water	Grams /1000g water	mmoles /1000g water	mOsm	Ratio of mOsm to mM	% of molecules disassociated
0.5	5	59	107	1.81	81
1.0	10	119	213	1.79	79
1.5	15	178	318	1.79	79
2.0	20	238	422	1.77	77
2.5	25	302	524	1.74	74
3.0	30	364	626	1.72	72
3.5	35	426	726	1.70	70
4.0	40	489	825	1.69	69
4.5	45	552	924	1.67	67
5.0	50	615	1021	1.66	66

[1] Adapted from Table on page D-298 assuming molecular weight is 84.01g/mole and solution is at 25 C.

Table 1.17. Summary of published sodium bicarbonate research with lactating cows on modest to high (≥30%) forage diets from 1960 to 1988 (adapted from Table 6 of Erdman, 1988a).

Table 1.17. Summary of published sodium bicarbonate research with lactating cows on modest to high (≥30%) forage diets from 1960 to 1988 (adapted from Table 6 of Erdman, 1988a).

	Control	Sodium bicarbonate	Significance, *P*
Experiments, n	55	55	-
Treatment means	55	65	-
Cow observations	1476	1739	-
Diet ADF%	20.7	20.7	NS[1]
Buffer intake, g/d	0	205	<0.001
DMI, kg/d	18.1	18.3	NS
DMI, % BW	3.21	3.22	NS
Milk, kg/d	26.8	27.0	NS
Milk fat, %	3.54	3.64	<0.01
FCM, kg/d	24.9	25.5	NS
Milk protein, %	3.21	3.17	NS
Rumen treatment means	27	28	-
Ruminal pH	6.38	6.43	NS
Ruminal A:P	2.45	2.65	<0.01
Ruminal Total VFA, mEq/L	94	95	NS

[1] $P > 0.05$

'e 1.18. Summary of published reports of studies with diets averaging 1.1% sodium bicarbonate for lactating cows from 1980 to 1989 (Adapted from Staples and Lough, 1989).

Table 1.18. Summary of published reports of studies with diets averaging 1.1% sodium bicarbonate for lactating cows from 1980 to 1989 (Adapted from Staples and Lough, 1989).

	Control		Sodium Bicarbonate		Net Gain	
	early lactation	mid lactation	early lactation	mid lactation	early lactation	mid lactation
Buffer, % of DM	0.0	0.0	1.1	1.1		
Milk, kg/d	29.7	24.4	30.5	25.3	+0.8	+0.9
Milk fat, %	3.38	2.98	3.54	3.28	+0.16	+0.30
4% FCM, kg/d	26.8	20.6	28.2	22.5	+1.4	+1.9

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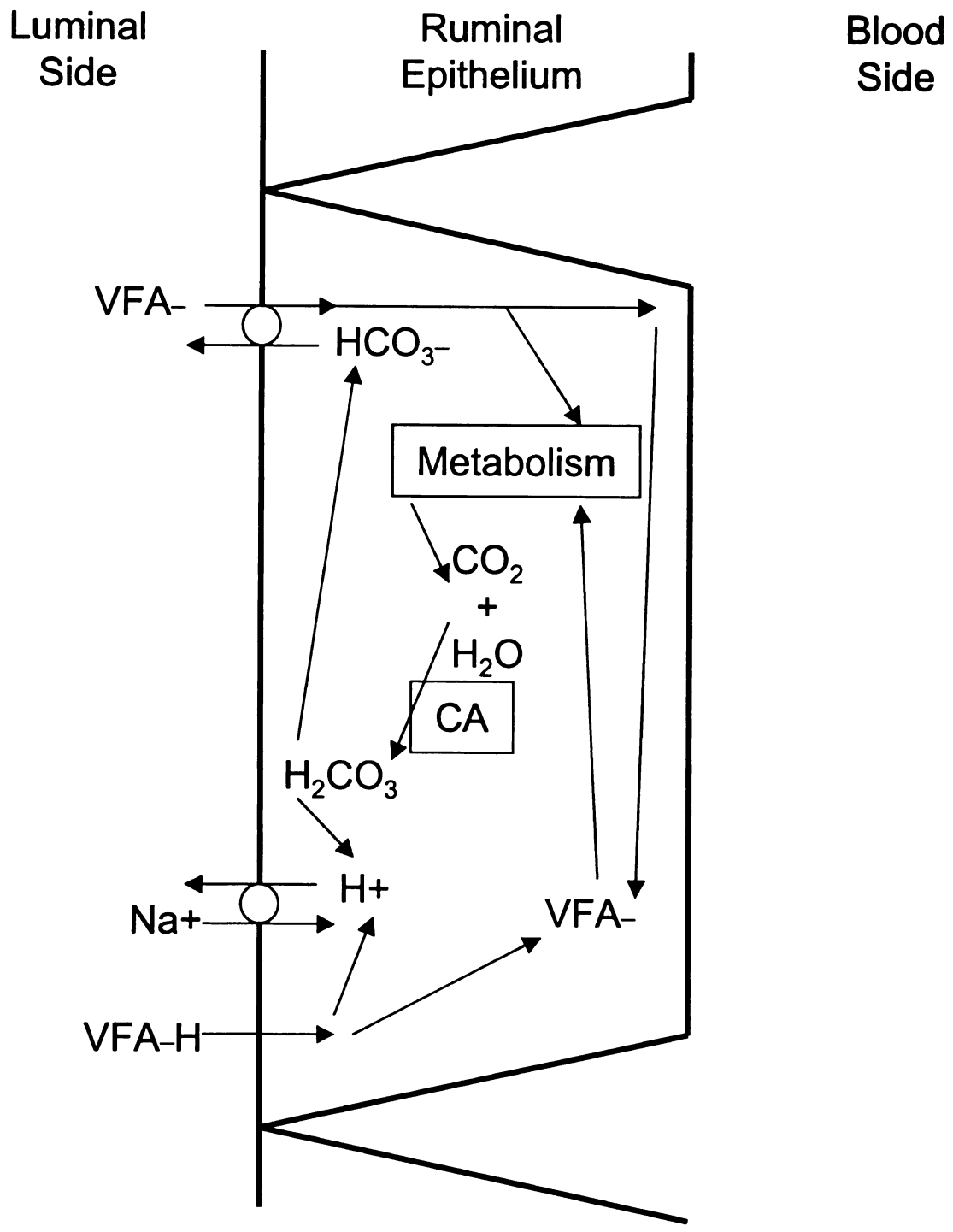

Figure 1.1. Hypothesized movement of VFA across the ruminal epithelium based on figures from Gaebel and Sehested (1997), Sehested et al. (1999b), and Leek (2004). CA is the enzyme carbonic anhydrase.

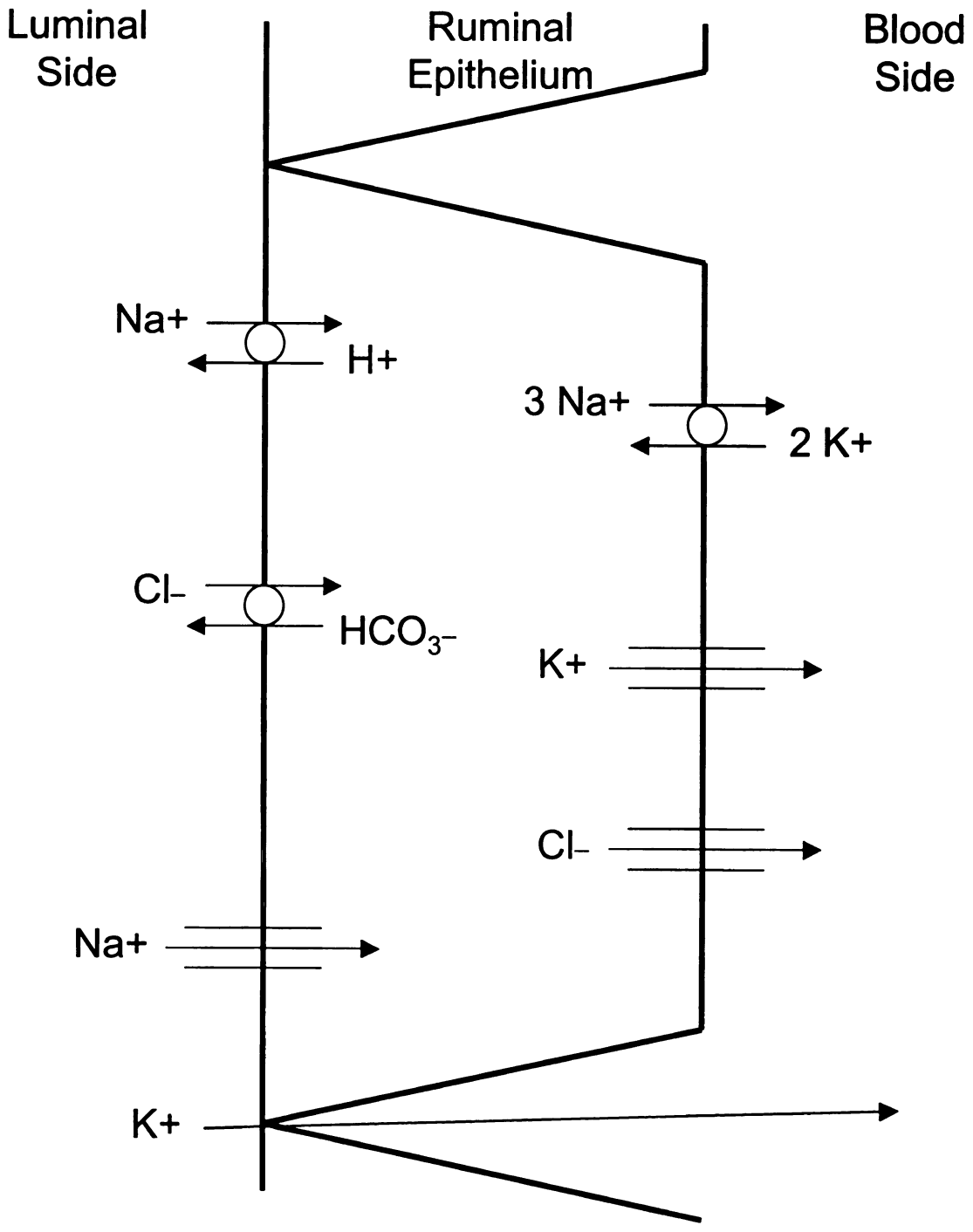

Figure 1.2. Hypothesized movement of sodium, potassium, and chloride across the ruminal epithelium based on figures from Gaebel and Sehested (1997), Sehested et al. (1999b), and Leek (2004).

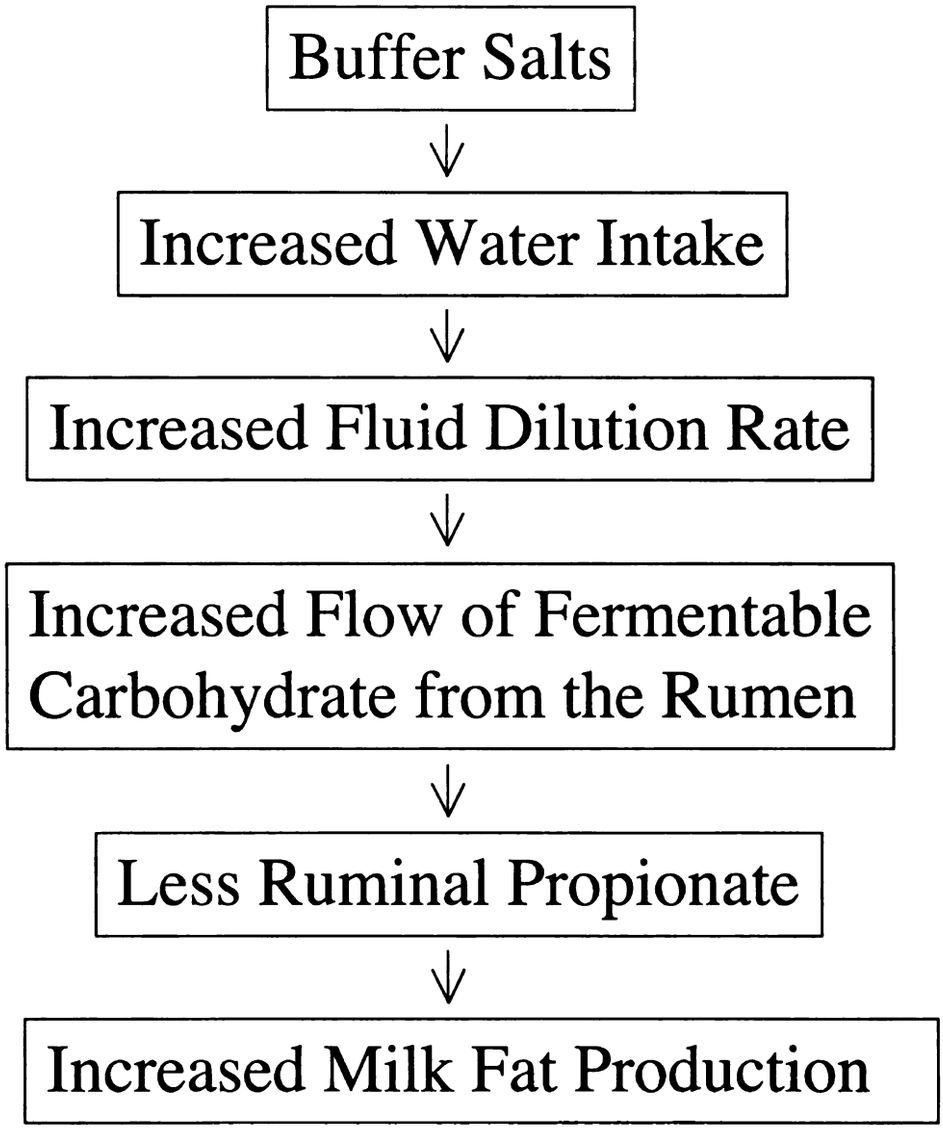

Figure 1.3. A hypothetical mechanism for increasing milk fat production with buffer salts (Adapted from Figure 1 in Russell and Chow, 1993).

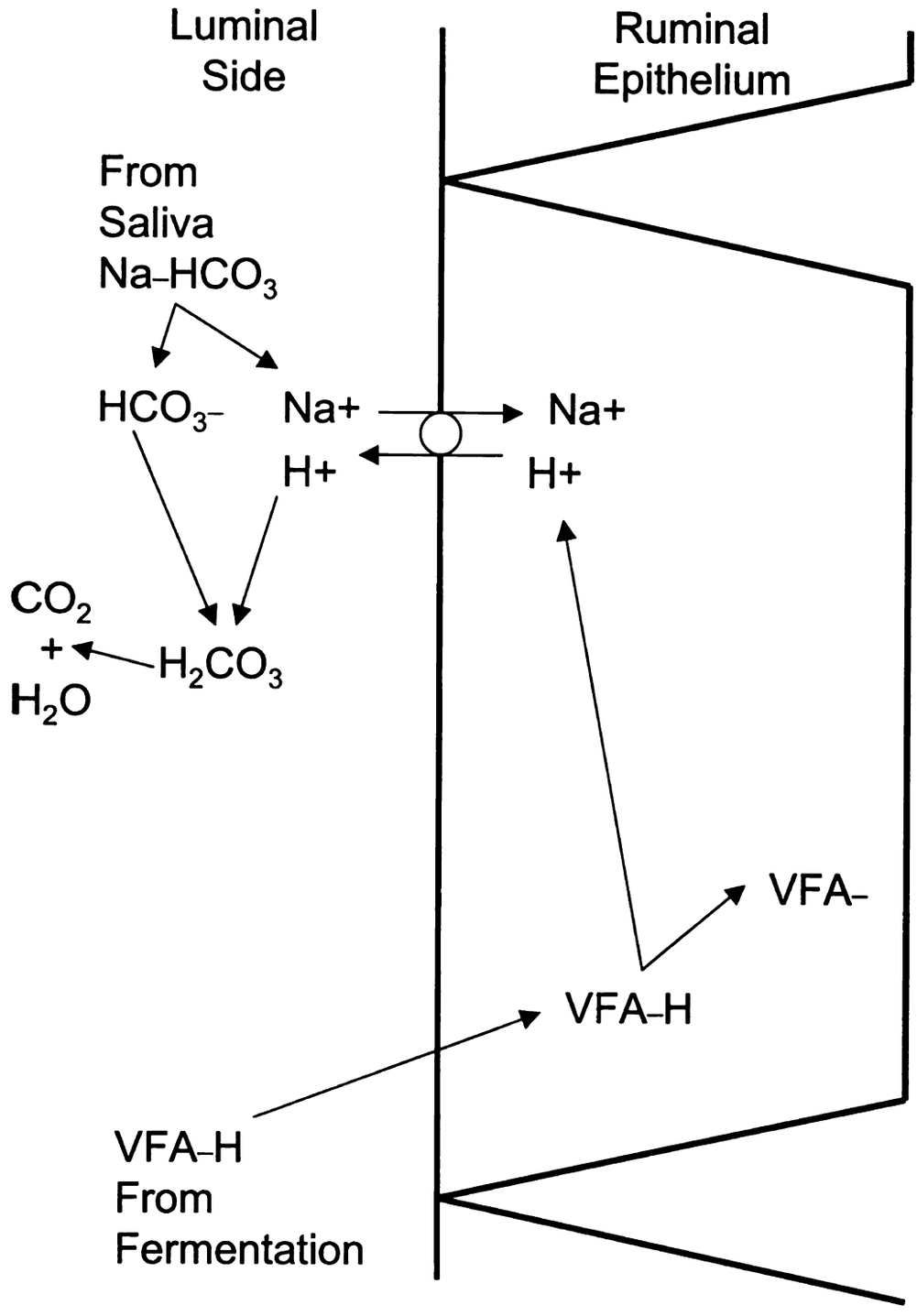

Figure 1.4. Proposed flow of proton with absorption of undisassociated VFA.

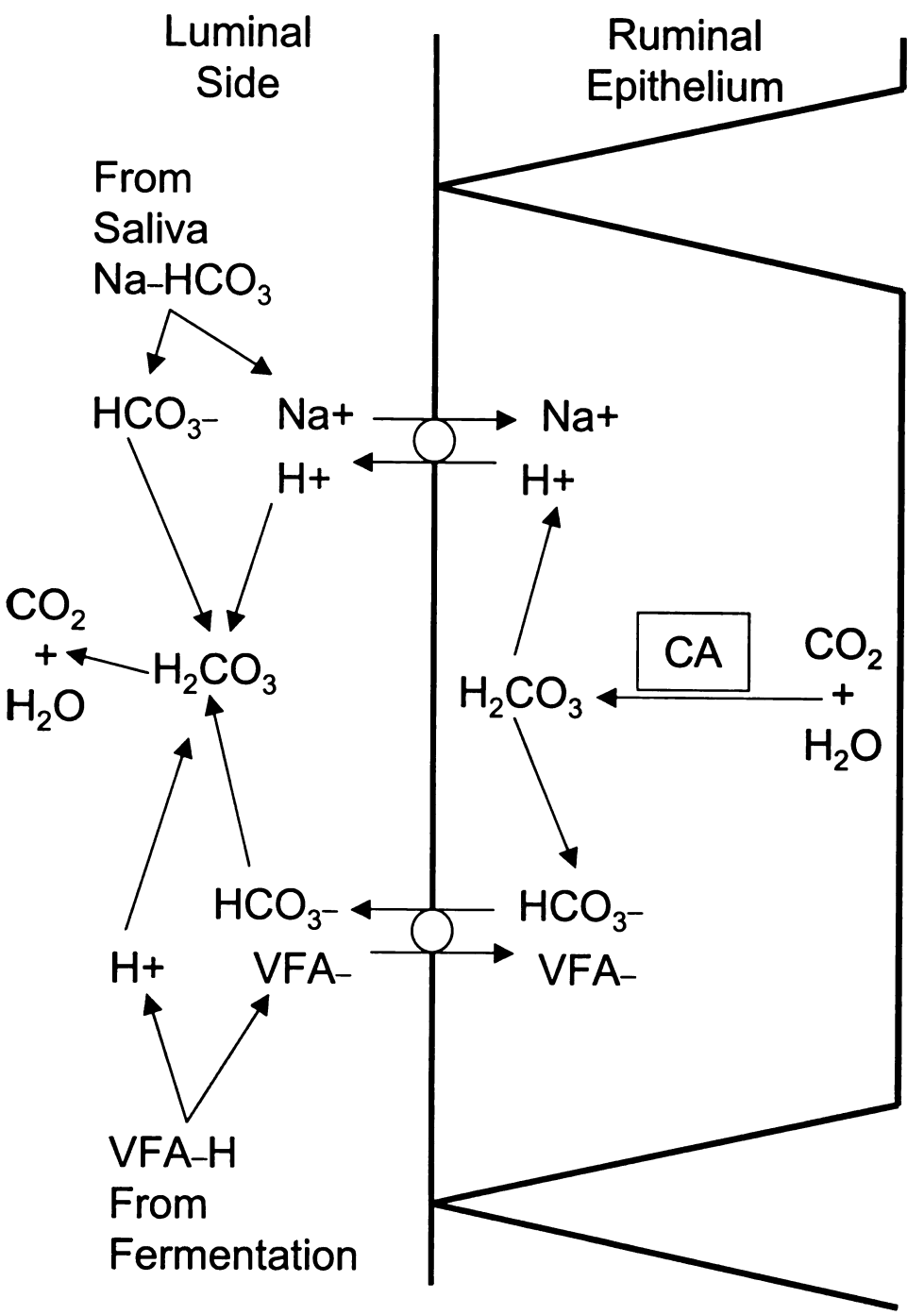

Figure 1.5. Proposed flow of proton with absorption of disassociated VFA. CA is the enzyme carbonic anhydrase.

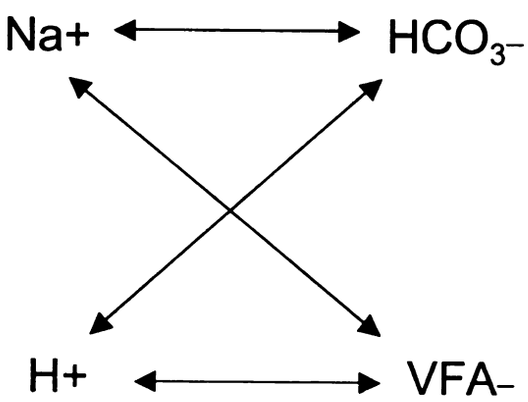

Figure 1.6. Proposed ion exchange to balance charges.

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CHAPTER 2: Effect of dietary strong ions on chewing activity and milk production in lactating dairy cows.

ABSTRACT

The objective of this study was to determine effects of strong ions on chewing activity and short-term lactational performance of dairy cows. Forty multiparous Holstein cows were used in a replicated 5 x 5 Latin square design with a 2 x 2 factorial arrangement of treatments of cations (sodium and potassium), anions (chloride and bicarbonate), plus a control diet. Periods were 14 d in length with the last 4 d for data and sample collection. Diets were formulated to 29% NDF and 17.5% CP. Sodium bicarbonate was included at 1% of DM in one treatment diet and other treatments (sodium chloride, potassium chloride, and potassium bicarbonate) were added to be equimolar to sodium bicarbonate in their respective diets. Chewing activity was recorded every 5 min for the last 24 h of each period. Cation treatments did not affect any measured variable ($P > 0.05$). Treatments did not affect DMI which averaged 27.9 kg/d across treatments. Bicarbonate treatments increased milk fat, milk lactose, and corrected milk yield and tended to increase milk yield when compared to chloride treatments. The four ion treatments reduced ruminating time per d when compared to the control by decreasing the length of rumination bouts. This effect was not specific to cations or anions suggesting a general osmotic effect. The additional ions are expected to tonically increase ruminal osmolality and, based on a threshold theory, possibly terminate rumination sooner.

Key words: buffers, osmolality, lactating cows

INTRODUCTION

Sodium, a strong ion, is required for the health of dairy cattle and is extensively regulated as the primary extracellular cation (NRC, 2001). As one of its many functions, sodium is a major component of saliva and charge-paired with bicarbonate and hydrogen phosphate to form the two major buffers of cattle saliva (Bailey and Balch, 1961b). These buffers are part of lactating dairy cows' system to regulate hydrogen ion concentration in the rumen (Allen, 1997). If endogenous ruminal buffering is lacking, exogenous sodium bicarbonate can be added to the diets of lactating dairy cows (Erdman, 1988a).

Solutions containing sodium compounds infused into the rumen may reduce rumination time. The infusion of 1.2 moles of sodium bicarbonate into the rumens of sheep suspended rumination approximately ten times longer than controls (444 vs. 48 min; Welch, 1982) even with a ruminal pH of greater than 6.5. An infusion of 3 L of 0.75 M sodium salts of VFA into the rumen over 5 min at the onset of spontaneous meals decreased rumination time by 28% during the 12 h test relative to control (Choi and Allen, 1999).

A reduction in rumination time could reduce total daily salivary flow to the rumen and, given the uniform concentration of sodium bicarbonate in the saliva, total ruminal buffer flow (Allen, 1997). An infusion of 110 g of sodium bicarbonate into the rumen of lactating cows over 2 h initially decreased hydrogen ion concentration, but, after several hours, increased ruminal hydrogen ion concentration (Hogue et al., 1991). A decrease in rumination is a possible explanation for this increase in ruminal hydrogen ion concentration, however, chewing activity was not reported. However, these infusions

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may generate ruminal conditions that are not representative of normal physiological or nutritional conditions. The effects of additional sodium on rumination under standard conditions remains to be quantified.

The objective of this experiment was to determine the effects of strong ions on chewing activity and short-term lactational performance in dairy cows. We hypothesized that the addition of sodium bicarbonate at 1% of diet DM would decrease rumination time per d compared to potassium or control diets.

Design

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MATERIALS AND METHODS

Design

Animal procedures were approved by the All University Committee on Animal Use and Care at Michigan State University (AUF# 11/00-150-00). Forty multiparous Holstein cows (126 ± 53 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to replicated 5 x 5 Latin squares balanced for carry over effects with a 2 x 2 factorial arrangement of equimolar treatments for cations (Na and K), anions (Cl and HCO_3) plus a control diet. Experimental periods were 14 d with the final 4 d used for sample and data collection. This design had the power to detect a ten minute difference in chewing activity according to a previous analysis (Dado and Allen, 1994) given previously reported variances (Mooney and Allen, 1997). A ten minute difference was the minimum thought to be meaningful given the method of behavior measurement and an expected saliva flow and composition (Appendix A.7 for basis for experimental design. Appendix A.8 for SAS script for power test.)

Treatments

Experimental diets contained corn silage (67% of forage DM), alfalfa silage (33% of forage DM), alfalfa hay, whole cottonseed, high moisture shelled corn, a premix of protein supplements (soybean meal, distillers grains, and blood meal), a premix of minerals and vitamins and a premix containing the treatment (Tables 2.1 and 2.2). All diets were formulated using the Spartan Dairy Ration Evaluator/Balancer (Version 2.10,

Spartan Software Laboratory, Department of Animal Science, Michigan State University, East Lansing, MI) for 17.5% dietary CP concentration with sufficient metabolizable protein, 29% dietary NDF concentration, and minimum NRC mineral and vitamin requirements (Table 2.3). The control diet was balanced for sodium and potassium then treatments were added as ground rice hulls were removed. Therefore, sodium and potassium on treatment diets were in excess of requirements. All ingredients except treatment mix were combined to form a base mix common to all diets. The base mix was combined daily with each treatment mix in a tumble mixer (Roll-A-Mix Mini-Mix, Model 690, Sand Mark Corporation, Marshfield, WI) for three minutes to form the five final experimental diets. Trace-mineral salt blocks were not available to cows for the duration of the experiment.

Data And Sample Collection

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1030 h) at 110% of expected intake. The amount of feed offered and refused (orts) was weighed daily for each cow. Samples of all dietary ingredients (0.5 kg) and orts (12.5%) were collected daily during the test phase of each period. Samples were frozen immediately after collection at –20° C.

Cows were moved to exercise lot twice daily (0300 and 1300 h) prior to milking. Cows were milked twice daily in a milking parlor (0430 and 1430 h). Milk yield at both milkings was measured and summed for a daily total on d 11-14 of each period. These daily totals were averaged across the test phase of each period. Milk was sampled at each milking on d 11, 12, 13, and 14 of each period and analyzed for fat, true protein, lactose, solids-not-fat, milk urea nitrogen (MUN) and somatic cell count (SCC) with infrared

spectroscopy by Michigan DHIA (East Lansing). Body weight was measured immediately prior to the start of the first period and following the morning milking on d 14 of each period. Body condition score (BCS) was determined (Wildman, 1982; five-point scale where 1 = thin to 5 = fat) by two trained investigators blinded to treatments immediately prior to the start of the first period and on the last day each period. Feeding behavior was monitored manually every 5 minutes for 24 h on d 14 of each period. Behavior was noted as eating, ruminating, drinking or idle for each cow at each time.

Sample Processing

Samples were thawed and composited to one sample per cow per period prior to drying. Diet ingredients, orts and fecal samples were dried in a 55° C forced-air oven for 72 h and DM concentration was determined. Forages and whole cottonseed samples were ground with a Wiley mill (1 mm screen; Authur H. Thomas, Philadelphia, PA). High moisture shelled corn and all premixes were ground with a UDY Cyclone Sample Mill (2 mm screen; Fort Collins, CO).

Sample Analysis

Samples were analyzed for DM, ash, CP, starch, and NDF. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Crude protein was analyzed according to Hach et al. (1987). Starch corrected for free glucose was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was measured using a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and absorbance was determined with micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA).

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Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Concentrations of all nutrients except for DM are expressed as percentages of DM determined by drying at 105° C in a forced-air oven for more than 8 h.

Feed samples were analyzed for sodium, potassium, chloride, and sulfur. Sodium and potassium were determined by digestion according to Hach et al. (1987) and measurement of the element in the supernate by atomic absorption according to manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA). Chloride was determined by extracting the feed with 1.0% nitric acid solution for one hour on a shaker (Orbimix 1010, Brinkman Instruments, Westbury, NY) and measuring chloride in the supernate by coulometric titration (Digital Chloridometer, Model 442-5000, Labconco Corporation, Kansas City, MO). Digests and dilutions were stored in polypropylene containers until analysis-either polypropylene specimen cups or polypropylene, round bottom, 13 x 100 mm, culture test tubes (Fisherbrand® Catalog No. 14-956-7A, Fisher Scientific, Pittsburgh, PA). Dried and ground samples of the base mix, rice hulls, and dried, ground corn were composited across periods and sent to Dairy One Forage Laboratory (Ithaca, NY) for sulfur analysis according to manufacturer's recommendation (LECO Application Note 203-601-229, 08/92, LECO Model SC-432, St. Joseph, MI).

Calculations

Dry-matter intake and nutrient intake were calculated by subtracting the amount refused from amount offered. The intake calculations assume that the diet was combined exactly as prescribed on the mix sheet. Orts were not analyzed for starch, Cl, Na, and K and therefore, intake calculations assume that concentrations of starch, Cl, Na, and K in

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the orts were equal to the concentrations in the DM offered. Change in empty body weight and body condition score were calculated by subtracting the beginning of period value from end of period value. Yield of solids-corrected milk (SCM) was calculated as per Tyrrell and Reid (1965) and yield of fat-corrected milk (FCM) was calculated as per NRC (2001). Somatic cell score (SCS) was calculated by taking the log (base 2) of somatic cell count (SCC).

Dietary cation anion difference (DCAD) as mEq/100g DM was calculated two ways: sodium plus potassium minus chloride (DCAD3) and sodium plus potassium minus chloride plus sulfur (DCAD4). Total diet concentrations for cations and anions were calculated from individual ingredient analyses and dietary proportions of the dry matter. (Appendix A.6 for actual equations.)

Sodium contribution from drinking water was not incorporated into either DCAD calculation because water intake was not measured in this experiment. However, the sodium concentration in the water from a common well was reported as 8 ppm by Michigan State University (2002). This concentration would deliver only 0.8 g of sodium to a cow drinking 100 L/d which is less than 1.3% of the sodium consumed in the control diet.

Manual observation of behavior data were summarized by a logic script in Igor Pro® (2002) to generate meal and bout information. Variables generated included number of meal bouts per d, interval between meals, number of ruminating bouts per d, interval between ruminating bouts, eating time per d, ruminating time per d, and total chewing time per d. (Appendix A.9 for logic used to summarize manual behavior to meals and bouts. Appendix A.10 for Igor Pro® script.)

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Three cow periods from two cows were excluded from the data set because of health problems unrelated to the experiment. One cow was replaced in period three and the other cow was dropped after period three. If a milk sample or weight was not obtained at an individual milking, the milk data for the entire cow day was removed and, therefore, 35 days of milk data out of a possible 800 were removed from the final data set (Appendix Table A.8).

Statistical Analysis

All data were analyzed using the fit model procedure of JMP® (Version 5.0.1.2; 2003) according to the following model for cow period means:

$$Y_{ijk} = \mu + C_i + P_j + T_k + O_{ij} + e_{ijk}$$

Where

μ = overall mean,

C_i = random effect of cow (i = 1 to 40),

P_j = fixed effect of period (j = 1 to 5),

T_k = fixed effect of treatment (k = 1 to 5),

O_{ij} = fixed effect of treatment carryover,

e_{ijk} = residual, assumed to be normally distributed.

Orthogonal contrasts were performed for effects of ion treatments, cation treatments, anion treatments, and interaction of cation and anion treatments. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects were declared at $P < 0.10$. When interactions of main effects were significant, treatment means were compared using

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Student's t-test and differences were declared significant at $P < 0.05$. Milk yield and composition cow period averages were weighted because of missing data for some days.

Residual plots were checked for the appearance of normality. All plots appeared normally distributed except for the SCC plot. SCC data were transformed to SCS and the associated residual plot appeared to be normally distributed. Period by treatment interaction was originally evaluated, but it was removed from the statistical model because interaction was not significant for response variables of primary interest.

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RESULTS

Postexperiment analysis (Table 2.3) showed experimental diets had less crude protein (0.3% of DM) and NDF (1.0% of DM) than planned due to lower postexperiment CP and NDF concentrations in some feeds. Postexperiment analysis also showed the control diet was adequate for sodium, potassium and chloride (NRC, 2001; assuming an experimental cow of 630 kg BW producing 37 kg of milk and consuming 28 kg DM). Measured DCAD4 ranged from 16.1 to 27.6 mEq/100g DM and was near the proposed optimal range of 20 to 50 mEq/100g DM (Sanchez and Beede, 2005). Treatments did not affect intake of DM and OM, averaging 27.9 kg and 25.0 kg, respectively. NDF intake for the control diet tended to increase when compared to the ion treatments (7.5 kg vs. 7.4 kg) because of its greater proportion of rice hulls. Sodium, potassium and chloride intakes were as expected according to the experimental design. A uniform cation intake was achieved across all treatment diets, averaging 3.3 moles/cow/d. (Table 2.4)

Ion treatments affected milk yield and composition (Table 2.5). Potassium treatments tended to increase yield of some milk components (milk fat, milk lactose, and milk SNF) and component-corrected milk yield (3.5% FCM, 4.0% FCM, and SCM) when compared with sodium treatments. Bicarbonate treatments increased milk fat (0.12% and 0.07 kg), milk lactose (0.06% and 0.06 kg), and SNF (0.05% and 0.10 kg) when compared to chloride treatments. Bicarbonate treatments tended to increase milk yield and increased corrected milk yield (1.5 kg 3.5% FCM, 1.4 kg 4.0% FCM, and 1.5 kg SCM) when compared with chloride treatments. The interaction among cations and anions was significant for SNF percentage ($P = 0.08$) and Student's t-test showed the two bicarbonate treatments (8.71%) were greater than the sodium chloride treatment (8.63%).

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Ion treatments did not affect SCC or SCS ($P > 0.30$). Bicarbonate treatments also increased efficiency of component corrected milk production (0.06 kg 3.5% FCM/kg DMI, 0.05 kg 4.0% FCM/kg DMI, and 0.05 kg SCM/kg DMI) when compared to chloride treatments (Table 2.6).

All experimental diets showed a gain in BCS and BW across the test periods (Table 2.6) suggesting these mid-lactation cows were in positive energy balance. Treatments did not affect change in BCS. However, an interaction among treatments ($P = 0.06$) was detected for BW; both sodium treatments resulted in similar BW gain (10.8 kg/period) but the potassium chloride treatment resulted in more than twice the gain as potassium bicarbonate treatment (15.1 kg/period vs. 7.0 kg/period).

Experimental diets affected chewing activity (Table 2.7). Ion treatments reduced rumination time by 23.0 min when compared to the control diet. Concurrently, ion treatments increased idle time by 27.0 min and decreased total chewing time 33.5 min when compared to the control diet. Anion treatments also affected behavior. Chloride treatments decreased eating time per d and increased drinking observations (6.4 observations vs. 5.7 observations) when compared to bicarbonate treatments. However, drinking was usually associated with eating and, when drinking and eating were summed within cow, no treatment effect was observed on this combined time per d.

Manual observation of eating, ruminating and total chewing activity was highly correlated with Igor Pro® summation ($r > 0.93$; Table 2.8). In this summary, ion treatments again decreased chewing activity (Table 2.9). Meals per d were similar across all diets (8.0 meals/d) and meal length was decreased with ion treatments (2.1 min/meal) but total eating time per d was similar across all diets (273.3 min/d). The number of

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ruminating bouts per d was similar across diets (14.3 bouts/d), but ion treatments decreased ruminating bout length (1.6 min/bout) and total ruminating time per d (26.2 min/d). Even when ruminating time (min/d) was corrected for intake (DM and NDF), the depression by ion treatments remained (1.0 min/kg DM and 2.5 min/kg NDF). Ion treatments had similar effects on total chewing time (min/d) with the decreases in eating and ruminating time resulting a decrease in total chewing time (28.2 min/d). No specific cation or anion effects were observed.

DISCUSSION

Two unique elements to the design of this experiment were the equimolar addition of cations and anions and the commonality of greater than 98% of the dietary DM across experimental diets. These, with the uniform DMI, allowed a separation of chemical and osmotic effects.

Production And Performance

The DMI and milk production recorded in this experiment are among the highest reported in a sodium bicarbonate study (Staples and Lough, 1989). Intake was similar across all experimental diets but performance differed. Bicarbonate diets increased FCM production and chloride diets were associated with the greatest weight gain. Both of these suggest either an increase in digestibility or a change in nutrient partitioning leading to an increase efficiency (i.e. the same nutrient intake utilized more efficiently).

Sodium bicarbonate is theorized to increase FCM in several possible ways. The sodium bicarbonate may increase ruminal pH and allow for greater fiber digestion in the rumen (Erdman, 1988a) or may increase water consumption that might increase liquid turnover in the RR (Russell and Chow, 1993). This increased turnover may, in turn, flush starch particles from the RR which will be digested in the intestine increasing digestible energy (Russell and Chow, 1993). Or, perhaps, sodium bicarbonate addition leads to a change in ruminal saturation of FA and this change is speculated to alter lipid metabolism (Bauman and Griinari, 2001). However, the mechanism of action can not be concluded from this experiment. Potassium bicarbonate has been shown to be as effective as sodium bicarbonate for relieving milk fat depression (Emery, 1976).)

Potassium treatments in this experiment increased yield of milk components and tended to increase component-corrected milk yields when compared to sodium treatments. Previous work (Oba and Allen, 2003d) showed ruminal infusion of sodium as sodium VFA (12 mmol/min for 14 h) increased milk component percentage but not yield (0.14% for milk protein and 0.29% for milk lactose) when compared to potassium as potassium VFA (12 mmol/min for 14 h). This difference in results between experiment is probably related to the difference in experimental design. In this experiment, the cations were fed and, in the previous work, cations were infused into the RR on a short term basis (14 h/d).

Potassium chloride had the highest BW gain per period and potassium bicarbonate and the control diet had the lowest. Because BW gain was not qualified, the question is whether these body weight gains were associated with tissue gain or with changes in water spaces within the body. In rats, increased chloride intake was associated with a net zero balance of chloride and no change in fluid compartments (Kaup et al., 1991). Whether this holds true in ruminants as well remains to be determined. In this experiment, each unit of BCS was related to 260 kg of BW (Figure 2.1). This result is much higher than the stated relationship of each unit of BCS equaling 80 to 85 kg of BW for lactating dairy cows (NRC, 2001) suggesting that BW changes are not solely based on body condition.

Chewing Activity

For eating behavior, meals were slightly shorter for treatment diets. Increasing ruminal osmolality has decreased intake (Bergen, 1972) and has caused satiety (Choi and

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Allen, 1999). However, in this experiment, the ion treatments would likely produce a much smaller and more chronic change in ruminal osmolality.

Ion treatments decreased chewing activity but the effect was general and not specific to cations or anions. Without a specific chemical element effect, these effects must be due to direct or indirect effects of increased ruminal osmolality. Increasing ruminal osmolality can cause rumination to cease. An osmotic threshold of the ruminal solution has been proposed for the termination of rumination (Welch, 1982) where rumination will cease until the ruminal osmolality returns to less than 350 mOsm in sheep.

All the components of the treatment (sodium, potassium, bicarbonate, and chloride) are osmotically active (Weast, 1978) and, with their consumption, are expected to increase the osmolality of the RR solution. In this experiment, cows consumed a mean of 28 kg DM/d in 8 meals for an average 3.5 kg DM/meal. The 3.5 kg meal would contain 0.420 moles of treatment as 35.0 g of sodium bicarbonate or 24.5 g of sodium chloride. Assuming 50 L of water in the RR (Andrews et al., 1995), 35.0 g of sodium bicarbonate would contribute 0.7 g/L and would have an osmotic index of 1.80 times the molar concentration and 24.5 g of sodium chloride would contribute 0.5 g/L and would have and osmotic index of 1.87 times the molar concentration (Weast, 1978). Assuming instantaneous consumption and mixing, all treatments could increase the ruminal solution 8 mmoles/L or 14 to 15 mOsm/L. Under real conditions, the increase would be less because of absorption and passage.

Overall, and with uniform nutrient intake, the inclusion of ion treatments should lead to a small but chronic increase in the ruminal osmolality. If a proposed threshold for

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rumination remains the same, this small increase should reduce potential ruminating time per d (Figure 2.2).

Cost And Gain Of Sodium Bicarbonate Addition

With the increase in milk production and loss of rumination, the concern must be the cost and gains associated with the addition of sodium bicarbonate to the diet. Cost and gain can be determined for economics as well as ruminal buffering (Table 2.10). The average consumption of 279 g/d costs $0.112/d (assuming a cost of $360/ton) but the return for increased production of 1.4 kg of milk is $0.372 (assuming $12/cwt of milk). Thus, the return is 332% of cost. Sensitivity analysis shows, at a range of typical costs and returns, that this sodium bicarbonate addition remains profitable.

Like economics, sodium bicarbonate addition shows a net gain for ruminal buffering (Table 2.10). A loss of 25 min/d of rumination time is expected to lead to a loss of 469 mEq of bicarbonate equivalent. However, the consumption of 279 g/d of sodium bicarbonate delivers 3298 mEq of bicarbonate equivalent to the RR for a net gain of 2829 mEq of bicarbonate equivalent. As a point of comparison, the measured chewing times of control diet in this experiment would generate saliva containing almost 40 Eq of bicarbonate equivalent (Table 2.11) given published valves for saliva flow (Cassida and Stokes, 1988; Maekawa et al. 2002A; Maekawa et al., 2002B) and bicarbonate equivalent composition (Erdman, 1988a).

CONCLUSION

The cows on this experiment responded to bicarbonate treatment as expected, increasing FCM yield while maintaining the same feed intake. The addition of ions to diets reduced rumination time per d with no differences among the ion treatments. Though sodium addition was expected to decrease rumination, the effect was not specific to sodium but was apparently through tonically increasing ruminal osmolality which ended rumination bouts. The decrease in rumination can lead to decreased saliva flow but, in this experiment, the gain in ruminal buffering with sodium bicarbonate addition was calculated to be greater than the loss from reduced rumination.

Table 2.1. Nutrient composition of ingredients used to formulate experimental diets (% of dietary DM).

Nutrient	Ingredients											
	CS[1]	AS[2]	Hay[3]	HMC[4]	WCS[5]	SMP[6]	SMV[7]	SBM[8]	SCM[9]	PBM[10]	PCM[11]	CntlM[12]
DM, 55°C	37.3	32.4	85.9	74.9	88.9	89.6	90.0	90.7	90.3	90.8	90.5	89.2
DM, 100°C	35.5	30.7	82.7	72.2	85.9	86.8	87.4	87.0	87.1	87.4	87.7	85.8

Table 2.1. Nutrient composition of ingredients used to formulate experimental diets (% of dietary DM).

	Ingredients											
Nutrient	**CS**[1]	**AS**[2]	**Hay**[3]	**HMC**[4]	**WCS**[5]	**SMP**[6]	**SMV**[7]	**SBM**[8]	**SCM**[9]	**PBM**[10]	**PCM**[11]	**CntlM**[12]
DM, 55°C	37.3	32.4	85.9	74.9	88.9	89.6	90.0	90.7	90.3	90.8	90.5	89.2
DM, 100°C	35.5	30.7	82.7	72.2	85.9	86.8	87.4	87.0	87.1	87.4	87.7	85.8
OM	96.4	90.1	89.1	98.6	96.3	93.8	69.9	81.4	78.0	75.7	75.2	88.9
Ash	3.6	9.9	10.9	1.4	3.7	6.2	30.1	18.6	22.0	24.3	24.8	11.1
CP	8.0	21.0	18.0	9.1	20.5	48.3	6.0	5.1	5.4	5.0	5.2	5.9
NDF	40.4	43.3	39.3	9.6	45.4	16.7	6.6	25.5	29.8	23.0	27.3	39.3
Starch	31.1	1.5	4.0	70.3	1.4	4.1	46.9	35.9	36.0	36.7	35.9	35.7
Ether extract	4.4	5.8	2.3	3.4	17.7	4.0	1.6	6.6	1.3	2.2	1.4	1.2
Na^+	0.09	0.07	0.05	0.04	0.07	0.10	3.39	5.37	5.40	0.07	0.23	0.06
K^+	0.78	2.49	3.02	0.41	0.78	1.10	0.46	0.40	0.32	7.97	9.67	0.39
Cl^-	0.17	0.54	0.56	0.06	0.05	0.07	5.07	0.07	8.19	0.08	8.46	0.09

[1] CS: Corn silage.
[2] AS: Alfalfa silage.
[3] Hay: Mixed mostly legume hay.
[4] HMC: High moisture corn.
[5] WCS: whole cottonseed.
[6] SMP: Protein Mix contained 75.6% soybean meal, 19.5% corn distillers grain, and 4.9% blood meal.
[7] SMV: Mineral and vitamin mix contained 69.4% dry ground corn, 10.5% dicalcium phosphate, 9.2% limestone, 8.1% trace mineral salt, 1.8% trace mineral premix, 0.4% magnesium oxide, 0.4% vitamin A, 0.3% vitamin D, and 0.1% vitamin E.
[8] SBM: Sodium bicarbonate treatment mix.
[9] SCM: Sodium chloride treatment mix.
[10] PBM: Potassium bicarbonate treatment mix.
[11] PCM: Potassium chloride treatment mix.
[12] CntlM: Control mix.

Table 2.2. Ingredient composition of experimental diets (% of dietary DM).

	Experimental Diets				
Diet Ingredients	***SBT***[1]	***SCT***[2]	***PBT***[3]	***PCT***[4]	***Cntl***[5]
Corn silage	*25.6*	*25.6*	*25.6*	*25.6*	*25.6*
Alfalfa silage	*12.7*	*12.7*	*12.7*	*12.7*	*12.7*

Table 2.2. Ingredient composition of experimental diets (% of dietary DM).

	Experimental Diets				
Diet Ingredients	**SBT**[1]	**SCT**[2]	**PBT**[3]	**PCT**[4]	**Cntl**[5]
Corn silage	25.6	25.6	25.6	25.6	25.6
Alfalfa silage	12.7	12.7	12.7	12.7	12.7
Hay	6.0	6.0	6.0	6.0	6.0
High moisture corn	23.5	23.5	23.5	23.5	23.5
Whole cottonseed	7.2	7.2	7.2	7.2	7.2
Protein mix[6]	15.1	15.1	15.1	15.1	15.1
Vitamin & mineral mix[7]	5.0	5.0	5.0	5.0	5.0
Treatment mix	5.0	5.0	5.0	5.0	5.0
Dried, ground corn	2.5	2.5	2.5	2.5	2.5
Ground rice hulls	1.5	1.8	1.3	1.6	2.5
NaCl	-	0.7	-	-	-
KCl	-	-	-	0.9	-
$NaHCO_3$	1.0	-	-	-	-
$KHCO_3$	-	-	1.2	-	-

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.
[6] Protein Mix contained 75.6% soybean meal, 19.5% corn distillers grain, and 4.9% blood meal.
[7] Mineral and vitamin mix contained 69.4% dry ground corn, 10.5% dicalcium phosphate, 9.2% limestone, 8.1% trace mineral salt, 1.8% trace mineral premix, 0.4% magnesium oxide, 0.4% vitamin A, 0.3% vitamin D, and 0.1% vitamin E.

Table 2.3. Composition of experimental diets (% of dietary DM).

	Experimental Diets				
	SBT[1]	**SCT**[2]	**PBT**[3]	**PCT**[4]	**Cntl**[5]
DM, 55°C	54.7	54.7	54.7	54.7	54.7
DM, 1005°C	52.5	52.5	52.5	52.5	52.5
OM					

Table 2.3. Composition of experimental diets (% of dietary DM).

	Experimental Diets				
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]
DM, 55°C	54.7	54.7	54.7	54.7	54.7
DM, 1005°C	52.5	52.5	52.5	52.5	52.5
OM	93.2	93.1	92.9	92.9	93.6
Ash	6.8	6.9	7.1	7.1	6.4
Starch	29.9	29.9	29.9	29.9	29.9
NDF	27.8	28.0	27.7	27.9	28.5
CP	17.2	17.2	17.2	17.2	17.2
Ether extract	4.8	4.5	4.6	4.5	4.5
Na^+	0.50	0.50	0.23	0.24	0.23
K^+	1.06	1.06	1.44	1.52	1.06
Cl^-	0.43	0.83	0.43	0.85	0.43
S^{-2}	0.14	0.14	0.14	0.14	0.14
Forage	44.3	44.3	44.3	44.3	44.3
Forage NDF	18.2	18.2	18.2	18.2	18.2
DCAD3, mEq/100g DM	36.7	25.2	34.8	25.6	25.1
DCAD4, mEq/100g DM	27.6	16.2	25.8	16.6	16.1

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.

Table 2.4. The effect of dietary strong ion treatment on component intakes.

	Experimental Diets						Significance, *P*			
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]	SE	TMT[6]	Cation[7]	Anion[8]	X[9]
DMI, kg	27.8	27.8	28.0	28.0	27.8	0.4	0.66	0.46	0.90	0.94
OMI, kg	24.9	24.9	25.0	25.0	25.0	0.4	0.76	0.64	0.82	0.98
NDFinta, kg										

Table 2.4. The effect of dietary strong ion treatment on component intakes.

	Experimental Diets						Significance, *P*			
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]	SE	TMT[6]	Cation[7]	Anion[8]	X[9]
DMI, kg	27.8	27.8	28.0	28.0	27.8	0.4	0.66	0.46	0.90	0.94
OMI, kg	24.9	24.9	25.0	25.0	25.0	0.4	0.76	0.64	0.82	0.98
NDF intake, kg	7.3	7.4	7.3	7.4	7.5	0.1	0.06	0.93	0.20	0.68
CP intake, kg	4.6	4.6	4.6	4.6	4.6	0.1	0.85	0.37	0.72	0.97
Estimated[10] starch intake, kg	8.0	8.0	8.0	8.0	8.0	0.1	0.59	0.37	0.83	0.82
Estimated[10] treatment intake, kg	1.4	1.4	1.4	1.4	1.4	0.0	0.66	0.46	0.90	0.93
Estimated[10] sodium intake, kg	0.133	0.134	0.063	0.065	0.062	0.001	<0.001	<0.001	0.07	0.34
Estimated[10] potassium intake, kg	0.284	0.282	0.385	0.409	0.284	0.006	<0.001	<0.001	0.009	0.004
Estimated[10] chloride intake, kg	0.115	0.223	0.115	0.228	0.115	0.002	<0.001	0.04	<0.001	0.07
Est. [10] treatment cation intake, moles	3.3	3.3	3.3	3.3	0.0	0.0	<0.001	0.74	1.00	0.93

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.
[6] TMT is the contrast of strong ion treatment and control.
[7] Cation is the contrast of sodium and potassium treatments.
[8] Anion is the contrast of bicarbonate and chloride treatments.
[9] X is the interaction of cations and anions.
[10] "Estimated" is based on offered diet concentrations only.

Table 2.5. The effect of dietary strong ion treatment on milk production and milk composition.

	Experimental Diets						Significance, *P*			
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]	SE	TMT[6]	Cation[7]	Anion[8]	X[9]
Milk, kg	37.3	36.3	37.7	37.0	36.4	1.2	0.16	0.16	0.05	0.69
Milk fat, %	3.88	3.74	3.89	3.80	3.83	0.07	0.87	0.32	0.001	0.53
Milk protein, %	3.12	3.12	3.10	3.13	3.12	0.03	0.89	0.72	0.25	0.19
Milk lactose, %	4.73	4.66	4.73	4.69	4.69	0.03	0.36	0.17	<0.001	0.22
Milk SNF[10,11], %	8.72[a]	8.63[b]	8.70[a]	8.69[ab]	8.68[ab]	0.06	0.70	0.39	0.04	0.08
Milk fat, kg	1.45	1.36	1.46	1.41	1.40	0.05	0.44	0.08	0.001	0.42
Milk protein, kg	1.16	1.12	1.16	1.15	1.13	0.03	0.17	0.23	0.10	0.48
Milk lactose, kg	1.77	1.70	1.79	1.74	1.72	0.06	0.16	0.10	0.06	0.53
Milk SNF, kg	3.25	3.13	3.28	3.21	3.16	0.10	0.17	0.12	0.02	0.48
3.5% FCM, kg	39.5	37.6	39.9	38.8	38.4	1.3	0.28	0.08	0.002	0.47
4.0% FCM, kg	36.6	34.9	37.0	36.0	35.6	1.2	0.28	0.08	0.002	0.40
SCM, kg	36.3	34.5	36.7	35.6	35.2	1.2	0.26	0.08	0.001	0.40
SCC, 1000's/ml	219	240	297	205	178	70	0.36	0.72	0.55	0.34
SCS	2.5	2.8	2.5	2.6	2.7	0.3	0.71	0.66	0.30	0.52

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.
[6] TMT is the contrast of strong ion treatment and control.
[7] Cation is the contrast of sodium and potassium treatments.
[8] Anion is the contrast of bicarbonate and chloride treatments.
[9] X is the interaction of cations and anions.
[10] SNF is solids, milk non-fat.
[11] Student's t-test, $P < 0.05$.

Table 2.6. The effect of dietary strong ion treatment on body weight, body condition and milk production efficiency.

	Experimental Diets						Significance, *P*			
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]	SE	TMT[6]	Cation[7]	Anion[8]	X[9]
Change in BW, kg/14 d[10]	10.7[ab]	10.9[ab]	7.0[b]	15.1[a]	7.6[b]	2.0	0.15	0.91	0.05	0.06
Change in BCS, change/14 d	0.08	0.11	0.06	0.05	0.04	0.03	0.26	0.16	0.90	0.46
Milk per DMI, kg/kg	1.34	1.30	1.35	1.33	1.31	0.04	0.27	0.27	0.08	0.76
3.5% FCM/DMI, kg/kg	1.42	1.35	1.43	1.39	1.38	0.04	0.41	0.18	0.01	0.59
4.0% FCM/DMI, kg/kg	1.31	1.25	1.33	1.29	1.28	0.04	0.41	0.18	0.01	0.59
SCM per DMI, kg/kg	1.30	1.24	1.31	1.28	1.27	0.04	0.41	0.17	0.008	0.50

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.
[6] TMT is the contrast of strong ion treatment and control.
[7] Cation is the contrast of sodium and potassium treatments.
[8] Anion is the contrast of bicarbonate and chloride treatments.
[9] X is the interaction of cations and anions.
[10] Tukey's HSD, $P < 0.05$.

Table 2.7. The effect of dietary strong ion treatment on chewing behavior based on manual observation.

	Experimental Diets						Significance, *P*			
	SBT[1]	**SCT**[2]	**PBT**[3]	**PCT**[4]	**Cntl**[5]	**SE**	**TMT**[6]	**Cation**[7]	**Anion**[8]	**X**[9]
I^{10}, *min/d*	674.6	683.4	675.9	671.4	649.5	12.8	0.003	0.50	0.78	0.39
D^{11}, *observations/d*	5.6	6.7	5.7	6.0	5.7	0.5	0.35	0.37	0.02	0.25

Table 2.7. The effect of dietary strong ion treatment on chewing behavior based on manual observation.

	Experimental Diets						Significance, *P*			
	SBT[1]	SCT[2]	PBT[3]	PCT[4]	Cntl[5]	SE	TMT[6]	Cation[7]	Anion[8]	X[9]
I[10], min/d	674.6	683.4	675.9	671.4	649.5	12.8	0.003	0.50	0.78	0.39
D[11], observations/d	5.6	6.7	5.7	6.0	5.7	0.5	0.35	0.37	0.02	0.25
E[12], min/d	250.7	240.0	248.8	242.5	251.3	5.8	0.15	0.94	0.02	0.54
D+E, min/d	278.5	273.5	277.5	272.5	280.0	6.5	0.14	0.77	0.18	0.91
R[13], min/d	486.5	483.4	486.7	495.9	510.8	9.9	0.001	0.31	0.63	0.32
R+E, min/d	737.2	723.3	735.5	738.3	762.0	12.5	0.001	0.39	0.47	0.28

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] PBT is potassium bicarbonate treatment diet.
[4] PCT is potassium chloride treatment diet.
[5] Cntl is control diet.
[6] TMT is the contrast of strong ion treatment and control.
[7] Cation is the contrast of sodium and potassium treatments.
[8] Anion is the contrast of bicarbonate and chloride treatments.
[9] X is the interaction of cations and anions.
[10] I is an observation of not drinking, eating or ruminating.
[11] D is an observation of drinking.
[12] E is an observation of eating.
[13] R is an observation of ruminating.

Table 2.8. Pearson Correlation between manual observation raw counts and Igor Pro® summation[1].

Igor Pro® Summation[1]	**Manual Counts**		
	E's[2]	**R's[3]**	**R+E[4]**
Eating Time, min/d	0.93	0.21	0.61
Ruminating Time, min/d	0.20	0.99	0.87

Table 2.8. Pearson Correlation between manual observation raw counts and Igor Pro® summation[1].

	Manual Counts		
Igor Pro® Summation[1]	**E's[2]**	**R's[3]**	**R+E[4]**
Eating Time, min/d	0.93	0.21	0.61
Ruminating Time, min/d	0.20	0.99	0.87
Total Chewing Time, min/d	0.64	0.85	0.98

[1] See Appendices A.9 and A.10 for logic and script.
[2] E is eating observations.
[3] R is rumination observations.
[4] R+E is rumination plus eating observations.

Table 2.9. The effect of strong ion treatment on Igor Pro® summarization of manually observed chewing behavior data.

Igor Pro® Summarization[1]	Experimental Diets						Significance, *P*			
	SBT[2]	SCT[3]	PBT[4]	PCT[5]	Cntl[6]	SE	TMT[7]	Cation[8]	Anion[9]	X[10]
Meals, n	7.9	8.1	8.1	8.3	7.8	0.2	0.18	0.29	0.29	0.87
Meal length, min	36.1	34.3	34.5	33.8	36.8	1.3	0.04	0.26	0.16	0.51

Table 2.9. The effect of strong ion treatment on Igor Pro® summarization of manually observed chewing behavior data.

	Experimental Diets						Significance, *P*			
Igor Pro® Summarization[1]	**SBT**[2]	**SCT**[3]	**PBT**[4]	**PCT**[5]	**Cntl**[6]	**SE**	**TMT**[7]	**Cation**[8]	**Anion**[9]	**X**[10]
Meals, n	7.9	8.1	8.1	8.3	7.8	0.2	0.18	0.29	0.29	0.87
Meal length, min	36.1	34.3	34.5	33.8	36.8	1.3	0.04	0.26	0.16	0.51
Eating time, min/d	273.9	270.4	274.6	272.6	275.0	7.2	0.67	0.74	0.54	0.87
Intermeal interval, min	113.2	111.4	109.5	111.1	115.5	3.3	0.20	0.50	0.97	0.56
Ruminating bout, n	14.4	13.9	14.4	14.4	14.4	0.3	0.52	0.27	0.33	0.27
Ruminating bout length, min	34.5	35.6	34.4	35.1	36.5	1.0	0.01	0.57	0.13	0.74
Ruminating time, min/d	490.2	486.4	489.2	497.1	516.9	10.2	<0.001	0.45	0.75	0.36
Interbout interval, min	64.0	64.9	65.0	63.0	61.5	1.7	0.06	0.72	0.65	0.25
Total chewing time, min/d	764.0	756.7	763.8	769.7	791.8	13.7	0.003	0.44	0.93	0.42
Eating time/DMI, min/kg	9.9	9.8	9.9	9.8	10.0	0.27	0.51	0.99	0.50	0.84
Ruminating time/DMI, min/kg	17.7	17.5	17.6	17.9	18.7	0.38	<0.001	0.68	0.87	0.26
Total chewing/DMI, min/kg	27.6	27.2	27.4	27.6	28.6	0.53	0.002	0.75	0.80	0.33
Eating time/NDF intake, min/kg	37.7	36.6	37.7	37.1	37.1	1.05	0.77	0.70	0.18	0.72
Ruminating time/NDF intake, min/kg	67.6	65.7	67.2	67.7	69.5	1.51	0.02	0.36	0.45	0.17
Total chewing/NDF intake, min/kg	105.3	102.3	104.9	104.8	106.5	2.09	0.10	0.36	0.19	0.22

[1] See Appendices A.9 and A.10 for logic and script.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] PBT is potassium bicarbonate treatment diet.
[5] PCT is potassium chloride treatment diet.
[6] Cntl is control diet.
[7] TMT is the contrast of strong ion treatment and control.
[8] Cation is the contrast of sodium and potassium treatments.
[9] Anion is the contrast of bicarbonate and chloride treatments.
[10] X is the interaction of cations and anions.

Table 2.10. Cost and gain to economics and ruminal buffering per cow per day with the addition of sodium bicarbonate to lactating dairy cows at 1% of the DM.

Item	Change	Units
Cost of 279 g/d of sodium bicarbonate	-0.112	dollars
Assumes a price of $360/ton		

Table 2.10. Cost and gain to economics and ruminal buffering per cow per day with the addition of sodium bicarbonate to lactating dairy cows at 1% of the DM.

Item		Change	Units
Cost of 279 g/d of sodium bicarbonate		-0.112	dollars
Assumes a price of $360/ton			
Gain of 1.4 kg of 3.5% FCM		+0.372	dollars
Assumes a price of $12/cwt			
	NET	**+0.260**	dollars
Cost of 25 min/d of lost ruminating time		-469	mEq of bicarbonate equivalent[1]
Assumes 125 ml/min loss of saliva flow changing from ruminating to idle			
Assumes 150 mEq/L of bicarbonate equivalent			
Gain of 279 g/d of sodium bicarbonate		+3298	mEq of bicarbonate equivalent
	NET	**+2829**	mEq of bicarbonate equivalent
Sensitivity Analysis			
Cost of 279 g/d if $340/ton		-0.105	dollars
Cost of 279 g/d if $360/ton		-0.112	dollars
Cost of 279 g/d if $380/ton		-0.118	dollars
Cost of 279 g/d if $400/ton		-0.124	dollars
Gain of 1.4kg/d of 3.5% FCM if $10/cwt		+0.310	dollars
Gain of 1.4kg/d of 3.5% FCM if $12/cwt		+0.372	dollars
Gain of 1.4kg/d of 3.5% FCM if $14/cwt		+0.434	dollars
Gain of 1.4kg/d of 3.5% FCM if $16/cwt		+0.496	dollars

[1] as defined by Erdman, 1988a.

Table 2.11. Estimated saliva flow on the control diet based on measured behavior and expected saliva flows.

Chewing behavior	Min/d	Saliva ml/min[1]	Saliva L/d
Eating	275	200	55
Ruminating	520	250	130
Idle	645	125	81

Table 2.11. Estimated saliva flow on the control diet based on measured behavior and expected saliva flows.

Chewing behavior	**Min/d**	**Saliva ml/min**[1]	**Saliva L/d**
Eating	275	200	55
Ruminating	520	250	130
Idle	645	125	81
Totals	**1440**	-	**266**[2]

[1] based on Cassida and Stokes, 1986, Maekawa et al., 2002A, and Maekawa et al., 2002B.
[2] If lactating cow saliva is 150 mEq/L of bicarbonate equivalent (See Erdman, 1988a for definition), then 266 L has 39,900 mEq of bicarbonate equivalent.

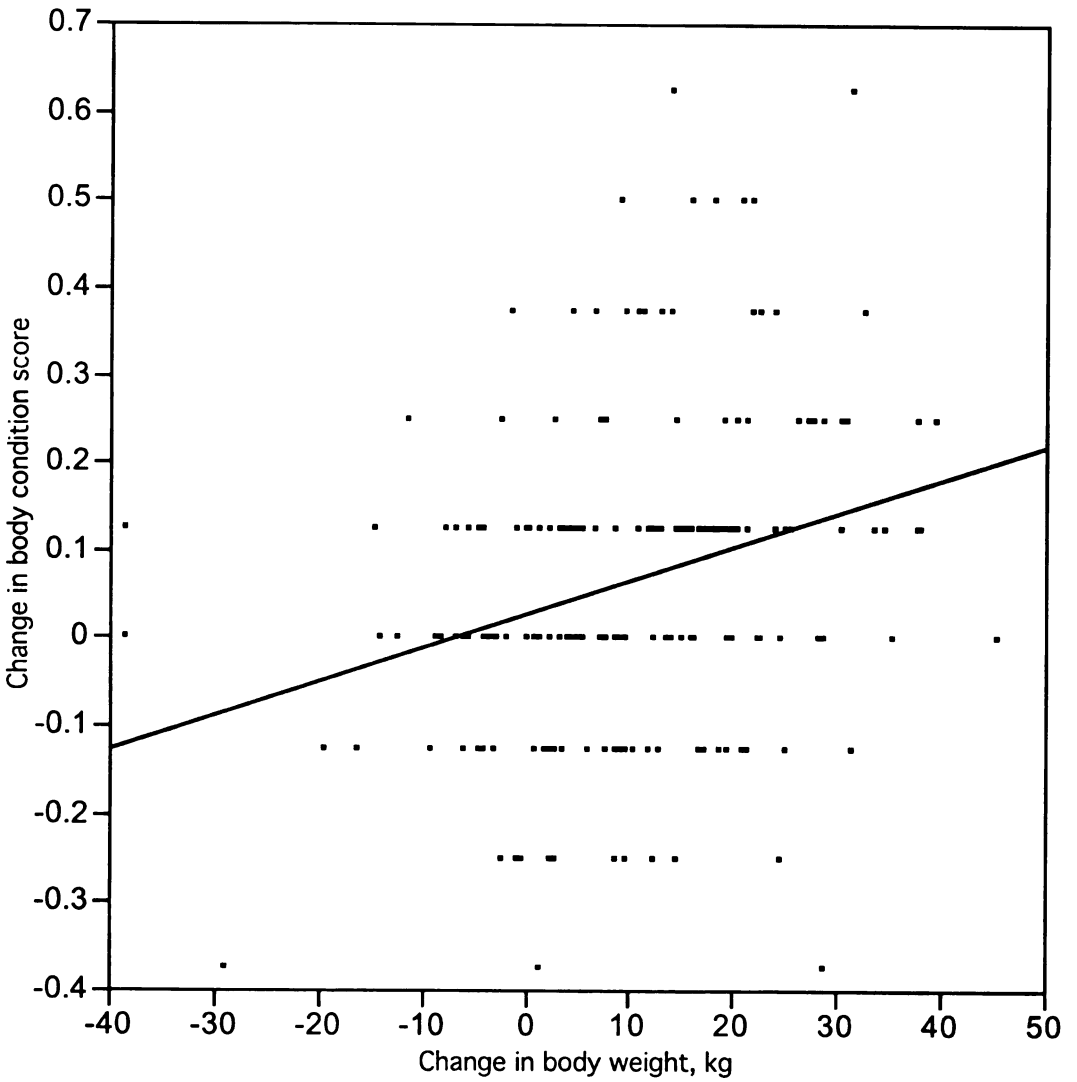

Figure 2.1. The relationship between change in body weight and change in body condition score across periods. Regression equation is (change in body condition score) = (0.03 + (0.004)(change in body weight, kg)). $R^2 = 0.07$, $P < 0.0001$.

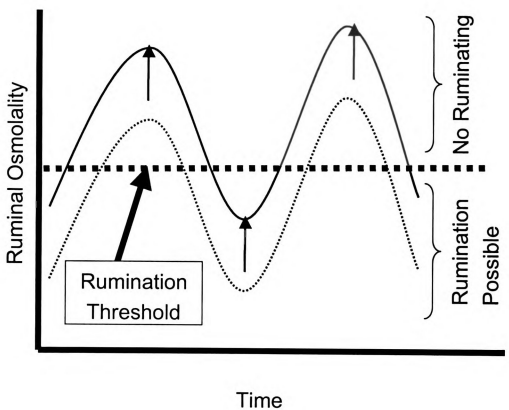

Figure 2.2. Proposed model of the decreased rumination due to ion treatment in this experiment. The addition of ions to the diet shifts the ruminal osmolality up (----- is ruminal osmolality before addition and — is ruminal osmolality after addition). With the addition ruminal osmolality stays above the rumination threshold longer resulting in shorter rumination bouts and longer inter-rumination intervals.

CHAPTER 3: Effects of sodium bicarbonate on site and extent of nutrient digestion, microbial efficiency, feeding behavior, and yield and composition of milk for mid- to late-lactation dairy cows

ABSTRACT

Six ruminally and duodenally cannulated, mid-lactation (180 ± 12 DIM, mean ± SD) Holstein cows were used in a replicated 3 x 3 Latin square design to evaluate effects of sodium bicarbonate on feeding behavior, nutrient digestion, and microbial protein production. Periods were 28 d in length with the last 14 d for data and sample collection. Treatments were control, sodium bicarbonate at 1% of dietary DM and an isomolar concentration of sodium chloride. Diets measured 19% forage NDF and 17.8% CP. Dry matter intake was not different across treatments (24.5 kg/d) nor were milk yield (36.7 kg) and composition. Mean ruminal pH was 6.20 and was not affected by treatment ($P >$ 0.62) nor were any other measures of pH (minimum, maximum, range, or standard deviation; $P > 0.42$). Both sodium treatments increased water intake compared to the control diet (103.8 L/d vs. 98.7 L/d, $P = 0.05$) but did not affect extent or site of starch digestion or liquid passage rate. Sodium treatments increased total tract NDF digestibility probably by slowing of passage of digesta from the RR because of an expansion of ruminal contents. Osmotic effects likely partially contribute to sodium bicarbonate effects on total tract NDF digestibility.

Key words: sodium, chloride, osmolality

INTRODUCTION

The use of sodium bicarbonate in diets of lactating dairy cows has changed over the years. Early research focused on adding sodium bicarbonate to diets of limited forage (<10% of DMI) and ad libitum grain as a possible method to alleviate milk fat depression (MFD, Emery and Brown, 1961; Emery et al., 1964; Emery et al., 1965; Muller and Kilmer, 1979). Sodium bicarbonate generally was offered at 454 g/d or approximately 5% of daily DM intake. Over the years, the recommended inclusion rate of sodium bicarbonate in the diets of lactating dairy cows has decreased to less than or equal to 1% of the dietary DM.

Currently, the addition of sodium bicarbonate is recommended as 0.6 to 0.8% of dietary DM (NRC, 2001). Exogenous sodium bicarbonate is added commonly to the diets of lactating dairy cows for the purpose of buffering the RR (Erdman, 1988a). Overall, the addition of sodium bicarbonate to lactating dairy cow diets often increases ruminal pH, can alter VFA profile in RR, does not affect DMI, increases milk fat percentage and FCM, and may increase fiber digestion in the gastrointestinal tract (Erdman, 1988a; Staples and Lough, 1989). Increases in milk yield or components without changing intake suggest increased efficiency of diet utilization for milk production. At higher inclusion rates (≥2.5%), water dynamics in the RR may be altered in cattle (Rogers et al., 1982; Rogers and Davis, 1982b). The effects of sodium bicarbonate inclusion generally are diet dependent (Erdman, 1988a; Staples and Lough, 1989).

Several mechanisms of sodium bicarbonate action in the ruminant have been proposed. One hypothesis proposed that the addition of sodium bicarbonate to the diet of

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lactating dairy cows causes an elevation in ruminal pH that leads to increased fiber digestibility and energy per unit of DM (Erdman, 1988a). Another states that the addition of sodium bicarbonate increases water consumption which increases liquid passage from the RR and shifts the site of starch digestion for the rumen to the intestines (Russell and Chow, 1993). Both of the hypotheses suggest an increased efficiency results from increased digestible energy density of the diet.

While hypotheses exist and empirical experiments have shown the addition of sodium bicarbonate to be beneficial, the mechanism of action of the addition of sodium bicarbonate has not been fully described. Intensive digestibility studies are limited and these studies are not usually representative of recommended for feeding lactating dairy cattle sodium bicarbonate. Investigations with multi-cannulated lactating dairy cows for the purpose of elucidating the mechanisms of sodium bicarbonate have not been reported. Therefore, intensive research is warranted to determine the mechanisms of action of sodium bicarbonate.

The objective of this study was to investigate the mechanism of action of sodium bicarbonate addition in lactating dairy cows. Dietary treatments were: a control, sodium bicarbonate at 1% of dietary dry matter and sodium chloride (an osmotic control) at a concentration isomolar to the sodium bicarbonate treatment. Digestion kinetics, microbial efficiency, feeding behavior, intake and milk yield and composition were compared among these treatments. The null hypothesis was that adding sodium bicarbonate at a recommended rate to diets of lactating dairy cows does not affect milk production, diet digestibility, MN production, ruminal liquid turnover, or chewing behavior.

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MATERIALS AND METHODS

Design

Animal procedures were approved by the All University Committee on Animal Use and Care at Michigan State University (AUF# 09/01-148-00). Six multiparous Holstein cows (180 ± 12 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to treatment sequence within a pair of 3 x 3 Latin squares balanced for carry over effects. Experimental periods were 28 d with the final 14 d used to collect samples and data. Cows were cannulated ruminally and duodenally prior to calving. Cows were fitted with a 10 cm ruminal cannulae (10 cm i.d.; Bar Diamond Inc., Parma, ID). Duodenal cannulas were soft gutter type made of tygon and vinyl tubing (Crocker et al., 1998). The duodenum was fistulated between the pylorus and the pancreatic duct and cannulas were placed between 10th and 11th ribs as described by Robinson et al. (1985). Both surgeries were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University.

The test phase of each period (d 15 through d 28) was divided into several subperiods. The subperiods were a digestibility determination (d 15 through d 17), an intensive 24 h collection of blood and ruminal fluid (d 19), feeding behavior monitoring (d 20 through d 24), ruminal valerate absorption and liquid passage determination (d 25 and d 26), and rumen evacuations for pool size determination (d 27 and d 28).

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Treatments

The three experimental diets were a control, sodium bicarbonate as 1% of dietary DM, and sodium chloride (an osmotic control) isomolar to sodium bicarbonate. Nutrient composition of diet ingredients and the three treatments appear in Tables 3.1 and 3.2. Experimental diets contained corn silage (67% of forage DM), alfalfa silage (33% of forage DM), high moisture shelled corn, a premix of protein supplements (70% soybean meal, 25% distillers grains, and 10% blood meal), a premix of minerals and vitamins and premix containing the treatment (Table 3.3). All diets were formulated using the Spartan Dairy Ration Evaluator/Balancer (Version 2.10, Spartan Software Laboratory, Department of Animal Science, Michigan State University, East Lansing, MI) for 19.0% dietary CP concentration with sufficient metabolizable protein, 20% dietary forage NDF concentration, and to meet minimum NRC mineral and vitamin requirements. The control diet was balanced for sodium then treatments were added and ground rice hulls were removed to form experimental treatments. Therefore, sodium concentration in treatment diets was projected to be in excess of requirements. All ingredients except treatment mix were combined to form a base mix common to all diets. The base mix was combined daily with each treatment mix in a tumble mixer (Roll-A-Mix Mini-Mix, Model 690, Sand Mark Corporation, Marshfield, WI) for three minutes to form the three final experimental diets. Trace mineral salt blocks were not available to cows for the duration of the experiment.

Data And Sample Collection

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1130 h) at 110% of expected intake. The amounts of feed offered and refused (orts) were

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weighed daily for each cow. Samples of all dietary ingredients (0.5 kg) and orts (12.5%) were collected daily during the test phase of each period. Cows were milked twice daily in their stalls from d 19 through d 24 and in a milking parlor for the rest of period. Stall milking times (0600 h and 1700 h) were slightly different than parlor milking times (0500 h and 1600 h) but the milking intervals were similar. Milk was sampled at each milking on d 19 through 24 of each period and analyzed for fat, true protein, lactose, solids-not-fat, milk urea nitrogen (MUN) and somatic cell count (SCC) with infrared spectroscopy by Michigan DHIA (East Lansing). Empty body weight was measured after evacuation of ruminal digesta immediately prior to the start of the first period and on d 28 of each period. Body condition score (BCS) was determined (Wildman, 1982; five-point scale where 1 = thin to 5 = fat) by three trained investigators blinded to treatments immediately prior to the start of the first period and on d 28 of each period. All samples collected during the experiment (feed, orts, digesta, fecals, milk, and plasma) were frozen immediately after collection at –20° C.

Days 15, 16, 17: Digestibility Determination

Indigestible NDF (iNDF) was used as a marker to estimate nutrient digestibility in the rumen and in the total tract. Duodenal samples (1,000 g) and fecal samples (500 g) were collected every 9 h from 15 to 17 d (A total of 8 samples per cow per period.) thus representing every 3 h of a 24 h period to account for diurnal variation. Also, at these collection times, reticular fluid (400 ml) was collected near the reticulo-omasal orifice to determine the ratio of microbial nitrogen to microbial purines, OM, and starch. Additional ruminal fluid (50 ml) samples were collected from 5 sites in the rumen for determination of pH and concentrations of VFA, lactate, and ammonia.

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Day 18: Preparation Day

On d 18, cows were prepared for intensive sample and behavior data collection. Cows were fitted with chewing halters (Dado and Allen, 1993a) for acclimation before measurement. Catheters (45 cm long, MRE 095 Renathane® tubing, Braintree Scientific, Inc., Braintree, MA) were installed in a jugular vein using sterile technique.

Day 19: Intensive 24 h Collection Of Blood And Ruminal Fluid

On d 19, a intensive 24 h collection of plasma and rumen fluid was coupled with feeding behavior data collection. Two whole blood samples and two ruminal fluid samples were collected every 20 min for 24 h by automated sample collection system (Allen et al., 2000b, 4.2% sodium citrate solution replaced saline containing heparin as anticoagulant), starting at 0930 h. Blood was sampled from a jugular vein through a catheter inserted 1 d prior to sample collection. Feeding behavior and ruminal pH was also monitored by a computerized data acquisition system (Dado and Allen, 1993a). This system successfully collected 99.5% and 95.8% of the total samples (2,592 each) for blood and ruminal fluid, respectively.

Ruminal fluid was centrifuged at 2,000 x g for 15 min immediately after collection, and supernatants were frozen at -20° C until analysis. Whole blood, collected in a tube containing lithium heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), was analyzed immediately for pH, pCO_2, hematocrit, pO_2, ionized calcium, sodium, potassium, and chloride by a blood gas analyzer (Stat Profile 4, Nova Biomedical, Waltham, MA) and ten other blood variables were calculated by manufacturer's equations. Whole blood was also collected in a tube with potassium oxalate and sodium fluoride as a glycolytic inhibitor (Becton Dickinson Vacutainer

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Systems, Franklin Lakes, NJ). Both whole blood samples were centrifuged at 2,000 x g for 15 min immediately after sample collection, and plasma was harvested and frozen at -20° C until analysis.

Days 20, 21, 22, 23, 24: Feeding Behavior Monitoring

Feeding behavior and ruminal pH were monitored from d 19 through d 24 (144 h) of each period by a computerized data acquisition system (Dado and Allen, 1993a). Data of chewing activities, feed disappearance, water consumption, and ruminal pH were recorded to computer file for each cow every 5 sec. Chewing activity for 24-h periods from feeding to feeding were deleted when chewing halters were out of adjustment or malfunctioning. Electrodes for ruminal pH determination were checked daily at pH 7 and pH 4 and calibrated as needed, and ruminal pH data were deleted for the entire day if pH deviated more than 0.1 unit at either pH 7 and pH 4. All pH data retained for data analysis had a deviation of less than 0.10 for both pH 7 and pH 4 and at least one with a deviation less than 0.05. The daily pH electrode check occurred for <1.5 h during the last 2 h of the d. The 1.5 h associated with the check was removed from each cow day and, therefore, daily pH data are representative of 22.5 h out the 24 h day. The system successfully collected 82.2% of the total chewing activity data and 81.1% of the total ruminal pH data (Appendix Tables A.10 and A.11, respectively).

Chewing activities were summarized as meal bouts, interval between meals, and meal size for eating behavior and as ruminating bouts and inter-ruminating interval for ruminating behavior. Ruminal pH data were summarized to daily mean, variance, median, minimum, maximum, range, the hours and area for which ruminal pH is below 6.0, 5.8, and 5.5. The minimum, maximum, and range were calculated using the daily

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2.5th and 97.5th percentiles for ruminal pH measured every 5 seconds. The area were calculated by determining the time below a specific ruminal pH (6.0, 5.8, or 5.5) and weighted that time by the deviation from the threshold.

Days 25, 26: Ruminal Valerate Absorption And Liquid Passage Determination

Rate of valerate absorption and rate of liquid passage was determined using a pulse dose of valeric acid and cobalt EDTA, respectively, 2 h after feeding on d 25 (Allen et al, 2000a). Ruminal fluid was sampled at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 12, 16, 20, 24, and 36 h after dosing, frozen and subsequently analyzed for valerate and cobalt. Rate of valerate absorption was used as a proxy for total VFA absorption rate.

Days 27, 28: Rumen Evacuations For Pool Size Determination

Ruminal contents were evacuated manually through the ruminal cannula at 1500 h (3.5 h after feeding) on d 27 and at 0830 h (3.0 h before feeding the following day) on d 28 of each period. Total ruminal content mass and volume were determined. During evacuation, a 10% aliquot of digesta was separated to allow accurate sub-sampling. The aliquot was squeezed through nylon mesh (1 mm) to separate it into primarily solid and liquid phases. Samples were taken from both phases for determination of pool size of digesta components in the rumen. Samples were immediately frozen at –20°C

Sample Processing

Daily samples of dietary ingredients and orts were thawed and composited by cow into experimental subperiods. Composites and individual fecal samples were dried in a

55° C forced-air oven for 72 h and DM concentration was determined. Forages samples were ground with a Wiley mill (1 mm screen; Authur H. Thomas, Philadelphia, PA). High moisture shelled corn and all premixes were ground with a UDY Cyclone Sample Mill (2 mm screen; Fort Collins, CO). After drying and an initial 6 mm grind, individual fecal samples were composited within cow and period on an equal 100°C DM basis. Microbial pellets were obtained by differential centrifugation of reticular fluid samples collected during the digestibility determination. The fluid was centrifuged at 500 x g for 15 minutes at 4°C was to remove feed particles. The supernate was centrifuged at 18,000 x g for 15 minutes at 4°C to form the microbial pellet. The supernant was discarded and the microbial pellet was resuspended with minimal 0.9% sodium chloride solution and frozen –20°C

Duodenal samples were thawed and composited by cow period. Composites were sieved through 1 mm mesh screen for an approximate liquid and solid separation. The fractions were mixed thoroughly, subsampled and refrozen in aluminum trays in preparation for lyphilization.

Microbial pellets, duodenal digesta liquid and solid subsamples, and ruminal solids and liquid samples from ruminal evacuation were lyphilized (Tri-Philizer ™ MP, FTS Systems, Stone Ridge, NY) and DM concentration was determined. Microbial pellets were ground with a mortis and pestle to eliminate clumping prior to sample analysis. Duodenal digesta solids were passed through screens of 4.75 mm and 1.18 mm by hand to remove stones and dry matter weights were corrected for the weight of the stones removed. After freeze-drying and grinding with a Wiley mill (6 mm screen; Authur H. Thomas, Philadelphia, PA), duodenal and ruminal liquids and solids were

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Sample Analysis

Samples were analyzed for DM, ash, CP, starch, NDF, and indigestible NDF (iNDF). Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Crude protein was analyzed according to Hach et al. (1987). Starch corrected for free glucose was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was measured using a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 240-h in vitro fermentation (Goering and Van Soest, 1970, reinoculation at 120 h). Ruminal fluid for the in vitro incubations was collected from a non-pregnant dry cow fed alfalfa hay only. Fraction of potentially digestible NDF (pdNDF) was calculated by difference (1.00 minus iNDF). Concentrations of all nutrients except for DM are expressed as percentages of DM determined by drying at 105° C in a forced-air oven for more than 8 h.

Ruminal fluid was analyzed for concentrations of major VFA and lactate. Samples were centrifuged at 26,000 × g for 15 min, and supernatant (600 μL) was mixed with 600 μL $Ca(OH)_2$ and 300 μL of $CuSO_4$ containing crotonic acid as an internal marker in 1.7 ml micro centrifuge tubes. Samples were centrifuged at 12,000 × g for 10 min, and

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supernatant (1000 μl) was taken and mixed with 28 μl of H_2SO_4 in 1.5 ml micro centrifuge tubes. Samples were frozen and thawed twice, and centrifuged at 12,000 × g for 10 min to precipitate and remove protein thoroughly. Supernatant was transferred to HPLC vials. Concentrations of VFA and lactate of the supernatant were determined by HPLC (Waters Corp., Milford, MA) according to (Oba and Allen 1999a). Cobalt concentration in ruminal fluid was determined by atomic absorption according to manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA).

Feed sodium and potassium concentration were determined by digestion according to Hach et al. (1987) and measurement of the element in supernate by atomic absorption according to manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA). Feed chloride concentration was determined by extracting the feed with 1.0% nitric acid solution for one hour on shaker (Orbimix 1010, Brinkman Instruments, Westbury, NY) and measuring supernate chloride by coulometric titration (Digital Chloridometer, Model 442-5000, Labconco Corporation, Kansas City, MO). Dried and ground samples of the base mix, rice hulls, and dried, ground corn were composited across periods and sent to Dairy One Forage Laboratory (Ithaca, NY) for sulfur analysis according to manufacturer's recommendation (LECO Application Note 203-601-229, 08/92, LECO Model SC-432, St. Joseph, MI).

Microbial pellets and duodenal digesta were analyzed for ash, OM, N, and starch as previously described and were also analyzed for purines. Total purines was measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and

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Owens, 1986). Ammonia concentrations were determined on supernate of centrifuged duodenal and ruminal fluid samples (Broderick and Kang, 1980).

Plasma samples were analyzed for concentrations of acetate, glucose, NEFA, insulin, and glucagon. Plasma was processed as described for ruminal fluid to quantify acetate concentration. Due to greater protein concentration for plasma samples, the first stage of sample processing was duplicated to obtain enough supernatant (1000 μl) to be mixed with 28 μl of H_2SO_4 in 1.5 ml micro centrifuge tubes. Commercial kits were used to determine plasma concentration of glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA C-kit; Wako Chemicals USA, Richmond, VA), insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA), and glucagon (Double Antibody, Diagnostic Products Corporation, Los Angeles, CA).

Most of the intensive 24 h blood and ruminal fluid collection was not analyzed due to nonsignificant differences and lack of funding. A subset of 6 to 8 samples per cow period (n = 72) was analyzed to determine plasma and ruminal fluid means.

Calculations

Dry matter intake and nutrient intake was calculated by subtracting the amount refused from the amount offered. The intake calculations assume that the diet was combined exactly as prescribed on the mix sheet. Orts were not analyzed for Cl, Na, and K and therefore, intake calculations assume that concentrations of Cl, Na, and K in the orts were equal to the concentrations in the DM offered. Change in empty body weight, body condition score, ruminal content weight, and ruminal content volume were calculated by subtracting the beginning of period value from end of period value. Milk

yield at both milkings was measured and summed for a daily total. Daily totals were averaged across the test phase of each period. Yield of SCM was calculated as per Tyrrell and Reid (1965) and yield of FCM was calculated as per NRC (2001). SCS was calculated by taking the log (base 2) of SCC.

Purine to nitrogen ratio for microbes collected in fluid near the reticulo-omasal orifice was used to calculate duodenal flux of microbial nitrogen while the ratio for microbes in rumen contents were use to calculate rumen pool size of microbial nitrogen because of potential differences in these microbial populations.

Duodenal flux was calculated for DM, OM, iNDF, pdNDF, starch, microbial N, non-ammonia non-microbial N (NANMN), and ammonia N using 240 h iNDF as flow marker. Duodenal flow of microbial OM was determined using the ratio of purines to OM (Oba and Allen 2003c), and true ruminally degraded OM (TRDOM) was calculated by subtracting duodenal flow of non-microbial OM from OM intake. Ruminal pool sizes (kg) of OM, NDF, iNDF, pdNDF, starch, microbial N, and NANMN was determined by multiplying the concentration of each component in rumen samples by the ruminal digesta DM mass (kg). Ruminal digestibility was determined for each fraction.

Rates of valerate and cobalt disappearance were determined by non-linear regression of the decline in their respective concentration in ruminal fluid over time after dosing, accounting for background (Allen et al., 2000b)

Dietary cation anion difference (DCAD) as mEq/100g DM was calculated two ways: sodium plus potassium minus chloride (DCAD3) and sodium plus potassium minus chloride plus sulfur (DCAD4). Total diet concentrations for cations and anions were

calculated from individual ingredient analyses and dietary proportions of the dry matter. (Appendix A.6 for equations.)

Sodium contribution from drinking water was not incorporated into either DCAD calculation. However, the sodium concentration in the water from a common well was reported as 9 ppm by Michigan State University (2003). This concentration would deliver only 0.9 g of sodium to a cow drinking 100 L/d which is less than 2% of the sodium consumed in the control diet.

Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) was calculated by the following equations:

Turnover rate in the rumen (%/h) =

(intake of component / ruminal pool of component) / 24 x 100

Particulate passage rate from the rumen (%/h) =

(duodenal flow of component / ruminal pool of component) / 24 x 100

Digestion rate in the rumen (%/h) =

turnover rate in the rumen (%/h) – passage rate from the rumen (%/h)

Turnover time in the rumen (h) was calculated as 1/(turnover rate in rumen(%/h)/100)

Indigestible NDF passage rate from the rumen was calculated as

iNDF passage rate from the rumen (%/h) =

(intake of iNDF / ruminal pool of component) / 24 x 100 (Dado and Allen 1995)

Energy values were calculated as follows:

NE_L of intake, Mcal/d = DMI, kg x (0.0245 x TDN%) (NRC, 1989)

NE_L of milk, Mcal/d = Milk yield, kg x ((0.0929 x fat%) + (0.0563 x

true protein%) + (0.0395 x lactose%)) (NRC, 2001)

NE_L for maintenance, Mcal/d = 0.080 x $BW^{0.75}$ (NRC, 2001)

NE_L balance, Mcal/d = NE_L of intake - NE_L for maintenance - NE_L for milk

Statistical Analysis

All data were analyzed using the fit model procedure of JMP® (Version 5.0.1.2; 2003) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

C_i = fixed effect of cow (i = 1 to 6),

P_j = fixed effect of period (j = 1 to 3),

T_k = fixed effect of treatment (k = 1 to 3),

e_{ijk} = residual, assumed to be normally distributed.

Period x treatment interaction was originally evaluated, but it was removed from the statistical model because interaction was not significant for response variables of primary interest. Cow period means for feeding behavior variables were weighted by the number of cow days included. Contrasts were performed for the control diet vs. both sodium treatments and sodium chloride vs. sodium bicarbonate. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects were declared at $P < 0.10$. A mixed model with cow as a random variable was not used because, for some variables, the estimate of parameter did not converge during iteration. Residual plots were checked for appearance of normality

and appeared normally distributed. One cow had clinical mastitis during period one on days 25, 26, and 27. All data from these days were removed from the final data sets.

RESULTS

This experiment had to two key elements to its design: the equimolar addition of cations and anions and the commonality of greater than 98% of the dietary DM. These, with the uniform DMI, allowed a focus on the separation of chemical and osmotic effects. The postexperiment differences in the CP and NDF concentrations of the diet ingredients only fostered the experimental challenge. This experiment is among the highest production and highest DMI of all sodium bicarbonate experiments reviewed by Staples and Lough (1989).

Postexperiment analysis (Table 3.4) showed experimental diets had less crude protein (1.2% of DM) and NDF from forage (0.9% of DM) than the diet was formulated for. Postexperiment analysis also showed the control diet was adequate for sodium, potassium and chloride (NRC, 2001; assuming an experimental cow of 620 kg BW producing 36 kg of milk and consuming 25 kg DM). Sodium and chloride intakes were according to experimental design. Calculated DCAD4 was 10 mEq/100g DM for sodium and control diets which was outside the optimum range of 20 to 50 mEq/100g DM proposed by Sanchez and Beede (2005).

Intakes were similar across experimental diets for each subperiod (Tables 3.5, 3.6, and 3.7). DMI averaged 24.5 kg/d for the experiment and was not affected by treatment ($P > 0.35$). Also, treatment did not affect intakes for OM, NDF, starch, crude protein, and forage. The intakes of sodium, chloride, and sometimes iNDF were reflective of the differences among treatment mixes.

Sodium treatments did not affect milk yield or composition (Table 3.8) with the exception of MUN. Sodium treatments tended to decrease MUN and chloride treatment

tended to decrease MUN when compared to bicarbonate treatment. However, with MUN <19 mg/ml, all treatment averages are considered acceptable for lactating dairy cows (NRC, 2001).

Sodium treatments did not affect change in empty BW and BCS ($P > 0.13$) and all experimental treatments showed gains in empty BW (14.1 kg) and condition (0.14; Table 3.9). However, when BW change was measured with the ruminal contents included, sodium treatments caused a BW gain (28.4 kg) while the control diet showed a BW loss (10.0 kg). Efficiency of milk production (1.48 kg of 3.5% FCM/ kg of DMI, 1.37 kg of 4.0% FCM/ kg of DMI, and 1.48 kg of SCM/ kg of DMI) was similar across experimental diets.

When iNDF was used as the digestibility and passage marker, sodium treatments increased total tract apparent digestibility for DM (2.4%) and OM (2.2%; Table 3.10). The difference in DM and OM digestibility is due to an increase in total tract pdNDF digestibility total tract (0.2 kg and 4.4%) as starch digestibilities were not affected by treatment ($P > 0.43$; Tables 3.11 and 3.12). And, more specifically, sodium treatments increased pdNDF digested total tract (7.8%; Table 3.12). Ruminal starch (21.5%/h) and pdNDF (2.2%/h) digestion rates were similar across all treatments but sodium treatments tended to decrease iNDF passage rate from the RR (0.4%/h; Table 3.13). With N fractions, sodium treatments tended to increase ammonia passage to duodenum and increase N digested (%) total tract (Table 3.14). (For the results of chromic sesquioxide (Cr_2O_3) as a marker to estimate nutrient digestibility in the rumen and in the total tract, see Appendix A.14 and Appendix Tables A.17, A.18, A.19, A.20, A.21, and A.22.)

Within the RR, turnover times (h) were similar across experimental diets for OM (11.2 h), starch (2.6 h), NDF (25.8 h), and pdNDF (22.3 h), however, even with less consumed, sodium treatments tended to increase ruminal iNDF turnover time (31.9 h vs. 27.7, $P = 0.09$; Table 3.15). Fractional rates for ruminal liquid passage and ruminal valerate absorption were similar for all experimental diets (0.155/h and 0.352/h, respectively; Table 3.13).

Ruminal pools of water, DM, OM, starch and NDF on the sodium treatments were numerically greater than ruminal pool of the control diet, however, ruminal pools across experimental diets were not statistically different ($P > 0.23$; Table 3.16). When change in ruminal contents across the experimental periods was considered, sodium treatments increased ruminal weight (+7.6 kg vs. –7.2 kg) and tended to increase ruminal volume (+8.8 L vs. –9.4 L) when compared with the control diet (Table 3.16). Taken together, these changes suggest sodium treatments expanded the ruminal contents. Bicarbonate treatment tended to increase mean ruminal volume (+9.5 L) and lowered ruminal content density (0.81 vs. 0.87 kg/L) when compared with chloride treatment (Table 3.16). Given the similar ruminal wet weight (82.2 kg), dry matter (12.2 kg), water (70.0 kg), and DM to water ratio (0.174) of the bicarbonate and chloride treatments, the differences in ruminal volume and, hence, density were caused by gas trapped in the ruminal mass from the decomposition of bicarbonate to carbon dioxide and water (Table 3.16).

During the digestibility determination (d15 – d17), ruminal VFA and associated parameters were measured (Table 3.17, n=144). Bicarbonate treatment had higher total VFA than chloride treatment (147.5 mM vs. 140.3 mM). This difference was accounted for largely by the greater ruminal propionate concentration for the bicarbonate treatment.

This difference, in turn, leads to a lower ruminal A:P for bicarbonate treatment compared to chloride (2.48 vs. 2.69, respectively). Ruminal pH measured at these collection times showed small but significant differences among the three experimental diets ($P < 0.05$). Chloride treatment had the highest mean ruminal pH at 6.00 and the control diet had the lowest at 5.94. Ruminal VFA and associated parameters were also measured with a subset from the intensive 24 h collection (Table 3.18, n=108). During this sampling, individual VFA, total VFA, ruminal acetate:propionate ratio, ruminal pH were not different among the experimental diets.

VFA profiles were determined on two sets of samples; those collected by grab sample and squeezing the liquid through a nylon mesh (Table 3.17) and those collected by computer-controlled pump drawing ruminal fluid through a fine mesh and into a collection tube (Table 3.18). The grab samples (Tables 3.17) contained greater than 50% more total VFA and had a lower mean ruminal pH than those collected by pump (Table 3.18). These differences are reflective of the difference between these two collection methods. With the squeezing and straining, more VFA is expressed from the particulate matter yielding higher VFA concentration and lower sample pH (Erdman, 1988a). Thus, the latter (Table 3.18) is probably more representative of the ruminal solution. Difference in sampling location may also be a factor.

Net energy for lactation (NE_L) associated with intake, maintenance, and milk yield was calculated (Table 3.19). Net energy measures were not different across experimental diets.

Sodium treatments did not affect eating or ruminating chewing activity (Table 3.20). Mean DMI kg/d was slightly less (0.3 kg) during this behavior subperiod (Table

3.20) than during digestibility trial subperiod (Table 3.6). This slight difference is probably an artifact due to the difference in calculations to produce these means with wetting of the orts being of primary concern (Table 3.20 is sum of as fed feed disappearance times percentage of TMR DM percentage vs. Table 3.6 is offered DM kg minus refused DM kg). It is also possible that the DMI was higher in the digestibility subperiod than the behavior subperiod because of compensation for removed digesta.

Sodium treatments affected water consumption (Table 3.21) by increasing daily water intake (5.1 L/d or 5.2%/d). Chloride tended to increase water consumed during rumination when compared to bicarbonate (5.1 L vs. 2.3 L).With bicarbonate treatment, cows tended to drink fewer times per d (1.6 bouts/d) but tended to consumed more water per bout (0.7 L) than with chloride treatment so daily total water consumed was similar (103.8 L). Across all experimental diets, more than half of average total daily water consumption was consumed while eating.

Sodium treatments did not affect ruminal pH (Table 3.22). Sodium treatments did not affect ($P > 0.21$) ruminal pH measured as mean (6.20), SD (0.24), median (6.20), minimum (5.78), maximum (6.62), or range (0.84). Also, treatments did not affect time and area of curve under pH 6.0.

Whole jugular blood was analyzed for gas and electrolyte concentrations (Tables 3.23). Treatments did not affect the measured variables of blood pH, partial pressure of oxygen, hematocrit, sodium concentration, or calcium concentration. Sodium treatments tended to increase calculated variables based on the partial pressure of carbon dioxide and blood pH: total carbon dioxide, base excess (ECF and blood), calculated bicarbonate, and standardized bicarbonate. Bicarbonate treatment shows higher whole

blood partial pressure of carbon dioxide and lower chloride when compared chloride treatment. These effects are likely probably related to treatment. Bicarbonate treatment also tended to decrease oxygen saturation, increase carbon dioxide content, and decrease potassium concentration when compared with chloride treatment. Compared to values reported in the literature, means were within normal ranges of measurement for bovine for venous pH, carbon dioxide measurements, bicarbonate, sodium, potassium, chloride, and anion gap. However, mean hematocrit was less than some reports and average calcium concentrations were less than half of expected values (Table 1.13a and 1.13b).

Plasma metabolites and hormones were measured (Table 3.24, n=108). Sodium treatments had no effect on plasma concentrations ($P > 0.16$) of glucose (56.4 mg/dl), NEFA (55.7 μEq/L), BHBA (6.0 mg/dl), or glucagon (109.0 pg/ml) but tended to increase plasma insulin (9.4 vs. 7.8 μIU/ml) suggesting a postabsorptive effect. No differences were seen between bicarbonate and chloride treatments.

In summary, sodium treatments increased NDF digestibility, expanded the ruminal content weight and volume and slowed passage from the RR. With sodium treatments, water consumption was increased but no effects were measured for starch digestibility (extent or site of digestion), ruminal microbial N production, or ruminal liquid turnover. Valerate absorption was not affected by treatments and no strong ruminal pH effects are measured. Intake was not different across experimental diets as was net energy intake and expenditure, milk yield and composition, and chewing activity. Sodium treatments caused some postabsorptive differences in blood gas, and electrolytes.

DISCUSSION

The beneficial effects of sodium bicarbonate feeding are well documented (Erdman, 1988a; Staples and Lough, 1989) but, the actions of sodium bicarbonate are probably more complex than the simple elevation of ruminal pH. Several hypotheses regarding the mechanism of sodium bicarbonate action have been proposed but verification with diets applicable to current lactating dairy cows have not been reported.

Two hypotheses of mechanism of sodium bicarbonate action in lactating dairy cows can be generalized as an increased efficiency of dietary DM use. Sodium bicarbonate inclusion in the diets of lactating dairy cows generally increases milk fat percentage and possibly FCM yield without changing DMI (Erdman, 1988a; Staples and Lough, 1989).

The first hypothesis is the addition of sodium bicarbonate directly increases ruminal pH which increases ruminal fiber digestibility. Lower ruminal pH can depress fiber digestibility in vitro (Hoover, 1986; Grant and Mertens, 1992). The addition of sodium bicarbonate often leads to increases in ruminal pH and ruminal A:P (Erdman, 1988a; Staples and Lough, 1989) and is associated with small increases in apparent digestibility of the DM (Erdman, 1988a) or, more specifically, an increase in ADF digestibility-usually in the RR (Staples and Lough, 1989). In this experiment, no effects on ruminal pH were detected. The mean ruminal pH of 6.2, which is near the pK_a of carbonic acid (Turner and Hodgetts, 1955a), suggests that the ruminal solutions were well buffered across all experimental diets. Yet without ruminal pH differences, differences in NDF digestibility were detected in this experiment.

Another hypothesis of the mechanism that sodium bicarbonate acts osmotically to increase liquid turnover in the RR (Russell and Chow, 1993). The addition of the sodium to the diet increases water intake which could lead to an increased flow of liquid from the RR and, with it, increased the flow of other components leaving the rumen such as particulate matter, rumen microbes and VFA. An increase in particulate matter escape should increase the flow of starch to the small intestine thus reducing starch fermented to VFA and increase starch digested and absorbed as glucose from the small intestine. Less starch fermentation in the RR might lead to the higher ruminal pH observed in some experiments. In this experiment, sodium treatments increased water consumption which would be expected given the increased sodium consumption (NRC, 2001). However, ruminal liquid passage and ruminal starch digestibility were not increased by treatment. Changes were not expected as these effects are usually associated with higher diet inclusion rates of sodium bicarbonate than used in this experiment (Rogers et al., 1982; Rogers and Davis, 1982b).

Given the results of the experiment, the mechanism of sodium bicarbonate action at recommended rates in the diets of lactating dairy cows appears to be, in part, a hybrid of the preceding ideas. Sodium bicarbonate has an osmotic effect within the RR and this effect may increase fiber digestibility. In the RR, sodium bicarbonate provides a sodium cation and bicarbonate anion and both of these ions are osmotically active. In solution, the sodium is not completely disassociated from the bicarbonate. At ruminal concentrations, this disassociation is probably 80 to 90% yielding an osmotic index of 1.8 to 1.9 times the sodium bicarbonate molar concentration (Weast, 1978). In the RR solution, sodium is strong ion and, chemically, an alkalizer (Stewart, 1983). Bicarbonate

in the solution is a weak acid anion and, depending on ruminal pH, an alkalizer or a buffer. The bicarbonate ion can combine with a proton and decompose to form water and carbon dioxide (Segel, 1976).

As the primary extracellular cation, sodium determines ECF volume in the body (Carlson, 1997). The amount of sodium is regulated and this cation is balanced with anions and these ions drawn water as water follows solute (Houpt, 2004). Similarly, across the RR wall, ion transport is regulated (Gaebel and Sehested, 1997; Sehested et al., 1999b) and water movement is restricted (Engelhart, 1970). The concentration of sodium plus potassium also seems to be maintained in the RR solution (Lang and Martens, 1999).

With the active regulation of the RR solution, a tonic addition of osmotically active particles with each meal could, over time, lead to expansion of ruminal solution (weight and volume) within the RR. This expansion, given a constant DMI, could lead to slowing of passage and turnover from the RR, possibly allowing fiber more time for microbial digestion.

For the sodium bicarbonate treatment in this experiment, the mean meal size was 2.8 kg which contained 28g of sodium bicarbonate. At 1.8 mOsm/mole, the sodium bicarbonate in the meal would contribute 600 mOsm to the RR. For the 70 kg of water in the RR, 56 kg is estimated to be in the ruminal solution assuming 10% of the water is within both the feed and microbes (for a total of 20% of water confined). Taking these assumptions and assuming instantaneous consumption and mixing, the sodium bicarbonate in the meal would be expected to raise ruminal osmolality by 11 mOsm. In this experiment, the increase would occur, on average, every 2.5 h. Under real

conditions, the increase would be smaller with passage, absorption, and the loss of bicarbonate ion in carbon dioxide and water.

In this experiment, sodium treatments increased BW but not empty BW. The difference is ruminal contents which, taken on their own, increased in weight over the experimental period with sodium treatments compared to control. Also, total tract pdNDF digested increased with sodium treatments. The link between these two responses is the change in ruminal dynamics. The slowing of ruminal fiber passage associated with sodium treatments may have lead to the more fiber digested in the RR and thus the total tract. However, amounts digested in the RR were numerically but not statistically different. Also, numeric but nonsignificant differences in ruminal pool sizes do not negate expansion. Although, not statistically significant, the increase in net energy lactation gained by increased pdNDF digestion is coincidentally equal to the increase in net energy expended in milk produced.

This proposal could explain why midlaction are more responsive than early lactation cows to the inclusion of sodium bicarbonate in their diets. Midlactation cows with sodium bicarbonate in their diets gain more milk fat and fat-corrected milk than early lactation cows compared to similar cows on control diets (Staples and Lough, 1989; Tucker et al., 1994). Midlactation cows are less likely to be limited by fill than early lactation cows and, therefore are more likely to expand RR contents with sodium bicarbonate addition.

The mechanism of sodium bicarbonate action in lactating dairy cows is likely partly osmotic. Feeding sodium bicarbonate increases weight of ruminal contents, decreases iNDF passage from the RR, and increases total tract digestibility of pdNDF. In

this experiment however, the lack of milk fat response suggests that only part of the mechanism of sodium bicarbonate action was apparent.

As a 3 x 3 Latin square, this experiment had lower statistical power to separate treatment means than some of other experiments in our lab. This reduction in power increases the likelihood of Type II error (i.e. failure to reject H_o).

Sodium treatments caused some postabsorptive differences in insulin, blood gas, and electrolytes. The tendency for increased insulin suggests greater propionate absorption (Oba and Allen, 2003b). The blood gas and electrolyte change are probably due to slight acid-base adjustments due to increased sodium and chloride flux through the cows.

The results of this experiment can be compared to previous work in our lab using similar methods and materials and cows with similar genetics. Compared to the average cows in several previous experiments, the cows on this experiment ate more, produced less, and weighed more (Table 3.25). These cows showed an increased starch digestion and passage and a decreased pdNDF digestion and passage compared with previous work.

The results of this experiment can be compared to a previous experiment (Chapter 2) with the same treatment (Table 3.26). Rumination and total chewing time per d was not affected in this experiment as it was previously, possibly due to this experimental design and its decreased power to separate means. This experiment can explain why the body weight change was disproportionally greater than the body condition score change.

CONCLUSION

Sodium bicarbonate inclusion increased total tract digestibility of pdNDF likely as a result of osmotic changes in the RR expanding the ruminal contents and decreasing passage rate. This osmotic effect is likely only part of the mechanism of sodium bicarbonate action in lactating dairy cows.

Table 3.1. Nutrient composition of ingredients used to formulate experimental diets (% of dietary DM).

	Ingredients							
Nutrient	**CS[1]**	**AS[2]**	**HMC[3]**	**SMP[4]**	**SMV[5]**	**SBM[6]**	**SCM[7]**	**CntlM[8]**
DM, 55°C	31.7	35.1	74.4	89.4	92.2	91.5	91.0	90.0
DM, 100°C	29.2	32.4	68.5	84.5	87.3	86.0	85.4	84.1
OM	96.2	89.6	98.4	93.5	63.2	81.2	77.2	88.0
Ash	3.8	10.4	1.6	6.5	36.8	18.8	22.8	12.0
CP	7.1	15.5	8.8	49.0	5.5	5.2	4.8	5.0
NDF	38.4	46.7	7.9	11.2	5.8	24.7	29.1	38.9
pdNDF	25.7	21.1	5.9	8.6	4.0	4.3	4.5	4.6
iNDF	12.7	25.6	2.0	2.6	1.8	20.4	24.6	34.3
pdNDF, % of NDF	66.9	45.1	74.4	77.1	69.5	17.4	15.5	11.7
iNDF, % of NDF	33.1	54.9	25.6	22.9	30.5	82.6	84.5	88.3
Starch	28.7	2.4	70.4	5.1	42.4	35.0	34.6	34.5
Free Glucose	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Ether extract	3.6	3.8	3.8	3.4	1.5	1.7	1.5	1.5
Na^+	0.08	0.06	0.03	0.09	3.33	5.32	5.71	0.10
K^+	0.63	1.94	0.33	1.22	0.25	0.28	0.28	0.36
Cl^-	0.25	0.45	0.06	0.06	4.93	0.15	8.37	0.11

[1] CS: Corn silage.
[2] AS: Alfalfa silage.
[3] HMC: High moisture corn.
[4] SMP: Protein Mix contained 75.1% soybean meal, 20.0% distillers grain, and 4.8% blood meal.
[5] SMV: Mineral and vitamin mix contained 64.8% dry ground corn, 13.6% limestone, 10.5% dicalcium phosphate, 8.1% trace mineral salt, 1.8% trace mineral premix, 0.4% magnesium oxide, 0.4% vitamin A, 0.3% vitamin D, and 0.1% vitamin E.
[6] SBM: Sodium bicarbonate treatment mix.
[7] SCM: Sodium chloride treatment mix.
[8] Cntl: Control treatment mix.

Table 3.2. Fermentation products of wet feeds (% of dietary DM).

Product	Corn Silage	Alfalfa Silage
Lactate	5.71	6.27
Total VFA	1.60	2.34
Acetate	1.57	2.28
Propionate	0.01	0.01
Butyrate	0.02	0.02
Ethanol	1.65	0.39

Table 3.3. Ingredient composition of experimental diets (% of dietary DM).

	Experimental Diets		
Ingredients	**SBT**[1]	**SCT**[2]	**Cntl**[3]
Corn silage	31.3	31.3	31.3
Alfalfa silage	15.6	15.6	15.6
High moisture corn	21.3	21.3	21.3
Protein mix[4]	21.8	21.8	21.8
Mineral and vitamin mix[5]	5.0	5.0	5.0
Treatment mix	5.0	5.0	5.0
Dried, ground corn	2.5	2.5	2.5
Ground rice hulls	1.5	1.8	2.5
NaCl	-	0.7	-
$NaHCO_3$	1.0	-	-

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] Protein Mix contained 75.1% soybean meal, 20.0% distillers grain, and 4.8% blood meal.
[5] Mineral and vitamin mix contained 64.8% dry ground corn, 13.6% limestone, 10.5% dicalcium phosphate, 8.1% trace mineral salt, 1.8% trace mineral premix, 0.4% magnesium oxide, 0.4% vitamin A, 0.3% vitamin D, and 0.1% vitamin E.

Table 3.4. Composition of experimental diets (% of dietary DM).

	Experimental Diets		
	SBT[1]	SCT[2]	Cntl[3]
DM, 55°C	48.1	48.1	48.1
DM, 1005°C	44.8	44.7	44.7
OM	92.6	92.4	92.9
Ash	7.4	7.6	7.1
Starch	29.2	29.2	29.2
NDF	24.8	25.0	25.5
pdNDF as a % of NDF	58.7	58.3	57.2
iNDF as a % of NDF	41.3	41.7	42.8
pdNDF	14.5	14.6	14.6
iNDF	10.2	10.4	10.9
Forage NDF	19.0	19.1	19.1
CP	17.8	17.8	17.8
Ether extract	3.5	3.4	3.4
Na^+	0.51	0.52	0.24
K^+	0.83	0.83	0.83
Cl^-	0.44	0.86	0.43
S^{-2}	0.15	0.15	0.15
Forage	46.9	46.9	46.9
DCAD3, mEq/100g DM	31.0	19.8	19.3
DCAD4, mEq/100g DM	21.4	10.1	9.7

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.

Table 3.5. The effect of dietary strong ion treatment on diet component intake during digestibility subperiod.

	Experimental Diets					Significance, *P*	
Intake, kg	**SBT[1]**	**SCT[2]**	**Cntl[3]**	**SE**	**n**	**TMT[4]**	**CvB[5]**
DMI	24.9	24.8	25.2	0.6	18	0.61	0.94
OMI	21.5	21.4	21.8	0.5	18	0.51	0.91
NDF intake	5.7	5.6	5.9	0.1	18	0.19	0.85
pdNDF intake	3.3	3.3	3.3	0.1	18	0.60	0.86
iNDF intake	2.4	2.4	2.6	0.1	18	0.05	0.85
Estimated[6] forage NDF intake	4.4	4.4	4.5	0.1	18	0.63	0.92
Starch intake	6.8	6.9	6.9	0.2	18	0.69	0.79
CP intake	4.2	4.2	4.3	0.1	18	0.54	0.89
Estimated[6] treatment intake	1.3	1.2	1.2	0.0	18	0.88	0.73
Estimated[6] sodium intake	0.11	0.11	0.05	0.00	18	<0.001	0.39
Estimated[6] potassium intake	0.18	0.18	0.18	0.00	18	0.76	0.54
Estimated[6] chloride intake	0.10	0.19	0.10	0.00	18	<0.001	<0.001

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] "Estimated" is based on offered diet concentrations only.

Table 3.6. The effect of dietary strong ion treatment on diet component intake during feeding behavior subperiod.

	Experimental Diets					Significance, *P*	
Intake, kg	**SBT[1]**	**SCT[2]**	**Cntl[3]**	**SE**	**n**	**TMT[4]**	**CvB[5]**
DMI	24.6	24.2	23.7	0.6	18	0.35	0.68
OMI	21.2	20.8	20.4	0.5	18	0.44	0.64
NDF intake	5.5	5.4	5.4	0.1	18	0.65	0.69
pdNDF intake	3.1	3.1	3.0	0.1	18	0.28	0.54
iNDF intake	2.4	2.4	2.4	0.1	18	0.60	0.95
Estimated[6] forage NDF intake	4.3	4.2	4.1	0.1	18	0.31	0.63
Starch intake	6.8	6.7	6.6	0.2	18	0.42	0.80
CP intake	4.1	4.0	4.0	0.1	18	0.47	0.74
Estimated[6] treatment intake	1.3	1.2	1.2	0.0	18	0.15	0.55
Estimated[6] sodium intake	0.12	0.12	0.05	0.00	18	<0.001	0.97
Estimated[6] potassium intake	0.19	0.19	0.18	0.01	18	0.41	0.65
Estimated[6] chloride intake	0.10	0.20	0.10	0.00	18	<0.001	<0.001

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] "Estimated" is based on offered diet concentrations only.

Table 3.7. The effect of dietary strong ion treatment on diet component intake during the entire test period.

	Experimental Diets					Significance, *P*	
Intake, kg	**SBT[1]**	**SCT[2]**	**Cntl[3]**	**SE**	**n**	**TMT[4]**	**CvB[5]**
DMI	24.6	24.5	24.1	0.5	18	0.49	0.90
OMI	21.1	21.0	20.8	0.4	18	0.61	0.85
NDF intake	5.5	5.5	5.5	0.1	18	0.89	0.96
pdNDF intake	3.2	3.2	3.1	0.1	18	0.46	0.79
iNDF intake	2.3	2.4	2.4	0.1	18	0.26	0.85
Estimated[6] forage NDF intake	4.3	4.3	4.2	0.1	18	0.44	0.84
Starch intake	6.7	6.8	6.6	0.2	18	0.47	0.94
CP intake	4.1	4.1	4.0	0.1	18	0.63	0.99
Estimated[6] treatment intake	1.2	1.2	1.2	0.0	18	0.22	0.71
Estimated[6] sodium intake	0.12	0.12	0.05	0.00	18	<0.001	0.53
Estimated[6] potassium intake	0.19	0.19	0.19	0.00	18	0.52	0.76
Estimated[6] chloride intake	0.10	0.20	0.10	0.00	18	<0.001	<0.001

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] "Estimated" is based on offered diet concentrations only.

Table 3.8. The effect of dietary strong ion treatment on milk production and composition during feeding behavior subperiod.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Milk, kg	36.1	36.1	34.9	0.8	18	0.27	0.98
Milk fat, %	3.47	3.54	3.52	0.05	18	0.73	0.34
Milk protein, %	3.05	3.05	3.01	0.04	18	0.42	0.97
Milk lactose, %	4.76	4.77	4.73	0.03	18	0.26	0.75
Milk SNF, %	8.76	8.77	8.68	0.05	18	0.13	0.83
Milk fat, kg	1.25	1.28	1.24	0.04	18	0.62	0.60
Milk protein, kg	1.10	0.10	1.05	0.03	18	0.24	0.93
Milk lactose, kg	1.73	1.73	1.65	0.04	18	0.18	0.98
Milk SNF, kg	3.17	3.17	3.03	0.08	18	0.19	0.98
3.5% FCM, kg	35.8	36.2	35.1	0.9	18	0.44	0.74
4.0% FCM, kg	33.2	33.6	32.6	0.8	18	0.44	0.74
SCM[6], kg	33.4	33.8	32.5	0.9	18	0.32	0.78
MUN[7], mg/dl	16.2	15.1	16.6	0.4	18	0.08	0.06
SCC[8], 1000's/ml	165	156	140	20	18	0.43	0.74
SCS	2.6	2.3	2.1	0.2	18	0.27	0.39

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] SCM is solids-corrected milk.
[7] MUN is milk urea nitrogen.
[8] SCC is somatic cell count and SCS is somatic cell score.

Table 3.9. The effect of dietary strong ion treatment on body weight and condition and milk production efficiency.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Empty Rumen BW[6] change, kg/28d	9.5	29.5	3.4	8.5	18	0.16	0.13
BW[6] change, kg/28d	14.4	42.4	-10.0	13.4	18	0.05	0.18
BCS[6], change/28d	0.04	0.21	0.17	0.07	18	0.66	0.15
3.5% FCM/DMI, kg/kg	1.45	1.49	1.48	0.03	18	0.92	0.37
4.0% FCM/DMI, kg/kg	1.35	1.39	1.37	0.03	18	0.92	0.37
SCM[7]/DMI, kg/kg	1.37	1.39	1.36	0.03	18	0.82	0.35

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] BW is body weight and BCS is body condition score.
[7] SCM is solids-corrected milk.

Table 3.10. The effect of dietary strong ion treatment on digestibility of dry matter (DM) and organic matter (OM)[1].

	Experimental Diets					Significance, *P*	
	SBT[2]	SCT[3]	Cntl[4]	SE	n	TMT[5]	CvB[6]
DMI, kg/d	23.2	23.1	23.5	0.6	18	0.61	0.94
DM apparently digested in total tract, kg/d	16.3	16.1	15.9	0.3	18	0.47	0.63
DM apparently digested in total tract, %	70.4	69.7	67.6	0.6	18	0.01	0.46
OMI, kg/d	21.5	21.4	21.9	0.5	18	0.51	0.91
OM apparently digested in rumen, kg/d	7.4	5.9	6.6	0.8	18	0.98	0.21
OM apparently digested in rumen, %	34.6	27.7	29.8	3.8	18	0.78	0.23
OM truly digested in rumen, kg/d	12.0	11.5	12.1	0.6	18	0.67	0.56
OM truly digested in rumen, %	55.8	53.9	54.7	3.1	18	0.96	0.69
Apparent OM passed to duodenum, kg/d	14.1	15.5	15.2	0.9	18	0.70	0.31
True OM passed to duodenum, kg/d	9.5	9.9	9.8	0.8	18	0.93	0.70
OM apparently digested in intestines, kg/d	8.0	9.3	8.5	0.8	18	0.90	0.32
OM apparently digested in intestines, % of intake	37.3	43.4	39.6	3.8	18	0.87	0.28
OM apparently digested in intestines, % of OM passed to duodenum	56.1	59.9	55.6	2.7	18	0.49	0.36
OM apparently digested in total tract, kg/d	15.4	15.2	15.1	0.3	18	0.68	0.58
OM apparently digested in total tract, %	71.9	71.2	69.4	0.6	18	0.03	0.44

[1] Based on iNDF flow.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.11. The effect of dietary strong ion treatment on digestibility of starch[1].

	Experimental Diets					Significance, *P*	
Starch	**SBT[2]**	**SCT[3]**	**Cntl[4]**	**SE**	**n**	**TMT[5]**	**CvB[6]**
Intake, kg/d	6.8	6.9	6.9	0.2	18	0.69	0.79
Digested in rumen, kg/d	3.8	3.7	3.6	0.3	18	0.64	0.79
Digestibility in rumen, %	56.6	55.0	50.9	4.8	18	0.43	0.83
Passage to duodenum, kg/d	3.0	3.1	3.3	0.4	18	0.56	0.72
Digested in the intestines, kg/d	2.5	2.7	2.9	0.4	18	0.55	0.78
Digested in the intestines, % of intake	36.9	38.0	42.4	4.9	18	0.44	0.88
Digested in the intestines, % of duodenal passage	82.4	83.2	84.4	2.2	18	0.57	0.79
Digested in total tract, kg/d	6.4	6.4	6.4	0.1	18	0.62	0.92
Digested in total tract, %	93.5	93.0	93.3	0.7	18	0.98	0.64

[1] Based on iNDF flow.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.12. The effect of dietary strong ion treatment on digestibility of NDF[1].

	Experimental Diets					Significance, *P*	
NDF	**SBT[2]**	**SCT[3]**	**Cntl[4]**	**SE**	**n**	**TMT[5]**	**CvB[6]**
Intake, kg/d	5.7	5.6	5.9	0.1	18	0.19	0.85
pdNDF intake, kg/d	3.3	3.3	3.3	0.1	18	0.60	0.86
Digested in rumen, kg/d	1.8	1.3	1.4	0.2	18	0.78	0.23
Digested in rumen, %	31.0	23.6	24.4	4.2	18	0.60	0.25
pdNDF digested in the rumen, %	53.5	40.2	43.3	7.2	18	0.70	0.23
Passage to duodenum, kg/d	3.9	4.3	4.5	0.3	18	0.38	0.35
pdNDF passage to duodenum, kg/d	1.5	2.0	1.9	0.2	18	0.66	0.26
Digested in the intestines, kg/d	0.3	0.7	0.3	0.2	18	0.59	0.28
Digested in the intestines, % of intake	5.3	12.0	6.2	4.0	18	0.62	0.27
Digested in the intestines, % of duodenal passage	6.4	14.1	7.4	5.1	18	0.66	0.32
pdNDF digested in the intestines, % of duodenal passage	11.3	29.8	15.2	13.0	18	0.74	0.34
Digested in total tract, kg/d	2.1	2.0	1.8	0.1	18	0.02	0.51
Digested in total tract, %	36.4	35.6	30.6	1.1	18	0.003	0.62
pdNDF digested in total tract, %	62.9	61.4	54.4	1.8	18	0.008	0.58

[1] Based on iNDF flow.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.13. The effect of dietary strong ion treatment on ruminal kinetics[1].

	Experimental Diets					Significance, *P*	
	SBT[2]	SCT[3]	Cntl[4]	SE	n	TMT[5]	CvB[6]
Ruminal starch passage rate, %/h	15.8	18.2	19.1	2.3	17	0.50	0.45
Ruminal starch digestion rate, %/h	21.8	22.1	20.7	2.1	17	0.65	0.91
Ruminal pdNDF passage rate, %/h	2.2	2.6	2.6	0.4	17	0.63	0.43
Ruminal pdNDF digestion rate, %/h	2.5	1.8	2.2	0.4	17	0.92	0.22
Ruminal iNDF passage rate, %/h	3.2	3.2	3.6	0.2	17	0.09	0.87
Ruminal liquid passage, /h	0.152	0.165	0.147	0.013	17	0.55	0.49
Ruminal valerate absorption, /h	0.386	0.348	0.323	0.027	17	0.24	0.32

[1] Based on iNDF flow where appropriate.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.14. The effect of dietary strong ion treatment on N metabolism[1].

	Experimental Diets					Significance, *P*	
	SBT[2]	SCT[3]	Cntl[4]	SE	n	TMT[5]	CvB[6]
TRDOM, kg/d	12.0	11.5	12.1	0.6	18	0.67	0.56
N Intake, g/d	667	670	682	17	18	0.54	0.89
Ruminal ammonia, mg/dl	25.2	23.7	23.7	0.6	18	0.34	0.13
Passage to duodenum							
Ammonia, g/d	24	25	20	2	18	0.09	0.72
NAN, g/d	590	642	601	34	18	0.73	0.31
NAN, % of intake	88.3	96.0	88.9	4.6	18	0.57	0.27
NANMN, g/d	200	175	141	26	18	0.19	0.52
NANMN, % of intake	30.4	26.1	21.0	3.8	18	0.16	0.45
NANMN, % of duodenal NAN	34.0	27.3	23.5	4.0	18	0.18	0.27
Microbial N, g/d	390	467	460	39	18	0.53	0.20
Microbial N, % of duodenal NAN	66.0	72.7	76.5	4.0	18	0.18	0.27
Microbial N, g/g of TRDOM	0.033	0.042	0.039	0.004	18	0.71	0.15
NAN, g/d digested in intestines	421	467	411	33	18	0.44	0.36
NAN, % of duodenal passage digested in intestines	70.5	72.6	68.2	2.1	18	0.22	0.48
N apparently digested in total tract, g/d	497	494	491	12	18	0.78	0.88
N apparently digested in total tract, %	74.5	73.9	72.0	1.0	18	0.10	0.66
Fecal N, g/d	169	175	190	9	18	0.17	0.66

[1] Based on iNDF flow.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.15. The effect of dietary strong ion treatment on ruminal turnover times.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
OM turnover time, h	11.7	11.3	10.7	0.4	17	0.19	0.61
Starch turnover time, h	2.7	2.5	2.5	0.1	17	0.60	0.33
NDF turnover time, h	26.3	26.7	24.3	1.1	17	0.16	0.81
pdNDF turnover time, h	22.2	23.1	21.5	1.1	17	0.47	0.57
iNDF turnover time, h	32.0	31.7	27.7	1.6	17	0.09	0.88

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.16. The effect of dietary strong ion treatment on ruminal measurements.

Ruminal measurements	Experimental Diets			SE	n	Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]			TMT[4]	CvB[5]
Wet weight, kg	83.2	81.3	77.6	2.7	17	0.23	0.62
Water, kg	70.8	69.3	66.2	2.3	17	0.24	0.65
DM, kg	12.4	12.0	11.4	0.5	17	0.26	0.51
OM, kg	10.4	10.1	9.6	0.4	17	0.29	0.60
Starch, kg	0.8	0.7	0.7	0.0	17	0.63	0.30
NDF, kg	6.2	6.3	5.9	0.3	17	0.41	0.93
pdNDF, kg	3.0	3.1	2.9	0.1	17	0.44	0.64
iNDF, kg	3.2	3.1	3.0	0.2	17	0.52	0.82
Volume, L	102.8	93.3	92.4	3.1	17	0.20	0.05
DM to water ratio	0.176	0.173	0.173	0.003	17	0.78	0.54
Content density, kg/L[6]	0.81	0.87	0.84	0.01	17	0.91	0.007
Change in ruminal weight, kg/28d	7.7	7.5	-7.2	4.8	16	0.04	0.97
Change in ruminal volume, L/28d	14.7	2.9	-9.4	6.7	16	0.06	0.25

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] Ruminal wet weight, kg divided by ruminal volume, L.

Table 3.17. The effect of dietary strong ion treatment on ruminal VFA profile and ruminal measurements during digestibility subperiod.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Acetate, mM	85.2	82.5	84.3	1.4	18	0.79	0.21
Propionate, mM	35.9	31.0	33.9	1.0	18	0.74	0.009
Butyrate, mM	19.5	20.0	19.1	0.9	18	0.60	0.70
Isobutyrate, mM	1.8	1.9	1.8	0.1	18	0.40	0.84
Valerate, mM	2.9	2.7	2.8	0.1	18	0.95	0.02
Isovalerate, mM	2.7	2.8	2.9	0.1	18	0.32	0.50
Total VFA, mM	147.5	140.3	144.4	2.1	18	0.86	0.04
Acetate: Propionate	2.48	2.69	2.55	0.08	18	0.74	0.09
Ruminal pH[6]	5.97	6.00	5.94	0.01	18	0.003	0.04
Ruminal ammonia, mM	14.8	13.9	13.9	0.4	18	0.34	0.13
Ruminal ammonia, mg/dl	25.2	23.7	23.7	0.6	18	0.34	0.13
Ruminal redox potential, mV[6]	-172	-174	-206	19	18	0.20	0.93

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] Measured at ruminal sampling.

Table 3.18. The effect of dietary strong ion treatment on ruminal VFA profile during intensive 24 h collection.

	Experimental Diets					Significance, *P*	
VFA, mM	**SBT**[1]	**SCT**[2]	**Cntl**[3]	**SE**	**n**	**TMT**[4]	**CvB**[5]
Acetate	55.5	55.1	52.6	1.5	18	0.19	0.89
Propionate	22.0	19.8	19.4	1.7	18	0.48	0.37
Butyrate	11.8	11.2	10.6	0.4	18	0.16	0.35
Isobutyrate	0.9	0.9	0.9	0.0	18	0.97	0.89
Valerate	1.7	1.5	1.4	0.1	18	0.11	0.18
Isovalerate	1.8	1.8	1.9	0.1	18	0.71	0.92
Total VFA	93.7	90.3	86.9	2.5	18	0.13	0.36
Acetate:Propionate	2.80	2.84	2.84	0.17	18	0.90	0.89
Ruminal pH[6]	6.03	6.11	6.15	0.06	18	0.33	0.41

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] Measured during behavior monitoring (d19).

Table 3.19. The effect of dietary strong ion treatment on net energy intake and expenditure.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
NE_L of intake, Mcal/d	39.6	39.0	38.5	0.8	18	0.47	0.62
NE_L for maintenance, Mcal/d	10.4	10.4	10.2	0.1	18	0.25	0.76
NE_L to milk, Mcal/d	24.6	24.9	24.0	0.6	18	0.34	0.77
NE_L balance, net Mcal/d	4.6	3.7	4.4	1.0	18	0.85	0.50
Milk energy to feed energy ratio	0.62	0.64	0.62	0.02	18	0.71	0.61

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.20. The effect of dietary strong ion treatment on chewing behavior during feeding behavior subperiod.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Meals, n	9.7	9.8	9.4	0.6	18	0.56	0.90
Meal length, min	31.6	27.4	28.8	2.8	18	0.85	0.33
Eating time, min/d	266.2	258.9	263.8	8.8	18	0.91	0.58
Meal size, kg DM/meal	2.8	2.5	2.6	0.2	18	0.72	0.44
DMI, kg/d	24.1	24.1	23.5	0.7	18	0.45	0.99
Intermeal interval, min	105.9	99.9	105.4	5.7	18	0.73	0.49
Ruminating bouts, bouts/d	14.8	14.9	14.2	0.4	18	0.27	0.86
Ruminating bout length, min	35.2	34.8	36.9	1.4	18	0.29	0.82
Ruminating time, min/d	505.7	508.1	517.2	10.7	18	0.44	0.88
Inter-ruminating interval, min	64.4	63.7	65.1	2.3	18	0.69	0.84
Total chewing time, min/d	771.9	767.0	781.0	13.2	18	0.48	0.80

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.21. The effect of dietary strong ion treatment on drinking behavior during feeding behavior subperiod.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Drinking bouts, n	16.7	18.3	17.1	0.6	18	0.63	0.08
Water consumed per drinking bout, L/bout	6.6	5.9	6.1	0.2	18	0.57	0.07
Water consumed, L/d	103.5	104.1	98.7	1.8	18	0.05	0.83
Interdrink interval, min	81.1	70.9	76.6	3.9	18	0.91	0.10
Water consumed while eating, L/d	55.3	53.0	57.9	3.0	18	0.33	0.60
Water consumed while ruminating, L/d	2.3	5.1	1.9	0.9	18	0.13	0.06

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.22. The effect of dietary strong ion treatment on ruminal pH during feeding behavior subperiod.

Measures of ruminal pH[1]	Experimental Diets			SE	n	Significance, *P*	
	SBT[2]	SCT[3]	Cntl[4]			TMT[5]	CvB[6]
Mean	6.19	6.23	6.18	0.05	18	0.67	0.62
SD	0.24	0.24	0.24	0.01	18	0.92	0.95
Median	6.19	6.23	6.18	0.05	18	0.67	0.60
Effective minimum[7]	5.78	5.80	5.77	0.05	18	0.75	0.80
Effective maximum[7]	6.60	6.66	6.61	0.05	18	0.67	0.42
Effective range[7]	0.82	0.86	0.83	0.04	18	0.90	0.49
Time below pH 6.0, h/d	6.73	6.26	6.32	1.21	18	0.91	0.79
Area of curve below pH 6.0, pH*30 seconds	155.64	155.22	128.97	37.68	18	0.58	0.99

[1] Ruminal pH was measured continuously and summarized to data file every 5 seconds and was reported for every 22.5 out of 24 h.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[7] Minimum, maximum, and range were calculated using the daily 95% confidence interval (2.5^{th} and 97.5^{th} percentiles) of ruminal pH measured every 5 seconds.

Table 3.23. The effect of dietary strong ion treatment on whole jugular blood gas and electrolyte concentrations at 38.5°C.

	Experimental Diets					Significance, *P*	
Whole blood at 38.5°C	**SBT**[1]	**SCT**[2]	**Cntl**[3]	**SE**	**n**	**TMT**[4]	**CvB**[5]
pH	7.451	7.452	7.441	0.006	18	0.19	0.92
Partial pressure of O_2, mm Hg	47.1	52.0	50.7	1.9	18	0.62	0.11
O_2 content, ml/dl	12.0	12.1	12.3	0.3	18	0.40	0.70
O_2 saturation, %	83.2	85.9	84.7	1.0	18	0.88	0.09
Partial pressure of CO_2, mm Hg	35.1	33.5	33.6	0.5	18	0.28	0.04
Total CO_2 content, mmol/L	25.8	24.7	24.2	0.4	18	0.08	0.10
Base excess of extracellular fluid, mmol/L	0.5	-0.5	-1.2	0.5	18	0.08	0.17
Base excess of blood, mmol/L	1.6	0.7	0.1	0.4	18	0.08	0.18
Hematocrit, %	31.1	30.5	31.5	0.4	18	0.22	0.28
Calculated hemoglobin[6], g/dl	10.4	10.2	10.5	0.1	18	0.22	0.28
Sodium, mmol/L	142.3	142.7	142.1	0.2	18	0.20	0.20
Potassium, mmol/L	4.17	4.24	4.20	0.03	18	0.94	0.09
Calcium, mmol/L	1.14	1.10	1.12	0.02	18	0.94	0.27
Ionized normalized calcium[7], mmol/L	1.17	1.14	1.15	0.02	18	0.72	0.23
Chloride, mmol/L	104.9	106.2	105.5	0.3	18	0.95	0.02
Calculated HCO_3^-, mmol/L	24.7	23.6	23.1	0.4	18	0.08	0.11
Standardized bicarbonate[8], mmol/L	25.6	24.9	24.3	0.4	18	0.08	0.21
Anion gap, mmol/L[9]	16.8	17.1	17.7	0.4	18	0.16	0.70

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[6] Assumes hematocrit is 1/3 hemoglobin.
[7] Normalized to pH 7.4.
[8] Standardized to partial pressure of carbon dioxide of 40 mm Hg.
[9] Anion Gap is (Na + K) – (Cl + bicarbonate) in mmol/L.

Table 3.24. The effect of dietary strong ion treatment on plasma metabolite and hormone concentration.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Glucose, mg/dl	56.4	57.1	55.5	1.2	18	0.41	0.67
NEFA, μEq/L	60.2	51.7	55.2	4.4	18	0.90	0.20
BHBA, mg/dl	6.4	5.6	5.9	0.5	18	0.91	0.33
Insulin, μIU/ml	9.4	9.5	7.8	0.7	18	0.07	0.87
Glucagon, pg/ml	114.2	103.5	109.3	4.7	18	0.94	0.15
Insulin:Glucagon	0.08	0.10	0.07	0.01	18	0.16	0.40

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table 3.25. Distributions from previous work at Michigan State University with ruminally and duodenally cannulated lactating Holstein dairy cattle compared experiment in Chapter 3 (Based on iNDF flow).

	Previous Work				02CSM2	
	Mean	SD	Median	n[1]	Mean	Approx. Percentile
DMI, kg	22.8	3.8	22.9	218	24.4	70
Milk yield, kg	38.5	8.4	38.4	218	35.7	40
FCM yield, kg	38.6	7.5	38.1	218	35.7	40
Empty Rumen BW, kg	564	66	561	188	578	60
Ruminal DM digestibility, %	30.6	13.0	31.0	180	25.1	30
Ruminal DM digestibility, kg/d	6.5	2.7	6.7	189	5.9	40
Ruminal apparent OM digestibility, %	35.2	11.7	35.5	189	30.7	35
Ruminal apparent OM digestibility, kg/d	7.1	2.5	7.2	190	6.6	40
Ruminal NDF digestibility, %	32.3	12.3	33.6	190	26.3	30
Ruminal pdNDF digestibility, %	55.6	15.1	56.0	191	45.6	25
Ruminal rate of pdNDF digestion, %/h	3.1	1.5	2.9	181	2.2	30
Ruminal rate of pdNDF passage, %/h	2.8	0.8	2.7	184	2.4	35
Ruminal rate of iNDF passage, %/h	3.2	1.1	3.2	184	3.3	55
Ruminal apparent starch digestibility, %	49.8	19.1	52.2	185	54.2	55
Ruminal rate of apparent starch digestion, %/h	15.6	8.8	15.4	185	21.5	80
Ruminal rate of starch passage, %/h	15.6	6.5	14.4	185	17.7	65
Microbial N Production, g/d	418	120	413	153	439	60
Microbial N production efficiency, g/kg TRDOM	36	9	35	152	38	60

[1] number of cow periods in the mean.

Table 3.26. Comparison of design and results of experiments in Chapter 2 and Chapter 3.

	Chapter 2	Chapter 3
Mean DMI, kg	28	25
Mean Milk, kg	37	36
Mean DIM	126	180
Diet ingredients		
Corn silage?	Yes	Yes
Alfalfa silage?	Yes	Yes
Alfalfa hay?	Yes	No
WFCS?	Yes	No
Protein mix?	Yes	Yes
High moisture corn?	Yes	Yes
Treatment mix?	Yes	Yes
Diet concentrations		
CP, %	17.2	17.8
NDF, %	28.1	25.1
Forage NDF, %	18.2	19.1
Sodium bicarbonate effects		
DMI, kg	0	0
Milk yield, kg	+	0
Milk fat, %	+	0
Number of rumination bouts	0	0
Length of rumination bout, min	-	0
Total rumination time, min/d	-	0
Change in BW	+?	+?
Experimental design		
Maximum cow periods in statistics	200	18
Experimental period length, d	14	28
Statistical power?	more	less

CHAPTER 4: Effects of dietary starch concentration and corn conservation method on ruminal and plasma ions in lactating dairy cows.

ABSTRACT

The objective of this study was to investigate relationships among strong ions in the rumen. Eight ruminally cannulated Holstein cows (55 ± 16 DIM; mean ± SD) were used in an experiment with a duplicated 4 x 4 Latin square design. A 2 x 2 factorial arrangement of treatments was used with main effects of dietary starch percentage (32% vs. 21%) and conservation method of corn grain (dry, 90% DM or high-moisture, 63% DM). Ruminal fluid samples were collected through a ruminal cannula every 20 min for 24 h per period during which feeding behavior and ruminal pH were monitored continuously. Dietary treatments did not affect mean ruminal pH (6.22) or total VFA (102 mM) but high starch treatments increased daily pH variability as measured by standard deviation and range ($P < 0.09$). High starch treatments increased ruminal sodium concentration (98.5 vs. 94.2 mEq/L) and decreased ruminal potassium and ammonium concentrations (38.3 vs. 43.0 mEq/L and 3.4 vs. 4.0 mEq/L, respectively). Ruminal potassium concentrations were influenced by treatment while ruminal sodium concentration was not reflective the uniform concentration of sodium across diets nor the expected decrease in saliva flow on the high starch treatment. The sum of sodium, potassium and ammonium (141.1 mEq/L) was not affected by treatment nor was strong ion difference defined as sodium plus potassium minus chloride (124.9 mEq/L). Dietary treatment did not affect mean ruminal osmolality (261 mOsm) but high starch treatment increased variability of ruminal osmolality. Ruminal pH was correlated positively (r >

0.72) with ruminal sodium concentration, the sum of ruminal sodium and potassium, and ruminal strong ion difference, and was correlated negatively ($r < -0.42$) with total VFA concentration, ruminal ammonium, and the sum of ruminal ammonium and potassium. Ruminal sodium concentration was negatively related to ruminal potassium concentration and ruminal ammonium concentration and more negatively related to the sum of potassium and ammonium concentrations. The alkalizing strong ion difference and charge balance in the rumen are likely regulated by modifying sodium flux across the ruminal epithelium.

Keywords: ruminal pH, strong ions, sodium

INTRODUCTION

Sodium, potassium and chloride are essential nutrients for lactating dairy cows (NRC, 2001) and are the primary strong ions in the ruminal solution (Bailey, 1961b, Bennick et al., 1978, Tucker et al., 1988). In solution, sodium and potassium are alkalogenic and chloride acidigenic and each of three has a single valence (Stewart, 1983). These ions also contribute equally to the osmolality of a solution (Weast, 1978). The sum of cations (sodium plus potassium as mEq/L) minus anion (chloride as mEq/L) is strong ion difference (SID) which is a determinant of the pH of solution (Stewart, 1983). Other strong ions can be included in the calculation of SID (Constable, 2000). In addition to SID, other determinants of blood pH include partial pressure of carbon dioxide, weak acid concentration and phosphate concentration (Stewart, 1983; Constable, 2000; Heisey and Adams, 2002).

In the rumen, sodium is usually the most abundant cation in the ruminal solution with a typical concentration of 120 mEq/L (Bailey, 1961b). The ruminal concentration of sodium is proportional to but lower than the concentration of sodium in saliva (Bailey, 1961b) and does not appear to be influenced by meals (Tucker et al., 1993). Sodium enters the reticulorumen (RR) in the diet or in saliva and can leave the ruminal lumen by passage to the omasum or by absorption across the ruminal epithelium (Stevens and Hume, 1995). Sodium is absorbed into the ruminal epithelium by sodium-hydrogen exchange and by the electrogenic diffusion of sodium through ion channels (Martens and Gaebel, 1988).

Ruminal potassium concentration is typically 25-40 meq/L (Bailey, 1961b; Tucker et al., 1988, Tucker et al., 1993). This concentration of potassium is usually

higher than that found in saliva (Bailey, 1961b) and is primarily a function of diet concentration (Bailey, 1961b; Bennick et al., 1978; Tucker et al., 1993). Potassium enters the RR from the diet and, to a lesser extent, in the saliva and will leave the ruminal lumen by paracellular absorption through the ruminal epithelium diffusing down the concentration gradient from lumen to the blood and by passage to the omasum (Stevens and Hume, 1995).

Ruminal chloride concentration usually ranges from 10 to 15 mEq/L (Bailey, 1961b; Tucker et al., 1988, Tucker et al., 1993) and enters the RR from the diet and saliva and leaves by absorption and passage (Stevens and Hume, 1995). Ruminal chloride is absorbed by the exchange of chloride anion for bicarbonate anion across the apical membrane of ruminal epithelial cell (Gaebel et al., 1991; Martens et al., 1991; Gaebel et al., 1993).

Sodium and chloride have been shown to be absorbed proportionally across the ruminal epithelium and the indirect link is either intracellular pH or intracellular carbonic acid where the sodium-hydrogen exchange is working in parallel with a chloride-bicarbonate exchange (Martens et al., 1991). Sodium and potassium concentrations in the RR have a reciprocal relationship in sheep (Sellers and Dobson, 1960; Scott, 1966; Stacy and Warner, 1966; Scott, 1967) and cattle (Bailey, 1961b). Based on in vitro experiments with sheep ruminal epithelium, this relationship is likely achieved by the potassium modulation of sodium absorption (Lang and Martens, 1999). The high potassium enhancement of sodium absorption keeps the sum of sodium and potassium relatively constant and this could be an effective mechanism for the regulation of both charge and osmolality in the RR (Lang and Martens, 1999). Also, based on in vitro

experiments with sheep ruminal epithelium, sodium and ammonium also may have a similar relationship as ammonium can promote sodium absorption (Abdoun et al., 2003) but the mechanism has not been determined. Therefore, in the ruminal solution, sodium, potassium, and chloride appear regulated and interacting.

The objective of this experiment was to determine the relationships among strong ion concentrations and ruminal pH in cows consuming diets varying in fermentibility. Given previous blood SID theories, in vitro ruminal epithelial and in vivo sheep experiments, sodium, potassium and chloride are expected to interact and be related to ruminal pH in lactating dairy cows in vivo. The null hypothesis was that sodium and other strong ions in the ruminal solution are not related to ruminal pH or each other.

MATERIALS AND METHODS

This paper is the fourth in a series of papers from one experiment that evaluated effects of corn grain conservation method at two dietary starch concentrations. This paper will focus on strong ions and their relationships in plasma and centrifuged ruminal fluid. Feeding behavior and productivity (Oba and Allen, 2003a), ruminal digestion kinetics (Oba and Allen, 2003b), and efficiency of microbial nitrogen production (Oba and Allen, 2003c) were reported previously (Table 4.1). Animal procedures were approved by the All University Committee on Animal Use and Care at Michigan State University (AUF# 05/96-037-00).

Design And Treatments

Eight multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to duplicated 4 x 4 Latin squares balanced for carry over effects with a 2 x 2 factorial arrangement of treatments. Treatments were dietary starch concentration (21% vs. 32%) and conservation method of corn grain [high moisture ground (HM) vs. dry ground (DG) corn]. Treatment periods were 21 d with the samples and data reported in this paper collected primarily on d 15. (Tables 4.2, 4.3, and 4.4)

Electronic Data And Automatic Sample Collection

On d 15, a 24 h intensive collection of plasma and rumen fluid was conducted simultaneously with measurement of feeding behavior. Whole blood samples and ruminal fluid samples were collected every 20 min for 24 h by automated sample collection

system (Allen et al., 2000b), starting at 1200 h. Feed doors were closed at 1200 h and reopened at 1400 h. Blood was sampled from a jugular vein through a catheter (45 cm long, MRE 095 Renathane® tubing, Braintree Scientific, Inc., Braintree, MA) inserted 1 d prior to sample collection using sterile technique. Ruminal fluid was drawn from the ventral rumen through tubing inserted through the stopper of the ruminal cannula (10 cm i.d.; Bar Diamond Inc., Parma, ID). This system successfully collected 99.5% and 97.9% of the total samples (4,308 each) for blood and ruminal fluid, respectively.

On d 15, feeding behavior and ruminal pH was also monitored by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, water consumption, and ruminal pH were recorded to computer file every 5 seconds for each cow. Chewing activities were summarized as meal bouts, interval between meals, and meal size for eating behavior and as ruminating bouts and inter-ruminating interval for ruminating behavior. Ruminal pH data were summarized to daily mean, variance, median, minimum, maximum, range, the hours and area for which ruminal pH was below pH 6.0. The minimum, maximum, and range were calculated using the daily 2.5^{th} and 97.5^{th} percentiles for ruminal pH measured every 5 seconds. The area was calculated by determining the time below ruminal pH 6.0 and weighting that time by the deviation from the threshold.

Sample Processing And Storage

Ruminal fluid was centrifuged at 2,000 x g for 15 min immediately after collection, and supernatants were frozen at -20° C until analysis. Whole blood collected in tubes with potassium oxalate and sodium fluoride as a glycolytic inhibitors (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Both whole blood samples were

centrifuged at 2,000 x g for 15 min after sample collection, and plasma was harvested and frozen at -20° C until analysis.

Sample Preparation

All feed and orts were frozen immediately after collection at and frozen at –20° C. (See Oba and Allen, 2003a for more detail.) Composites were dried in a 55° C forced-air oven for 72 h and DM concentration was determined. Samples were ground with a Wiley mill (1 mm screen; Authur H. Thomas, Philadelphia, PA).

Rumen evacuations For Pool Size Determination

Ruminal contents were evacuated manually through the ruminal cannula at 1800 h (4 h after feeding) on d 20 and at 1000 h (4 h before feeding the following day) on d 21 of each period. Total ruminal content mass and volume were determined. During evacuation, a 10% aliquot of digesta was separated to allow accurate sub-sampling. The aliquot was squeezed through nylon mesh (1 mm pore size) to separate it into primarily solid and liquid phases. Samples were taken from both phases for determination of pool size of digesta components in the rumen. Samples were frozen immediately at –20°C

Sample Analysis

Before centrifuging and freezing, whole blood, collected in a tube containing lithium heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), was analyzed immediately for pH, pCO_2, hematocrit, pO_2, calcium, sodium, potassium, and chloride by a blood gas analyzer (Stat Profile 4, Nova Biomedical, Waltham, MA) and ten other blood variables were calculated by manufacturer's equations assuming a

temperature of 38.5°C (Appendix A.5). Feed samples were processed and analyzed for DM, ash, CP, starch, NDF, and indigestible NDF (iNDF) as described by Oba and Allen (2003a, 2003b, and 2003c). Feed samples were analyzed for sodium, potassium, and chloride concentrations. Sodium and potassium concentrations were determined by digestion according to Hach et al. (1987) and measurement of the element in supernate by atomic absorption according to manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA). Chloride concentration was determined by extracting the feed with 1.0% nitric acid solution for one hour on shaker (Orbimix 1010, Brinkman Instruments, Westbury, NY) and measuring supernate chloride concentration by coulometric titration (Digital Chloridometer, Model 442-5000, Labconco Corporation, Kansas City, MO). Digests and dilutions were stored in either polypropylene specimen cups or polypropylene test tubes (Round bottom, 13 x 100 mm, culture test tubes, Fisherbrand® Catalog No. 14-956-7A, Fisher Scientific, Pittsburgh, PA) until analysis.

Centrifuged rumen fluid was analyzed for sodium, potassium, chloride, and ammonia concentrations. Sodium and potassium concentrations were determined by atomic absorption using AOAC procedures for beer (1990, #987.03 for sodium and #987.02 for potassium) adapted to the manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA). Chloride concentration was determined by coulometric titration (Digital Chloridometer, Model 442-5000, Labconco Corporation, Kansas City, MO). Ammonia concentration was determined for ruminal fluid samples according to Broderick and Kang (1980).

Ruminal fluid was analyzed for concentrations of major VFA and lactate. Samples were centrifuged at 26,000 × g for 15 min, and supernatant (600 μL) was mixed with 600 μL $Ca(OH)_2$ and 300 μL of $CuSO_4$ containing crotonic acid as an internal marker in 1.7 ml micro centrifuge tubes. Samples were centrifuged at 12,000 × g for 10 min, and supernatant (1000 μl) was taken and mixed with 28 μl of H_2SO_4 in 1.5 ml micro centrifuge tubes. Samples were frozen and thawed twice, and centrifuged at 12,000 × g for 10 min to precipitate and remove protein thoroughly. Supernatant was transferred to HPLC vials. Concentrations of VFA and lactate of the supernatant were determined by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (1999a).

Osmolality of plasma and centrifuged rumen fluid was measured on the Advanced™ Osmometer (Model 3D3, Advanced Instruments, Inc., Lab Products Division, Norwood, MA, buzzpoint of 3000 for plasma and 2500 for centrifuged rumen fluid).

A subset of plasma samples was randomly selected across all experimental periods (n=47) and analyzed for sodium and potassium using atomic absorption according to the manufacturer's recommendation (SpectrAA 220FS, Atomic Absorption Spectrometer, Varian Analytical Instruments, Walnut Creek, CA) to confirm whole blood electrolyte analysis by blood gas analysis. Within sample, measurements recorded had mean differences of –9.0% for sodium and –1.2% for potassium.

Calculations

Total diet concentrations for cations and anions were calculated from individual ingredient analyses and dietary proportions of the dry matter. Dietary cation anion

difference in feeds as mEq/100g DM was calculated as sodium plus potassium minus chloride (DCAD3). (Appendix A.6)

Strong ion difference (SID) in solution was calculated as concentrations of sodium plus potassium minus chloride.

Estimated associated VFA concentration in the ruminal solution as mM was calculated as (Total VFA concentration, mM)(1/(10^(ruminal pH minus 4.8) + 1). Estimated disassociated VFA concentration in the ruminal solution as mM was calculated as total VFA minus estimated associated VFA.

Calculated ruminal bicarbonate in the ruminal solution as mEq/L was calculated as 10^(ruminal pH minus 7.74) times 500 (assuming carbon dioxide concentration of 0.5 atm; Kohn and Dunlap, 1998).

Turnover rate in the rumen (/h) = (intake of component / ruminal pool of component)/24.

Statistical Analysis

All data were analyzed using the fit model procedure of JMP® (Version 5.0.1.2; 2003) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period (j = 1 to 4),

T_k = fixed effect of treatment (k = 1 to 4),

e_{ijk} = residual, assumed to be normally distributed.

Period by treatment interaction was originally evaluated, but it was removed from the statistical model because interaction was not significant for response variables of primary interest. Orthogonal contrasts were performed for effects of starch concentration, corn moisture, and interaction of starch concentration and corn moisture. Treatment effects and their interaction were declared significant at $P < 0.05$ and $P < 0.10$, respectively, and tendency for treatment effects were declared at $P < 0.10$. When interactions of main effects were significant, treatment means were compared using Student's t-test and differences were declared significant at $P < 0.05$. Residual plots were checked for appearance of normality and appeared normally distributed. Ruminal pH data for Cow 2946, Period 3 were lost due to pH probe failure.

Cow period means were used to generate Pearson correlation coefficients in JMP® (Version 5.0.1.2; 2003). Individual ion and metabolite measures within sample were used to develop linear regression equations and calculated adjusted R^2 values in JMP® (Version 5.0.1.2, 2003). Changes in ion concentration from the beginning to end of meal, bouts, and drinks were generated using a logic script (Appendix A.15) in Igor Pro® (2002).

RESULTS

As previously reported, dietary treatments affected DMI, meal size, starch digestibility, microbial nitrogen production, ruminal pH, chewing time, and milk production (Table 4.1). Measurements in this paper were generated primarily from data from an intensive collection on d 15 of each period so some of the results may not match previous behavioral measurements which were based on d 16 through 19 inclusive. Dietary sodium, potassium, and chloride concentrations were 0.5%, 1.2%, and 0.3%, respectively for the 32% starch diets and 0.5%, 1.4%, and 0.4%, respectively for the 21% starch diets. With regards to ruminal strong ions, this experiment uniquely combines measurements in the conditions of in vivo, in cattle, and under standard dietary conditions.

Dietary treatments affected feeding behavior of cows (Table 4.5). High starch treatments increased daily water intake 6.8 L by tending to increase the frequency of drinking bouts. High starch treatments decreased meal length by 4 min (13%). With an interaction among the treatments, DMI was greatest on the high starch, DG treatment (20.9 kg/d) and lowest on the low starch, DG treatment (17.8 kg/d). These differences are attributed to changes in number of meals, meal length, and intermeal interval which are proposed to be caused by differences in ruminal starch fermentation as previously reported (Oba and Allen, 2003a). With a significant interaction, eating time per d was highest on the low starch, HM corn treatment (254.2 min/d) and lowest on the high starch, HM corn and low starch, DG corn treatments (230.4 and 241.9, min/d, respectively). High starch treatments decreased ruminating time per d (48.0 min) and this

decrease was attributed in part to an increased inter-ruminating interval (7.8 min). High starch treatments also decreased total chewing time per d (63.7 min/d).

Ruminal content weight at the end of the experimental period was lower for high starch treatments and, with a similar amount of DM, this is attributed to less total water in the RR (Table 4.6). The distribution of water within the ruminal contents was not determined (i.e. within digesta, within the microbial mass, or free ruminal solution).

Dietary treatment did not affect mean ruminal pH (6.22; Table 4.7). However, high starch treatments increased pH variability as measured by standard deviation and range. Similarly, dietary treatment did not affect ruminal total VFA concentrations (101.6 mM; Table 4.8) but high starch treatments increased concentration of some individual VFA (propionate, iso-butyrate, valerate, and iso-valerate). With a similar ruminal pH and total VFA concentration across dietary treatments, similar concentrations of associated and disassociated total VFA were estimated among dietary treatments.

Overall mean ruminal ion concentrations were 97.7 mEq/L for sodium, 39.8 mEq/L for potassium, and 12 mEq/L for chloride (Table 4.9). Sodium and potassium concentrations were similar to those previously report by Emery et al. (1960) and lower in sodium and higher in potassium than more recent reports with cattle (Tucker et al., 1988; Tucker et al., 1993). Chloride concentrations were similar to recent reports with cattle (about 140 mEq/L for sodium and 27 mEq/L for potassium, respectively; Tucker et al., 1988; Tucker et al., 1993). (Distributions for other experimental measures are located in Appendix Tables A.23 and A.24).

High starch treatments increased ruminal sodium concentration (98.5 vs. 94.2 mEq/L) and decreased ruminal potassium and ammonium concentrations (38.3 vs. 43.0

mEq/L and 3.4 vs. 4.0 mEq/L, respectively; Table 4.10), as well as the sum of potassium and ammonium concentrations. Ruminal chloride concentration was highest on the low starch treatments (13 mEq/L), intermediate on the high starch, DG corn diet (12 mEq/L), and lowest on the high starch, HM corn diet (11 mEq/L). The higher ruminal concentrations of chloride and potassium in low starch treatments were proportional to the dietary concentrations of these ions. Ruminal ammonium may also be higher on low starch treatments because they contain more alfalfa silage but dietary soluble protein was not measured. Ruminal sodium concentrations decreased with low starch treatments which did not reflect the uniform concentration of sodium across diets or with the expected increase in saliva flow associated with a 10% increase in total chewing time per d. Lower ruminal sodium concentrations were associated with higher ruminal potassium and ammonium concentrations. In contrast to individual ion concentrations, dietary treatment did not affect the sum of sodium and potassium (136.9 mEq/L), the sum of sodium, potassium and ammonium (141.1 mEq/L), the sum of chloride and estimated disassociated VFA (107.5 mEq/L), the sum of chloride, estimated disassociated VFA, and calculated ruminal bicarbonate (131.7 mEq/L), or strong ion difference (124.9 mEq/L) suggesting regulation of the ruminal environment (Table 4.10).

Dietary treatment did not affect whole blood sodium concentrations (142.4 mmol/L), potassium concentrations (4.1 mmol/L), chloride concentrations (106.0 mmol/L), or anion gap (16.4 mmol/L; Tables 4.11 and 4.12). High starch treatments tended to decrease calcium concentration (0.1 mmol/L) but not normalized calcium concentrations (1.3 mmol/L). High starch treatments increased whole venous blood pH (7.428 vs. 7.417) and lowered partial pressure of oxygen (2.8 mm Hg) and oxygen

saturation (2%) leading to greater oxygen content (0.4 ml/dl). These differences are probably related as decreased pH is associated with greater disassociation of oxygen from hemoglobin (Rhoades and Tanner, 1995).

Dietary treatments did not affect mean ruminal and plasma osmolalities (Table 4.13). Ruminal osmolality averaged 261 mOsm as measured and 286 mOsm corrected for bicarbonate. Plasma osmolality averaged 284 mOsm as measured and 309 mOsm corrected for bicarbonate. Ruminal osmolality SD was greater on high starch treatments than low starch treatments (20.5 vs. 17.2). Plasma osmolality SD was similar across all dietary treatments ($P > 0.47$). Mean difference of plasma minus ruminal osmolality was similar across dietary treatments whether as measured or with inclusion of respective bicarbonate measures (22 mOsm). Across all treatments, measured plasma osmolality was greater than ruminal osmolality measured at the same time more than 85% of the time. With plasma osmolality relatively constant (Figure 4.1), ruminal osmolality exceeded plasma osmolality only when ruminal osmolality was highest.

Osmolalities were adjusted for bicarbonate that was lost during sample processing and storage. Both ruminal and whole blood samples were centrifuged, frozen, thawed, mixed and measured. Over this extended time between sampling and measurement, samples equilibrated with the carbon dioxide poor atmosphere (Table 1.6) and a significant decrease in osmolality is possible (Dobson, 1970). Plasma osmolality was adjusted by adding the bicarbonate concentration from the blood gas measurement. Whole blood bicarbonate measurements averaged 24.0 mmol/L and were normally distributed. Ruminal osmolality was adjusted with a calculation of bicarbonate concentration (Kohn and Dunlap, 1998). This calculation assumed a carbon dioxide

concentration of 50% in the gas phase above the ruminal mass. The ruminal bicarbonate calculation averaged 23.8 mmol/L but had a skewed distribution. The actual bicarbonate is, however, dependent of the specific ruminal carbon dioxide at the time of sampling. Ruminal carbon dioxide is very variable within the day (Table 1.5) and specific measures of mean and variability for contemporary lactating dairy cows have not been reported. Calculated ruminal bicarbonate is sensitive to the choice of carbon dioxide percent and this 50% assumption should be tested in lactating dairy cows.

Among cow period mean ruminal measurements (n=32; Table 4.14), ruminal pH was correlated positively with ruminal sodium concentration (r = 0.74), the sum of ruminal sodium and potassium (r = 0.74), and ruminal strong ion difference (r = 0.72), and was correlated negatively with total VFA concentration (r = -0.42), ruminal ammonium (r = -0.69), and the sum of ruminal ammonium and potassium (r = -0.49). Ruminal pH was not significantly related to estimated disassociated VFA concentration, ruminal potassium concentration, or ruminal chloride concentration. Ruminal pH was not related to ruminal osmolality (r = -0.32) but was positively related to ruminal osmolality plus calculated bicarbonate (r = 0.45). Ruminal osmolality was positively related to total VFA concentration (r = 0.60) and ruminal osmolality adjusted for bicarbonate was positively related to ruminal sodium concentration (r = 0.44) and other measures containing the ruminal sodium concentration. Ruminal sodium concentration was negatively related to ruminal potassium (r = -0.68) and ruminal ammonium (r = -0.79) concentrations. Ruminal ammonium was also negatively related to ruminal SID (r = -0.72).

Among mean whole blood measurements (n=32; Table 4.15), partial pressure of carbon dioxide was negatively related to SID (r = 0.83). Sodium concentration was positively related to both plasma osmolality measures (r > 0.67). Blood bicarbonate concentration was negatively related to chloride concentration (r = -0.82). Blood SID was negatively related to chloride (r = -0.86) and positively related to bicarbonate (r = 0.93). These relationships among whole blood measures are consistent with the strong ion theory (Stewart, 1983). Across mean blood and ruminal measurements (Table 4.16), a negative relationship was recorded for blood pH and ruminal potassium concentration (r = -0.58).

Ruminal pH was regressed against several ruminal ion measurements using individual measurements (n > 2272; Table 4.17). Ruminal pH was related positively to ruminal sodium concentration ($R^2 = 0.42$, $P < 0.0001$, Figure 4.2) but less related to SID ($R^2 = 0.23$, $P < 0.0001$, Figure 4.3). Ruminal pH was negatively related to ruminal ammonium concentration ($R^2 = 0.26$, $P < 0.0001$, Figure 4.4) and ruminal potassium concentration ($R^2 = 0.21$, $P < 0.0001$, Figure 4.5) but ruminal chloride concentration explained negligible variation in ruminal pH ($R^2 = 0.01$, $P < 0.0001$, Figure 4.6).

Stronger relationships exist among concentrations of sodium, potassium, and ammonium (n > 2246; Table 4.17). Ruminal sodium concentration had a moderate negative relationship with ruminal potassium concentration ($R^2 = 0.46$, $P < 0.0001$, Figure 4.7) and ruminal ammonium concentration ($R^2 = 0.45$, $P < 0.0001$, Figure 4.8). However, it had a stronger negative relationship with the sum of potassium and ammonium concentrations ($R^2 = 0.56$, $P < 0.0001$, Figure 4.9) suggesting an interaction among these cations. (Table 4.17)

Ruminal pH was positively related to ruminal sodium, ruminal sodium plus potassium, and strong ion difference which is expected given that sodium is associated with ruminal buffering and that cations are alkalogenic. Ruminal pH has a negative relationship with total VFA, ruminal ammonium, and ruminal potassium as expected as they all increase with the ingestion and fermentation of a meal.

Changes were measured from beginning to the end of meals (n=351, mean meal size was 1.8 kg), drinks (n=445, mean drink size was 7 L), and rumination bouts (n=480, mean rumination bout was 30 min; Table 4.17). Ruminal sodium concentration difference was related negatively to meal size ($R^2 = 0.41$, $P < 0.0001$, Figure 4.10) while ruminal potassium concentration difference was related positively ($R^2 = 0.69$, $P < 0.0001$, Figure 4.11) and ruminal sodium plus potassium difference was not related($R^2 = 0.01$, $P < 0.04$, Figure 4.12). Change in ruminal osmolality was related positively to meal size ($R^2 = 0.28$, $P < 0.0001$, Figure 4.13) while change in ruminal pH was related negatively ($R^2 = 0.28$, $P < 0.0001$, Figure 4.14). Drinking, as expected, generally lowered ruminal sodium, and potassium concentrations and ruminal osmolality (Table 4.17). Length of rumination bout was not highly related to change in concentrations of ions (Table 4.17).

DISCUSSION

High starch treatments were more fermentable (Oba and Allen, 2003a) and a greater production of osmotically active particles is expected. The cows on these treatments drank more but had less total water in their rumens. The high starch treatments decreased ruminal potassium, chloride, and ammonium concentrations which are reflective of the diet or fermentation and increased ruminal sodium which was not reflective of the uniform diet concentration. Treatments were not different in ruminal pH and osmolality but the variability of both was greater on the high starch treatments. Treatments were also not different for total VFA concentration and various sums of concentrations of cations and anions.

Ruminal potassium and ammonium were negatively related to ruminal sodium concentration. However, their sum explained more of the variation in ruminal sodium concentration than either ruminal concentration did alone. Within the ruminal solution, charges must balance. Given unknowns of the diet, lactating dairy cows could possibly regulate one or more ions to control the overall composition of the ruminal solution. Such a mechanism would help balance charges and limit osmolality while maintaining strong ion difference in response to the varying cation entry into the ruminal solution.

For the cations in this experiment, potassium and ammonium are likely related to the meals or fermentation which leaves the sodium as the likely candidate for modulation and regulation by lactating cows. The experimental means and relationships suggest that sodium concentration is regulated through sodium flux across the ruminal epithelium which provides a means of controlling strong ion difference and charge balance in the rumen. These results agree with concentration relationships reported in vitro work with

sheep ruminal epithelium (Lang and Martens, 1999; Abdoun et al., 2003). As the primary extracellular cation, sodium determines extracellular fluid volume in the body (Carlson, 1997). The amount of sodium is regulated and this cation is balanced with anions and the total ion concentration draws water as water follows solute (Houpt, 2004). In the ruminal solution, sodium appears to have a similar role.

Ruminal osmolality was examined to determine completeness of ruminal ion measurement. Within each osmotic measurement, half of contributing particles will be negatively charged and half will be positively charged to satisfy charge balance of a solution (Stewart, 1983). Ruminal concentrations of sodium, potassium, ammonium, chloride, estimated disassociated VFA, and calculated bicarbonate were summed and then divided by ruminal osmolality plus calculated bicarbonate; the measured ions accounted for 95.1% of the ruminal osmolality. Overall, 50% of the sums ranged between 90 to 100% of the ruminal osmolality plus calculated bicarbonate suggesting a reasonable completeness of the measurement of osmotically active particle in the ruminal solution. If the three anion sum is subtracted from the three cation sum, the average difference is +10 mEq/L and, given the need for the balance of charges, this suggests that all anions have not been measured. Among the ruminal anions previously measured (Bailey, 1961b), hydrogen phosphate is likely an important missing anion.

As a dependent variable (Stewart, 1983), ruminal pH is probably not managed by cows; cows likely manage the independent variables that determine pH. In the blood, examples of independent variables are SID, weak acid concentrations, and partial pressure of carbon dioxide (Stewart, 1983; Constable, 2001; Heisey and Adams, 2002). In the RR, lactating cows probably manage variables such as these rather than the pool of

hydrogen ions which is several orders of magnitude less. Also, osmolality of all bodily solutions must be in balance and the sum of positive and sum of negative charges within these solutions must also be balanced. Cows must transport and regulate ions to maintain these balances.

In this experiment, SID of the ruminal solution, defined as sodium plus potassium minus chloride, only explained 23% of the variation in ruminal pH but additional ions may be included in the calculation of SID. In descending order of concentration, the key cations to be considered are sodium, potassium, ammonium, calcium, magnesium and the key anions to be considered are disassociated VFA, bicarbonate, hydrogen phosphate, chloride, lactate, and feed. Adapting criteria of Constable (2001) to ions at ruminal pH, the strong cations to be summed should be sodium, potassium and ammonium and the strong anions to be summed should be chloride and lactate if present. (In this experiment, lactate was detected in 268 out of 2304 ruminal fluid samples with 70 samples showing concentrations of >5 mM.) Other ions are not likely present in concentrations high enough to warrant inclusion in the calculation of SID but this has be verified.

A better accounting of the ions used in the SID calculation may tighten the relationship with pH but SID is still only one component that determines pH. The pK_a of VFA and hydrogen phosphate are close enough to the expected range of ruminal pH to produce significant pools for undisassociated and disassociated molecules and they warrant separate consideration. Partial pressure of carbon dioxide in the RR is required by the theory but is unknown in lactating dairy cows. Carbon dioxide proportion in the free ruminal gas is quite variable within day (Table 1.5) but an estimate of 50% is a reasonable starting point until more definitive research is done. Ruminal pH is likely by

determined by SID as the sum of the concentrations of sodium, potassium, and ammonium minus the sum of the concentrations of chloride and lactate, total VFA concentration, hydrogen phosphate concentration, and partial pressure of carbon dioxide (Table 4.18). Feed anions have been considered in the context of cation exchange capacity (McBurney et al., 1983; Jasaitis et al., 1987) and further investigation is needed to conclude whether it should be included as a factor in this ruminal pH theory.

Lactating dairy cows have the ability to generate and regulate a large VFA load in their RR. VFA and ions have transporters in the ruminal epithelium that require energy (Gaebel and Sehested, 1997; Sehested et al., 1999b) and the movements of water (Engelhart, 1970) and ions (Gemmell and Stacy, 1973) across the ruminal wall are restricted and regulated. Overall, the forestomach of lactating dairy cows limits diffusion of water while removing osmotically active particles from the ruminal solution. In a manner that appears analogous to the kidney, the RR actively removes ions from the ruminal solution with a recycling bicarbonate system.

The sodium bicarbonate secreted in the saliva is important for the removal of VFA (Gaebel and Sehested, 1997) and osmolality (Welch, 1982) from the ruminal solution. Compared to other species, ruminant saliva is rich in sodium bicarbonate (McDougall, 1948). Lactating dairy cows produce large amounts of saliva each day and this measurement has increased over time (Table 1.4). It could be argued that lactating dairy cows have been selected for their ability to produce saliva and its associated sodium bicarbonate. Overall, cows in this experiment would be predicted to produce about 38 Eq of sodium bicarbonate equivalent each day based on a predicted mean saliva production (254 L/d) and 150 mEq of bicarbonate equivalent per L (Erdman, 1988a).

In the context of this relationship, a net sodium bicarbonate cycle can be predicted in lactating dairy cows (Figure 4.15). With sodium bicarbonate in solution, sodium provides a positive charge and bicarbonate provides a negative charge and both ions are osmotically active (Weast, 1978). In contrast to sodium, bicarbonate is ephemeral and this provides a mechanism for the removal of a proton. Sodium bicarbonate is secreted by the salivary glands and passes in solution to the RR. In the ruminal solution, bicarbonate neutralizes a proton and decomposes to carbon dioxide and water leaving the charged pair of sodium and VFA. The sodium and VFA are absorbed across the ruminal wall and, with metabolism of the VFA in the ruminal wall or elsewhere in the body, a net bicarbonate ion is regenerated. The net bicarbonate and sodium can then be recycled through the salivary gland. This cycle is similar in charge balance to the alkaline and acid tides from the lower digestive tract.

The charges of sodium bicarbonate balance and exchange with the charges of a disassociated VFA produced in the ruminal fermentation (Figure 1.6). The overall net result of this scheme is that osmotic particles are removed from ruminal solution with a balance of charge. Within this scheme, sodium is just as important as bicarbonate in the regulation of acids produced by the microbial mass in the RR. Indeed, ruminal VFA concentration has been linked to ruminal sodium absorption in vitro (Gaebel et al., 1987a; Uppal and Martens, 2002; Uppal et al., 2003).

Bicarbonate enters the RR through several paths: from the saliva (Bailey and Balch, 1961b), from the ruminal epithelium (Gaebel and Sehested, 1997; Sehested et al., 1999b), and from gas over the ruminal solution (Kohn and Dunlap, 1998) but only the bicarbonate from the salivary glands can produce the net removal of a proton.

Bicarbonate in the ruminal solution recycles and is probably best considered as an expanding and contracting pool. In contrast, sodium is a element, stable and unchanging within the body of the lactating dairy cow.

Beyond ruminal concentrations, this experiment provides the data necessary to calculate the amounts of certain ions moving through the RR and, with this data, steady-state ruminal turnovers for sodium, potassium, chloride, water and VFA can be calculated with reasonable confidence. Given measurements of daily chewing times, diet composition, water intake, and ruminal pools and concentrations and the assumptions of ruminal VFA production (Oba and Allen, 2003a), saliva composition (Bailey and Balch, 1961), and salvia flow (Cassida and Stokes, 1986; Maekawa et al., 2002), predictions can be made by treatment for amounts of ions and water entering the RR and ruminal pool sizes (Table A.25).

Calculating turnovers, VFA and sodium turnovers were the highest and water and chloride turnovers were intermediate (Table 4.19). Potassium turnover was similar to reported liquid passage rates. Across dietary treatments, VFA and sodium turnover might be viewed as similar and perhaps VFA and sodium absorption are connected in vivo. If this is true, then total sodium absorbed each day across the ruminal wall would have to be equal to the VFA crossing the ruminal wall unmetabolized to maintain charge balance and bicarbonate must be actively recycled and regenerated. This proposal remains to be tested but this experiment is consistent with the possibility that sodium absorption is associated with VFA absorption.

SUMMARY AND CONCLUSION

In this experiment, ruminal pH was positively related to ruminal sodium concentration and negatively related to ruminal potassium and ammonium concentrations. Ruminal sodium concentration was negatively related to ruminal potassium and ammonium concentrations and more strongly to their sum. The sum of ruminal sodium, potassium, and ammonium concentrations was similar across diets despite dietary treatment differences. Across all treatments, an alkalizing (positive) SID was maintained in the ruminal solution. The alkalizing (positive) strong ion difference and charge balance in the rumen are likely regulated by modification of sodium flux across the ruminal epithelium. Sodium and VFA absorption may be linked in vivo which is consistent with observations in vitro.

Table 4.1. Selected descriptive results for this experiment published previously in Oba and Allen (2003a), Oba and Allen (2003b), and Oba and Allen (2003c).

	High Starch		Low Starch				Significance, *P*		
	HM[1]	DG[2]	HM	DG	SE	n	Starch[3]	Corn[4]	INT[5]
DMI, kg/d	20.8[b]	22.5[a]	19.7[b]	19.6[b]	0.5	32	<0.001	0.12	0.07[6]
Meals size, kg	1.9[b]	2.3[a]	2.1[ab]	2.0[ab]	0.1	32	0.53	0.21	0.06[6]
TRDOM[7], kg/d	11.3	10.3	9.3	7.7	0.6	32	<0.001	0.03	0.60
Starch intake, kg/d	6.2[b]	7.0[a]	3.9[c]	4.1[c]	0.1	32	<0.001	<0.001	<0.01[6]
Starch digested in the rumen, kg/d	4.3	3.3	2.4	1.9	0.2	32	<0.001	<0.001	0.14
Microbial N, g/d	434	484	354	347	30.7	32	<0.01	0.48	0.37
Daily mean pH	6.12	6.13	6.25	6.32	0.05	32	<0.01	0.41	0.48
Estimated VFA, moles/d	68.8	52.0	58.0	43.7	5.7	32	0.13	0.01	0.89
Eating time, min/d	253.2	258.9	300.4	287.0	9.2	32	<0.001	0.77	0.38
Ruminating time, min/d	427.1	438.2	493.4	478.3	12.5	32	<0.001	0.87	0.31
Total chewing, min/d	680.2	697.0	793.8	765.3	16.2	32	<0.001	0.77	0.22
Milk yield, kg/d	38.8	38.4	33.4	34.3	0.9	32	<0.001	0.78	0.45
3.5% FCM yield, kg/d	35.7	38.7	35.7	35.4	1.0	32	0.12	0.21	0.14

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Student's *t*-test was conducted if $P < 0.10$ for interaction of main effects. Treatment means in a row with different superscript letters differ ($P < 0.05$).
[7] TRDOM = true ruminally degraded organic matter.

Table 4.2. Strong ion composition of ingredients used to formulate experimental diets (% of dietary DM).

Strong ion, % of DM	CS[1]	AS[2]	DC[3]	HM[4]	SMP[5]	SMV[6]
Na^+	0.16	0.24	0.17	0.19	0.24	7.68
K^+	0.88	2.00	0.40	0.42	1.90	0.20
Cl^-	0.15	0.54	0.07	0.06	0.09	3.53

[1] CS: Corn silage.
[2] AS: Alfalfa silage.
[3] DC: Dry corn.
[4] HM: High moisture corn.
[5] Protein mix contained 70.2% soybean meal, 26.9% distillers grain, and 2.9% blood meal.
[6] Vitamin & mineral mix contains 50.0% dry ground corn, 15.6% sodium bicarbonate, 11.3% limestone, 10.4% dicalcium phosphate, 4.9% salt, 4.5% magnesium oxide, 2.5% trace mineral premix, 0.85% Vitamin ADE mix.

Table 4.3. Ingredient composition of experimental diets (% of dietary DM).

	High Starch		Low Starch	
Diet Ingredient	**HM[1]**	**DG[2]**	**HM**	**DG**
Dry ground corn	...	31.6	...	10.8
High moisture corn	32.0	...	11.0	...
Corn silage	20.8	20.9	31.8	32.0
Alfalfa silage	22.2	22.3	34.0	34.1
Protein mix[3]	21.4	21.5	19.5	19.5
Vitamin & mineral mix[4]	3.6	3.7	3.7	3.6

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Protein mix contained 70.2% soybean meal, 26.9% distillers grain, and 2.9% blood meal.
[4] Vitamin & mineral mix contains 50.0% dry ground corn, 15.6% sodium bicarbonate, 11.3% limestone, 10.4% dicalcium phosphate, 4.9% salt, 4.5% magnesium oxide, 2.5% trace mineral premix, 0.85% Vitamin ADE mix.

Table 4.4. Composition of experimental diets (% of dietary DM).

	High Starch		Low Starch	
	HM[1]	DG[2]	HM	DG
DM	48.8	53.0	42.8	43.8
OM	93.4	93.5	92.2	92.3
Starch	31.1	32.2	21.0	21.3
NDF	23.1	24.2	30.1	30.5
ADF	15.2	15.4	20.8	20.9
Lignin	2.2	2.2	3.3	3.3
iNDF[3]	10.9	11.2	14.6	14.7
CP	18.0	18.0	18.3	18.3
Ether extract	5.2	5.5	4.8	4.9
Forage NDF	16.5	16.5	25.3	25.4
Corn grain starch, % of dietary starch	68.8	69.8	35.0	36.2
Na^+	0.47	0.47	0.48	0.47
K^+	1.18	1.17	1.39	1.39
Cl^-	0.32	0.32	0.39	0.38
DCAD3, mEq/100g DM[4]	41.8	41.5	45.5	45.1

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Indigestible NDF: estimated after 120-h in vitro ruminal fermentation.
[4] DCAD3 is sodium plus potassium minus chloride.

Table 4.5. The effect of dietary treatment on feeding behavior.

	High Starch		Low Starch				Significance, *P*		
	HM[1]	DG[2]	HM	DG	SE	n	Starch[3]	Corn[4]	INT[5]
Water intake, L/d	94.2	91.8	84.0	88.4	5.1	32	0.10	0.79	0.40
Drinking bouts, /d	15.6	14.3	13.3	12.5	1.8	32	0.07	0.33	0.77
Water intake, L/bout	7.3	7.0	6.9	8.5	1.2	32	0.56	0.48	0.34
Meals, /d	11.9	11.1	11.6	9.8	0.7	32	0.23	0.06	0.40
Meal size, kg DM	1.7	1.9	1.7	1.9	0.1	32	0.96	0.13	0.84
DMI, kg/d	19.8^{ab}	20.9^{a}	19.6^{ab}	17.8^{b}	0.9	32	0.03	0.68	0.06[6]
Meal length, min/meal	25.8	26.8	28.7	32.0	2.4	32	0.03	0.22	0.53
Intermeal interval, min	89.8^{b}	83.4^{b}	87.9^{b}	107.7^{a}	6.3	32	0.08	0.27	0.04[6]
Eating time, min/d	230.4^{b}	244.2^{ab}	264.2^{a}	241.9^{b}	8.3	32	0.03	0.54	0.02[6]
Ruminating bouts, /d	14.8	14.4	16.1	14.8	1.0	32	0.24	0.24	0.49
Ruminating time, min/bout	29.5	30.8	30.7	33.7	2.4	32	0.33	0.30	0.68
Inter-ruminating interval, min	65.3	64.4	56.3	57.9	4.4	32	0.06	0.92	0.74
Ruminating time, min/d	415.8	434.9	467.8	478.7	23.2	32	0.006	0.34	0.79
Total chewing time, min/d	646.2	679.1	732.0	720.6	26.8	32	0.003	0.57	0.24

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Student's *t*-test was conducted if P was < 0.10 for interaction of main effects. Treatment means in a row with different superscript letters differ ($P < 0.05$).

Table 4.6. The effect of dietary treatment on ruminal pools.

	High Starch		Low Starch				Significance, *P*		
Ruminal pool	**HM**[1]	**DG**[2]	**HM**	**DG**	**SE**	**n**	**Starch**[3]	**Corn**[4]	**INT**[5]
Volume, L	93.6	98.5	104.3	99.8	6.2	31	0.12	0.97	0.21
Weight, kg	72.5	75.1	82.1	78.8	5.2	31	0.02	0.89	0.28
DM, kg	8.8	9.6	9.5	9.3	0.5	31	0.60	0.37	0.18
Water, kg	63.7	65.5	72.7	69.6	4.8	31	0.01	0.78	0.31

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.

Table 4.7. The effect of dietary treatment on ruminal pH.

	High Starch		Low Starch				Significance, *P*		
Ruminal pH	**HM**[1]	**DG**[2]	**HM**	**DG**	**SE**	**n**	**Starch**[3]	**Corn**[4]	**INT**[5]
Mean	6.17	6.21	6.20	6.31	0.12	31	0.22	0.19	0.53
SD	0.34	0.34	0.33	0.27	0.03	31	0.09	0.20	0.19
Effective maximum[6]	7.05	7.20	6.96	6.99	0.10	31	0.04	0.22	0.44
Effective minimum[6]	5.48	5.44	5.50	5.54	0.11	30	0.31	0.95	0.48
Effective range[6]	1.62	1.76	1.46	1.45	0.13	30	0.02	0.50	0.42
Area under pH 6.0, ph*30seconds	387	304	307	179	145	31	0.13	0.12	0.73
Time under pH 6.0, h/d	8.8	7.2	8.4	4.8	2.7	31	0.30	0.07	0.46

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Minimum, maximum, and range were calculated using the daily 95% confidence interval (2.5th and 97.5th percentiles) of ruminal pH measured every 5 seconds.

Table 4.8. The effect of dietary treatment on volatile fatty acid concentration of ruminal fluid.

	High Starch		Low Starch				Significance, *P*		
Ruminal VFA	**HM**[1]	**DG**[2]	**HM**	**DG**	**SE**	**n**	**Starch**[3]	**Corn**[4]	**INT**[5]
Total VFA, mM	101.8	105.4	102.3	96.8	5.5	32	0.18	0.75	0.14
Acetate, mM	58.4	60.5	62.4	60.4	2.9	32	0.24	0.93	0.14
Propionate, mM	25.9	27.3	22.3	20.7	2.3	32	0.005	0.98	0.35
Butyrate, mM	12.3	12.5	12.6	11.3	0.8	32	0.34	0.25	0.14
Iso-butyrate, mM	1.1	1.1	1.2	1.2	0.1	32	0.02	0.63	0.50
Valerate, mM	2.4	2.2	1.9	1.6	0.3	32	0.01	0.28	0.97
Iso-valerate, mM	1.8	1.8	1.9	2.0	0.2	32	0.05	0.97	0.85
Total branched chain VFA, mM	2.9	2.9	3.2	3.2	0.2	32	0.03	0.84	0.94
Estimated Associated VFA[6], mM	8.0	6.6	6.6	4.9	1.9	31	0.15	0.16	0.85
Estimated Disassociated VFA[6], mM	95.8	98.7	95.7	91.9	4.6	31	0.17	0.87	0.18

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Assumes an aggregate VFA pKa of 4.8.

Table 4.9. Distribution of ruminal ions measured across cows and periods.

	percentile							
Ruminal ions, mEq/L	**97.5**	**75**	**50**	**25**	**2.5**	**Mean**	**SD**	**n**
Sodium	122.1	107.0	97.7	86.4	65.6	96.3	14.7	2260
Potassium	63.8	45.4	39.8	34.4	25.6	40.7	9.3	2249
Ammonia	16.1	5.4	2.9	1.6	0.5	4.2	3.9	2268
Chloride	17	14	12	10	8	12	2	2265

Table 4.10. The effect of dietary treatment on ruminal ion concentration.

	High Starch		Low Starch				Significance, *P*		
Ruminal ions, mEq/L	**HM**[1]	**DG**[2]	**HM**	**DG**	**SE**	**n**	**Starch**[3]	**Corn**[4]	**INT**[5]
Na^+	99.5	97.5	93.6	94.7	3.8	32	0.03	0.80	0.39
K^+	37.6	39.0	42.8	43.1	2.3	32	0.007	0.57	0.74
NH_4^+	3.0	3.8	5.3	4.6	1.0	32	0.03	0.93	0.24
Cl^-	11^c	12^b	13^a	13^a	1	32	<0.001	0.07	0.10[6]
Na^+ and K^+	137.1	136.4	136.3	137.9	2.8	32	0.73	0.65	0.28
K^+ and NH_4^+	40.6	42.8	48.1	47.7	2.8	32	0.003	0.61	0.48
Na^+, K^+ and NH_4^+	140.0	140.3	141.6	142.4	2.3	32	0.14	0.67	0.79
Cl^- and VFA^{-7}	106.2	110.5	108.5	104.9	4.5	31	0.52	0.89	0.13
Cl^-, VFA^{-7}, and bicarbonate[8]	129.4	135.0	132.1	130.4	5.5	31	0.76	0.54	0.25
Strong ion difference[9]	126.5	124.7	123.5	125.0	3.0	32	0.17	0.89	0.11

[1] HM: High moisture corn.

[2] DG: Dry ground corn.

[3] Starch: Effect of dietary starch content.

[4] Corn: Effect of conservation method of corn grain.

[5] INT: Interaction of dietary starch content and conservation method of corn grain.

[6] Student's *t*-test was conducted if $P < 0.10$ for interaction of main effects. Treatment means in a row with different superscript letters differ ($P < 0.05$).

[7] Assumes an aggregate VFA pKa of 4.8.

[8] Calculated ruminal bicarbonate based on Kohn and Dunlap (1998) assuming ruminal pCO_2 of 0.5 atm.

[9] Strong Ion Difference is Na + K – Cl in mEq/L.

Table 4.11. The effect of dietary treatment on whole blood measurements.

	High Starch		Low Starch				Significance, *P*		
Whole blood at 38.5°C	**HM[1]**	**DG[2]**	**HM**	**DG**	**SE**	**n**	**Starch[3]**	**Corn[4]**	**INT[5]**
Sodium, mmol/L	142.3	142.8	142.3	142.3	0.3	32	0.42	0.37	0.35
Potassium, mmol/L	4.0	4.1	4.1	4.1	0.1	32	0.58	0.47	0.63
Chloride, mmol/L	105.7	106.1	106.2	106.1	0.7	32	0.61	0.83	0.67
Calcium, mmol/L	1.2	1.3	1.3	1.3	0.0	32	0.06	0.67	0.99
Ionized normalized calcium[6], mmol/L	1.3	1.3	1.3	1.3	0.0	32	0.23	0.80	0.84
Anion gap[7], mmol/L	16.4	16.3	16.4	16.5	0.3	32	0.78	0.97	0.59
Hematocrit, %	31	31	31	31	0	32	0.71	0.37	0.92
Calculated hemoglobin[8], g/dl	10.4	10.5	10.3	10.4	0.2	32	0.71	0.37	0.92

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Normalized to pH 7.4.
[7] Anion Gap is (Na + K) – (Cl + bicarbonate) in mmol/L.
[8] Assumes hematocrit is 1/3 hemoglobin.

Table 4.12. The effect of dietary treatment on whole blood gases and related measures.

	High Starch		Low Starch				Significance, *P*		
Whole blood at 38.5°C	**HM**[1]	**DG**[2]	**HM**	**DG**	**SE**	**n**	**Starch**[3]	**Corn**[4]	**INT**[5]
pH	7.430	7.425	7.417	7.417	0.008	32	0.02	0.59	0.51
Partial pressure of CO_2, mm Hg	36.1	36.8	36.3	36.5	0.9	32	0.94	0.42	0.60
Base excess extracellular fluid, mmol/L	-0.4	-0.2	-1.0	-0.9	0.9	32	0.19	0.78	0.85
Base excess of blood, mmol/L	0.7	0.9	0.1	0.2	0.8	32	0.18	0.79	0.86
Total CO_2 content, mmol/L	25.2	25.6	24.9	24.9	0.8	32	0.30	0.69	0.76
Calculated HCO_3^-, mmol/L	24.1	24.4	23.7	23.8	0.8	32	0.28	0.71	0.76
Standardized bicarbonate[6], mmol/L	24.8	25.0	24.4	24.4	0.7	32	0.19	0.82	0.82
Partial pressure of O_2, mm Hg	52.7	53.3	50.4	49.9	1.1	32	0.008	0.94	0.60
O_2 saturation, %	84.2	84.1	82.7	81.8	0.6	32	0.001	0.33	0.47
O_2 content, ml/dl	12.1	12.2	11.9	11.8	0.2	32	0.02	0.76	0.63

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] Standardized to partial pressure of carbon dioxide of 40 mm Hg.

Table 4.13. The effect of dietary treatment on ruminal fluid and plasma osmolality.

	High Starch		Low Starch				Significance, *P*		
Osmolality, mOsm/kg	**HM[1]**	**DG[2]**	**HM**	**DG**	**SE**	**n**	**Starch[3]**	**Corn[4]**	**INT[5]**
Ruminal	262	265	263	258	4	32	0.47	0.84	0.30
SD[6] of ruminal	21.6	19.4	17.6	16.7	1.6	32	0.02	0.27	0.65
Ruminal plus calculated bicarbonate[7]	287	290	286	284	5	31	0.33	0.98	0.45
Plasma	283	285	285	285	1	32	0.47	0.37	0.50
SD[6] of plasma	4.5	4.4	4.3	4.0	0.4	32	0.48	0.86	0.50
Plasma plus measured bicarbonate[8]	308	310	309	309	1	32	0.81	0.30	0.44
Plasma minus ruminal	21	20	22	27	4	32	0.34	0.62	0.40
Plasma minus ruminal (with bicarbonate added)[7, 8]	20	20	22	25	5	31	0.30	0.61	0.63

[1] HM: High moisture corn.
[2] DG: Dry ground corn.
[3] Starch: Effect of dietary starch content.
[4] Corn: Effect of conservation method of corn grain.
[5] INT: Interaction of dietary starch content and conservation method of corn grain.
[6] SD is standard deviation.
[7] Calculated ruminal bicarbonate based on Kohn and Dunlap (1998) assuming ruminal pCO_2 of 0.5 atm.
[8] Measure bicarbonate from blood gas analysis.

Table 4.14. Pearson correlation coefficients[1] among mean cow period ruminal measurements (n≥31).

Ruminal Measures	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**	**(9)**	**(10)**	**(11)**
(1) pH	1.00										
(2) Est.[2] Disassociated VFA, mM	-0.12	1.00									
(3) Total VFA, mM	-0.42	0.95	1.00								
(4) Na^+, mEq/L	0.74	-0.05	-0.26	1.00							
(5) K^+, mEq/L	-0.31	0.20	0.28	-0.68	1.00						
(6) NH_4^+, mEq/L	-0.69	0.11	0.31	-0.79	0.47	1.00					
(7) Cl^-, mEq/L	-0.14	-0.30	-0.25	-0.26	0.15	0.36	1.00				
(8) Na^+ plus K^+, mEq/L	0.74	0.08	0.13	0.79	-0.10	-0.69	-0.23	1.00			
(9) K^+ plus NH_4^+, mEq/L	-0.49	0.20	0.33	-0.81	0.95	0.72	0.24	-0.31	1.00		
(10) Strong ion difference[3], mEq/L	0.72	0.15	-0.06	0.79	-0.12	-0.72	-0.44	0.98	-0.34	1.00	
(11) Osmolality, mOsm	-0.32	0.52	0.60	-0.08	0.14	0.37	0.05	0.00	0.24	-0.01	1.00
(12) Osmolality plus calculated bicarbonate[4], mOsm	0.45	0.33	0.20	0.44	-0.11	-0.17	-0.07	0.53	-0.14	0.50	0.69

[1] Significant correlation ($P < 0.01$) for variables greater than 0.45 or less than -0.45. Significant correlation ($P < 0.05$) for variables greater than 0.35 or less than -0.35.
[2] Assumes an aggregate VFA pKa of 4.8.
[3] Strong Ion Difference is Na + K – Cl in mEq/L.
[4] Calculated ruminal bicarbonate based on Kohn and Dunlap (1998) assuming ruminal pCO_2 of 0.5 atm.

Table 4.15. Pearson correlation coefficients[1] among mean cow period blood measurements (n=32).

Whole blood at 38.5°C	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**	**(9)**	**(10)**
(1) pH	1.00									
(2) pCO_2, mm Hg	-0.02	1.00								
(3) pO_2, mm Hg	-0.38	-0.15	1.00							
(4) Na^+, mEq/L	0.02	0.32	0.21	1.00						
(5) K^+, mEq/L	-0.07	0.04	0.09	0.38	1.00					
(6) Cl^-, mEq/L	-0.39	-0.69	0.37	0.18	0.38	1.00				
(7) Bicarbonate[2], mEq/L	0.48	0.87	-0.32	0.26	-0.05	-0.82	1.00			
(8) Anion gap[3], mEq/L	-0.38	-0.40	0.29	0.09	-0.12	0.22	-0.53	1.00		
(9) Strong ion difference[4], mEq/L	0.39	0.83	-0.25	0.34	-0.10	-0.86	0.93	-0.18	1.00	
(10) Plasma osmolality, mOsm	-0.03	0.05	0.25	0.67	0.29	0.25	0.01	0.22	0.10	1.00
(11) Plasma osmolality plus measured bicarbonate[2], mOsm	0.27	0.56	0.00	0.69	0.20	-0.30	0.61	-0.15	0.65	0.79

[1] Significant correlation ($P < 0.01$) for variables greater than 0.45 or less than -0.45. Significant correlation ($P < 0.05$) for variables greater than 0.35 or less than -0.35.
[2] Measure bicarbonate from blood gas analysis.
[3] Anion Gap is (Na + K) – (Cl + bicarbonate) in mmol/L.
[4] Strong Ion Difference is Na + K – Cl in mEq/L.

Table 4.16. Pearson correlation coefficients[1] between mean cow period blood and ruminal measurements (n≥31).

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Blood pH	1.00								
(2) Blood Na^+, mEq/L		1.00							
(3) Blood K^+, mEq/L			1.00						
(4) Blood Cl^-, mEq/L				1.00					
(5) Plasma osmolality, mOsm					1.00				
(6) Ruminal pH	0.09	0.17	0.04	-0.17	0.17	1.00			
(7) Ruminal Na^+, mEq/L	0.26	0.42	0.34	-0.18	0.31		1.00		
(8) Ruminal K^+, mEq/L	-0.58	-0.44	-0.47	0.16	-0.31			1.00	
(9) Ruminal Cl^-, mEq/L	0.05	-0.03	0.06	-0.05	0.19				1.00
(10) Ruminal osmolality, mOsm	0.07	0.11	-0.15	-0.30	0.02				

[1] Significant correlation ($P < 0.01$) for variables greater than 0.45 or less than -0.45. Significant correlation ($P < 0.05$) for variables greater than 0.35 or less than -0.35.

Table 4.17. Linear regression results summary.

X	Y	Equation	Adjusted R^2	Significance, *P*
Ruminal [Na^+]	Ruminal pH	Y = 4.30 + 0.02X	0.41	<0.0001
Ruminal [K^+]	Ruminal pH	Y = 7.14 – 0.02X	0.21	<0.0001
Ruminal [Cl^-]	Ruminal pH	Y = 6.44 – 0.02X	0.01	<0.0001
Ruminal [SID]	Ruminal pH	Y = 3.78 + 0.02X	0.23	<0.0001
Ruminal [NH_4^+]	Ruminal pH	Y = 6.47 - 0.06X	0.27	<0.0001
Ruminal [K^+]	Ruminal [Na^+]	Y = 140.08 – 1.08X	0.46	<0.0001
Ruminal [NH_4^+]	Ruminal [Na^+]	Y = 106.76 – 2.50X	0.45	<0.0001
Ruminal [K^+] + [NH_4^+]	Ruminal [Na^+]	Y = 137.79 – 0.93X	0.56	<0.0001
Meal size, kg DM	Change in [Na^+]	Y = 2.38 – 3.53X	0.41	<0.0001
Meal size, kg DM	Change in [K^+]	Y = -2.48 + 3.03X	0.69	<0.0001
Meal size, kg DM	Change in [Na^+] + [K^+]	Y = -0.15 – 0.48X	0.01	0.0386
Meal size, kg DM	Change in mOsm	Y = -5.37 + 6.16X	0.28	<0.0001
Meal size, kg DM	Change in ruminal pH	Y = 0.02 – 0.08X	0.28	<0.0001
Drink size, L	Change in [Na^+]	Y = 0.80 – 0.32X	0.45	<0.0001
Drink size, L	Change in [K^+]	Y = 0.35 – 0.06X	0.09	<0.0001
Drink size, L	Change in [Na^+] + [K^+]	Y = 1.14 – 0.38X	0.43	<0.0001
Drink size, L	Change in mOsm	Y = 2.55 – 0.66X	0.39	<0.0001
Rumination bout length, min	Change in [Na^+]	Y = -1.15 + 0.11X	0.10	<0.0001
Rumination bout length, min	Change in [K^+]	Y = 0.04 – 0.04X	0.07	<0.0001
Rumination bout length, min	Change in [Na^+] + [K^+]	Y = -1.08 + 0.07X	0.04	<0.0001
Rumination bout length, min	Change in mOsm	Y = -0.15 – 0.06X	0.00	0.1455
Rumination bout length, min	Change in ruminal pH	Y = -0.02 + 0.004X	0.06	<0.0001

Table 4.18. Potential ions contributing to ruminal pH according to the strong ion difference theory.

Ions in probable descending order of concentration in RR	99MO01 measured concentration, mEq/L	Estimated ruminal concentration, mEq/L	Into RR?[1]	Out of RR?[1]	Controlled by cow?	Include In Ruminal Theory?
Cation						No
Sodium	96		F, S	W, P	Yes	SID[2]
Potassium	41		F, S	W, P	No	SID
Ammonia	4		F, S, W	W, P, F	No	SID
Calcium, Magnesium		?	F, S	P	No	No?
Other?		?	?	?	No	No
Hydrogen	0		F, R	W, P, R	No	No
Anions						
Disassociated VFA	96		F, R	W, P, R	Yes?	Weak acid
Bicarbonate		24	F, S, W, C	W, P, R, C	Yes	pCO_2
Hydrogen Phosphate		?	F, S	P, R	Yes	Weak acid
Chloride	12		F, S	W, P	No?	SID
Lactate	5 when present		F, R	W, P	No	SID
Feed-		?	F	P, R	No	No?
Other?		?	?	?	No	No
Hydroxyl		0	F	P, R	No	No

[1] RR is reticulorumen. F is "from feed", S is "in saliva", R is "from fermentation", W is "moves across the ruminal wall", C is "formed from excess carbon dioxide", and P is "passage to lower tract".
[2] Strong ion difference.

Table 4.19. Influx, pool size and ruminal turnover of sodium, potassium, chloride, water and VFA in the rumen[1].

	High Starch		Low Starch	
	HM[2]	DG[3]	HM	DG
Entering the RR each day[4]				
Sodium, mEq	44178	44954	45677	45174
Potassium, mEq	7521	7821	8570	7928
Chloride, mEq	3557	3680	3990	3790
Water, L	364	363	368	369
VFA, mEq	59722	39444	54167	29583
Estimated ruminal pool				
Sodium, mEq	5071	5109	5444	5273
Potassium, mEq	1916	2044	2489	2400
Chloride, mEq	535	613	744	718
Water, L	51	52	58	56
VFA, mEq	5188	5523	5956	5390
Turnover rate, /h				
Sodium	0.36	0.37	0.35	0.36
Potassium	0.16	0.16	0.14	0.14
Chloride	0.28	0.25	0.22	0.22
Water	0.30	0.29	0.26	0.28
VFA	0.48	0.30	0.38	0.23

[1] Ruminal DM (kg), ruminal water (L), diet composition, and animal behavior are as measured assuming 10% of ruminal water is the microbial mass and 10% is in the feed and daily VFA production is as per Oba and Allen (2003a).
[2] HM: High moisture corn.
[3] DG: Dry ground corn.
[4] Saliva flows are 0.125 L/min at rest, 0.200 L/min during eating, and 0.250 L/min while ruminating. Saliva composition is 161 mEq sodium/L, 6 mEq potassium/L, and 7 mEq chloride/L as per Bailey and Balch (1961b).

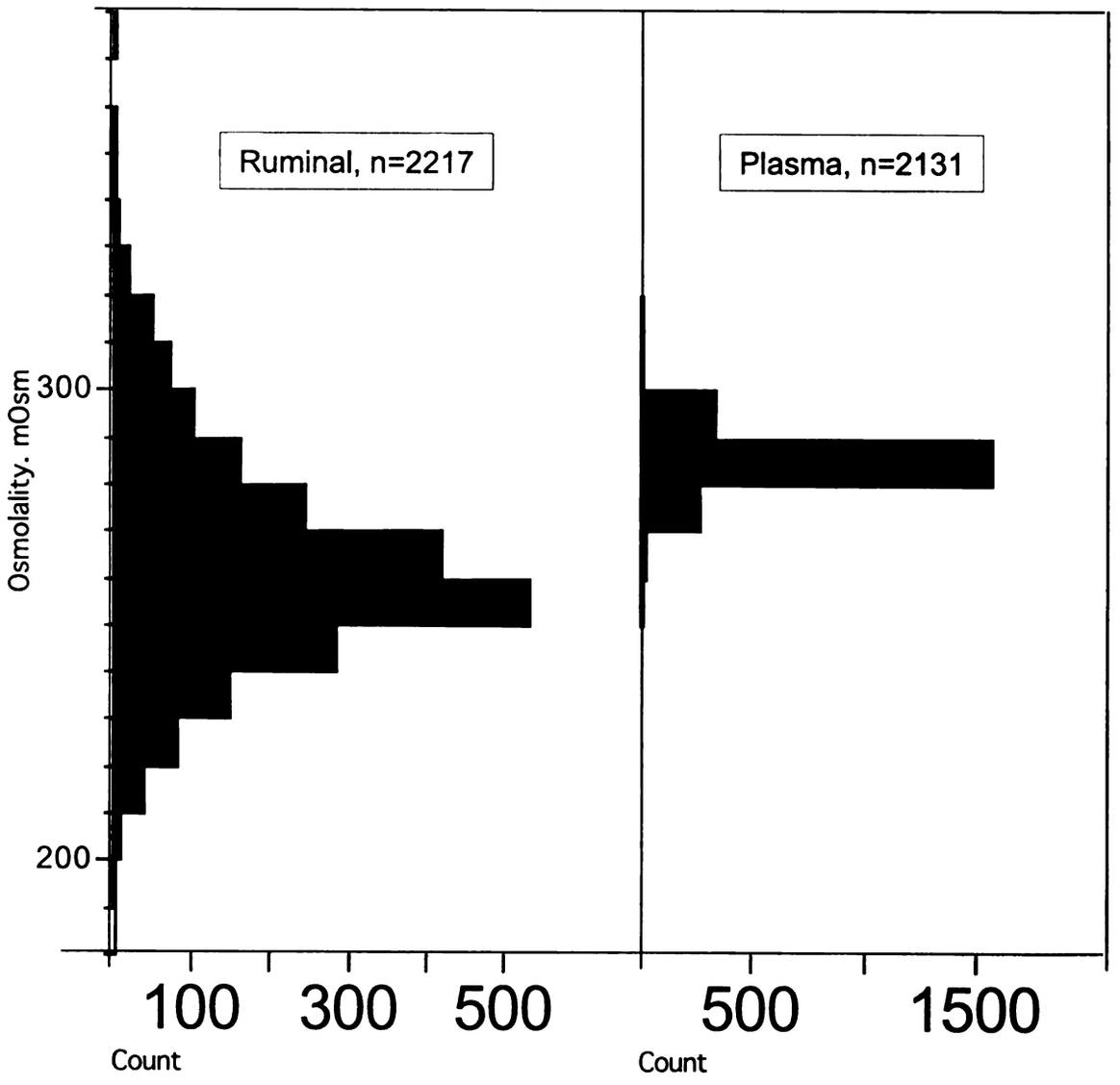

Figure 4.1. A comparison of ruminal and plasma osmolality distributions.

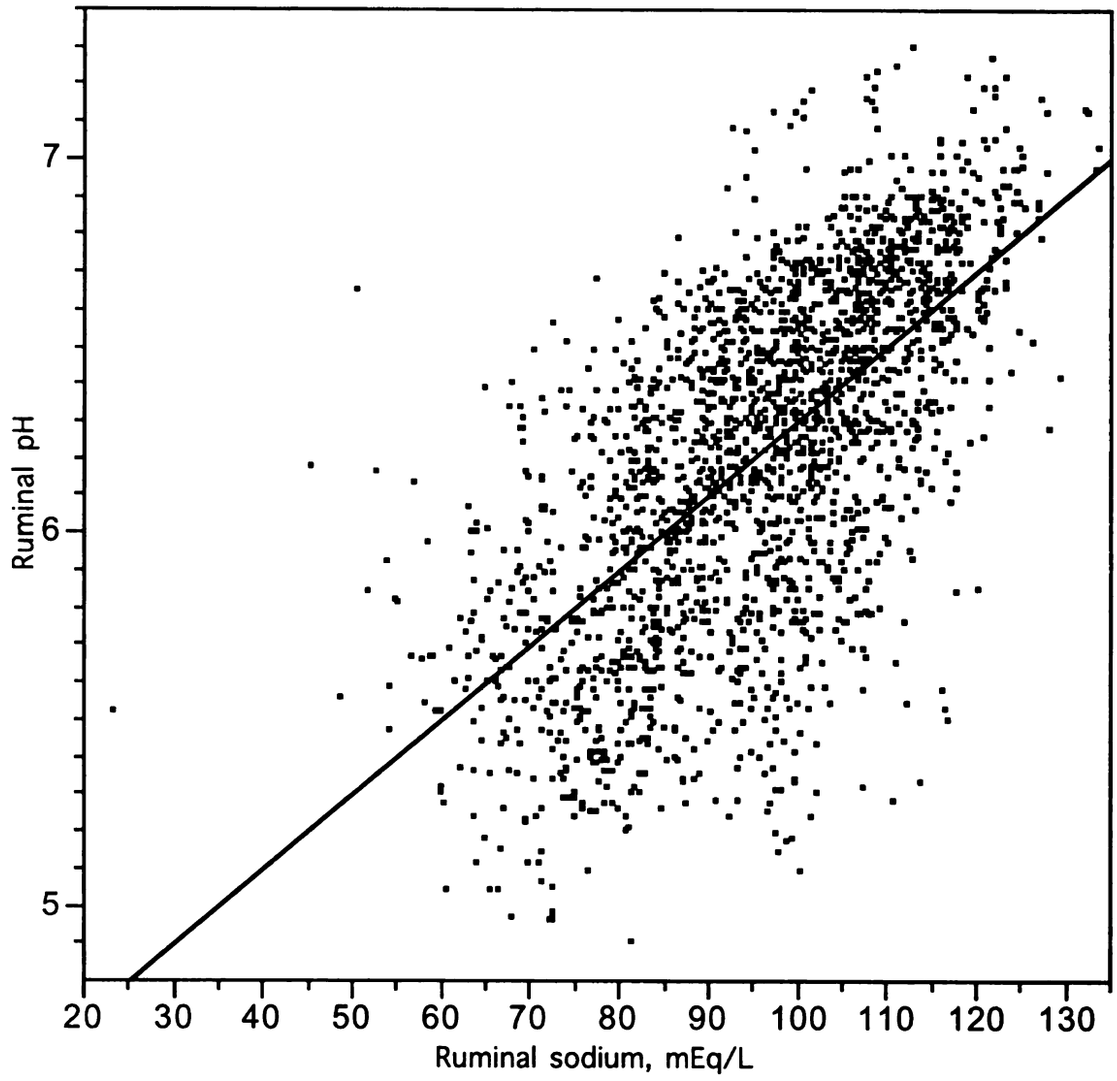

Figure 4.2. The relationship between ruminal sodium and ruminal pH. $R^2 = 0.42$, $P < 0.0001$.

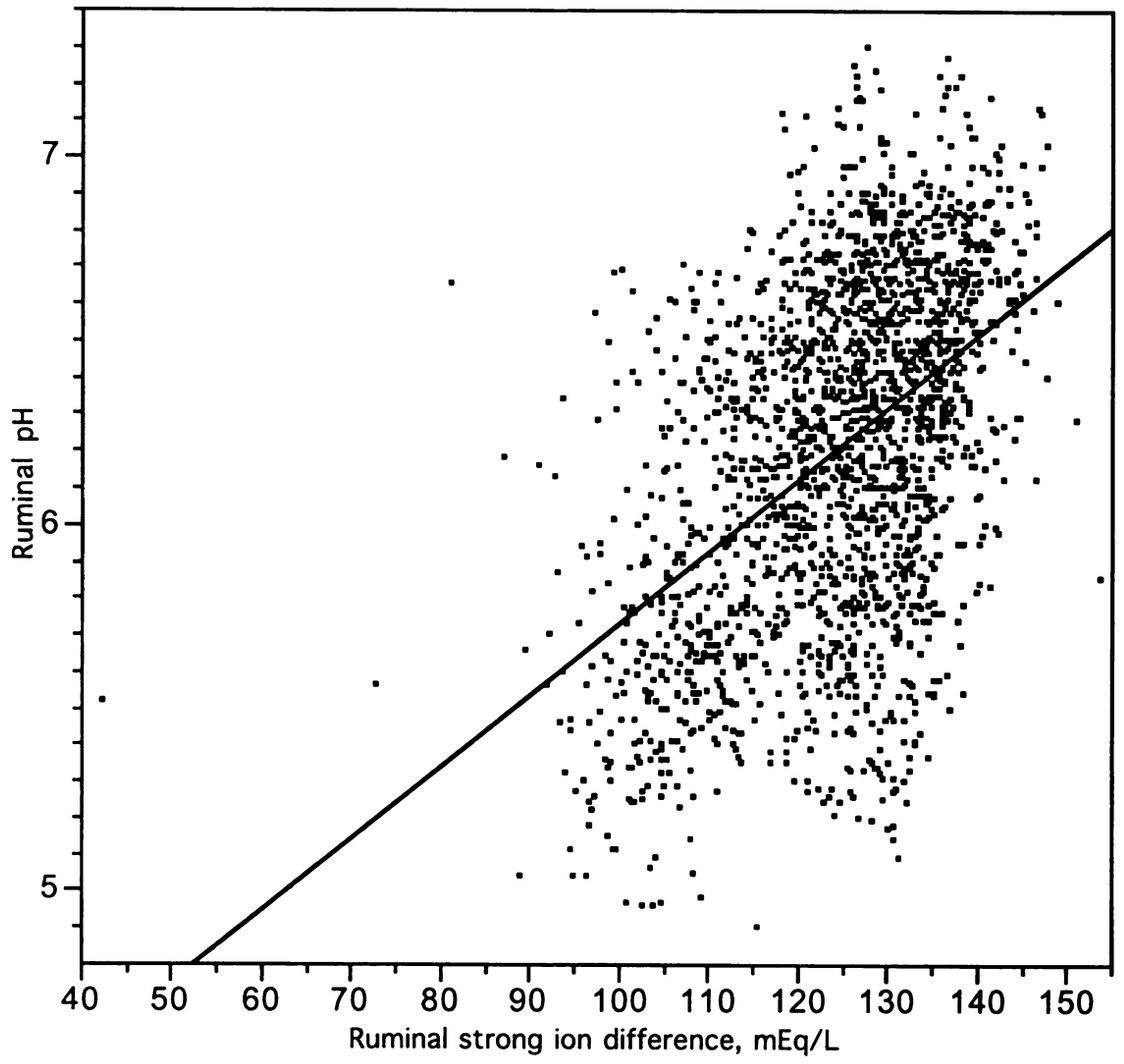

Figure 4.3. The relationship between ruminal SID and ruminal pH. $R^2 = 0.23$, $P < 0.0001$.

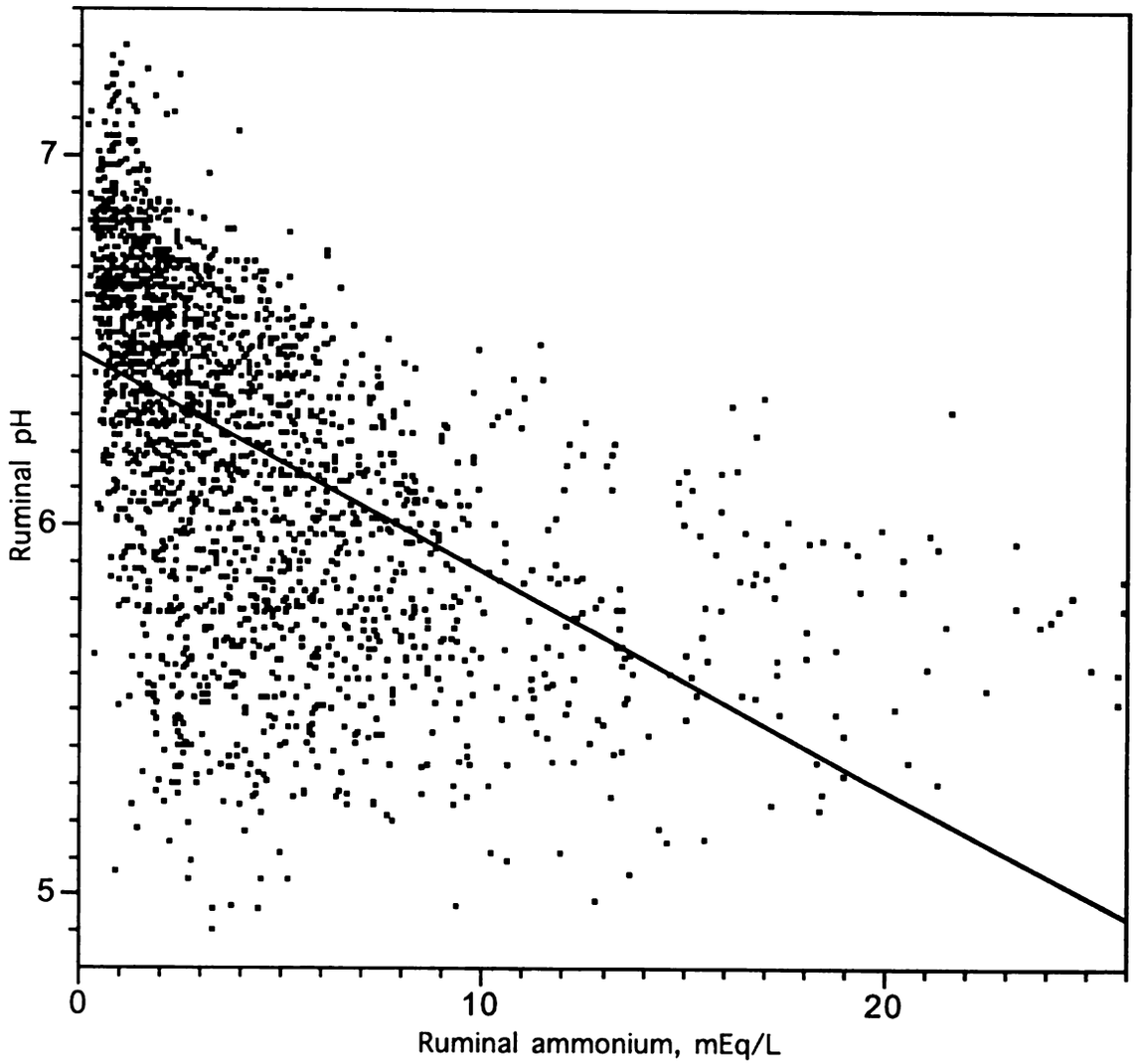

Figure 4.4. The relationship between ruminal ammonia and ruminal pH. $R^2 = 0.27$, $P < 0.0001$.

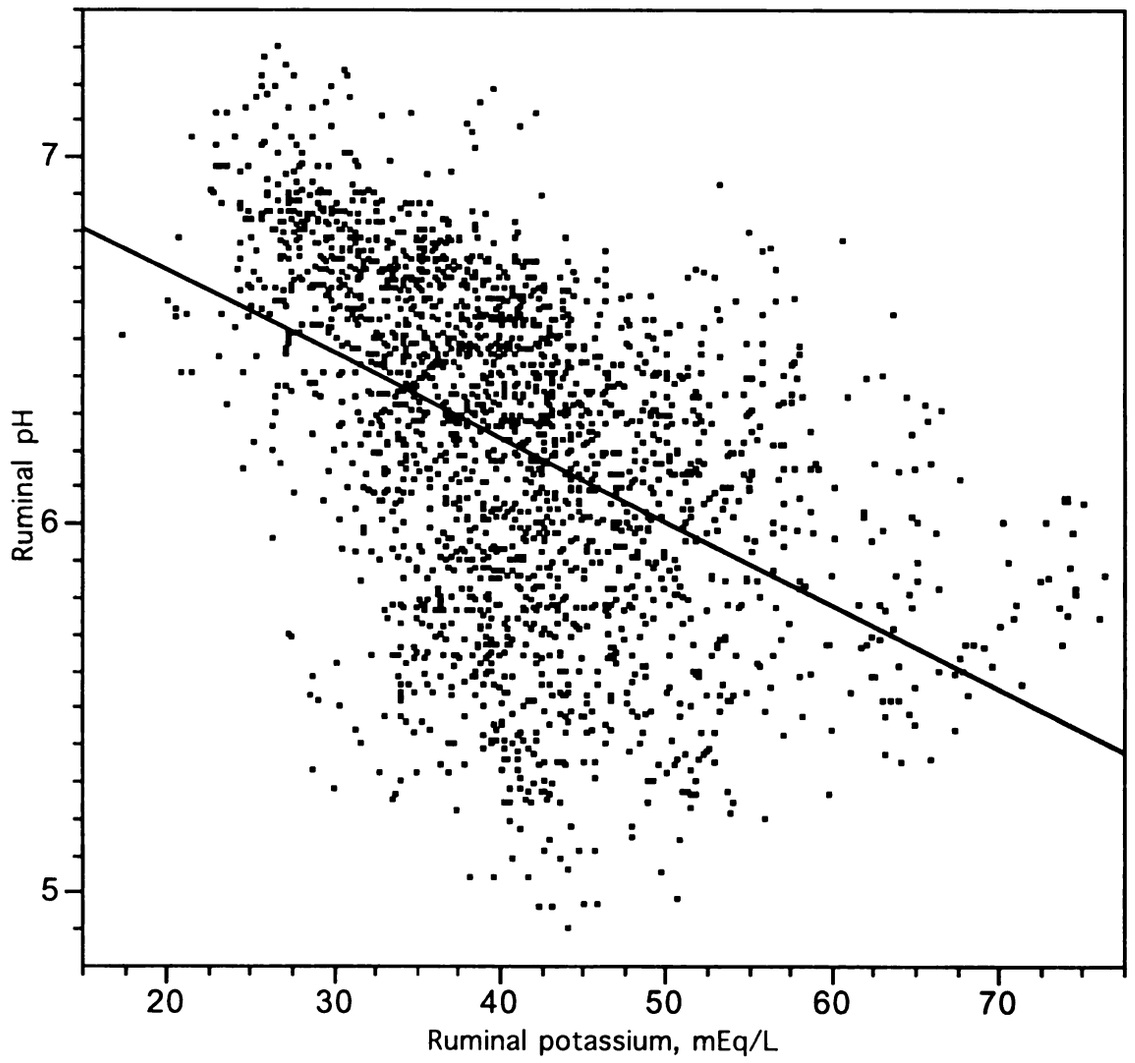

Figure 4.5. The relationship between ruminal potassium and ruminal pH. $R^2 = 0.21$, $P < 0.0001$.

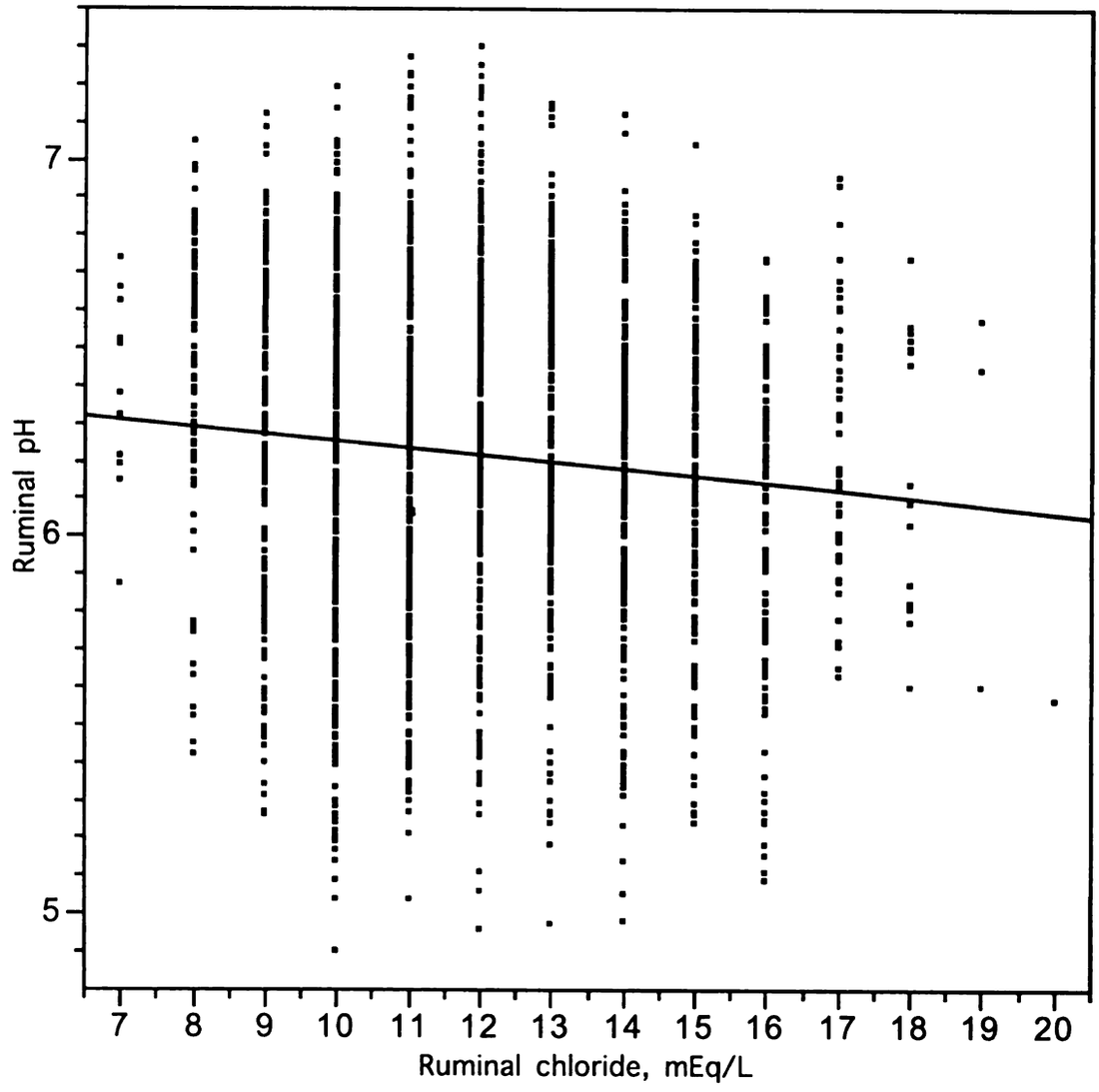

Figure 4.6. The relationship between ruminal chloride and ruminal pH. $R^2 = 0.01$, $P < 0.0001$.

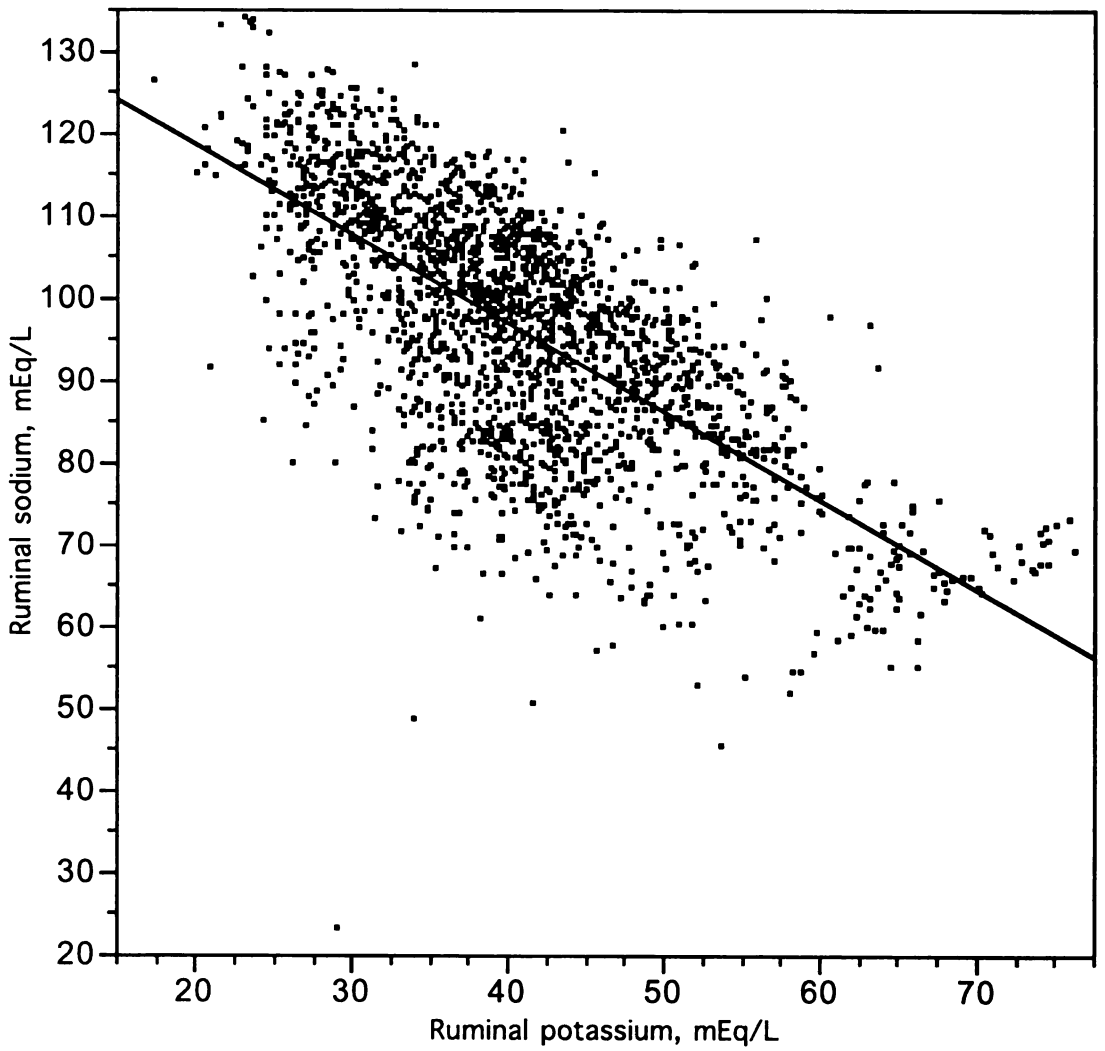

Figure 4.7. The relationship between ruminal potassium and ruminal sodium. $R^2 = 0.46$, $P < 0.0001$.

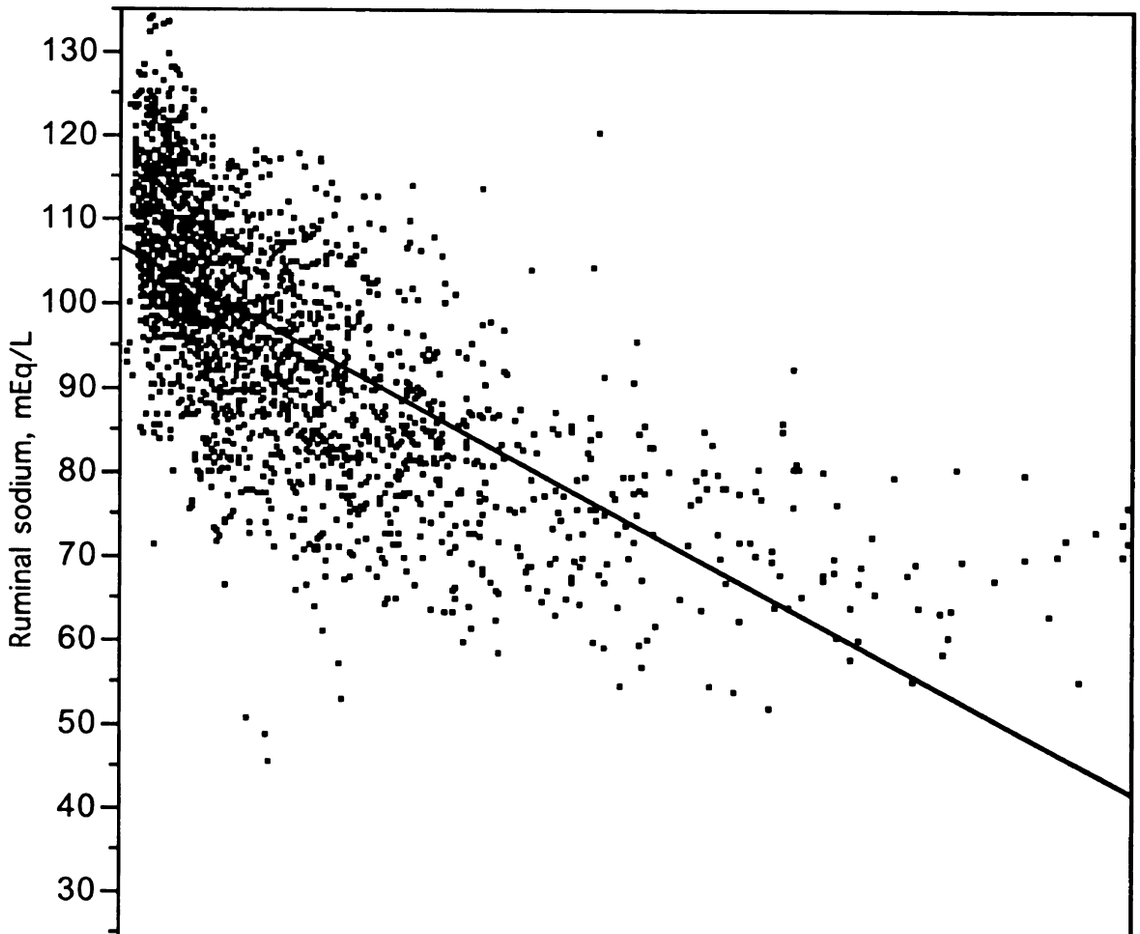

Figure 4.8. The relationship between ruminal ammonia and ruminal sodium. $R^2 = 0.45$, $P < 0.0001$.

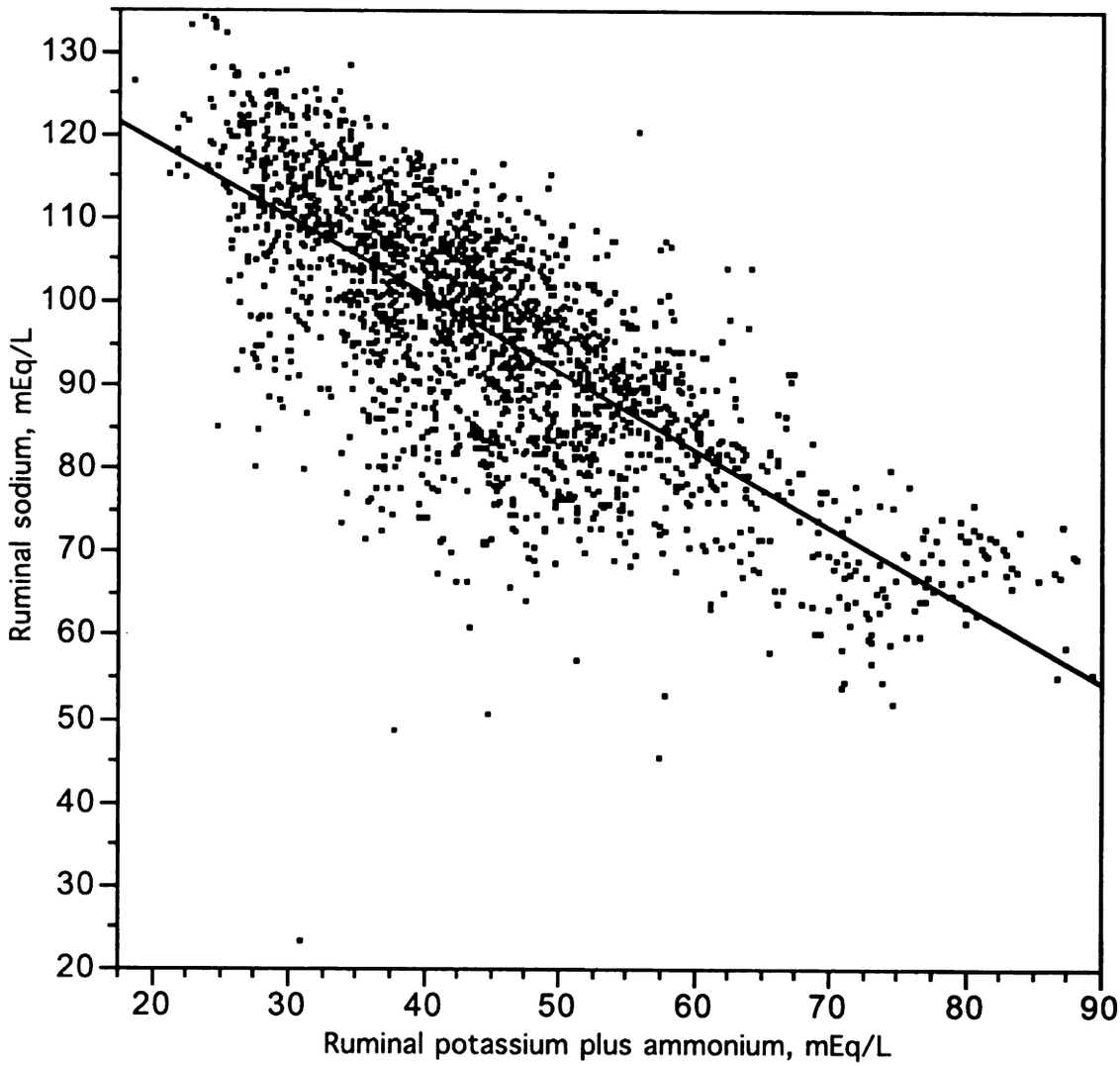

Figure 4.9. The relationship between ruminal ammonia plus potassium and ruminal sodium. $R^2 = 0.56$, $P < 0.0001$.

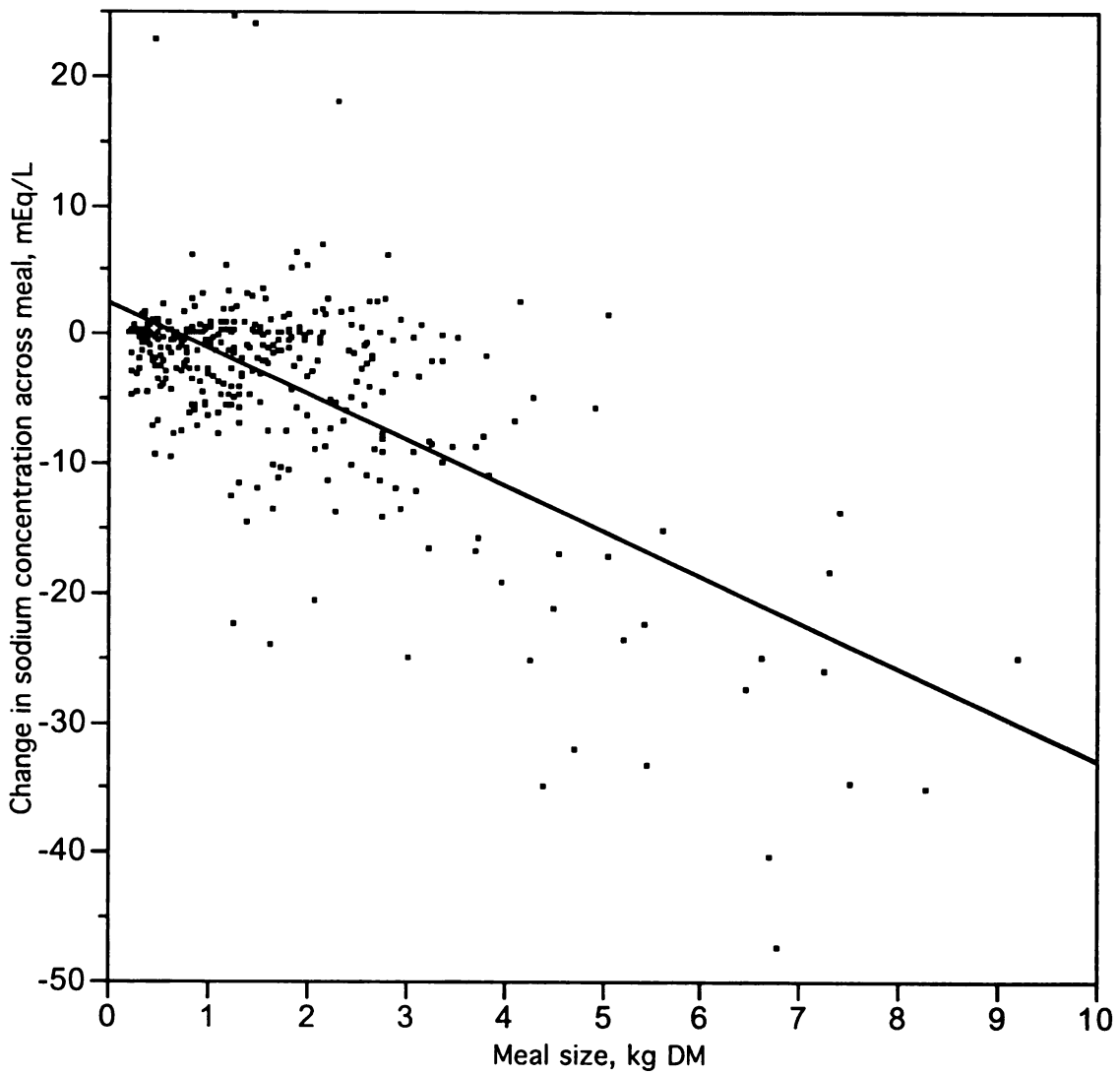

Figure 4.10. The relationship between meal size (kg) and ruminal sodium concentration difference. Overall mean meal was 1.8 kg. $R^2 = 0.42$, $P < 0.0001$.

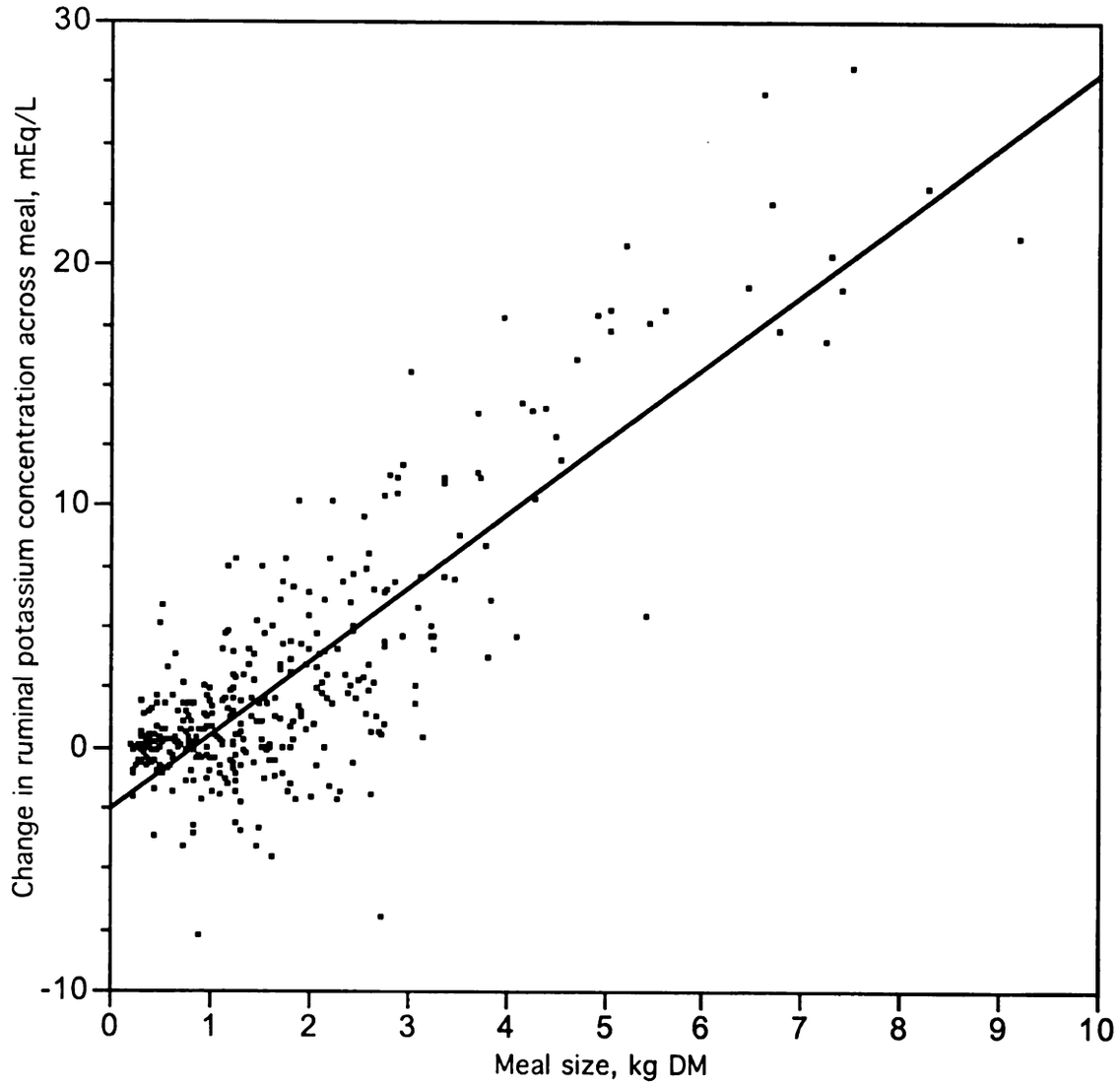

Figure 4.11. The relationship between meal size (kg) and ruminal potassium concentration difference. Overall mean meal was 1.8 kg. $R^2 = 0.69$, $P < 0.0001$.

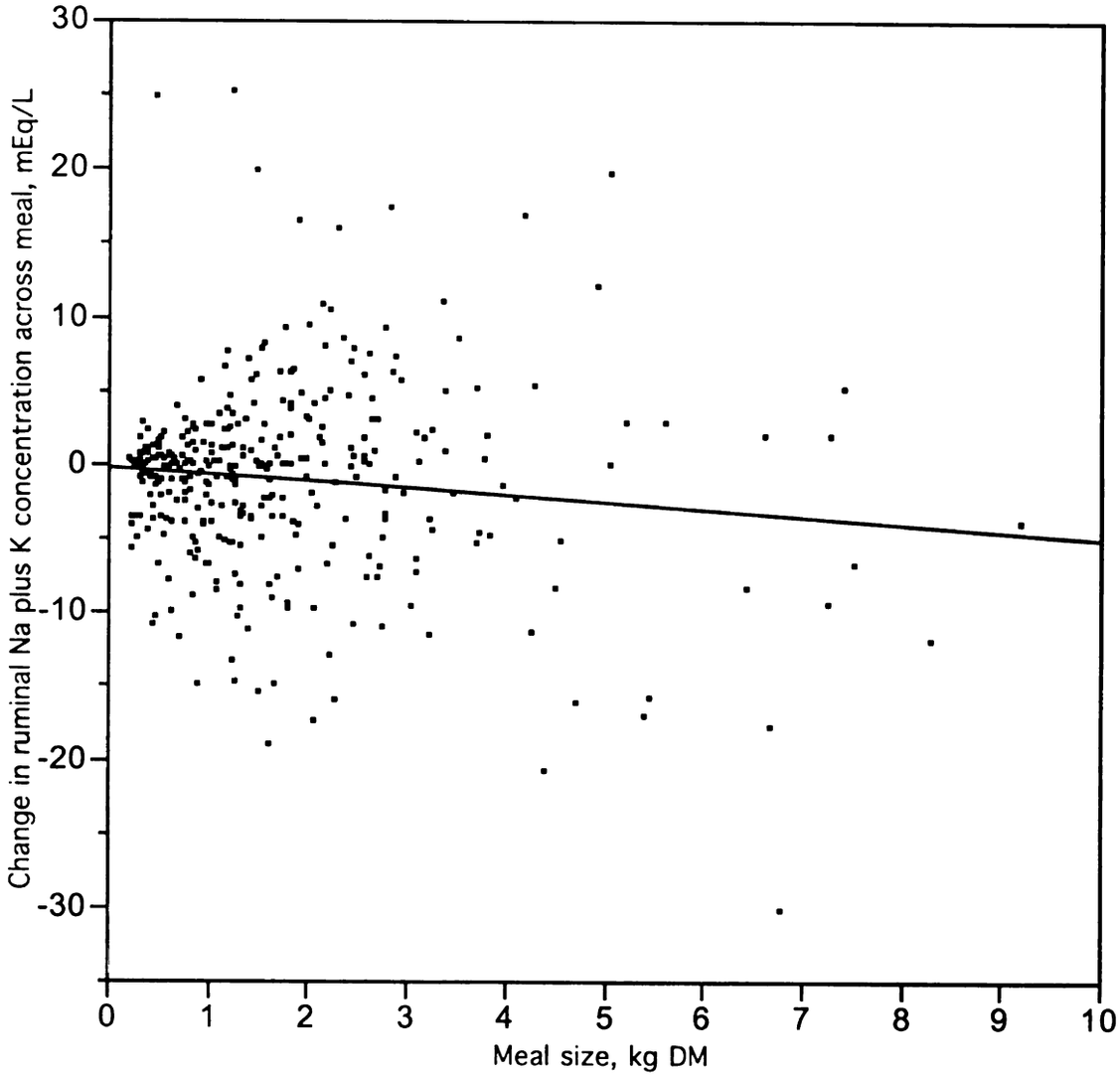

Figure 4.12. The relationship between meal size (kg) and ruminal sodium plus potassium concentration difference. Overall mean meal was 1.8 kg. $R^2 = 0.01$, $P < 0.0386$.

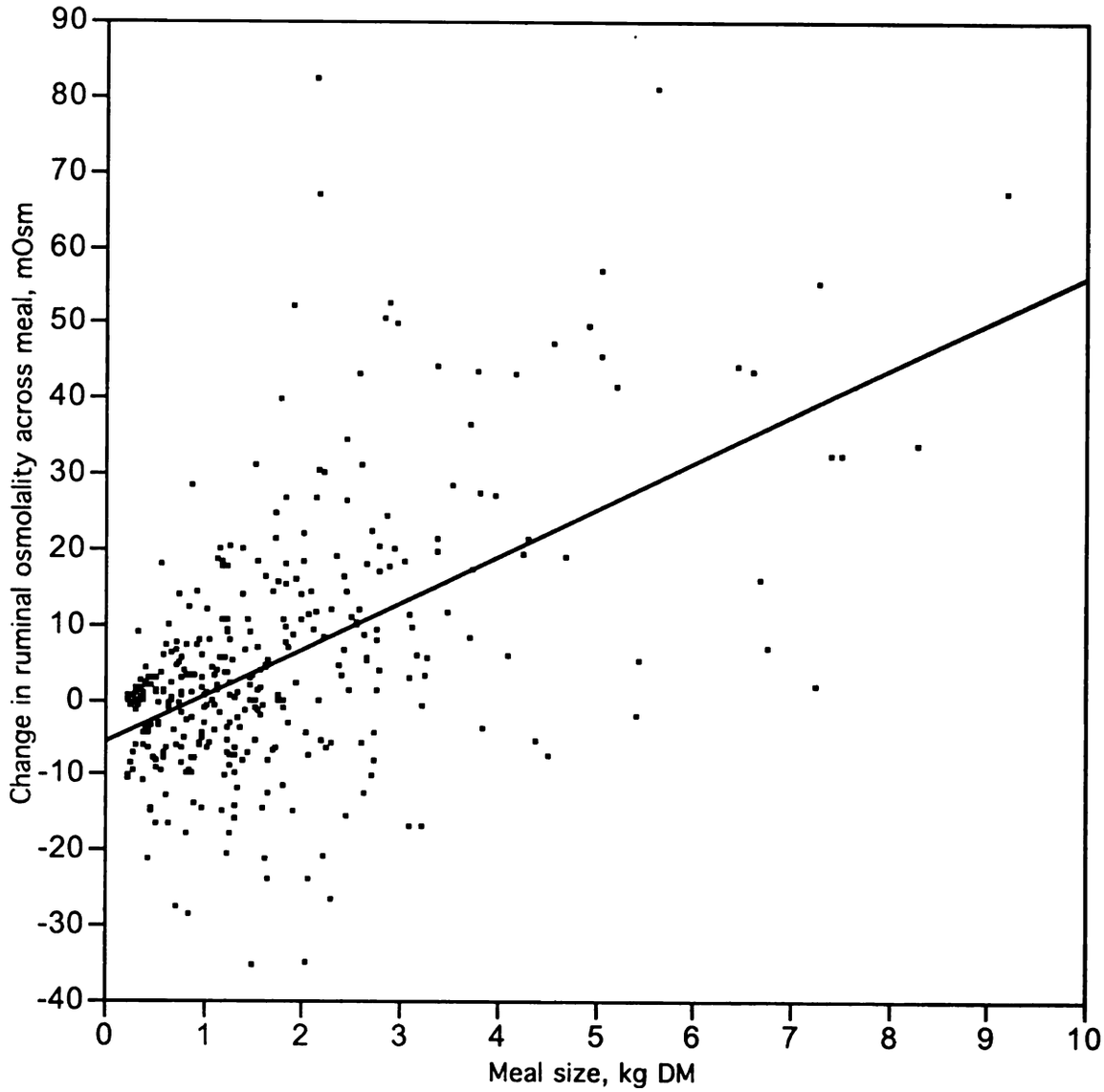

Figure 4.13. The relationship between meal size (kg) and ruminal osmolality difference. Overall mean meal was 1.8 kg. $R^2 = 0.28$, $P < 0.0001$.

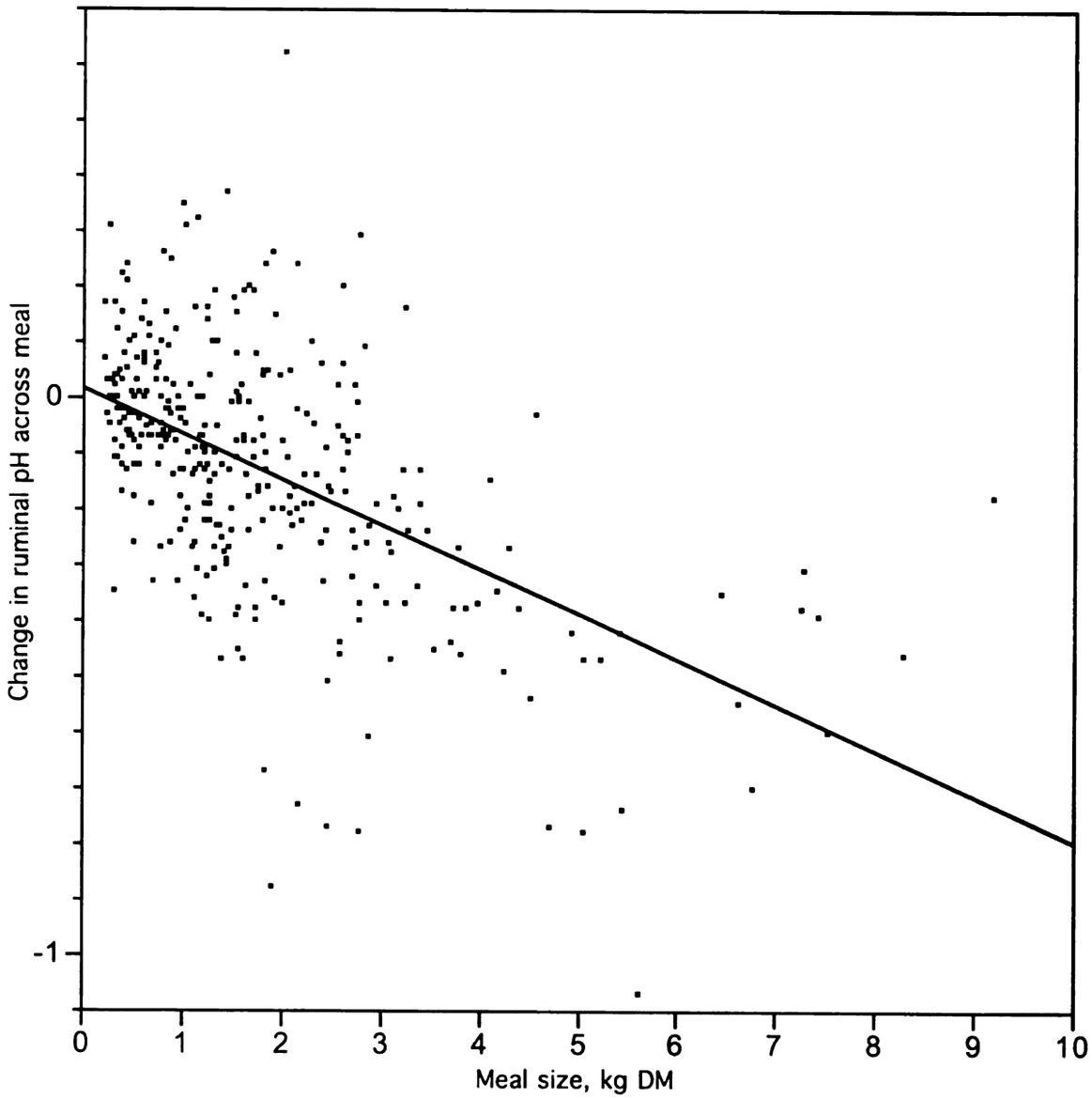

Figure 4.14. The relationship between meal size (kg) and ruminal pH difference. Overall mean meal was 1.8 kg. $R^2 = 0.28$, $P < 0.0001$.

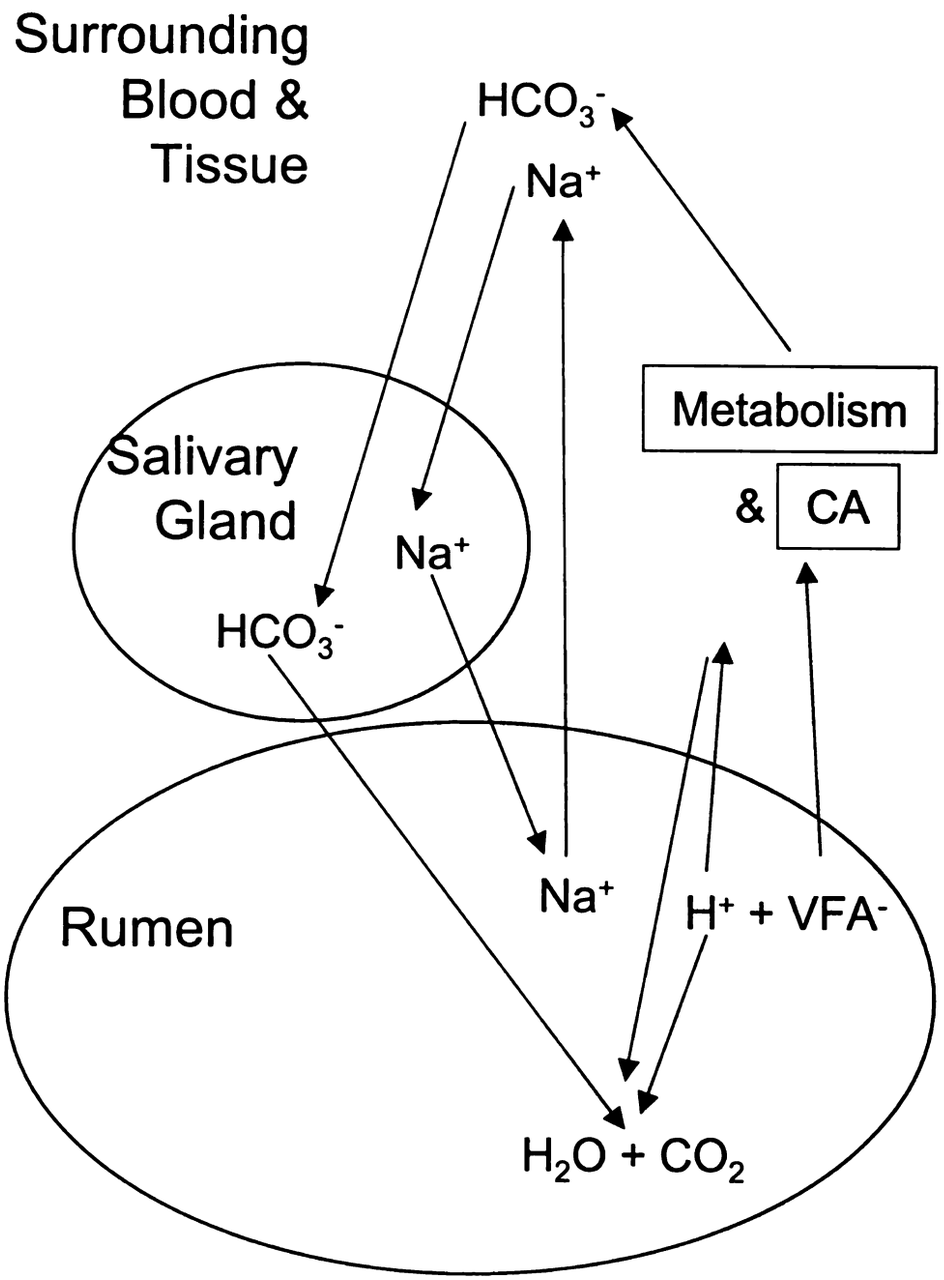

Figure 4.15. Proposed net sodium bicarbonate recycling in lactating dairy cows. Sodium bicarbonate is secreted from the salivary glands and flows in the saliva to the rumen. The proton (H^+) and bicarbonate (HCO_3^-) combine to form carbon dioxide and water. Sodium (Na^+) and the volatile fatty acid anion (VFA^-) then move across the ruminal epithelium as a charged pair. With metabolism and the action of carbonic anhydrase (CA), the volatile fatty acid is removed and the bicarbonate regenerated. This bicarbonate and sodium can be recycled through the salivary gland.

CHAPTER 5: Implications

Sodium bicarbonate added to the diet at recommended rates increased milk components and decreased rumination time (Chapter 2). It also increased ruminal digesta mass and decreased ruminal iNDF passage (Chapter 3). Sodium concentration in the RR is likely regulated by the cow to maintain an alkalizing strong ion difference and a constant ruminal osmolality (Chapter 4). In solution, sodium adds a positive charge, is osmotically active, and is alkalogenic and all these qualities are important in the ruminal environment. These results suggest sodium is important in the regulation the ruminal environment and that sodium should not be considered just for minimum requirement but for its balance and interaction with other cations within the ruminal solution.

Lactating cows appear to actively maintain a concentration of ruminal cations at approximately 150 mEq/L. Sodium is the major cation in saliva and, therefore, the most abundant cation in the ruminal solution. Dietary cations appear to modify the flux of sodium across the ruminal wall. This change in flux is likely the mechanism that maintains the constant total cation concentration. The maintenance of the total cation concentration also controls ruminal osmolality. The total cation concentration helps set the large strong ion difference in the rumen which counters the disassociated VFA charge and helps maintain the bicarbonate pool.

The forestomach of lactating dairy cows regulates the ruminal environment. The processes involved in this regulation have been traditionally studied with reductionist approach. The sodium, bicarbonate, protons, and VFA have been studied empirically and individually or in pairs. A true understanding of the regulation of the ruminal environment will come when all four of moieties are studied simultaneously.

The strong ion difference theory for the regulation of pH is one way to accomplish this goal. A strong ion theory of the ruminal solution has been proposed (Chapter 4). This proposal needs to be tested and verified and the unmeasured ions need to be quantified. Partial pressure of carbon dioxide above the ruminal solution needs to quantified for current lactating dairy cows and their diets. When sampling the ruminal solution, multiple sites should be sampled given the potential heterogeneity of the ruminal contents. More intensive but shorter term collections are needed to evaluate the trends and variance in ruminal solution concentrations. Specifically, high-frequency collections through meals and ruminating bouts are needed. The ionic changes associated with acute changes in ruminal fermentibility are of particular interest. With a more complete understanding, the ruminal environment can be challenged to test new hypotheses that arise.

Another way to accomplish this goal is to explore the sodium, bicarbonate, and VFA fluxes and turnovers in the bodies of lactating dairy cows. Sodium and VFA absorption may be linked in vivo. In vitro work has shown associations between sodium and VFA absorption, possibly connected by the intracellular pH of the ruminal epithelium. Turnovers of sodium and VFA in the ruminal solution may be similar (Chapter 4), but whether they are connected mechanistically awaits further experimentation. If sodium and VFA absorption are linked in lactating dairy cows, further experimentation must investigate aspects of the association. If one limits absorption the other, then this association must be incorporated into the diet formulation of lactating dairy cows. The interactions of ruminal sodium, potassium, ammonium, and possibly, other cations must also be included.

The sodium turnover rates calculated are highly dependent on the estimates of saliva composition and flow. Measurements of saliva composition and saliva flow during rumination are particularly needed for lactating dairy cows. The dairy cow is unique in its ability to process its fermentation acid load and the genetic selection for greater milk production also may have selected cattle with a greater daily flow of sodium bicarbonate to the RR so new measurements are warranted.

Quantitative flows of anions and cations across the ruminal wall are an area of needed research, particularly for how the cows maintain charge balance from the ruminal solution to the portal blood. The quantitative flow of water across the ruminal wall of lactating dairy cows also is unknown. Dietary sodium bicarbonate can be used a research model of the forementioned work.

Dose-response feeding of sodium bicarbonate would be the next step in the study of sodium bicarbonate research in both for production and digestion trials. In both cases, finding the breakpoint where the benefits are lost to costs would be beneficial to the industry. The literature suggests that changes in water dynamics in the RR begin when sodium bicarbonate is included at >2.5% of the dietary DM but the exact percentage for lactating dairy cows would be found in these dose response trials.

Overall, this dissertation investigated the role of strong ions (particularly sodium) in the solutions in the forestomach of lactating dairy cows. A more mechanistic and comprehensive understanding of strong ions will lead to improved diet formulation for lactating dairy cows, one that focuses on relationships among cations and not solely on minimum requirements.

APPENDIX TABLES

Table A.1. Projected concentrations based on book values.[1]

Experiment 01CSM1	% Na	% K	% Cl
Base Mix	0.3	1.2	0.3
SMV[2]	3.4	0.3	5.2
SCM[3]	5.0	0.2	7.7
PCM[4]	0.1	8.7	7.9
SBM[5]	5.0	0.2	0.1
PCM[6]	0.1	8.7	0.1
CntlM[7]	0.1	0.2	0.1

Experiment 02CSM2	% Na	% K	% Cl
Base Mix	0.2	1.3	0.3
SMV	3.5	0.3	5.2
SCM	0.1	0.1	0.1
SBM	5.0	0.1	7.7
CntlM	5.0	0.1	0.1

[1]Assumes no contribution from ricehulls and corn grain.
[2] SMV: Mineral and vitamin mix contained 69.4% dry ground corn, 10.5% dicalcium phosphate, 9.2% limestone, 8.1% trace mineral salt, 1.8% trace mineral premix, 0.4% magnesium oxide, 0.4% vitamin A, 0.3% vitamin D, and 0.1% vitamin E.
[3] SBM: Sodium bicarbonate treatment mix
[4] SCM: Sodium chloride treatment mix
[5] PBM: Potassium bicarbonate treatment mix
[6] PCM: Potassium chloride treatment mix
[7] CntlM: Control mix

Table A.2. Treatment assignments[1,2,3,4,5,6,7] for individual cows for 01CSM1.

CowID	Stall	Block	Latin Square	TMTSequence WithinSquare	Period One	Period Two	Period Three	Period Four	Period Five
3258	1	1	A	2	3	4	1	2	5
3465	2	1	A	3	2	3	5	1	4
3468	3	1	A	1	4	5	2	3	1
3429	4	1	A	4	1	2	4	5	3
3104	5	1	A	5	5	1	3	4	2
3788	6	2	B	1	4	1	5	3	2
3373	7	2	B	2	3	5	4	2	1
3238	8	2	B	3	2	4	3	1	5
3403	9	2	B	4	1	3	2	5	4
3065	10	2	B	5	5	2	1	4	3
3790	11	3	A	2	3	4	1	2	5
3467	12	3	A	5	5	1	3	4	2
3390	13	3	A	3	2	3	5	1	4
3212	14	3	A	4	1	2	4	5	3
3374	15	3	A	1	4	5	2	3	1
3435	16	4	B	1	4	1	5	3	2
3499	17	4	B	5	5	2	1	4	3
3160	18	4	B	2	3	5	4	2	1
3780	19	4	B	4	1	3	2	5	4
3380	20	4	B	3	2	4	3	1	5

[1] Treatment One is Sodium Chloride Treatment
[2] Treatment Two is Potassium Chloride Treatment
[3] Treatment Three is Sodium Bicarbonate Treatment
[4] Treatment Four is Potassium Bicarbonate Treatment
[5] Treatment Five is Control
[6] Pairs of balance Latin squares were located for a uniform barn environment
[7] Cows were randomly assigned to stalls and treatment sequences were randomly assigned to stalls within a block

Table A.2. Treatment assignments[1,2,3,4,5,6,7] for individual cows for 01CSM1 (continued).

CowID	Stall	Block	Latin Square	TMTSequence WithinSquare	Period One	Period Two	Period Three	Period Four	Period Five
3493	21	5	A	4	1	2	4	5	3
2943	22	5	A	2	3	4	1	2	5
3486	23	5	A	1	4	5	2	3	1
3282	24	5	A	5	5	1	3	4	2
3785	25	5	A	3	2	3	5	1	4
3455	26	6	B	2	3	5	4	2	1
3470	27	6	B	1	4	1	5	3	2
3787	28	6	B	3	2	4	3	1	5
2847	29	6	B	4	1	3	2	5	4
3784	30	6	B	5	5	2	1	4	3
3782	31	7	A	1	4	5	2	3	1
3109	32	7	A	2	3	4	1	2	5
3007	33	7	A	4	1	2	4	5	3
3779	34	7	A	5	5	1	3	4	2
3375	35	7	A	3	2	3	5	1	4
3439	36	8	B	4	1	3	2	5	4
3278	37	8	B	5	5	2	1	4	3
3783	38	8	B	3	2	4	3	1	5
3424	39	8	B	2	3	5	4	2	1
3438	40	8	B	1	4	1	5	3	2

[1] Treatment One is Sodium Chloride Treatment
[2] Treatment Two is Potassium Chloride Treatment
[3] Treatment Three is Sodium Bicarbonate Treatment
[4] Treatment Four is Potassium Bicarbonate Treatment
[5] Treatment Five is Control
[6] Pairs of balance Latin squares were located for a uniform barn environment
[7] Cows were randomly assigned to stalls and treatment sequences were randomly assigned to stalls within a block

Table A.3. Individual cow descriptors at the beginning of 01CSM1.

CowID	Stall #	DIM	7 d average milk, kg	BW, kg	Average BCS
3258	1	167	35	649	2.50
3465	2	117	35	638	2.88
3468	3	80	54	579	2.38
3429	4	169	32	603	2.88
3104	5	216	42	715	2.25
3788	6	128	41	630	2.25
3373	7	79	46	582	1.75
3238	8	56	44	658	2.13
3403	9	215	31	654	2.38
3065	10	191	49	627	2.00
3790	11	105	44	556	2.00
3467	12	98	46	610	2.50
3390	13	86	51	683	2.25
3212	14	169	38	719	2.25
3374	15	63	45	671	2.50
3435	16	158	41	681	3.00
3499	17	64	54	602	2.00
3160	18	255	32	692	3.50
3780	19	143	43	593	2.38
3380	20	76	43	627	2.13
3493	21	61	45	588	2.75
2943	22	233	39	700	2.63
3486	23	62	49	543	1.88
3282	24	74	42	768	3.25
3785	25	138	33	556	3.13
3455	26	59	51	584	2.25
3470	27	97	46	597	2.13
3787	28	134	42	597	2.13
2847	29	85	52	630	2.25
3784	30	132	40	760	3.88
3782	31	140	34	641	2.00
3109	32	68	47	607	2.88
3007	33	173	55	579	1.50
3779	34	141	36	580	2.38
3375	35	109	49	691	3.38
3439	36	82	43	561	2.00
3278	37	111	47	601	2.13
3783	38	139	35	676	3.50
3424	39	210	29	607	2.13
3438	40	140	43	598	2.50

Table A.4. Average status of 40 experimental cows at the beginning of 01CSM1.

Parameter	Mean	SD
DIM	126	53
7 d average milk, kg	43	7
BW, kg	631	55
BCS	2.46	0.53

Table A.5. Treatment[1,2,3,4] assignments for individual cows for 02CSM2.

CowID	Stall	Treatment Period One	Treatment Period Two	Treatment Period Three	Square
3353	3	1	2	3	1
3581	4	3	2	1	2
3623	5	3	1	2	1
3499	6	2	3	1	1
3238	7	1	3	2	2
3184	8	2	1	3	2

[1] Treatment One is Control
[2] Treatment Two is Sodium Chloride Treatment
[3] Treatment Three is Sodium Bicarbonate Treatment
[4] Cows were randomly assigned to stalls and treatment sequences were randomly assigned to stalls within a block

Table A.6. Individual cow descriptors at the beginning of 02CSM2.

CowID	Stall #	DIM	7 d average milk, kg	Empty Rumen BW, kg	Rumen Content, kg	Average BCS
3353	3	184	33.8	512	70	1.92
3581	4	169	37.5	508	55	2.17
3623	5	174	39.3	534	75	2.17
3499	6	172	38.8	539	59	1.92
3238	7	202	41.7	608	86	2.00
3184	8	181	35.5	599	64	2.08
Average	-	**180**	**39.4**	**550**	**68**	**2.04**

Table A.7. Visual representation of the 99MO01 experimental design.

Square	Stall	Cow ID	April 7, 1999 Period One	April 27, 1999 Period Two	May 18, 1999 Period Three	June 8, 1999 Period Four
One	1	3098	HSM[1]	HSD[2]	LSD[3]	LSM[4]
One	2	3283	HSD	LSM	HSM	LSD
One	3	3304	LSM	LSD	HSD	HSM
One	4	3297	LSD	HSM	LSM	HSD
Two	5	3300	HSM	HSD	LSD	LSM
Two	6	2946	HSD	LSM	HSM	LSD
Two	7	3106	LSM	LSD	HSD	HSM
Two	8	3159	LSD	HSM	LSM	HSD

[1]HSM High starch content AND hi moisture ground corn
[2]HSD High starch content AND dry ground corn
[3]LSD Low starch content AND dry ground corn
[4]LSM Low starch content AND hi moisture ground corn

Table A.8. Data removed from 01CSM1 data set.

CowID	Period	Day	What was removed?	Why removed?
3238	One	11	Milk data	Bad milk sample
3788	One	11	Milk data	Bad milk sample
3788	One	13	Milk data	Bad milk meter
3258	One	14	Milk data	Bad milk sample
3429	Two	11	All Data	Back Injury (slipped on ice, sold)
3429	Two	12	All Data	Back Injury (slipped on ice, sold)
3429	Two	13	All Data	Back Injury (slipped on ice, sold)
3429	Two	14	All Data	Back Injury (slipped on ice, sold)
3007	Two	11	Milk data	Bad milk sample
3783	Two	11	Milk data	Bad milk meter
3435	Two	12	Milk data	Bad milk sample
3438	Two	12	Milk data	Bad milk sample
3782	Two	12	Milk data	No milk sample
3782	Two	13	Milk data	Bad milk meter
3212	Two	13	Milk data	Bad milk sample
3258	Two	13	Milk data	Bad milk meter
3374	Two	13	Milk data	Bad milk meter
3424	Two	13	Milk data	Bad milk meter
3238	Three	11	Milk data	Bad milk sample
3375	Three	11	Milk data	Bad milk sample
3467	Three	11	Milk data	Bad milk sample
3007	Three	12	Milk data	No milk sample
2847	Three	13	Milk data	Bad milk meter
3380	Four	11	All data	Fluid on Heart (Hardware Disease?)
3380	Four	12	All data	Fluid on Heart (Hardware Disease?)
3380	Four	13	All data	Fluid on Heart (Hardware Disease?)
3380	Four	14	All data	Fluid on Heart (Hardware Disease?)
3380	Five	11	All data	Sold (Hardware Disease?)
3380	Five	12	All data	Sold (Hardware Disease?)
3380	Five	13	All data	Sold (Hardware Disease?)
3380	Five	14	All data	Sold (Hardware Disease?)
3007	Five	12	Milk data	Bad milk meter
3258	Five	13	Milk data	Bad milk meter
3787	Five	13	Milk data	Bad milk sample
3788	Five	14	Milk data	Bad milk meter

Table A.9. Start and stop times used in 01CSM1 behavior data sets.

	Period				
	One	**Two**	**Three**	**Four**	**Five**
Start time	6:40am	6:45am	6:30am	6:10am	6:00am
End time	6:35am	6:40am	6:25am	6:05am	5:55am
Hours feed doors closed	2:15h	2:50h	2:45h	2:40h	2:40h
Approx. P.M. time away from stall	2:05h	1:45h	1:55h	1:50h	1:45h
Approx. A.M. Time away from stall	2:30h	1:45h	2:00h	1:55h	1:55h
Approx. hours away from feed	6:45h	6:20h	6:40h	6:25h	6:30h

Table A.10. Number of days used in statistics for 02CSM2 feeding behavior data set.

CowID	Period	DrnkDaysOK?[1]	ChewDaysOK?[2]	pHDaysOK?[3]
3184	1	5	3	3
3184	2	5	3	4
3184	3	5	4	4
3238	1	5	5	4
3238	2	5	5	3
3238	3	5	2	4
3353	1	4	4	4
3353	2	5	3	5
3353	3	5	2	5
3499	1	5	5	2
3499	2	5	4	3
3499	3	5	5	3
3581	1	5	5	5
3581	2	5	5	4
3581	3	5	5	5
3623	1	5	5	5
3623	2	5	5	5
3623	3	5	4	5

[1] Number of days in behavior subperiod were water consumption was recorded satisfactorily.
[2] Number of days in behavior subperiod were chewing activity was recorded satisfactorily.
[3] Number of days in behavior subperiod were ruminal pH was recorded satisfactorily.

Table A.11. Data removed from 02CSM2 feeding behavior data set.

CowID	Period	Day	DrnkOK?[1]	ChewOK?[2]	pHOK?[3]
3184	1	1	y[4]	no, halter problem	y
3184	1	2	y	no, halter problem	no, malfunction
3184	1	3	y	y	y
3184	1	4	y	y	no, uncalibrated
3184	1	5	y	y	y
3184	2	1	y	no, halter problem	y
3184	2	2	y	y	no, malfunction
3184	2	3	y	y	y
3184	2	4	y	y	y
3184	2	5	y	no, halter problem	y
3184	3	1	y	y	y
3184	3	2	y	y	no, uncalibrated
3184	3	3	y	y	y
3184	3	4	y	y	y
3184	3	5	y	no, interference	y
3238	1	1	y	y	y
3238	1	2	y	y	y
3238	1	3	y	y	no, malfunction
3238	1	4	y	y	y
3238	1	5	y	y	y
3238	2	1	y	y	y
3238	2	2	y	y	y
3238	2	3	y	y	y
3238	2	4	y	y	no, malfunction
3238	2	5	y	y	no, malfunction
3238	3	1	y	y	y
3238	3	2	y	no, malfunction	y
3238	3	3	y	no, malfunction	no, uncalibrated
3238	3	4	y	y	y

[1] Was water consumption recorded satisfactorily?
[2] Was chewing activity recorded satisfactorily?
[3] Was ruminal pH recorded satisfactorily?
[4] y is "yes, recorded satisfactorily."

Table A.11. Data removed from 02CSM2 feeding behavior data set (continued).

CowID	Period	Day	DrnkOK?[1]	ChewOK?[2]	pHOK?[3]
3353	1	1	y[4]	y	y
3353	1	2	y	y	y
3353	1	3	y	y	y
3353	1	4	y	y	y
3353	1	5	no, pre-mastitis	no, pre-mastitis	no, pre-mastitis
3353	2	1	y	no, halter problem	y
3353	2	2	y	y	y
3353	2	3	y	y	y
3353	2	4	y	y	y
3353	2	5	y	no, malfunction	y
3353	3	1	y	no, malfunction	y
3353	3	2	y	no, malfunction	y
3353	3	3	y	no, malfunction	y
3353	3	4	y	y	y
3353	3	5	y	y	y
3499	1	1	y	y	y
3499	1	2	y	y	no, malfunction
3499	1	3	y	y	no, malfunction
3499	1	4	y	y	no, malfunction
3499	1	5	y	y	y
3499	2	1	y	y	y
3499	2	2	y	y	y
3499	2	3	y	y	y
3499	2	4	y	y	no, malfunction
3499	2	5	y	no, halter problem	no, malfunction
3499	3	1	y	y	y
3499	3	2	y	y	y
3499	3	3	y	y	no, malfunction
3499	3	4	y	y	no, uncalibrated
3499	3	5	y	y	y

[1] Was water consumption recorded satisfactorily?
[2] Was chewing activity recorded satisfactorily?
[3] Was ruminal pH recorded satisfactorily?
[4] y is "yes, recorded satisfactorily."

Table A.11. Data removed from 02CSM2 feeding behavior data set (continued).

CowID	Period	Day	DrnkOK?[1]	ChewOK?[2]	pHOK?[3]
3581	1	1	y[4]	y	y
3581	1	2	y	y	y
3581	1	3	y	y	y
3581	1	4	y	y	y
3581	1	5	y	y	y
3581	2	1	y	y	y
3581	2	2	y	y	y
3581	2	3	y	y	y
3581	2	4	y	y	y
3581	2	5	y	y	no, malfunction
3581	3	1	y	y	y
3581	3	2	y	y	y
3581	3	3	y	y	y
3581	3	4	y	y	y
3581	3	5	y	y	y
3623	1	1	y	y	y
3623	1	2	y	y	y
3623	1	3	y	y	y
3623	1	4	y	y	y
3623	1	5	y	y	y
3623	2	1	y	y	y
3623	2	2	y	y	y
3623	2	3	y	y	y
3623	2	4	y	y	y
3623	2	5	y	y	y
3623	3	1	y	y	y
3623	3	2	y	y	y
3623	3	3	y	y	y
3623	3	4	y	y	y
3623	3	5	y	no, halter problem	y

[1] Was water consumption recorded satisfactorily?
[2] Was chewing activity recorded satisfactorily?
[3] Was ruminal pH recorded satisfactorily?
[4] y is "yes, recorded satisfactorily."

Table A.12. Lack of effect of halter redesign on chewing activity: comparing first half of behavior subperiod to last half.

CowID	Period	Change in eating time, min/d	Change in ruminating time, min/d	Change in total chewing time, min/d
3184	1	10	-12	-2
3184	2	-26	-51	-77
3184	3	22	13	35
3238	1	11	-39	-28
3238	2	24	75	99
3238	3	10	88	98
3353	1	14	0	14
3353	2	-11	2	-9
3353	3[1]	.	.	.
3499	1	-27	-55	-82
3499	2	24	-1	23
3499	3	20	31	52
3581	1	11	6	16
3581	2	-6	21	16
3581	3	-6	5	-1
3623	1	-16	-10	-26
3623	2	1	24	26
3623	3	-26	0	-26
	AVERAGE	**2**	**6**	**7**

[1] Information could not be calculated.

Table A.13. The effect of dietary strong ion treatment on milk production and composition during feeding behavior monitoring (d20-d24). Statistics calculated using cow day as the response variable in Chapter 3.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Milk, kg	36.1	36.1	34.9	0.4	90	0.008	0.96
Milk fat, %	3.47	3.54	3.52	0.04	90	0.76	0.21
Milk protein, %	3.05	3.05	3.01	0.02	90	0.10	0.92
Milk lactose, %	4.76	4.77	4.73	0.01	90	0.006	0.45
Milk SNF, %	8.76	8.77	8.67	0.02	90	0.002	0.66
Milk fat, kg	1.25	1.28	1.24	0.02	90	0.34	0.31
Milk protein, kg	1.10	1.10	1.05	0.01	90	0.005	0.84
Milk lactose, kg	1.73	1.73	1.65	0.02	90	0.001	0.95
Milk SNF, kg	3.17	3.17	3.03	0.03	90	0.001	0.96
3.5% FCM, kg	35.8	36.2	35.1	0.4	90	0.09	0.47
4.0% FCM, kg	33.2	33.6	32.6	0.4	90	0.09	0.47
SCM, kg	33.4	33.8	32.5	0.4	90	0.02	0.52
MUN, mg/dl	16.2	15.1	16.6	0.2	90	<0.001	<0.001
SCC, 1000's/ml	165	157	141	15	90	0.29	0.68
SCS	2.6	2.3	2.1	0.1	90	0.005	0.04

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.14. The effect of dietary strong ion treatment on chewing behavior during feeding behavior monitoring (d20-d24). Statistics calculated using cow day as the response variable in Chapter 3.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Meals, n	9.7	9.8	9.4	0.3	74	0.26	0.81
Meal length, min	31.6	27.4	28.8	1.4	74	0.69	0.04
Eating time, min/d	266.2	258.9	263.8	5.2	74	0.85	0.33
Meal size, kg DM/meal	2.8	2.5	2.6	0.1	74	0.45	0.10
DMI, kg/d	24.1	24.1	23.5	0.4	74	0.15	0.97
Intermeal interval, min	105.9	99.9	105.4	3.9	74	0.60	0.29
Ruminating bouts, bouts/d	14.8	14.9	14.2	0.3	74	0.10	0.80
Ruminating bout length, min	35.2	34.8	36.9	0.8	74	0.06	0.70
Ruminating time, min/d	505.7	508.1	517.2	8.7	74	0.32	0.85
Inter-ruminating interval, min	64.4	63.7	65.1	1.9	74	0.62	0.80
Total chewing time, min/d	771.9	767.0	781.0	10.6	74	0.37	0.75

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.15. The effect of dietary strong ion treatment on drinking behavior during feeding behavior monitoring (d20-d24). Statistics calculated using cow day as the response variable in Chapter 3.

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Drinking bouts, n	16.7	18.3	17.1	0.5	89	0.60	0.04
Water consumed per drinking bout, L/bout	6.6	5.9	6.1	0.2	89	0.47	0.01
Water consumed, L/d	103.5	104.1	98.7	2.1	89	0.05	0.84
Interdrink interval, min	81.1	70.9	76.6	2.7	89	0.86	0.009
Water consumed while eating, L/d	55.3	53.0	57.9	2.8	74	0.28	0.57
Water consumed while ruminating, L/d	2.3	5.1	1.9	0.8	74	0.07	0.02

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.16. The effect of dietary strong ion treatment on ruminal pH during feeding behavior monitoring (d20-d24). Statistics calculated using cow day as the response variable in Chapter 3.

	Experimental Diets					Significance, *P*	
Measures of ruminal pH[1]	**SBT**[2]	**SCT**[3]	**Cntl**[4]	**SE**	**n**	**TMT**[5]	**CvB**[6]
Mean	6.19	6.23	6.18	0.03	73	0.47	0.41
SD	0.24	0.24	0.24	0.01	73	0.90	0.94
Median	6.19	6.23	6.18	0.03	73	0.49	0.40
Minimum[7]	5.78	5.80	5.77	0.04	73	0.66	0.73
Maximum[7]	6.60	6.66	6.61	0.03	73	0.52	0.21
Range[7]	0.82	0.86	0.83	0.04	73	0.89	0.44
Time below pH 6.0, h/d	6.73	6.26	6.32	0.78	73	0.86	0.68
Time below pH 5.8, h/d	2.79	2.74	2.20	0.51	73	0.35	0.94
Time below pH 5.5, h/d	0.13	0.35	0.06	0.08	73	0.06	0.05
Area of curve below pH 6.0, pH*30 seconds	155.6	155.2	129.0	24.9	73	0.38	0.99
Area of curve below pH 5.8, pH*30 seconds	42.5	48.4	28.2	9.4	73	0.14	0.67
Area of curve below pH 5.5, pH*30 seconds	2.5	4.9	2.9	1.4	73	0.63	0.23

[1] Ruminal pH was measured continuously and summarized to data file every 5 seconds and was reported for every 22.5 out of 24 h.
[2] SBT is sodium bicarbonate treatment diet.
[3] SCT is sodium chloride treatment diet.
[4] Cntl is control diet.
[5] TMT is the contrast of control and two sodium treatments.
[6] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.
[7] Minimum, maximum, and range were calculated using the daily 2.5th and 97.5th percentiles of ruminal pH measured every 5 seconds.

Table A.17. Table 3.10C. The effect of dietary strong ion treatment on digestibility of DM and OM (Based on Cr_2O_3 flow).

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
DMI, kg/d	23.2	23.1	23.5	0.6	18	0.61	0.94
DM apparently digested in total tract, kg/d	15.5	15.4	15.5	0.4	18	0.94	0.85
DM apparently digested in total tract, %	67.2	66.8	66.0	1.0	18	0.45	0.80
OMI, kg/d	21.5	21.4	21.9	0.5	18	0.51	0.91
OM apparently digested in rumen, kg/d	5.1	6.3	5.2	0.7	18	0.58	0.25
OM apparently digested in rumen, %	23.7	29.4	23.8	3.0	18	0.46	0.21
OM truly digested in rumen, kg/d	10.4	11.7	11.1	0.7	18	0.90	0.21
OM truly digested in rumen, %	48.2	54.9	50.7	3.0	18	0.83	0.16
Apparent OM passed to duodenum, kg/d	16.4	15.1	16.6	0.7	18	0.35	0.27
True OM passed to duodenum, kg/d	11.1	9.7	10.7	0.8	18	0.74	0.24
OM apparently digested in intestines, kg/d	9.6	8.3	9.6	0.6	18	0.45	0.18
OM apparently digested in intestines, % of intake	44.1	39.0	45.1	2.7	18	0.55	0.15
OM apparently digested in intestines, % of OM passed to duodenum	57.9	54.7	57.5	1.7	18	0.58	0.23
OM apparently digested in total tract, kg/d	14.8	14.6	14.8	0.4	18	0.77	0.80
OM apparently digested in total tract, %	68.8	68.4	67.9	1.0	18	0.58	0.78

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.18. Table 3.11C. The effect of dietary strong ion treatment on digestibility of starch (Based on Cr_2O_3 flow).

	Experimental Diets					Significance, *P*	
Starch	**SBT**[1]	**SCT**[2]	**Cntl**[3]	**SE**	**n**	**TMT**[4]	**CvB**[5]
Intake, kg/d	6.8	6.9	6.9	0.2	18	0.69	0.79
Digested in rumen, kg/d	3.3	3.8	3.3	0.3	18	0.42	0.27
Digestibility in rumen, %	48.7	56.0	46.2	4.2	18	0.27	0.26
Passage to duodenum, kg/d	3.5	3.1	3.7	0.3	18	0.36	0.39
Digested in the intestines, kg/d	3.0	2.5	3.2	0.3	18	0.35	0.36
Digested in the intestines, % of intake	46.7	36.4	44.0	4.5	18	0.27	0.26
Digested in the intestines, % of duodenal passage	81.3	80.4	85.1	3.0	18	0.28	0.85
Digested in total tract, kg/d	6.3	6.3	6.4	0.1	18	0.55	0.90
Digested in total tract, %	92.8	92.4	92.9	0.7	18	0.70	0.71

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.19. Table 3.12C. The effect of dietary strong ion treatment on digestibility of NDF (Based on Cr_2O_3 flow).

	Experimental Diets					Significance, *P*	
NDF	**SBT**[1]	**SCT**[2]	**Cntl**[3]	**SE**	**n**	**TMT**[4]	**CvB**[5]
Intake, kg/d	5.7	5.6	5.9	0.1	18	0.19	0.85
pdNDF intake, kg/d	3.3	3.3	3.3	0.1	18	0.60	0.86
Digested in rumen, kg/d	1.1	1.4	1.0	0.2	18	0.31	0.25
Digested in rumen, %	19.5	25.5	17.7	3.3	18	0.27	0.23
pdNDF digested in the rumen, %	47.6	42.3	39.4	5.4	18	0.42	0.50
Passage to duodenum, kg/d	4.6	4.2	4.9	0.2	18	0.13	0.32
pdNDF passage to duodenum, kg/d	1.7	1.9	2.0	0.2	18	0.32	0.55
Digested in the intestines, kg/d	0.6	0.2	0.6	0.2	18	0.36	0.17
Digested in the intestines, % of intake	9.5	3.8	9.8	2.6	18	0.43	0.14
Digested in the intestines, % of duodenal passage	10.5	3.1	11.0	3.3	18	0.32	0.14
pdNDF digested in the intestines, % of duodenal passage	18.8	20.7	20.1	8.2	18	0.97	0.87
Digested in total tract, kg/d	1.7	1.7	1.6	0.1	18	0.69	0.96
Digested in total tract, %	29.4	29.3	27.2	1.9	18	0.38	0.99
pdNDF digested in total tract, %	59.0	57.9	52.6	1.8	18	0.03	0.66

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.20. Table 3.13C. The effect of dietary strong ion treatment on ruminal kinetics and pools (Based on Cr_2O_3 flow).

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
Ruminal starch digestion rate, %/h	19.1	22.5	18.1	1.9	17	0.30	0.21
Ruminal pdNDF digestion rate, %/h	2.3	1.9	1.9	0.3	17	0.59	0.39
Ruminal OM passage rate, %/h	6.6	6.2	7.6	0.3	17	0.01	0.38
Ruminal starch passage rate, %/h	18.5	17.8	21.7	2.1	17	0.23	0.811
Ruminal NDF passage rate, %/h	3.1	2.8	3.6	0.2	17	0.01	0.19
Ruminal pdNDF passage rate, %/h	2.4	2.5	2.9	0.3	17	0.22	0.86
Ruminal iNDF passage rate, %/h	3.2	3.2	3.6	0.2	17	0.09	0.87

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.21. Table 3.14C. The effect of dietary strong ion treatment on N metabolism (Based on Cr_2O_3 flow).

	Experimental Diets					Significance, *P*	
	SBT[1]	SCT[2]	Cntl[3]	SE	n	TMT[4]	CvB[5]
TRDOM, kg/d	10.4	11.7	11.1	0.7	18	0.90	0.21
N Intake, g/d	667	700	682	17	18	0.54	0.89
Ammonia in the rumen, mg/dl	25.2	23.7	23.7	0.6	18	0.34	0.13
Passage to duodenum							
Ammonia, g/d	27	24	22	1	18	0.08	0.20
NAN, g/d	672	626	651	19	18	0.93	0.12
NAN, % of intake	101.2	93.6	95.6	2.8	18	0.62	0.10
NANMN, g/d	224	171	152	27	18	0.21	0.21
NANMN, % of intake	34.1	25.5	22.3	4.0	18	0.16	0.17
NANMN, % of duodenal NAN	34.0	27.3	23.5	4.0	18	0.18	0.27
Microbial N, g/d	449	455	500	23	18	0.13	0.86
Microbial N, % of duodenal NAN	66.0	72.7	76.5	4.0	18	0.18	0.27
Microbial N, g/g of TRDOM	0.045	0.040	0.045	0.003	18	0.51	0.22
NAN, g/d digested in intestines	484	435	453	16	18	0.76	0.06
NAN, % of duodenal passage digested in intestines	71.8	69.5	69.4	1.0	18	0.36	0.14
N apparently digested in total tract, g/d	477	478	483	14	18	0.78	0.97
N apparently digested in total tract, %	71.7	71.4	70.7	1.1	18	0.54	0.89
Fecal N, g/d	189	191	198	9	18	0.45	0.85

[1] SBT is sodium bicarbonate treatment diet.
[2] SCT is sodium chloride treatment diet.
[3] Cntl is control diet.
[4] TMT is the contrast of control and two sodium treatments.
[5] CvB is the contrast of sodium chloride treatment and sodium bicarbonate treatment.

Table A.22. Table 3.25C. Distributions from previous work at Michigan State University with ruminally and duodenally cannulated lactating Holstein dairy cattle compared experiment in Chapter 3 (Based on Cr_2O_3 flow).

	Previous Work				02CSM2	
	Mean	SD	Median	n[1]	Mean	Approx. Percentile
DMI, kg	22.8	3.8	22.9	218	24.4	70
Milk yield, kg	38.5	8.4	38.4	218	35.7	40
FCM yield, kg	38.6	7.5	38.1	218	35.7	40
Empty Rumen BW, kg	564	66	561	188	578	60
Ruminal DM digestibility, %	30.6	13.0	31.0	180	19.7	20
Ruminal DM digestibility, kg/d	6.5	2.7	6.7	189	4.6	25
Ruminal apparent OM digestibility, %	35.2	11.7	35.5	189	25.6	20
Ruminal apparent OM digestibility, kg/d	7.1	2.5	7.2	190	5.5	25
Ruminal NDF digestibility, %	32.3	12.3	33.6	190	20.9	20
Ruminal pdNDF digestibility, %	55.6	15.1	56.0	191	43.1	25
Ruminal rate of pdNDF digestion, %/h	3.1	1.5	2.9	181	2.0	25
Ruminal rate of pdNDF passage, %/h	2.8	0.8	2.7	184	2.6	45
Ruminal rate of iNDF passage, %/h	3.2	1.1	3.2	184	3.3	65
Ruminal apparent starch digestibility, %	49.8	19.1	52.2	185	50.3	45
Ruminal rate of apparent starch digestion, %/h	15.6	8.8	15.4	185	19.9	75
Ruminal rate of starch passage, %/h	15.6	6.5	14.4	185	19.3	75
Microbial N Production, g/d	418	120	413	153	468	70
Microbial N production efficiency, g/kg TRDOM	36	9	35	152	43	80

[1] number of cow periods in the mean.

Table A.23. Distribution of whole blood measures in Chapter 4.

	Percentile							
Whole Blood at 38.5°C	**97.5**	**75**	**50**	**25**	**2.5**	**Mean**	**SD**	**n**
pH	7.487	7.443	7.422	7.402	7.353	7.423	0.033	2279
Partial pressure CO2, mmHg	45.4	39.8	36.7	33.0	26.8	36.4	4.8	2282
Partial pressure pO2, mmHg	90.1	59.5	46.3	40.0	34.3	51.6	15.5	2280
Hematocrit, %	34	32	31	30	28	31	2	2279
Sodium, mmol/L	146.3	143.6	142.3	141.2	139.1	142.4	1.8	2282
Potassium, mmol/L	4.6	4.3	4.1	3.8	3.4	4.0	0.3	2282
Chloride, mmol/L	110.7	107.9	106.4	104.5	99.7	106.0	2.8	2282
Calcium, mmol/L	1.4	1.3	1.3	1.2	1.1	1.3	0.1	2282
Bicarbonate, mmol/L	30.6	26.2	24.2	21.8	17.2	24.0	3.4	2279
Anion Gap, mmol/L	19.7	17.4	16.4	15.3	13.6	16.4	1.6	2279

Table A.24. Distribution of ruminal VFA measures on d 15 in Chapter 4.

	percentile							
Ruminal VFA, mM	**97.5**	**75**	**50**	**25**	**2.5**	**Mean**	**SD**	**n**
Acetate	89.8	70.2	61.1	51.8	28.6	60.2	15.3	2268
Propionate	48.1	28.2	22.8	17.9	8.6	24.0	9.6	2268
Iso-butyrate	2.0	1.4	1.2	0.9	0.3	1.2	0.4	2231
Butyrate	20.1	14.8	12.2	9.5	4.7	12.1	3.9	2268
Iso-valerate	3.2	2.3	1.9	1.5	0.6	1.9	0.6	2257
Valerate	4.7	2.5	1.9	1.3	0.5	2.0	1.1	2248
Total VFA	**159.4**	**119.5**	**101.9**	**84.6**	**45.8**	**101.4**	**27.8**	**2268**

Table A.25. Results used to determine influx, pool size and ruminal turnover of sodium, potassium, chloride, water and VFA in the rumen[1].

	High Starch		Low Starch	
	HM[2]	DG[3]	HM	DG
DMI, kg/d	19.8	20.9	19.6	17.8
TRDOM, % of DMI	54.3	45.8	47.2	39.3
TRDOM, kg/d	10.8	9.6	9.3	7.0
Estimated Saliva Production, L/d[4]	249	253	258	258
VFA Produced, Eq/d	59.7	39.4	54.2	29.6
Water in feed, L/d	21	19	26	23
Sodium Consumed, mEq/d	4048	4273	4092	3639
Sodium Secreted in saliva, mEq/d	40130	40681	41585	41535
Potassium Consumed, mEq/d	5976	6254	6968	6328
Potassium Secreted in saliva, mEq/d	1545	1567	1601	1599
Chloride Consumed, mEq/d	1787	1886	2156	1958
Chloride Secreted in saliva, mEq/d	1770	1794	1834	1832

[1] Ruminal DM (kg), ruminal water (L), diet composition, and animal behavior are as measured assuming 10% of ruminal water is the microbial mass and 10% is in the feed and daily VFA production is as per Oba and Allen (2003a).
[2] HM: High moisture corn.
[3] DG: Dry ground corn.
[4] Saliva flows are 0.125 L/min at rest, 0.200 L/min during eating, and 0.250 L/min while ruminating. Saliva composition is 161 mEq sodium/L, 6 mEq potassium/L, and 7 mEq chloride/L as per Bailey and Balch (1961b).

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