The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism

Michel MUBIAYI BEYA

Thesis is presented in partial fulfillment of the requirements for the degree

Master of Science in Agriculture

(Animal Science)

at Stellenbosch University

Supervisor: Prof. C. W. Cruywagen

Stellenbosch December 2007

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature..

Date....27/11/2007.....

Abstract

Title: The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism

Name: Michel Mubiayi Beya

Supervisor: Prof. C.W. Cruywagen

Institution: Department of Animal Sciences, Stellenbosch University

Degree: MScAgric

The objective of this study was to evaluate the effect of different buffers in dairy cow diets on milk production and composition and on selected rumen metabolism parameters. A high concentrate TMR, formulated to be potentially acidotic, was used to construct three dietary treatments in which Acid Buf (the skeleton remains of the sea weed *Lithothamnium calcareum*) was compared against limestone (control) and sodium bicarbonate plus limestone. One basal diet was formulated and treatment diets contained either 4 g/kg DM of Acid Buf, 3.7g/kg DM of limestone + 8 g /kg of sodium bicarbonate or 3.5 g/kg DM limestone (control), respectively. The response to treatment was measured using 6 ruminally cannulated lactating Holstein cows allocated to treatments according to a 3 x 3 (n=2) balanced Latin square design, with three treatments and three periods. The total experiment period was 66 days in which every cow received each diet for a period of 15 days prior to the data collection period of 7 days. Rumen fluid was collected for volatile fatty acid (VFA), lactic acid and ammonia concentration. Rumen pH was monitored continuously every 10 minutes for two days using a portable data logging system and in-dwelling electrodes. During each data collection period, milk was collected and analyzed for its solid and mineral contents. Feed consumption was recorded individually. The impact of acidity was clearly visible, especially from the period from mid-day to mid-night when rumen pH dropped below 5.5 for a longer period (13 h) in the control (limestone) treatment than in the sodium bicarbonate (7.7 h) and Acid Buf (4 h) treatments. The minimum rumen pH was lower for the control (5.14) than for the Acid Buf treatment (5.42), while the pH in the sodium bicarbonate treatment (5.37) did not differ from other treatments. The dietary buffers did not have a significant impact on rumen VFA, lacticacid and ammonia concentrations. Daily milk yield was higher for the Acid Buf (31.8

kg) treatment than for the sodium bicarbonate (29.1 kg) and control (27.6 kg), treatments. Milk fat content was higher for the Acid Buf (42.1 g/kg) and sodium bicarbonate (41.8 g/kg) treatments and control (38.6 g/kg) treatments. Treatment had no effect on milk crude protein content (34.7 g/kg, 33.8 g/kg and 34.3 g/kg for the Acid Buf, sodium bicarbonate and control treatments, respectively). The trial indicated that supplementing high concentrate diets for lactating dairy cows with Acid Buf at a level of 90 g/cow per day has a greater impact on rumen pH, milk production and milk composition than 180 g/cow per day of sodium bicarbonate and that sub-clinical acidosis could reduce daily milk input by 4 kg/cow.

Key words: Acid Buf, Buffer, Rumen metabolism, Milk production

Uittreksel

Titel: Die invloed van melkkoeidiëte wat met voerkalk, Acid Buf of natriumbikarbonaat gebuffer is, op produksierespons en rumenmetabolisme

Naam: Michel Mubiayi Beya

Studieleier: Prof. C.W. Cruywagen

Instansie: Departement Veekundige Wetenskappe, Universiteit van Stellenbosch

Graad: MScAgric

Die doel van die studie was om die invloed van verskillende buffers in melkbeesdiëte op melkproduksie en melksamestelling, asook op bepaalde rumenparameters, na te gaan. 'n Volledige dieet met 'n hoë kragvoerinhoud, wat geformuleer is om potensieel asidoties te wees, is gebruik om drie dieetbeehandelings saam te stel waarin Acid Buf (die skeletoorblyfsels van die seegras *Lithothamnium calcareum*) vergelyk is met voerkalk (kontrole) en natriumbikarbonaat plus voerkalk. Een basale dieet is dus geformuleer en die behandelingsdiëte het onderskeidelik 4 g/kg DM Acid Buf, of 3.7g/kg DM voerkalk + 8 g/kg natriumbikarbonaat, of 3.5 g/kg DM voerkalk (kontrole) bevat. Ses rumengekannuleerde lakterende Holsteinkoeie is ewekansig aan die behandelings toegeken in 'n gebalanseerde 3 x 3 (n=2) Latynse vierkantontwerp met drie behandelings en drie periodes. Die totale eksperimentele periode was 66 dae, waartydens elke koei elke behandeling vir 15 dae ontvang heet voor die datakolleksieperiode van 7 dae. Rumennloeistof is versamel vir die bepaling van vlugtige vetsure (VVS), melksuur en ammoniakbepalings. Rumen pH is voortdurend, elke 10 minute, oor 'n twee-dae periode gemeet met behulp van draagbare dataloggers en pH elektrodes wat binne-in die rumen gesetel was. Melkmonsters is gedurende elke datakolleksieperiode versamel en ontleed vir totale vastestof- en mineraalinhoud. Voerinname is individueel bepaal. Die invloed van rumen-suurheid is duidelik waargeneem, veral gedurende die middag- tot middernagperiode toe die rumen pH in die kontrolebehandeling vir langer periodes (13 ure) tot onder 5.5 gedaal het as in die geval van die natriumbikarbonaat-behandeling (7.7 ure) en Acid Buf-behandeling (4 ure). Die minimum rumen pH was laer vir die kontrolebehandeling (5.14) as vir die Acid Buf-behandeling (5.42), terwyl dié van die natriumbikarbonaatbehandeling (5.37) nie van die ander behandelings verskil het nie. Die dieetbuffers het nie 'n betekenisvolle invloed op die rumen VVS-, melksuur- of ammoniak-

konsentrasies gehad nie. Daaglikse melkproduksie was hoër vir die Acid Buf-behandeling (31.8 kg) as vir die natriumbikarbonaat- (29.1 kg) en kontrolebehandelings (27.6 kg). Die melkvetinhoud was hoër vir die Acid Buf- (42.1 g/kg) en natriumbikarbonaatbehandelings (41.8 g/kg) as vir die kontrolebehandeling (38.6 g/kg). Behandeling het egter nie 'n betekenisvolle invloed op die melkproteïeninhoud gehad nie (34.7 g/kg, 33.8 g/kg and 34.3 g/kg vir die Acid Buf-, natriumbikarbonaat- en kontrolebehandelings, onderskeidelik). Die studie het aangedui dat die aanvulling van hoë-kragvoerdiëte vir melkkoeie met Acid Buf teen 90 g/koei per dag 'n groter impak op rumen pH, melkproduksie en melksamestelling het as natriumbikarbonaat teen 180 g/koei per dag en dat sub-kliniese asidose die melkproduksie met soveel as 4 kg per dag kan verlaag.

Sleutelwoorde: Acid Buf, Buffer, Rumen metabolisme, Melkproduksie

Table of contents

Acknowledgments

I wish to express my sincere thanks to the following people who played a role ensuring the successful completion of this study:

- Jehovah God for the strength, endurance and motivation He gave me throughout my study.
- Prof. C. W. Cruywagen for the guidance and support.
- Dr. Lucia Holtshausen for her enthusiastic advice.
- Dr. Florance Nherera for her valuable criticism during the writing of this thesis.
- Dr. Scotney Watt for his encouragement and moral support.
- Celtic Sea Minerals (Cork, Ireland) for financial support.
- The entire staff of the Department of Animal Science at Stellenbosch University for their support.
- My family for their encouragement.
- My friends and fellow postgraduate students for their encouragement.

Chapter one

General introduction

1.1 Introduction

Dairy cattle play an important role in human nutrition as they provide protein through milk production. Nutrition of dairy cattle is the key in enhancing performance and production of these animals. In dairy cow production, the feeding of potentially acidotic diets is a concern not only for economic reasons, but also for animal welfare. High-producing cows are commonly fed highly digestible diets containing a high proportion of rapidly degradable carbohydrates to meet the energy requirements for lactation (NRC, 2001). Feeding a high concentrate diet is usually associated with a decline in rumen pH, an increase in propionate and lactate production which affect the rumen fermentation negatively (Krause and Oetzel 2006).

Energy and fibre requirements of dairy cows in early lactation are not easily met, because the requirement for lactation exceeds the energy supply (NRC, 2001). Diets high in starch and low in fibre content are usually fed to increase intake of energy, but such diets predispose the cow to ruminal acidosis (Krause *et al.*, 2002) and saliva production is low (Zebeli *et al.*, 2006). Saliva plays a role in buffering the rumen environment and maintaining optimal conditions for rumen microbial growth (Mertens, 1997). It is estimated that salivary buffers account for about 30 to 40% of the neutralization of fermentation acids in the rumen (Allen, 1997). The addition of dietary buffers, if in sufficient quantities, compensate for decreased saliva output in high concentrate diets, thereby maintaining rumen conditions conducive for normal fermentation.

Buffers can affect rumen conditions by increasing the pH and thus providing a more favorable environment for microbial activity (Harrison *et al.*, 1989). Researchers (Erdman, 1988) have concluded that the responses to dietary buffers occur via reduced rumen acidity and subsequent improvement in the systemic acid-base balance, particularly during sudden ration changes. Apart

from improving rumen pH, dietary buffers have additional, non-buffering effects which result in an increase in rumen osmotic pressure and liquid dilution rate (Rogers *et al.*, 1982). These, in turn, increase the influx of water and accelerate the flow of liquid digesta from the rumen (Rogers *et al.*, 1985). The high liquid phase outflow passage rate is associated with increased efficiency of fibre digestion, microbial protein synthesis (Rogers *et al*, 1982), and utilization of organic matter (Roderick and Bryan, 1989). Improved feed utilization optimizes production of metabolizable protein and energy and increases the milk protein (Escobosa *et al.*, 1984) and milk fat contents (West *et al.*, 1986).

Sodium bicarbonate is commonly added as a buffer to high concentrate diets for high yielding dairy cows to increase and stabilize rumen pH (Solorzano *et al.*, 1989; Thomas *et al.*, 1984). The buffering capacity of sodium bicarbonate is, however, limited (Russell and Chow, 1993). Acid Buf, the calcified remains of the seaweed *Lithothamnium calcareum*, is extensively used as a buffer in ruminant nutrition. Cruywagen *et al.* (2004) reported that the positive influence of Acid Buf appears to be related to its buffering capacity in the rumen and thereby promoting volatile fatty acid production. However, its effect on rumen metabolism and milk production, in dairy cows, remains a subject of research. The objectives of this study were to compare the effects of Acid Buf and sodium bicarbonate on rumen metabolism and milk production in dairy cows.

1.2. References

Allen, M. S., 1997. Relationship between fermentation acid production in the rumen and the requirement of physically effective fiber. J. Dairy Sci. 80:1447-1462.

Cruywagen, C.W., Swiegers, J.P. & Taylor, S., 2004. The effect of Acid Buf in dairy cow diets on production response and rumen parameters. J. Dairy Sci. 87 (Suppl. 1): 46.

Erdman, R. A., 1988. Dietary Buffering Requirements of the Lactacting Dairy Cow : A Review. J. Dairy Sci. 71: 3246-3266.

Escobosa, A., Coppock, C. E., Rowe, L. D., Jenkins, & Gate, C. E., 1984. Effect of dietary sodium bicarbonate and sodium chloride on physiological responses of lactating dairy dairy cows in hot wheather. J. Dairy Sci. 67:574-584.

Harrison, J. H., Reley, R. E. & Loney, K. A., 1989. Effect of type and amount of buffer addition to grasse silage-based total mixed rations on milk production and composition. J. Dairy Sci. 72:1824-1830.

Krause, K. M., Combs, D. K. & Beauchemin, K. A., 2002. Effects of Forage Particle Size and Grain Fermentability in Midlactation Cows. I. Milk Production and Diet Digestibility. J. Dairy Sci.85:1936-1946.

Krause, K. M, Garret Oetzel, R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Techn. 126: 215-236.

Mertens, D. R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

NRC., 2001. Nutrient requirements of Dairy cattle, seventh ed. (rev.) Natl. Acad. Sci., Washigton, DC, USA.

Roderick, I. M. and Bryan A. W., 1989. Rumen microbial ecology and nutrient. J. Dairy Sci. 73:2971-2995.

Rogers, J. A. David, C. L. & Clark, J. H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilisation with mineral salts in dairy cows. J. Dairy Sci. 65:577-586.

Rogers, J. A., L. D. Muller, C. L. Davis, W. Chalupa, D. S. Kronfeld, L. F. Karcher, K. R. Cummings., 1985. Response of dairy cows to sodium bicarbonate and limestone in early laction. J. Dairy Sci. 68:646.

Russell, J. B. Chow, J. M., 1993. Another theory for the action of ruminal buffers salts: decreased starch fermentation and propionate production. J. Dairy Sci.78:826-830.

Solorzano, L. C., Armentano, L. E., Grummer, R.R. and Dentine, M. R., 1989. Effects of Sodium Bicarbonate and Sodium sesquicarbonate on lactating Holstein cows. J. Dairy Sci. 72:453-461.

Thomas, J. W., Emery, R. S., Breaux, J. K. & Liesman, J. S., 1984. Response of milking cows fed high concentrate, low roughage diet plus sodium bicarbonate, magnesium oxide or magnesium hydroxide. J. Dairy Sci. 67:2532-2545.

West, J. W., Coppock, C. E., Nave, D. H. & Schelling, G. T., 1986. Effects of potassium buffers on feed intake in lacting dairy cows and on rumen fermentation in vivo and in vitro. J. Dairy Sci. 69:124-134.

Zebeli, Q., Tafaj, M., Steingrass, H., Metzler, B., Brochner, W., 2006. Effect of physically effective fiber on digestive processes and milk fat content in early lactation dairy cow fed total mixed rations. J. Dairy Sci. 84:651-668.

Chapter two

Literature Review

2.1. Introduction

The demand for meat and milk is increasing at a high rate in the developing countries in Sub-Saharan Africa as well as other parts of the world (FAO 2006 report). It is therefore imperative that beef and dairy cattle, which are the main sources of meat and milk for human consumption, be supplied with adequate feed to improve production. However, feeding high energy diets to meet the nutritional requirements for maximal production has other consequences such as predisposing animals to rumen acidosis.

Ruminants are adapted to digesting predominantly forage-based diets; hence consumption of feeds high in easily fermentable carbohydrates may result in ruminal fermentation disorders. Krause and Oetzel (2006) reported that feeding easily fermentable carbohydrates increased the occurrence of liver abscesses, lactic acidosis and reduced acetate to propionate ratios. Allen (1997) demostrated that low ruminal pH has direct, negative effects on energy intake and absorbed protein, which are primary factors limiting production of high producing dairy cows. There are also economic concerns because of feeding energy dense diets. Donovan (1997) reported that sub-acute ruminal acidosis (SARA) cost the United States dairy industry over US$ 400 million per annum.

A major consideration when replacing forage with easily fermentable carbohydrate is the relatively low potential for stimulating chewing activity. Energy and fiber requirements of dairy cows in early lactation are not easily met because the requirement for lactation exceeds the energy supply (NRC, 2001). Feeds that are high in starch and low in fibre content are usually fed to increase intake of energy. In many cases it results in a failure in the regulation of ruminal pH,

which can have serious effects on animal health and productivity (Davis, 1979). The use of dietary buffers may therefore be justified under these circumstances.

2.2. Dairy cow diets

Dairy cows need adequate structural fibre to stimulate digesta stratification and rumination, which affect ruminal buffering, fiber digestion, digesta turnover, feed intake and nutrient utilization (NRC, 2001; Zebeli *et al.*, 2006). Feeding diets low in structural fiber to meet energy and nutrient requirements is a problem in dairy herds, and can lead to a low utilization of nutrients and an increased risk of ruminal acidosis (Krause and Oetzel, 2006). Conversely, diets that have high levels of structural fiber may decrease feed intake and lower the feed efficiency ratio due to reduced microbial protein synthesis (Yang and Beauchemin, 2006).

Several trials, including studies by Rode *et al.* (1985), Yang *et al.* (2001, 2002) and Krause and Combs (2003), were carried out to characterize the effects of forage particle size and the amount of fermentable carbohydrates in the ration on chewing activity, rumen fermentation and metabolism. It is generally accepted that elevating the dietary concentrate level (>500 g/kg) increases the risk of metabolic disorders. Krause and Combs (2003) showed that particle size was positively related to chewing activity and ruminal pH. Yet, Yang *et al.* (2001) and Kononoff and Heinrichs (2003) reported that dietary particle size was poorly related to ruminal pH. The discrepancy of ruminal pH response to dietary particle size was also reflected by inconsistent measures of efficiency of rumen microbial protein synthesis. Reduced forage particle size was reported to positively affect rumen microbial protein synthesis in dairy cows (Rode *et al.*, 1985; Yang and Beauchemin, 2006), whereas Yang *et al.* (2002) found a negative relationship in lactating Holstein cows.

The complexity of the interactions between forage particle size and dietary factors, such as the amount of ruminally degradable concentrate, often leads to difficulties in characterization of effects of diet type on rumen fermentation (Russell, 1998). Furthermore, the level of feed intake

may determine important qualitative changes, particularly on reticulo-rumen passage and digestion kinetics, which may confound effects of dietary particle size on rumen microbial activity. Russell (1988) stated that microbial protein synthesis is affected by the interaction of ruminal pH and retention time which alter nitrogen and metabolizable energy availability, and hence efficiency of microbial growth.

2.3. Ruminal metabolism

2.3.1. Rumen microorganisms

The rumen is warm, moist, anaerobic, and filled with food, conditions that support the growth of microorganisms. The rumen microbial ecosystem contains hundreds of species of anaerobic bacteria, protozoa, and fungi, and each species occupies a particular niche. Rumen microorganisms have evolved over millions of years and there are numerous interrelationships among them. Commensalism, mutualism, competition, and predation all occur within the rumen (Hungate, 1966). All species digest some component of the food and together produce the microbial protein and volatile fatty acid (VFA) that are used as nutrients by the host animal.

The rumen bacteria are adapted to live under pH conditions of 5.5 to 7.0, in the absence of oxygen, at a temperature of 39^0C and at the expense of the ingesta provided by the ruminant (Hungate, 1975). There are facultative and obligate anaerobic bacteria. The steady supply of food and continuous removal of fermentation products and food residues maintain relatively constant conditions in which an extremely dense population develops (William and Withhers, 1991). The constant conditions restrict the number of niches relative to other anaerobic situations, such as soil and sludge fermentation. Furthermore, the species are limited to those that will compete with the turnover of rumen contents. Very slow growing strains may be eliminated or limited by washout, depending on the rate of passage.

More than 200 species of bacteria have been identified, and most of them are non-spore-forming anaerobes. The rumen contains between 10^9 and 10^{10} bacteria/mL of rumen contents (McDonald *et al.*, 2002); the number varies, depending on animal and dietary conditions. The principal species that digest fiber are *Fibrobacter ruminococcus, F.succinogenes, Prevotella, Streptococcus, Ruminococcus and Lachnospira.* There are two main groups of carbohydrate utilizing rumen bacteria, the structural carbohydrate (SC) digesters and those that ferment non-structural carbohydrates (NSC). The total number of bacteria and the relative proportion of any species vary with the animal's diet. For example, diets rich in readily fermentable carbohydrates promote high total counts of NSC bacteria and encourage proliferation of lactobacilli. Rumen bacteria need a balance of protein and energy supply to promote feed digestion. However, when the supply of energy and protein are not synchronized or balanced, there is energy wastage as the microbes break down protein to meet energy requirements (Clark *et al.*, 1992). The NSC bacteria are more sensitive to changes in rumen pH compared to the SC bacteria.

Protozoa are ciliates and belong to two families. The Isotrichdae are ovoid organisms covered with cilia and they include the genera *Isotricha* and *Dastricha.* The Ophryoscolecidae, or oligotrichs, include many species that vary considerably in size, shape, and appearance and include the genera *Entodinium, Diplodinium, Epidinium* and *Ophryoscolex.* Protozoa occur in the rumen in much smaller numbers (10^6 per mL) than bacteria, but are much larger in size compared to bacteria (McDonald *et al.*, 2002). The effects of protozoa on cell-wall carbohydrate degradation in the rumen are not clearly understood. However, protozoa have been implicated in bacteria predation. Williams and Coleman (1997) showed that bacterial concentrations are generally lower in the rumen contents of faunated animals, while the bacterial population increased when the animal were defaunated. A possible exception would be that those bacteria attached to particulate matter would be less likely to be ingested by protozoa (Prins *et al.*, 1983). The use of high starch diets or defaunating agents such as copper sulfate in the total mixed ration of ruminants can be helpful to eradicate the existing population of protozoa and, therefore, the predation of bacteria (William and Withhers, 1991).

Rumen fungi are strictly anaerobic (Hungate, 1966). At least 12 species have been identified, typically those belonging to the genus *Neocallimastix*. The life cycle of fungi includes a mobile phase and a vegetative phase. During the vegetative phase, they become attached to food particles by rhizoids, which can penetrate cell walls. The rumen fungi are capable of utilizing most polysaccharides and many soluble sugars. Some carbohydrates not used by these fungi are pectin, polygalacturonic acid, arabinose, fructose, mannose and galactose (Prins *et al.*, 1983). Fungi therefore use up nutrients that would otherwise benefit the microbes and the host animal. Fungi, however, play an important role in fibre digestion and their numbers tend to proliferate when diets are rich in digestible fiber (Hungate, 1966).

2.3.2. Carbohydrate fermentation in the rumen

Diets of rangeland ruminants contain considerable quantities of fiber and less starch and water-soluble carbohydrates; whilst cattle on intensive systems tend to receive feeds high in starch and soluble carbohydrates. Cellulose and hemicelluloses are the main carbohydrate components of plant cell walls comprising 40-50% and 20-30% of the dry matter of vascular plants, respectively (Ward, 1981). The rumen microorganisms attack all the carbohydrates, but not lignin; the principal bacterial species involved are *Fibrobacter succinogenes* and *Ruminococci*. Fungal enzymes are also assumed to play an important role (McDonald *et al.*, 2002).

The major substrates for fermentation are complex carbohydrates originating from plant cells. The principal products of this fermentation are short chain volatile fatty acids (VFA), especially acetic acid, propionic acid and butyric acid, and gases, carbon dioxide and methane (Owens, 1998). The molar proportion of acetate to propionate to butyrate usually found in the bovine rumen under normal conditions is 65:20:15 (Murphy *et al.*, 1982). The relative concentrations of VFA's are often assumed to represent their relative rate of production, but this may be misleading if individual VFA's are absorbed at different rates (Begman, 1966). The total concentration of VFA varies widely according to the animal's diet and time since the previous meal, but is normally in the range of 70-150 mmol/litre (Begman, 1966). Production of VFA by

rumen microflora occurs at variable rates during the day and is influenced by the nature of the diet and feeding patterns of the animal (Van Soest 1982; Czerkawski, 1986).

Feeding concentrates usually increase milk production because of the increased supply of glucose coming from propionate, but acetic acid for fat synthesis may be in short supply (Owens *et al.*, 1998). This shortage of acetic acid due to reduced fiber digestion is associated with the reduction in milk fat production. In fact, excess propionate relative to acetate often results in the cow using the available energy for fatty tissue deposition rather than milk synthesis (Bergman and Wolff, 1971). Butyrate is a ketogenic VFA, as it is metabolized to β-hydroxybutyrate during absorption across the rumen epithelium (Owens *et al.*, 1998). β -hydroxybutyrate has been demonstrated to increase hepatic glucogenic activity through its metabolism to acetyl-CoA, an allosteric activator of pyruvate (Bergman, 1990).

Sugars, such as glucose, are fermented to lactate (McDonald *et al.*, 2002). Under normal rumen pH conditions lactate does not accumulate beyond 5 μ M, hence ruminal concentrations of lactate exceeding 40 mM indicate severe acidosis. Ruminal microbes produce two types of lactate, namely the D and L forms. The L form is easily metabolizable by the liver and heart tissue, whereas D-lactate and VFA predispose the animal to acidosis occurrence (Koers *et al.*, 1976; Slyter and Rumsey, 1976). *Streptococcus bovis* and lactobacilli, which ferment carbohydrates to lactic acid, as well as coliforms and the amino acid degrading microbes associated with tyramine and histamine production also, contribute to ruminal acidosis (Slyter and Rumsey, 1976).

2.3.3. Nitrogen metabolism in the rumen

Microbes synthesized in the rumen and amino acids from dietary protein that is not degraded in the rumen but is intestinally digestible (Tamminga, 1996) are available to meet the metabolizable protein and amino acid requirements of ruminants. Dietary protein is divided into rumen degradable (RDP) and undegradable protein (RUP), with RDP composed of non-protein N and

true protein nitrogen. True protein is degraded to peptides and amino acids (AA) and eventually deaminated to ammonia or incorporated into microbial protein. Non-protein N is composed of N present in DNA, RNA, and ammonia. Digesta passing from the rumen via the abomasum to the duodenum contains ammonia N, undegraded protein, and microbial protein (McDonald *et al.*, 2002). When dietary RDP is in excess of the amount required by ruminal microorganisms, the protein is degraded to ammonia N, absorbed, metabolized to urea in the liver, and excreted in the urine (Tamminga, 1996).

Rumen microbial proteolytic activity is associated with bacterial cells and protozoa have little activity toward soluble proteins (Nugent and Magan, 1981). However, the protozoa play an important role in the engulfment of bacteria and particulate matter and hence degradation of insoluble proteins (Coleman, 1979). Many bacteria are proteolytic. Most of the protease-producing bacteria in the rumen are Gram-negative, including species of *Bacteriodes, Selenomonas,* and *Butyrivibrio* (Cotts and Hespell, 1986). Other species, probably of less significance, are *Megasphaera elsdenii*, *Streptococcus bovis*, *Clostridium* spp., *Eubacteriumn* spp., *Lachnospira multiparus*, *Succinivibrio dextrinosolvens*, and *Spirochaetes* (Allison, 1970). Russell and Rychlik (2001) demonstrated that the Gram-positive *S. bovis* played a predominant role in ruminal proteolysis, especially on high concentrate diets.

Protein degradation in the rumen involves attachment of bacteria to feed particles, followed by activity of cell-bound microbial proteases (Brock *et al.*, 1982). Approximately 70 to 80% of ruminal microorganisms attach to undegraded feed particles in the rumen (Craig *et al.* 1987), and 30 to 50% of those have proteolytic activity (Prins *et al.*, 1983). Large numbers of different microbial species form a consortium that attaches to feed particles, acting symbiotically to degrade and ferment nutrients, including protein. The rate and extent at which the protein degradation occurs will depend on proteolytic activity of the ruminal microflora and the type of protein (Wallace *et al.,* 1997). Peptides and amino acids are formed from extracellular rumen proteolytic activity and are transported inside microbial cells. Peptides can be further degraded by peptidase into amino acids, and the latter can be incorporated into microbial protein or further

deaminated to ammonia, VFA and CO_2 (Tamminga, 1979). The fate of absorbed peptides and amino acid once inside the microbial cell will depend on the availability of energy. If energy is available, AA will be transaminated or used directly for microbial protein. However, if energy is limiting, AA will be deaminated and their carbon skeleton will be fermented into VFA.

Ruminal microbial protein synthesis depends on the supply of an adequate amount and type of carbohydrate as an energy source for synthesis of peptide bonds. Several *in vitro* (Stern and Hoover, 1979; Henning *et al.*, 1991) and *in vivo* (Casper and Schingoethe, 1989; Cameron *et al.*, 1991) studies demonstrated that infusion of increasing amounts of readily fermentable carbohydrates decreased ammonia N concentrations because of improved N uptake by ruminal microbes. In addition to adequate supplies of carbohydrate and N source, as well as other nutritional factors, such as sulfur supply, other non-nutritional factors, such as ruminal pH and dilution rate and dilution rate, also play an important role in microbial protein synthesis.

The optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0 (Kopecny and Wallace, 1982). Cardozo *et al.* (2000, 2002) compared high forage and high concentrate diets at pH values ranging from 4.9 to 7.0 and demonstrated that protein degradation was reduced as pH decreased. Although amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Wallace *et al.*, 1997), protein degradation in the studies of Cardozo *et al.* (2000, 2002) was consistently lower with high concentrate diets. In addition, Lana *et al.* (1998) reported that a decrease in ruminal pH from 6.5 to 5.7, after feeding of concentrate diets, reduced ruminal ammonia concentration only when bacteria were obtained from cattle on forage diets.

2.4. Ruminal environment

2.4.1. Ruminal osmolality

Ruminal osmolality normally ranges from 240 to 265 mOsm for animals on roughage diets and 280 to 300 mOsm on concentrate diets (Garza *et al.*, 1989). Owens *et al.* (1998) reported ruminal

osmolality as high as 515 mOsm under low ruminal pH conditions. Minerals, VFA, lactate, and glucose are the primary solutes in ruminal fluid and contribute to the increase in ruminal osmolality. In blood dissolved protein contributes substantially to osmotic pressure, which normally ranges from 285 to 310 mOsm. When ruminal osmolality is greater than blood osmolality, water from blood is drawn rapidly inward through the rumen wall. Rapid influx to neutralize osmotic pressure swells the ruminal papillae and can pull patches of the ruminal epithelium into the rumen by stripping the internal surface layers of the ruminal wall from the underlying layers (Eadie and Mann, 1970). Damage to the rumen wall or small intestine due to high osmotic pressure, detected later as sites of abscesses, is a result of this rapid influx of water.

An elevation of osmotic pressure in the rumen is sensed by the wall of the reticulo-rumen leading to an inhibition in feed intake (Carter and Gravum, 1990). Osmotic pressures above 350 mOsm inhibit bacterial digestion of fiber and starch, causing the rumen contents to become stagnant. Higher osmolality (>300 mOsm), combined with distention of the abomasum through inhibition of outflow, complicates removal of fluid and acid from the rumen (Scott, 1975). Contributors to osmotic pressure, such as dietary buffers or water, can be changed to alter osmolality. Salt at 5% of the diet increased ruminal osmolality to 344 mOsm and reducing minerals intake might reduce ruminal osmolality slightly (Owens *et al.*, 1998). Increasing input of saliva, at approximately 255 mOsm, also reduces ruminal osmotic pressure. High moisture diets or increased intake of water probably does not reduce ruminal osmolality because fermented diets often have a high osmolality, and drinking water may partially flush past the rumen.

2.4.1. Ruminal pH

Kohn *et al.* (2000) reported that ruminal pH is a function of digestion and absorption, changes in strong ion concentration, and changes in partial pressure of CO_2 in the rumen fluid. Ruminal pH drops below physiological levels when ruminants consume excessive amounts of rapidly fermentable carbohydrates. The rate of ruminal pH decline is faster as meal size increases and as dietary NDF decreases (Dado and Allen, 1993). The total intake of ruminally fermentable carbohydrates depends equally on total DM intake and the density of NSC in the diet. High

intake of NSC is associated with low rumen pH (Oetzel and Nordlund, 1988). This suggests that ruminal acidosis may be a more common problem in dairy herds (Krause and Oetzel, 2006).

Ruminal pH varies considerably during the course of a day. Increasing the frequency of feeding might decrease the variation in post-feeding ruminal pH, but can also lead to increased DM intake and ultimately result in low mean ruminal pH (Oetzel and Nordlund, 1999). However, the mean ruminal pH value is not dramatically affected by large dietary changes. Kennely *et al.* (1999) reported mean ruminal pH values of 6.31 and 6.15 for cows fed diets containing 50 and 75% concentrate; respectively, whereas the lowest pH appeared to be different (5.9 versus 5.5) for the same diets. Krause and Combs (2003) found that partially replacing alfalfa silage with corn silage did not affect mean ruminal pH, but it did decrease the lowest pH significantly. Cattle are generally able to maintain the pH within the physiological limits by their own regulation of intake, endogenous buffer production, microbial adaptation, and VFA absorption. However, the amounts of carbohydrate may yield more acid than the system can accommodate.

Low ruminal pH during sub-acute ruminal acidosis reduces the number of bacterial species in the rumen, although the metabolic activity of the bacteria that remain is very high (Garry, 2002). Protozoa populations also do not survive extended exposure to pH below 5.5. When fewer species of bacteria and protozoa are present, the ruminal microflora is less stable and less able to maintain pH during periods of sudden dietary changes (Garry, 2002). Thus, pre-existing sub-acute ruminal acidosis could increase the risk for acute ruminal acidosis in the event of accidental ingestion of excessive amounts of grain.

2.4.2. Ruminal acidosis

Acidosis is an imbalance in the acid-base system of the animal. Acidic conditions may occur in blood (metabolic acidosis), the rumen (rumen acidosis) or both in the rumen and blood (Krause and Oetzel, 2006). Dairy cattle are commonly fed *ad-libitum* to maximize potential dry matter

intake and milk yield. However, excessive intake of rapidly fermentable carbohydrates is the most obvious cause of ruminal acidosis in dairy cows.

Acute and sub-acute ruminal acidosis (SARA) is share a similar aetiology but is completely different clinical diseases. In acute ruminal acidosis an excessive intake of highly fermentable carbohydrates results in a sudden and uncompensated drop in rumen pH. Rumen pH drops below 5.5 as lactic acid concentrations increase (Owens *et al.*, 1998). Clinical signs of acute ruminal acidosis include complete anorexia, abdominal pain, tachycardia, tachypnea and diarrhea (Garry, 2002). Cows that survive the initial systemic effect of acute ruminal acidosis may later succumb to complications from severe mycotic or bacterial rumenitis (Radostits *et al.*, 1994).

SARA is defined as periods of moderately depressed ruminal pH (about 5.5 - 5.0) that is between acute and chronic in duration (Garrett *et al.*, 1997). Lactic acid does not consistently accumulate in the rumen fluid of dairy cattle affected with SARA (Oetzel *et al.*, 1999). However, transient spikes of ruminal lactate up to 20 mM can be discovered if ruminal lactate concentrations are measured frequently during the day (Kennelly *et al.*, 1999). The depression of ruminal pH in cows with SARA is apparently due to the total accumulation of volatile fatty acids and is not due to lactic acid accumulation (Owens *et al.*, 1998). Krause and Oetzel (2006) reported that milk yield dropped by 3.5 kg/d in cows experiencing SARA and remained lower during the recovery period. Dry matter intake decreased by 0.8 kg/d but there was no change in milk fat yield and milk protein.

2.5. Methods of alleviating ruminal acidosis

Dietary buffers cannot eliminate the causes of ruminal acidosis, but it can help to manage the problem. The response to feeding buffers depends upon the type of forage fed and its physical structure. However, the addition of dietary buffers is probably more likely to be beneficial in diets containing marginal amounts of effective fibre. Dairy cattle have diffrent primary ways of preventing a drop in ruminal pH due to either acid ingested or that produced by rumen

microorganisms. These include buffers that occur naturally in saliva and buffering capacity of ingested feed. The addition of a dietary buffer is considered only when the salivary buffers and natural feed buffering capacity fail to prevent a drop in pH.

2.5.1. Salivary buffer

Eating and ruminating stimulate the secretion of saliva (Baily and Blach, 1961) and so increase the buffering capacity of the rumen fluid. Chewing activity is highly affected by the nature of the feed. Welch and Smith (1969) demonstrated that rumination increased with the cell-wall content of coarse forages. Physical properties include length (Santini *et al.*, 1983; Jaster and Murphy, 1983; Lu, 1987), specific fragility (DesBordes and Welch, 1984) and rate at which specific gravity increases due to hydration and ion exchange (Hooper and Welch, 1985). All these factors play a role in the separation and differential clearance of particles from the reticulo-rumen (Sutherland, 1987).

Diet formulation based on neutral detergent fibre (NDF) as a percentage of the ration DM has been recommended because of the positive relationship between NDF and rumen fill and the negative relationship between NDF and energy density of the diet (Mertens, 1994). However, NDF level is usually not used alone as such, hence, Mertens (1997) introduced the concept of physically effective fiber (peNDF), which is primarily related, to particle size of the fiber. Increasing forage particles size increases salivary flow that is rich in sodium bicarbonate and acts as the primary buffer. Estimation of saliva flow as a function of DM intake ranged from 9.6 to 32.3 L/kg DM with a mean of 18.2 L/kg DM (Erdman, 1988). The NRC (2001) recommends that the minimum proportion (22%) of the recommended level of dietary NDF come from forage. Others, (Pitt *et al.*, 1996) have adopted the concept of effective NDF (eNDF) to describe the ability of a feed to replace forage such that milk percentage is maintained.

2.5.2. Feed buffering capacity

Feed buffering capacity is the inherent buffering capacity of the diet and is largely explained by dietary cation-anion difference (DCAD). Diets high in Na^+ and K^+ relative to Cl^- and S^- have high DCAD values, tend to support higher ruminal pH, and increase dry matter intake and milk yield (Sanchez *et al.*, 1994; Block and Sanchez, 2000). The optimal DCAD for an early lactation diet is about +40 mequiv/100 g of DM $(Na^+ + K^+) - (Cl^- + S^-)$ (Block and Sanchez, 2000). Mid-lactation cows require an optimal DCAD of about +27.5 to +40 mequiv/100 g of diet DM. Formulating diets with a high DCAD requires buffer supplements such as sodium bicarbonate or potassium carbonate. Alfalfa forages tend to have a higher DCAD than corn silage, although this depends considerably on the mineral composition of the soil on which they were grown (Erdman, 1988). Concentrate feeds typically have a low or negative DCAD, which adds to their already high potential to cause ruminal acidosis because of their fermentable carbohydrate.

2.5.3. Ionophores

Carboxylic polyether ionophore antibiotics are produced by various strains of Streptomyces and include monensin, lasalocid, salinomycin and narasin. Monensin exerts its effects primarily by altering the ruminal fermentation pattern, including a decrease in acetate and butyrate production and an increase in propionate production. Grummer (1995) also reported that monensin lowered DMI when fed at 200 mg/d but reduced beta-hydroxybutyrate production and the incidence of ketosis. Began and Bates (1984) found that in diets containing high levels of NSC, ionophores depressed feed intake, but body weight was not reduced.

2.5.4. Yeast cultures

Yeast culture is a live culture of yeast and the media on which it was grown (Williams and Withhers, 1991). Yeast cultures are valuable in stimulating microbial growth in the rumen, thereby increasing the supply of energy and protein to the animal. Yeasts remove oxygen in rumen environment, preventing toxicity to anaerobic microbes and they also decrease lactic acid

production by feeding by suggar; thus helping to prevent the occurrence of ruminal acidosis (Williams and Withhers, 1991).

2.5.5. Commercial buffers

Several chemicals have been evaluated for their buffering capacity and proved to be effective buffers of ruminal fluid (Herod *et al.*, 1978). Many compounds are currently being used as buffering agents, but would not be considered as chemical buffers. For example, magnesium oxide (MgO) is frequently referred to as a buffering agent and is a common additive in dairy rations, but it is rather an acid-neutralizing agent. Some of the buffers that have been evaluated in the literature include limestone (Rogers *et al.*, 1985), disodium phosphate (Hart and Polan, 1984), potassium carbonate (West *et al.*, 1987), potassium bicarbonate (West *et al.*, 1986), sodium sesquicarbonate (Coppock *et al.*, 1986), magnesium oxide (Xin *et al.*, 1989), Acid Buf (Cruywagen *et al.*, 2004) and sodium bicarbonate, which is the most extensively used buffer as it is easily available and less costly.

2.5.5a Sodium bicarbonate

$NaHCO_3$ is the most common buffer on the market and first appeared in a dairy cattle feeding experiment in the early 1960's. Muller *et al.* (1985) added sodium bicarbonate to the concentrate portion at 2.6 - 4.6% of the DM and noticed that it improved milk fat percent, but reduced DM intake compared to the cows fed the unsupplemented diets. A summary of 28 experiments (Staple and Lough, 1988) showed that cows consuming $NaHCO_3$-supplemented diets in early lactation produced an average of 0.8 kg/d more milk (30.5 vs. 29.7 kg/d) with 0.16% more milk fat (3.54 vs. 3.38%) than unsupplemented cows. This resulted in an average of 1.4 kg more 4% fat-corrected milk (FCM) yield daily for cows consuming $NaHCO_3$ over control cows (28.2 vs. 26.8 kg/d). Cows in mid-lactation (Staples and Lough, 1988) also responded to $NaHCO_3$ supplementation by producing 0.9 kg/d more milk (25.3 vs. 24.4) with 0.30% higher milk fat (3.28 vs. 2.98) than control cows. This resulted in an average increase of 1.9 kg yield of 4% FCM by cows receiving supplemental $NaHCO_3$ (22.5 vs. 20.6 kg/d). The $NaHCO_3$ was fed at an average level of 1.1% of dietary DM, regardless of stage of lactation.

2.5.5b Refined sodium sesquicarbonate (S-Carb)

Sodium sesquicarbonate is a mixture of $NaHCO_3$ and Na_2CO_3. A refined product has all inert materials removed and one is sold under the label "S-Carb". Muller and Sweeney (1985) reported no improvement in 4% FCM yield for cows consuming either S-carb or $NaHCO_3$-supplemented diets compared to animals on the control diets. In the early stage of lactation, S-carb has been proven to be effective as a dietary buffering compound by increasing 4% FCM yield compared to cows on control diets. The average increase in 4% FCM was approximately 1.7 kg/cow per day (Cassida *et al.*, 1988). There was little response in 4% FCM when S-carb was included at $\geq$ 1.25% (Poos-Floyd and Coyle, 1986). Cows consuming S-carb consumed more DM (0.9kg/d) and produced milk with a higher fat content (0.24% units) than cows receiving control diets (Cassida *et al.*, 1988). S-Carb also resulted in higher milk fat percentages than did $NaHCO_3$. This is because S-carb has some alkalizing and buffering potential, as it contains Na_2CO_3, as well as $NaHCO_3$.

2.5.5c Unrefined sodium sesquicarbonate (Alkaten)

Alkaten differs from S-carb in that it contains almost 6% inert material and is slightly lower in sodium than S-carb. Harris and Ventura (1985) reported that Alkaten and sodium bicarbonate were equally effective in increasing 4% FCM yield, although milk fat percent was lower for heifers consuming buffer-supplemented corn silage diets. However, Coppock *et al.* (1986) demonstrated that the performance of cows consuming Alkaten was similar to control cows and inferior to those fed sodium bicarbonate. Briceno *et al.* (1986) fed either Alkaten or sodium bicarbonate, and found that Alkaten was more effective in elevating milk fat percent than sodium bicarbonate, but high levels of Alkaten were required to increase 4% FCM yield above that of sodium bicarbonate (1.2 vs. 0.6% of diet DM).

2.5.5d Multi-element buffer (RumenMate)

RumenMate is a dietary buffer, consisting mainly of sylvite (KCl) and northupite ($MgCO_3.Na_2CO_3.NaCl$). Other components include potassium, magnesium, sodium and chloride.

Unlike other buffers it contains less sodium, only 8.8%. Staples and Lough (1988) reported on a trial where cows in mid-lactation were supplemented with 1% $NaHCO_3$, 1% RumenMate or 3% RumenMate. Sodium bicarbonate improved milk fat with 0.25% units, while RumenMate increased the fat test with 0.46 and 0.69% units when supplemented at 1 and 3%, respectively. Solorzano *et al.* (1989) also found RumenMate to have a positive, linear effect on milk fat percentage when fed at levels up to 4.5% of diet DM.

2.5.5e Magnesium Oxide

Magnesium oxide (MgO) is also used as a buffer. Teh *et al.* (1985) reported positive reactions in milk fat percentage when corn silage-based diets were supplemented with MgO at levels of 0.4 to 0.8% of diet DM. O`Connor *et al.* (1988) improved milk yield by increasing dietary MgO from 0.22 to 0.48% with no change in milk fat percent, but supplementing MgO at 0.60% of the diet DM reduced milk yield. Teh *et al.* (1985) demonstrated that 0.4% dietary MgO significantly increased milk yield, but when MgO was fed at 0.8% of the diet DM, milk yield declined. Erdman *et al.* (1980) also increased dietary Mg from 0.15 to 0.61% by feeding MgO at 0.8% of diet DM and observed no changes in milk yield or fat percentage. However, the same researchers in later experiments (Erdman *et al.*, 1982) found that 0.8% dietary MgO (which increased dietary Mg from 0.24 to 0.67%) significantly increased milk fat percentage, therefore, possibly illustrating its alkalizing characteristic.

2.5.5f Acid Buf

Acid Buf (*Lithothamnium calcareum*) is a natural dietary buffer derived from calcified seaweed that is harvested off the Irish Coast. It is rich in calcium and magnesium, plus all important trace minerals. It is used as a buffer in ruminant nutrition. Cruywagen *et al.* (2004) found that milk yield was significantly increased by increasing Acid Buf from 0.125 to 0.3% of the diet and also reported that milk fat and 4% FCM yield was highest at 0.3% Acid Buf in the diet. In the same study, milk protein percentage increased by 0.6%. Acid Buf can be used as a top dressing on silages or added to total mixed rations. Alternatively, Acid Buf can be included in mineral packs

or formulated into compound feeds. Acid Buf degrades slowly under acidic conditions and operate at a pH level of approximately 6.0, so preventing the rumen becoming too acidic and helps to maintain conditions for optimal microbial activity. Because Acid Buff is distinctly different from other buffers, it is important to investigate its contribution as a buffering agent in diets of high yielding dairy cows.

2.6. References

Allen, M. S., 1997. Relationship between acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447-1462.

Allison, M. J., 1970. Nitrogen metabolism in the rumen microorganism. Page 456 in Physiology of digestion and metabolism in the ruminant. Phillipson, A. T., Amison, E. F., Amstrong, D. G., Blach, C. C., Caroline, R. S., Hardy, R. N. Hobbson, P. N. and Knenes,R. D., ed. Oriel Press, Newcastle upon Tyne, UK.

Bailey, C. B. & Blach, C. C., 1961. The composition and rate of secretion of parotid saliva in a small steer. Br. J. Nutr., 15:371-382.

Bergen, W. G. & D. B. Bates., 1984. Ionophores: Their effect on productionefficiency and mode of action. J. Anim. Sci. 58:1465-1483.

Bergman, E. N., 1990. Energy contribution of volatile fatty acids from the gastrointestinal tract in various species. Am. Physiol. Soc. 70:567-590.

Bergman, E. N. & Wolff, J. E., 1971. Metabolism of volatile fatty acids by liver and portal-brained viscera in sheep. Am. J. Physiol. 221:586-592.

Bergman, E. N., Ried, R. S., Murray, M. G., Brockway, J. M. & Whitelow, F. G., 1966. Interconversion and production of volatile fatty acids in the sheep rumen. J. Biochim. 97:53-58.

Block, E. & Sanhez, W. K., 2000. Is it important to adjust the dietary cation-anion difference for lactating cow? In: Proceedings of the three states Dairy Nutrition Conference, Ohio State cooperative extension service, Columbus, Ohio, pp.27-39.

Briceno, J. V., Beede, D. G. & Staples, C. R., 1986. Additions of sodium bicarbonate and sodium sesquicarbonate (trona) to corn silage or cottonseed hull containing diets: lactation performance of midlactation Holstein cows. University of Florida, Gainesville, unpublished report.

Brock, F. M., Forsberg, C. W. & Buchanan Smith, J. G., 1982. Proteolytic activity of rumen microorganism. Appl. Environ. Microbiol. 44:561.

Cameron, M. R., Klusmeyer, T. H., Lynch, G. L., Clark, J. H. & Nelson, D. R., 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74:1321-1336.

Cardozo, P., Calsamiglia, S. & Ferret, A., 2000. Effect of pH on microbial fermentation in a dual flow continuous culture system. J. Dairy Sci. 83 (Suppl. 1):265.

Cardozo, P., Calsamiglia, S. & Ferret, A., 2002. Effect of pH on nutrient digestion and microbial fermentation in a dual flow continous culture system fed a high concentrate diet. J. Dairy Sci. 85 (Suppl. 1):182.

Carter, R. R. & Grovum, W. L., 1990. A review of the physiological significance of hypertonic body fluids on feed intake and ruminal fuction: Salivation, motility and microbes. J. Anim. Sci. 68: 2811-2832.

Cassida, K. A., Muller, L. D. & Sweeney, T. F., 1988. Sodium sesquicarbonate for early lactation dairy cows fed corn silage-based diets. J. Dairy Sci.71:381-387.

Casper, D. P. & Schingoethe, D. J., 1989. Lactational response of dairy cows to diets varying in ruminal solubility of carbohydrate and crude protein. J. Dairy Sci. 72:928-941.

Clark, J. H., Klusmeyer, T. H. & Cemeron, M. R., 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323.

Coleman, G. S., 1979. Rumen ciliate protozoa. Page 381 *in* Biochemistry and physiology of protozoa. 2nd ed. Vol. 2. Academic Press, New York, NY.

Coppock, C. E., Shelling, G. T., Byers, F. M., West, J. W., Labore, J. M. & Gates, C. E., 1986. A naturally occurring mineral as buffer in the diet of lactating dairy cows. J. Dairy Sci. 69:111-123.

Cotts, M. A. & Hespell, R. B., 1986. Protein and amino acid metabolism of rumen bacteria. Page 122 *in* control of digestion and metabolismin ruminants. L. P. Milligan, W. L. Grovum, and A. Dobson, ed. Butterworths, Washington, DC.

Craig, W. M.., Broderick, G. A. & Ricker, D. B., 1987. Quantification of microorganisms associated with particulate phase of ruminal ingesta. J. Nutr. 117:56-62.

Cruywagen, C.W., Swiegers, J.P. & Taylor, S., 2004. The effect of Acid Buf in dairy cow diets on production response and rumen parameters. J. Dairy Sci. 87 (Suppl 1): 46.

Czerkawski, J. W., 1986. An introduction to rumen studies. Pergamon press, London.

Dado,R. G. & Allen, M. S., 1993. Relationship between eating behavior and ruminal pH for dairy cow consuming low or high fiber diets. Page 18 in Abstr. Conf. Ruman function, Cornell Univ., Ithaca, NY.

Davis, C. L., 1979. The use of buffer in the ration of dairy lactating cows. Page 51 in regulation of acid base balance. W. H. Hale and P. Meinhardt, ed. Church and Dwight, Co., Inc., Pascalway, NJ.

Desborders, C. K. & Welch, J. G., 1984. Influence of specific gravity on rumination and passage of digestible particles. J. Anim. Sci. 59:470-475.

Donovan, J., 1997. Subacute acidosis is costing us millions. Hoard`s Dairyman 142, 666.

Eadie, J. M. & Mann, S. O., 1970. Developpment of microbial population: High starch diets and instability. In: A. T. Phillipson (Ed.) Physiology of digestion and metabolism in the ruminant. Pp 335-347. Oreil press, Newcastle upon Tyne, U.K.

Erdman, R. A. Botts, R. L., Hemken, R. W. & Bull, L. S., 1980. Effect of dietary sodium bicarbonate and magnesium oxide on production and physiology in early lactation. J. Dairy Sci. 63:923-930.

Erdman, R. A., R. W. Hemken, and L. S. Bull., 1982. Dietary sodium bicarbonate and maganesium oxide for early postpartum lactating dairy cow: effects on production, acid-base metabolism, and digestion. J. Dairy Sci. 65:712-731.

Erdman, R. A., 1988. Dietary buffering Requirements of the lactating Dairy Cow: A Review: J. Dairy Sci. 71:3246-3266.

FAO report, 2006. http://www.fao.org/newsroom/en/news/2006/1000349/index.html

Garrett, E. F., Nordlund, K. V., Goodger, W. J., Oetzel, G. R., 1997. A cross-sectional field study investigating the effect of periparturient dietary management on ruminal pH in early lactating cows. J. Dairy Sci. 80(Suppl 1) 169.

Garry, F. B., 2002. Ingestion in ruminants. In: Smith, B. P. (Ed.), large animal internal medicine, Mosby-Year Book. Mosby, St. Louis, Missouri, pp. 722-747.

Garza, G. D., Owens, F. N. & Breazzile., 1989. Effects of diet on ruminal fluid and on blood serum osmolality and hematocrit in feedlot heirfers. Okla. Agric. Exp. Stn. MP. 127:68-76.

Grummer, R. R., 1995. Impact of changes in organic nutrient metabolismon feeding the transition dairy cattle. J. Anim. Sci. 73:2820–2833.

Harris, B., Jr. & Ventura, M., 1985. Evaluation of Alkaten and Sodium bicarbonate in total mixed diets fed to primiparous Holestein cows. University of Florida, Gainesville, Unpublished report.

Hart, S. P. & C. E. Polan., 1984. Effect of sodium bicarbonate and disodium phosphate on animal performance, rumen metabolism, digestion, and rate of passage in ruminating calves. J. Dairy Sci. 67:2356-2379.

Henning, P. H., Styen, D. G. & Meissner, H. H., 1991. The effect of energy and nitrogen supply parttern on rumen bacterial growth in vitro. Anim. Prod: 165-175.

Herod, E. L., Bechtle, R. M., Bartley, E. E. & Dayton, A. D., 1978. Buffering ability of several compounds in vitro and the effect of the selected buffer combination on ruminal acid production in vivo. J. Dairy Sci. 61:1114-1122.

Hooper, A. P. & Welch, J. G., 1985. Effects of particle size and forage composition on functional specific gravity. J. Dairy Sci. 68:1181-1188.

Hungate, R. E., 1975. The rumen microbial ecosystem. Ann. Rev. Ecol. System. 6:39-66.

Hungate, R. E., 1966. The rumen and its microbes. Academic press, New York.

Jaster, E. H. & Murphy, M. R., 1983. Effect of varying particles size and forage composition and chewing behavior of dairy heifers. J. Dairy Sci. 66:802-810.

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

Koers, W. C., Britton, R., Klopfenstein, T. J. & Woods, W. R., 1976. Ruminal histamine, lactate and animal performance. J. Anim. Sci. 43:684-691.

Kohn, R. A., 2000. Three conditions of the ruminal milieu that determine pH. Proc. 25th Conf. Rumen function, Chicago, IL. Department of animal science, Michigan state University, East Lansing. Online. Avalaible http://www.msu.edu/rumen/index.htm .

Kononoff, P. J., Heinrichs, A. J., 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. J. Dairy Sci. 86:1445-1457.

Kopecney, J. & Wallace, R. J., 1982. Cellular location and properties of proteolytics enzymes of rumen bacteria. Appli. Environ. Microbial. 43:1026-1033.

Krause, K. M. & Combs, D. K., 2003. Effect of forage particle size, forage source and fermentability on performance and ruminal pH in midlactation cows. J. Dairy Sci. 86:1382-1397.

Krause, K. M., Oetzel, G. R., 2006. Understanding and preventing ruminal acute acidosis in dairy herds: a review. Anim. Feed Sci. Technol. 126:215-236.

Lana, R. P., Russell, J. B. & van Amburgh, M. E., 1998. The role of pH in regulating ruminal methane and ammonia production. J. Anim. Sci. 76:2190-2196.

Lu, C. D., 1987. Implication of forage particle length on chewing activities and milk production in dairy goats. J. Dairy Sci. 70:1411-1416.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. & Morgan, C. A., 2002. Animal nutrition. Sixth ed. pp. 163-198.

Mertens, D. R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R., 1994. Regulation of forage intake. In: Fahey Jr., G. C., Collins, M., Mertens, D. R., Moser, L. E. (Eds.)., Forage quality, evaluation, and utilization. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Am. Madison, Wisconsin, pp. 450-493.

Muller, L. D. & Sweeney,T. F., 1985. Sodium sesquicarbonate and sodium bicarbonate for midlactation Holstein cows. In: Penn state dairy and livestock Res. Rep., Pennsilvania State University, University Park, pp. 45-46.

Murphy, M. R., Baldwin, R. L. & Koong, L. J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55:411-421.

NRC., 2001. Nutrient requirements of Dairy cattle, seventh ed. (rev.) Natl. Acad. Sci., Washigton, DC, USA.

Nugent, J. H. A. & Mangan., 1981. Characteristics of the rumen proteolysis of protein 1 (18S) leaf protein from lucern (*Medicago sativa* L.). Br. J. Nutr. 46:39-58.

O`Connor, A. M., Beede, D. K. & Wilcox, C. J., 1988. Lactation responses to dietary magnesium potassium, and sodium during winter in Florida. J. Dairy Sci. 71:971-981.

Oetzel, G. R., Nordlund, K. V. & Garrett, E. F., 1999. Effect of ruminal pH and stage of lactation on ruminal lactate concentrations in dairy cows. J. Dairy Sci.82(suppl. 1):38.

Oetzel, G. R. & Nordlund, K. V. 1988. Effect of dry matter intake and feeding frequency on ruminal pH in lactating dairy cows. J. Dairy Sci. 81 (suppl.1): 297.

Owens, F. N., Secrist, D. S., Hill, W. J. & Gill, D. R., 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275-286.

Pitt, R. E., Van Kassel, J. S., Fox, D. G., Pell, A. N., Barry, M. C. & Van Soest, P. J., 1996. Prediction of ruminal volatile fatty acids and pH within the carbohydrate net protein system. J. Anim. Sci. 74:226-244.

Poos-Ployd, M. & Coyle, C. A., 1986. Effect of level of sodium sesquicarbonate on production and ruminal parameters of lactating Holstein cows. J. Dairy Sci., 69 (suppl.1): 241.

Prins, R. A., van Rheenen, D. L. & van`t Klooster, A. T. 1983. Characterisation of of microbial proteolytic enzymes in the rumen. Antonie Leeuwenboek. J. Microbiol. 49:585.

Radostits, O. M, Blood, D. C. & Gay, C. C., 1994. Acute carbohydrate engorgement of ruminants (rumen overload). In: Radostits, O. M., Blood, D. C., Gay, C. C. (Ed.), Veterinary medicine. W. B. Saunders, Philadelphia, P. A, pp. 262-269.

Rode, L. M., Weakley, D. C. & Satter, L. D., 1985. Effect of forage amount and particle size in diets of lactating dairy cows on site of digestion and microbial protein synthesis. Can. J. Anim. Sci. 65:101-111.

Rogers, J. A., Muller, L. D., Davis, C. L., Chalupa, W., Kronfeld, D. S., Karcher, L. F. & Cummings, K. R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early laction. J. Dairy Sci. 68:646-660.

Russell, J. B., 1988. Strategies that ruminal bacteria use to handle excess carbohydrate. J. Dairy Sci. 76:1955-1963.

Russell, J. B. & Rychlik, J. L., 2001. Factors that alter rumen microbial ecology. Science 292:1119-1122.

Sanchez, W. K., Beede, D. K. & Delorenzo, M. A., 1994. Mcromineral element interelationships and lactional performance: empirical models from a large data set. J. Dairy Sci. 77:3096-3110.

Santini, F. J., Hardie, A. R., Jorgensen, M. A. & Finner, M. F., 1983. Proposed use of adjusted intake based on forage particles length of calculation of roughage indexes. J. Dairy Sci. 66:811-820.

Scott, D., 1975. Changes in minerals, ater and acid-base balance associated with feeding and diet. In: I. W. McDonald and A. C. I. Waner (Ed.) Digestion and metabolism in the ruminant. Pp 205-215. University of new England Printing Unit, Armidale, N. S. W., Australia.

Staples, C. R. & Lough, D. S., 1988. Efficacy of supplemental dietary neutralizing agents for lactating dairy cows. A review. Anim. Feed Sci. Technol. 23: 277-303.

Stern, M. D. & H. Hoover., 1979. Microbial growth and flows as influenced by dietary manipulations. J. Dairy Sci. 70: 425-441.

Sutherland, T. M., 1987. Particle separation in the forestomach of sheep. In: A. Dobson and Dobson, M. J. (Editors), Aspect of digestive Physiology in ruminants. Comstock Publishing Assoc., Ithaca, NY, pp. 43-73.

Slyter, L. L. & Rumsey, T. S. 1976. Influence of acidosis on rumen function. J. Amin. Sci. 43:910-929.

Solorzano, L. C., Armentano, L. E., Grummer, R.R. & Dentine, M. R., 1989. Effects of Sodium Bicarbonate and Sodium sesquicarbonate on lactating Holstein cows. J. Dairy Sci. 72:453-461.

Tamminga, S., 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49:1615.

Tamminga, S., 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74:3112-3124.

Teh, T. H., Hemken, R. W. & Hamon, R. J., 1985. Dietary magnesium oxide interaction with sodium bicarbonate on cows in early lactation. J. Diary Sci. 68:881-890.

Van Soest, P. J., 1982. Nutritional ecology of the ruminant. O and B books, Inc., Corvallis, Oregon, USA, P. 252.

Wallace, R. J., Onodera, R. & Cotta, M. A., 1997. Metabolism of nitrogen-containing compounds. Page 283-328 in the rumen microbial Ecosystem. 2nd ed. P. N. Hobson and Stwart. Ed. Chapman and Hall, London, UK.

Ward, J. K., 1981. Non-competitive source of carbohydrate for animal feeding. Page 253 in new protein foods. Vol. Academic Press, New York, NY.

Welch, J. G. & Smith, A. M., 1969. Effect of varying amount of forage intake on rumination. J. Anim. Sci., 28:827-830.

West, J. W., Coppock, C. E., Nave, D. H. & Schilling, G. T., 1986. Effect of potassium buffers on feed intake in lactating dairy cows and on rumen fermentation in vivo and in vitro. J. Dairy Sci. 69:124-134.

West, J. W., Coppock, C. E., Nave, D. H., Labore, J. M., Greene, L. W. & Odom, T. W., 1987. Effects of potassium carbonate on rumen function in lactating Holstein cows. J. Dairy Sci. 70:81-90.

Williams, A. G., & Withhers, S. E., 1991. Effect of ciliate protozoa on the activity of polysaccharide-grading enzymes and and fibre breakdown in the rumen ecosystem. Appl. Bacteriol. 70:144-155.

Williams, A. G. & Coleman, G. S., 1997. The rumen protozoa. Pages 73-139 in the rumen microbial ecosystem. 2nd ed. P. N. Hobson & Steward, ed. Chapman & Hall, London, UK.

Williams, P. E. V., Tait, C. A. G., Innes, G. M. & Newbold, C. J., 1991. Effect of inclusion of yeast culture (Saccharomyces cereviciae plus growth medium) in the diet of dairy

cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Xin, Z., Tucker, W. B. & Hemken, R. W., 1989. Effect of reactivity rate and particle size on magnesium availability, acid-base balance, mineral metabolism, and milking perfomance of dairy cows. J. Dairy Sci. 72:462-470.

Yang, W. Z. & Beauchemin, K. A., 2006. Increasing the physically effective content of dairy cow diets may lower efficiency of feed use. J. Dairy Sci. 89:2694-2704.

Yang, W. Z., Beauchemin, K. A. & Rode, L. M., 2001. Barley processing forage: concentrate ratio, and forage length effects on rumen pH and digestion by dairy cows. J. Dairy Sci. 84:2203-2216.

Yang, W. Z., Beauchemin, K. A. & Rode, L. M., 2002. Effects of particle size of alfalfa based cow diets on site and extent of digestion. J. Dairy Sci. 85:1958-1968.

Zebeli, Q., Tafaj, M., Steingrass, H., Metzler, B. & Brochner, W. 2006. Effect of physically effective fiber on digestive processes and milk fat content in early lactation dairy cow fed total mixed rations. J. Dairy Sci. 84:651-668.

Chapter three

Effects of Acid Buf and sodium bicarbonate on rumen fermentation of lactating dairy cows fed high concentrate diets.

Abstract

The effects of Acid Buf and sodium bicarbonate on rumen fermentation of lactating dairy cows were tested in this experiment. High concentrate TMR, formulated to be potentially acidotic, was used to construct three dietary treatments in which Acid Buf, the skeleton remain of sea weed (Lithothamnium calcareum), was compared against limestone (control) and sodium bicarbonate plus limestone. The diet contained 4 g/kg DM of Acid Buf or 3.5 g/kg DM limestone (control) or 3.7g/kg DM of limestone plus 8 g/ kg DM of sodium bicarbonate, respectively. The response to treatment was measured using 6 ruminally cannulated lactating Holstein cows allocated to treatments according to a balanced 3 x 3 (n=2) Latin square design, with three treatments and three periods. The total experimental period was 66 days during which every cow received each diet for a period of 15 days prior to the data collection period of 7 days. Rumen fluid was collected for VFA, lactic acid or ammonia analysis. The concentration of VFA, lactic acid and ammonia in the ruminal fluid was not affected by buffer supplement to the TMR. Ruminal pH was monitored continuously every 10 minutes for two days using a portable data logging system and in-dwelling electrodes. The impact of acidity was clearly visible, especially for the period from mid-day to mid-night when ruminal pH dropped below 5.5 for a longer period (13 h) in the control (limestone) treatment than in the sodium bicarbonate (7.7 h) and Acid Buf (4 h) treatments. The minimum ruminal pH was lower for the control (5.14) than Acid Buf treatment (5.42), while the pH of the sodium bicarbonate treatment (5.37) did not differ from the other treatments. The experiment showed that supplementing the diet of dairy cows with approximately 90 g/day of Acid Buf may reduce the time period that rumen pH is below 5.5.

Key words: Acid Buf, Sodium bicarbonate, Buffers, pH, Rumen fermentation.

3.1. Introduction

Dietary buffers are utilized widely on a commercial basis to ameliorate the detrimental effects of high concentrate diets in the rumen of lactating dairy cows. The change in rumen fermentation via buffer supplementation is influenced by the type of buffer and the amount supplied as well as the composition of the basal diet (Rogers *et al.*, 1982). In a review of buffer research, Erdman (1988) reported that forage type and concentrate in the diet affect the responses to dietary buffers. Positive responses to dietary buffers in an acidotic diet have been demonstrated repeatedly (Erdman, 1988).

Several buffers and alkalinizing agents (i.e. neutralizing agents) have improved the rumen condition of dairy cows fed high concentrate diets. Marie (2006) reported that feeding high concentrate diets increased the occurrence of liver abscess, lactic acidosis and reduced acetate to propionate ratio. These disorders were described as resulting from alterations in the rumen milieu accompanying fermentation of soluble carbohydrates, including rumen acidity and lactate production and reduced degradation of cellulose (Wales, 2004). Dietary buffers reduce rumen acidity and normalize cellulose digestion and acetate to propionate ratios in the rumen (Erdman, 1982) hence, they may prevent metabolic disorders associated with high concentrate diets. Buffers evaluated for buffering the rumen environment include limestone (Rogers, 1985), disodium phosphate (Hart, 1984), potassium carbonate (West, 1987), potassium bicarbonate (West, 1986), sodium sesquicarbonate (Coppock, 1986), magnesium oxide (Xin, 1989), Acid Buf (Cruywagen, 2004) and the most extensively used, sodium bicarbonate (Erdman, 1982).

Rumen buffering agents such as sodium bicarbonate are included in high concentrate diets to increase and stabilize rumen pH and improve acetate to propionate ratio (Solorzano *et al.*, 1989; Thomas *et al.*, 1984). Nevertheless, Hogue *et al.* (1991) reported that although $NaHCO_3$ increased rumen pH and buffering capacity, its effects were transient, diminishing soon after cessation of infusion. Russell and Show (1993) also noted the limited capacity of sodium bicarbonate to buffer the rumen and suggested that the primary effects of feeding sodium

bicarbonate may be mediated through increased water intake, decreased rumen fluid osmolality, increased flow of starch from the rumen, and decreased rumen propionate production.

Acid Buf is a natural buffer used in ruminant nutrition; it is obtained from the skeletal remains of seaweed (*Lithothamnium calcareum*) harvested off the Irish Coast. Cruywagen *et al.* (2004) reported that the positive influence of Acid Buf appears to be related to its buffering capacity in the rumen and increased volatile fatty acid production. However, its effect on rumen metabolism is still the subject of research. The objective of the current study was to evaluate the effect of Acid Buf and the commonly used buffer sodium bicarbonate, as a 'positive control', in the rumen metabolism of lactating dairy cows.

3.2. Materials and methods

3.2.1. Animals and housing

Six ruminally cannulated, mature, lactating Holstein cows in mid lactation, weighing 700 ± 50 kg, were used in the trial. The cows were kept at the Stellenbosch University`s experimental farm, Welgevallen. Animals were housed individually in covered pens (3 x 5 m) with cement floors and sand bed sleeping areas. Cows were machine milked twice daily at 0600 and 1500h. Daily milk yield was recorded individually at each milking and noted for the data collection period and the average daily milk production was about 30 kg/ cow.

3.2.2. Experimental design and treatments

Cows were assigned to treatments in a balanced 3 x 3 Latin square design (n=2) with three treatments and three periods. The treatments were as follows:

Treatment 1: Basal diet plus Acid Buf included at 0.4% of the dietary DM

Treatment 2: Basal diet plus sodium bicarbonate included at 0.8% of the dietary DM plus 3.7 g/kg DM Limestone

Treatment 3: Basal diet without any buffer (control), but with limestone at 3.5 g/kg DM

Table3.1. Ingredient composition of the experimental basal diet supplemented with Acid Buf and Sodium bicarbonate as well as control diet.

Ingredient (Inclusions as g/kg DM)	**Acid Buf Treatment**	**$NaHCO_3$ Treatment**	**Control Treatment**
Oat hay	176	176	176
Lucerne hay	176	176	176
Wheat bran	38	37	30
Soybean meal	74	74	74
Cottonseed meal	37	37	37
Fish meal	26	26	26
Ground corn	405	405	405
Urea	4.3	4.3	4.3
Molasses	30	30	30
Megalac	25	25	25
Minvit	2	2	2
Limestone	3.5	-	3.7
Salt	3	3	3
Acid Buf	4	-	-
Sodium bicarbonate	-	8	-

Minvit: Minerals and Vitamin

All cows received all three treatments. The diet composition of the experimental diet is indicated in Tables 3.1 and 3.2. The basal diet was the same for all the treatments; the difference between the treatments was in terms of Acid Buf, and sodium bicarbonate inclusion. The Acid Buf was included at a level of 0.4% of the dietary dry matter to ensure an intake of 90 g/cow per day and the sodium bicarbonate was included at a level of 0.8 % of dietary dry matter to ensure an intake of 180 g/day. Feed was offered at a level of 25 kg/cow/ day. Daily and clean water was available ad libitum. Feed was supplied twice per day at a rate of approximately 5% in excess of appetite at 0730 and at 1600 h. The total experimental period was 66 days with three periods of 22 days. Each 22 days period consisted of an adaptation period of 15 days and a data collection period of 7 days.

Table3.2. Chemical composition of the experimental basal diets

Item	**Amount**
Neutral Detergent Fibre (g/kg)	262
Non Fibre Carbohydrate (g/kg)	471
Lignin (g/kg)	31
Crude Protein (g/kg)	172
Rumen Undegradable Protein (g/kg)	374
Fat (g/kg)	55.6
Metabolizable Energy (kJ/kg DM)	12.1
Calcium (g/kg)	8.7
Phosphor (g/kg)	4.5

3.2.3. Data collection and chemical analyses

Rumen fluid was collected via the ruminal cannula on the 16th day of each period. Sampling times were at 0600, 1000, 1400 and 1800 h for subsequent VFA, NH_3 and lactic acid analyses. Rumen fluid was filtered through two layers of cheesecloth to remove feed particles before transferring to the container. The following samples size applied: 9 ml for VFA, 9 ml for NH_3 and 10 ml for lactic acid. The rumen fluid samples for VFA and NH_3 analyses were preserved with 1 ml of NaOH (1 N) and H_2SO_4 (1 N), respectively. No preservative was used for lactic acid analysis. All the rumen fluid samples were immediately frozen at -10°C, pending analysis. Samples for VFA and lactic acid were analyzed using an HPLC method for fermentation products. Ruminal ammonia concentration was determined based on the method of Broderick (1980).

The pH was measured with the aid of WTW 340i pH data loggers (Supplied by Merck, Cape Town) and in-dwelling probes (WTW Sentinx 41 Electrodes). The pH loggers were housed in aluminium cases fitted directly on the rumen cannulae. The electrodes were housed in specially designed stainless steel capsules, attached to the cannula with water tight hoses and fittings. The arrangement was such that the electrodes resided more or less in the centre of the rumen. The pH loggers and probes were fitted at 0630 h on day four of the sampling period. Cannulae and capsules were removed at 0600 h of the following day to check if the area between the electrodes tips and stainless steel openings were not clogged up with fine feed particles, cleaned if necessary and replaced. All the pH loggers and electrodes were removed at 0630 h on day six of the experimental period and the pH data was downloaded onto a computer for further analysis.

Fecal grab samples were collected in the morning and afternoon of the last day of each experimental period and were analyzed for particles size distribution. The fecal samples were washed through a set of sieve with the following mesh sizes: 4 mm (coarse), 2.2 mm (medium), 1.12 mm (medium-fine) and 0.5 mm (fine). After washing, the fractions were dried at 60^{0}C for 48 h and each fraction was weighed to determine its contribution to the total washed sample. The fine and medium-fine fractions were expressed as g/kg of total sample dry matter.

3. 2. 4. Statistical analysis

Data were analysed with the GLM procedure of SAS according to a balanced 3 x 3 Latin square design. Fixed effects were cow, treatment and period. Pairwise t-tests were done to compare treatment means and differences were considered significant at $P < 0.05$.

3. 3. Results

3. 3. 1. Rumen pH

Rumen pH results are shown in Table 3.3. The mean, maximum and minimum pH were not affected by buffer supplementation to the basal diet. The basal diet was formulated to be acidotic and both the mean and maximun pH were below 6.2, a condition that limits microbial protein production and digestion of fibre (Pitt *et al.*, 1996). Treatment had no effect on mean and maximum pH, but animals on the control treatment tended toward the lower minimum pH (P = 0.111). However, the time period of pH below 5.5 was affected by the treatments (P = 0.02). The period time of pH below pH 5.5 was intermediate for the sodium bicarbonate treatment and did not differ from the other two treatments, although it tended to differ from the control treatment. Figure 3.1 illustrates rumen pH fluctuation over a period of 24 hours.

3.3.2. Effects of buffering on rumen fermentation and fecal particle size.

Results on fecal particle size are presented in Table 3.4. Treatment had no effect on fine and medium fecal particle size to suggest any differences in fiber digestion due to the addition of buffers. However, fine plus medium fecal particle size was affected by the treatment (*P=0.48*). Total VFA concentrations in ruminal fluid were not affected by the treatments. Treatment also had no effect on the acetate to propionate ratio. Ruminal ammonia concentration was not different among the treatments. The lactic acid production in the rumen was low and not different among treatments. However, lactic acid concentration tended to be higher for the control treatment (P = 0.13) than for the other treatments.

Table3.3. Effect of Acid Buf and sodium bicarbonate on mean, minimum, maximum ruminal pH and period time of pH below 5.5.

Parameters	Acid Buf	$NaHCO_3$	Control	SEm	P-value
Mean daily pH	5.58	5.48	5.51	0.02	0.5648
Maximum pH	6.08	6.06	6.10	0.07	0.8420
Minimum pH	5.42	5.37	5.14	0.02	0.1114
Time that pH was below 5.5 (h)	4.00^{a}	8.70^{ab}	13.00^{b}	10.87	0.0226

[a,b] Values with different superscripts (within rows) differed significantly ($P < 0.05$).

SEm: Standard Error of the mean

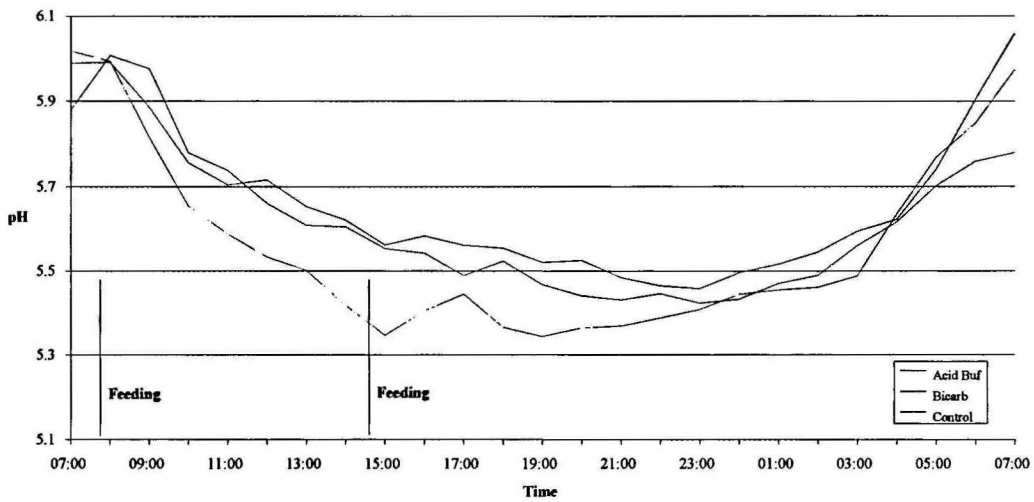

Figure3.1. Rumen pH fluctuation in cows supplemented with diets buffered with Acid Buf and sodium bicarbonate in high concentrate diets.

Table3.4. Effect of Acid Buf on rumen pH, ammonia, VFA, Acetate to propionate ratio, lactic acid and fecal particle size

Parameters	Acid Buf	$NaHCO_3$	Control	SEm	P-value
Total volatile fatty acid (mmol/L)	131.01	91.30	114.85	15.32	0.154
Acetate:Propionate ratio	3.47	5.17	3.53	0.98	0.426
Acetate (post feeding - mmol/L)	82.01	58.67	71.20	10.00	0.308
Propionate (postfeeding- mmol/L)	26.24	18.00	22.93	3.62	0.128
Lactic acid (mmol/L)	0.66	0.59	1.54	0.32	0.132
Ammonia (post-feeding - mmol/L)	11.66	11.13	11.11	0.84	0.874
Fecal-Fine particle (g/kg)	222.6	231.4	224.6	2.26	0.959
Fecal-Medium particle (g/kg)	569.9	456.4	504.7	3.74	0.161
Fecal-Medium- fine particle (g/kg)	792.5	649.2	729.4	3.37	0.048

SEm: standar error of the mean

3.4. Discussion

When cows were fed the control diet, they were visibly panting and appeared to be suffering from acidosis problems. Cows on other treatments appeared to be more comfortable. The ruminal pH curves (Figure 3.1) confirm the acidotic conditions in the rumen. A decline in ruminal pH was observed after the morning feeding, a slow increase through the evening and following night, followed by a rapid return to the pre-feeding level. It is proposed that the buffers have a clear impact on the pH profile up to 0300 h, and that the change in pH after 0300 h is related to substrate availability. The inclusion of either buffer reduces the rate of pH decline after the first feeding, with the minimum pH being reached late in the evening. The pH for cows on the Acid Buf treatment remained higher than for those on the bicarbonate treatment and it is

suggested that the slow release feature of this buffer prevented the pH from going as low as was apparent with the soluble buffer.

During the complete daily cycle, rumen pH remained below 6.2 in all animals, regardless of treatment. Pitt *et al.* (1996) found that there was a steep decline in microbial protein and fibre digestibility when pH is below 6.2; a condition that existed in all our diets. Of particular concern is the 13 hours period that the control animals experienced pH levels below 5.5. Buffering with Acid Buf seemed to reduce this time to about 4 hours. Cruywagen *et al.* (2004) proposed that milk production may be optimized by preventing pH from dropping or declining too low during the evening hours when bicarbonate flow form saliva is reduced. The production response reported here may be correlated with the extent to which the rumen pH dropped below 5.5 in the evening period, this being 13 hours for control, compared with 8.7 hours for the soluble buffer and 4 hours for the slow release buffer.

The lactate production in the ruminal fluid varied among treatments, although difference among treatments was not statistically significant. It is hypothesised that lactic acid was removed fairly quickly, resulting in the variation that prevented the 1.54 mmol/L value to be different form the other treatments. The depression of ruminal pH in dairy cows suffering from subacute ruminal acidosis is apparently largely due to accumulation of volatile fatty acids and not to lactic acid accumulation (Oetzel *et al.*, 1999). Under "normal" or SARA conditions, lactate does not accumulate, in contrast, ruminal concentration of lactate exceeding 40 mmol/ L are indicative of severe acidosis (Owens *et al.*, 1998). In this experiment, cows on unbuffered diets seemed to suffer from acidosis, probably due to VFA concentration rather than lactic acid production whereas the cows on Acid Buf and sodium bicarbonate treated diets appeared to be comfortable.

The concentration of ruminal VFA was higher for cows fed the Acid Buf treated diet, throughout the post-feeding interval even though there was no significant difference among the treatments. According to Russell and Chow (1993) sodium bicarbonate increases the animal's water intake,

the rate of dilution and flow of starch from the rumen and thereby decreasing propionate production. Hogue *et al.* (1991) also reported that the total concentration of VFA was at least numerically lower for $NaHCO_3$ than for the control throughout the post-feeding interval. Erdman *et al.* (1982) showed that total VFA was reduced on $NaHCO_3$ supplemented diets. Rogers and Davis (1982) also noticed that acetate to propionate ratio increased with dietary sodium bicarbonate supplementation, which agrees with results from this study. The increase of acetate to propionate ratio for sodium bicarbonate may be attributed to the decrease in propionate production, which is linked to the feeding of sodium bicarbonate (Russell and Chow, 1993).

Ammonia overflow occurs when ammonia N exceeds 5mg/100 ml of rumen fluid (Satter and Raffler, 1975). The point of ammonia overflow may not have been reached, in this experiment, because easily degradable carbohydrates were probably available during this time to facilitate greater utilization of ammonia for protein synthesis by rumen bacteria. Lana *et al.* (1998) reported that ammonia concentration could also be correlated with the deamination rate of the bacteria and noticed that cows fed high concentrate diets had low ruminal ammonia concentrations compared to those fed forages. However, Schaefer *et al.* (1989) suggested that ruminal bacteria are efficient scavengers of ammonia and they can grow on relatively low concentrations of ammonia in ruminal fluid.

Optimal fiber digestion usually results in a large amount of fine particles in the feces. The average fine and medium particle size of indigestible materials in the feces did not differ among treatments, although fine plus medium particle size were significantly different among tretment. One would have expected a greater percentage of fine particles in the feces for cows fed buffered diets than for those fed unbuffered or control diets. However, fine plus medium particles were highest for Acid Buf treated diets (79.25%) and lowest for sodium bicarbonate diets (64.92%). The kinetic changes due to addition of buffers to the total mixed ration are difficult to ascertain, in this experiment, without resorting to a model that integrate discrete lag and digestion rate and rate of passage. However Lund *et al.* (2006) showed that when cows ingest the slowly

degradable fibre originating from large feed particles is selectively retained in the rumen at the expense of older, small, and more digested particles.

3.5. Conclusion

Buffering with Acid Buf or sodium bicarbonate did not result in a significant improvement in mean rumen pH. There was, however, a reduction in the time period of ruminal pH below 5.5, which is very important as low pH has negative impacts on fibre digestion. Volatile fatty acid, ruminal ammonia concentration and lactic acid production were not affected by addition of buffers to the basal acidotic TMR. The effectiveness of Acid Buf was in its inclusion at half of the sodium bicarbonate concentration in the TMR.

3.6. Acknowledgments

Celtic Sea Mineral (Cork, Ireland) is acknowledged for financing the study.

3. 7. References

Coppock, C. E., Schelling, G. T., Byers, F. M., West, J. W.M. & Labore, J. M., 1986. A naturally occurring mineral as a buffer in the diet of lactating dairy cows. J. Dairy Sci. 69:111-123.

Broderick, G. A. & Kang, J. H., 1980. Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and In Vitro Media. J. Dairy Sci. 63: 64-75.

Cruywagen, C.W., Swiegers, J.P. & Taylor, S., 2004. The effect of Acid Buf in dairy cow diets on production response and rumen parameters. J. Dairy Sci. 87 (Suppl 1), 46.

Erdman, R. A., 1988. Dietary buffering Requirements of the lactating Dairy Cow: A Review: J. Dairy Sci. 71:3246-3266.

Erdman, R. A., Hemken, R. W. & L. S. Bull., 1982. Dietary sodium bicarbonate and maganesium oxide for early postpartum lactating dairy cow: effects on production, acid-base metabolism, and digestion. J. Dairy Sci. 65:712-731.

Hart, S. P. & C. E. Polan., 1984. Effect of sodium bicarbonate and disodium phosphate on animal performance, rumen metabolism, digestion, and rate of passage in ruminating calves. J. Dairy Sci. 67:2356-2379.

Hogue, J. F., Tucker, W. B., Koevering, M. T., Vernon, R. K. & Adams, G. D., 1991. Controlled ruminal infusion of sodium bicarbonate. 1. Influence of postfeeding infusion Interval on ruminal milieu. J. Dairy Sci. 74:1615-1683.

Lana, R. P., Russell, J. B. & Amburgh, M. E., 1998. The role of pH in regulating ruminal methane and ammonia production. J. Anim. Sci. 76:2190-2196.

Lund, P., Weisbjerg, M. R. & Hvelplund, T. 2006.Digestible NDF is selectively retained in the rumen of dairy cows compared to indigestible NDF. Anim. Feed Sci. and techno. 143:1-17.

Krause, K. M. & Garret Oetzel, R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Techn. 126: 215-236.

Oetzel, G. R., Nordlund, K. V. & Garrett, E. F., 1999. Effect of ruminal pH and stage of lactation on ruminal lactate concentrations in dairy cows. J. Dairy Sci.82(suppl. 1):38.

Owens, F. N., Secrist, D. S., Hill, W. J. & Gill, D. R., 1998. Acidosis in cattle: Areview. J. Anim. Sci. 76:275-286.

Pitt, R. E., Van Kassel, J. S., Fox, D. G., Pell, A. N., Barry, M. C. & Van Soest, P. J., 1996. Prediction of ruminal volatile fatty acids and pH within the carbohydrate net protein system. J. Anim. Sci. 74:226-244.

Rogers, J. A., Davis, C. L. & Clark, J. H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrients utilization with minerals salts in dairy cows. J. Dairy Sci. 65:577-586.

Rogers, J. A., Muller, L. D., Davis, C. L., Chalupa, W., Kronfeld, D. S., Karcher, L. F. & Cummings, K. R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early laction. J. Dairy Sci. 68:646-660.

Russell, J. B. & Chow, J. M., 1993. Another theory for the action of ruminal buffers salts: decreased starch fermentation and propionate production. J. Dairy Sci.78:826-830.

Satter, L. D. & Roffler., 1975. Nitrogen requirement and utilisation in dairy cattle. J. Dairy Sci. 58:1219-1237.

Schaefer, D. M., Davis, C. L. & Bryant, M. P., 1980. Ammonia saturation constants for predominant species of rumen bacteria. J. Dairy Sci. 63:1248-1263.

Solorzano, L. C., Armentano, L. E., Grummer, R.R. & Dentine, M. R., 1989. Effects of Sodium Bicarbonate and Sodium sesquicarbonate on lactating Holstein cows. J. Dairy Sci. 72:453-461.

Thomas, J. W. Emery, R. S., Breaux, J. K. & Liesman, J. S., 1984. Response of milking cow fed high concentrate , low roughage diet plus sodium bicarbonate, magnesium oxide, or magnesium hydroxide. J. Dairy Sci. 67:2532-2545.

Wales, W. J, Kolver, E. S. Thome P. L. & Egan, A. R., 2004. Diurne variation in ruminal pH on the digestibility of highly digestible perennial Ryegrass during continuous culture fermentation. J. Dairy Sci. 87:1864-1871.

West, J. W., Coppock, C. E., Nave, D. H. & Schilling, G. T., 1986. Effect of potassium buffers on feed intake in lactating dairy cows and on rumen fermentation in vivo and in vitro. J. Dairy Sci. 69:124-134.

West, J. W., Coppock, C. E., Nave, D. H., Labore, J. M., Greene, L. W. & Odom, T. W., 1987. Effects of potassium carbonate on rumen function in lactating Holstein cows. J. Dairy Sci. 70:81-90.

Xin, Z., Tucker, W. B. & Hemken, R. W., 1989. Effect of reactivity rate and particle size on magnesium availability, acid-base balance, mineral metabolism, and milking perfomance of dairy cows. J. Dairy Sci. 72:462-470.

Chapter four

The effect of Acid Buf and sodium bicarbonate as dietary buffers on milk production and milk composition of lactating dairy cows.

Abstract

A high concentrate TMR, formulated to be potentially acidotic, was used to construct three dietary treatments in which Acid Buf, the skeleton remain of sea weed Lithothamnium calcareum, was compared against limestone (control) and sodium bicarbonate plus limestone. The diet contained 4 g/kg DM of Acid Buf or 3.5 g/kg DM limestone (control) or 3.7g/kg DM limestone plus 8 g/ kg DM of sodium bicarbonate, respectively. The response to treatment was measured using 6 ruminally cannulated lactating Holstein cows allocated to treatments according to a balanced 3 x 3 (n=2) Latin square design, with three treatments and three periods. The total experimental period was 66 days in which every cow received each diet for a period of 15 days prior to a data collection period of 7 days. During each data collection period milk was collected and analyzed for its solid and mineral contents. Feed dry matter consumption was also recorded. Daily milk yield was 27.6, 29.1 and 31.6 liters /cow for the control, sodium bicarbonate and Acid Buf treatments, respectively, with milk containing 38.6, 41.8 and 42.1 g/kg fat and 34.3, 33.8 and 34.7 g/kg protein. The trial indicated that supplementing the diet of dairy cows with approximately 90 g/day of Acid Buf may have a greater impact on milk production than 180 g/day of sodium bicarbonate and resulted in increase in daily milk input production of 4 liters/cow/day.

Key words: Acid Buf, Sodium bicarbonate, Buffers, Milk production, Milk composition.

4. 1. Introduction

Lactating dairy cows are normally fed diets containing high levels of concentrates. Feeding large amount of concentrate increases milk yield per cow due to a high energy density of the diet (Kennelly *et al.*, 1999). The increase in energy density is beneficial because cows in early lactation are unable to consume enough nutrients to meet the lactation nutrient requirements (Moore *et al.*, 1992). However, feeding high concentrate diets has been linked to problems of rumen acidosis (Krause *et al.*, 2006), a reduction in fibre digestibility (Wales *et al.*, 2004) and milk fat depression (Martens, 1997). Subsequent long-term health problems such as anorexia, abomasal displacement, milk fever and ketosis (Belibasakis *et al.*, 1991) may also manifest in cows that have rumen acidosis; thus decrease in milk production. The addition of dietary buffers to high concentrate dairy cow diets could be beneficial for milk production while maintaining the welfare of the animal (Erdman, 1988).

Dietary buffers and alkalizers include sodium bicarbonate ($NaHCO_3$), Acid Buf, potassium bicarbonate ($KHCO_3$), sodium sesquicarbonate ($Na_2CO_3.NaHCO_3.2H_2O$), magnesium oxide MgO, potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3) and multi-element buffers (e.g. RumenMate). Most dietary buffers have been reported to improve dry matter intake (Erdman *et al.*, 1982; Rogers *et al.*, 1985; West *et al.*, 1987; Xin *et al.*, 1989), increase ruminal pH, rumen fluid dilution rate, the digestibility of dietary nutrients and acetate to propionate ratio (Russell, 1998). The rate of passage of fluid from the rumen is also enhanced (Rogers *et al*, 1982) by dietary buffer inclusion. The improvement in digestion and passage rate result in optimized metabolic functions for milk production in dairy cows, subsequently increasing milk yield (Boiclair *et al.*, 1987; Canale *et al.*, 1988, Cruywagen *et al.*, 2004), milk fat yield (Rogers *et al.*, 1985; Solorzano *et al.*, 1989; Xin *et al.*, 1989; Cruywagen *et al.*, 2004) and milk protein percentage (Escobosa *et al.*, 1984). However, the responses to dietary buffer additions have been variable depending on their nature, the amount used, as well as the type of the basal diet fed (Erdman, 1988).

Sodium bicarbonate is widely used as a buffer in high concentrate diets for high yielding dairy cows to alleviate the detrimental effects of easily fermentable carbohydrate diets. This usually results in an increase in rumen pH and in the acetate to propionate ratio. Nevertheless, Hogue *et al.* (1991) found that the buffering capacity of sodium bicarbonate was transient and diminished soon after cessation of infusion. Russell *et al.* (1993) suggested that sodium bicarbonate is primarily used for its non-buffering effects rather than as a buffer. Thus, dietary buffers such as Acid Buf (*Lithothamnium calcareum*) are preferable to sustain the metabolic function in the rumen and thereby improving milk production and composition. Cruywagen *et al.* (2004) reported that the inclusion of Acid Buf in concentrate diet improved fibre digestibility and also milk yield and composition. However, more research needs to be done on Acid Buf to clearly understand its effect on milk production. The objective of the current study was to evaluate the effect of Acid Buf and sodium bicarbonate, the common buffer, on milk yield and milk composition.

4. 2. Materials and methods

4.2.1. Animals and housing

Six mature ruminally cannulated, lactating Holstein cows in mid lactation, weighing 700 ± 50 kg, were used in the trial. The cows were kept at the Stellenbosch University`s experimental farm, Welgevallen. Animals were housed individually in covered pens (3 x 5 m) with cement floors and sand bed sleeping areas.

4.2.2. Experimental design and treatments

Cows were assigned to treatments in a balanced 3 x 3 Latin square design (n=2) with three treatments and three periods. The treatments were as follows:

Treatment 1: Basal diet plus Acid Buf included at 0.4% of the dietary DM

Treatment 2: Basal diet plus sodium bicarbonate included at 0.8% of the dietary DM plus 3.7 g/kg DM of limestone

Treatment 3: Basal diet without any buffer (control), but with 3.5 g /kg DM of Limestone

Table4.1. Ingredient composition of the experimental basal diet supplemented with Acid Buf and Sodium bicarbonate as well as control diet.

Ingredient (Inclusions as g/kg DM)	**Acid Buf Treatment**	**$NaHCO_s$ Treatment**	**Control Treatment**
Oat hay	176	176	176
Lucerne hay	176	176	176
Wheat bran	38	37	30
Soybean meal	74	74	74
Cottonseed meal	37	37	37
Fish meal	26	26	26
Ground corn	405	405	405
Urea	4.3	4.3	4.3
Molasses	30	30	30
Megalac	25	25	25
Minerals and vitamins	02	02	02
Limestone	3.5	-	3.7
Salt	3	3	3
Acid Buf	4	-	-
Sodium bicarbonate	-	8	-

The diet composition of the experimental diet is indicated in Tables 4.1 and 4.2. The basal diet was the same for all the treatments; the difference between the treatments was in terms of Acid Buf and sodium bicarbonate inclusion. The Acid Buf was included at a level of 0.4% of the dietary dry matter to ensure an intake of 90g/cow per day and the sodium bicarbonate was included at a level of 0.8 g of dietary dry matter to ensure an intake of 180g/cow/day. Feed was offered at a level of 25 kg/cow daily and clean water was available ad libitum. The daily feed allocation was offered in two feedings at 0730 and 1600 h. The total experimental period was 66 days with three periods of 22 days. Each 22 days period consisted of an adaptation period of 15 days and a data collection period of 7 days.

Table4.2. Chemical composition of the experimental basal diets

Item	**Amount**
Neutral Detergent Fibre (g/kg)	262
Non Fiber Carbohydrate (g/kg)	471
Lignin (g/kg)	31
Crude Protein (g/kg)	172
Rumen Undegradable Protein (g/kg)	374
Fat (g/kg)	55.6
Metabolizable Energy (KJ/Kg DM)	12.1
Calcium (g/kg)	8.7
Phosphor (g/kg)	4.5

4.2.3. Data collection and chemical analysis

Feed intake was recorded daily during the data collection period by monitoring the amount of feed supplied and refusals weighed back. Samples of feed and refusals were taken for DM analysis to determine daily DMI. Cows were machine milked twice daily at 0600 and 1500h. Daily milk yield was recorded individually at each milking and noted for the data collection period. Milk samples were collected from each cow immediately after each milking and preserved with $K_2Cr_2O_3$ (Sanchez *et al.*, 1997) separately in 100 mL vials and stored at 4^0C. The milk samples were analyzed for fat, protein, lactose, total solids and solids-not-fat using an infrared analyzer (Milko-scan 605, Foss Electric, Hillerod, Denmark) at the Dairy Laboratory of the Agricultural Research Council, Elsenburg. Calcium content in the feed and milk was determined according to the AOAC method (2000) and Mars Xpress Macrowave, respectively. The detection was made via Icap 6000 series ICP.

4.2.4. Statistical analysis

Data were analysed with the GLM procedure of SAS according to a balanced 3 x 3 Latin square design. Fixed effects were cow, treatment and period. Pairwise t-tests were done to compare treatment means and differences were considered significant at $P < 0.05$.

4.3. Results

Results on feed intake and milk production are presented in Table 4.3. Dry matter intake averaged 23.5 kg/cow/day and was not affected by treatment. Milk yield was different (P = 0.01) among treatments, with the highest yield for cows on the Acid Buf treatment. Fat corrected milk (FCM) appeared to be high for Acid Buf treated diet and the difference was statistically different among treatments (P=0.006).

Table4.3: The effect of Acid Buf and sodium bicarbonate in high concentrate diets on feed intake and milk production of Holstein cows.

Item	Acid Buf	$NaHCO_3$	Control	SEm	P-level
Daily intake (kg DM/day)	23.3	24.2	23.1	1.434	0.863
Milk production (kg/day)	31.8^{a}	29.1^{b}	27.6^{b}	4.560	0.010
4% Fat corrected milk (kg/day)	32.8^{a}	29.9^{ab}	26.9^{b}	2.245	0.006

[ab] Value with different superscripts (within rows) differed significantly; SEm= Standard error of the mean

Milk composition, as affected by the dietary buffered treatments, is indicated in Table 4.4. Milk fat content was higher for the two buffer treatments than for the control. This resulted in milk fat yield also being higher for these treatments, with the Acid Buf treatment yielding 25% more fat than the control treatment and the sodium bicarbonate treatment 15% more. Buffers supplements in the basal diet did not impact on the milk protein content; however, due to differences in milk yield among treatments, Acid Buf appeared to have the highest milk protein yield / day over sodium bicarbonate and control treatments. Milk lactose was similar for all treatments. However, due to milk yield differences, the lactose yield was higher for the cows in the Acid Buf treatment than for those in the control treatment. Total solids and SNF percentages did not differ between treatments, but their yields tended to be higher for the Acid Buf cows than for the control cows. No differences between treatments were observed regarding milk Ca content. Calcium balance (difference between Ca intake and Ca output) also did not differ between treatments.

Table4.4: Effects of Acid Buf and sodium bicarbonate treatments on composition of milk components

Item	Acid Buf	$NaHCO_3$	Control	SEm	P-level
Milk fat (%)	4.21[a]	4.18[a]	3.86[b]	0.040	0.057
Milk fat (kg/day)	1.33[a]	1.22[a]	1.06[b]	0.005	0.007
Milk protein (%)	3.47	3.38	3.43	0.016	0.554
Milk protein (kg/day)	1.09[a]	0.98[ab]	0.93[b]	0.004	0.032
Milk lactose (%)	4.58	4.59	4.57	0.002	0.766
Milk lactose (kg/day)	1.46[a]	1.34[a]	1.26[b]	0.003	0.008
Total solids (%)	12.9	13.0	12.9	0.063	0.689
Total solid (kg/day)	4.08[a]	3.77[ab]	3.54[b]	0.044	0.025
SNF (%)	8.69	8.81	9.01	0.047	0.136
SNF (kg/day)	2.75[a]	2.55[ab]	2.47[b]	0.023	0.077*
Ca content (%)	1.01	1.04	1.04	0.13	0.87
Calcium balance (g/day)	170.60	180.28	172.27	12.20	0.850

[ab] Values within rows with different superscripts differed significantly ($P < 0.05$);

* P-values marked with an asterisk indicate tendencies only ($P < 0.10$);

SEm= Standard error of the mean

SNF: Solid none fat

Calcium balance: difference between calcium intake in the feed and calcium output in the milk

4.4. Discussion

In this study, dry matter intake was not affected by treatment. In others studies, a decrease or increase has been reported. Cassida *et al.* (1988) observed a small, but non significant increase in DMI when dietary buffers were used to improve ruminal acid-base status. Contrary to this, Coppock *et al.* (1986) reported that the sudden addition of a buffer (Trona) at 15 g/kg of the diet resulted in a decrease of 3 kg/cow/day in feed intake, although it didn't differ significantly from the control treatment or cows fed $NaHCO_3$ supplemented diets. However, increases in feed intake have been greater for buffer additions in others studies (Vicini *et al.*, 1988; Xu *et al.*, 1994). Feed intake is usually not affected by the addition of dietary buffers when animals are consuming a total mixed ration.

Milk production was about 4 L higher for the Acid Buf treatment compared to the control treatment, and about 2.5 L higher than for the sodium bicarbonate treatment (Table 4.4). The fact that cows on the Acid Buf diet produced more milk than those on the sodium bicarbonate diet may be due to its high, and slow releasing, buffering capacity characteristics to maintain the ruminal pH above 5.5 for a longer period of time (Chapter three). Kennelly *et al.* (1999) found that the addition of buffers resulted in increased milk yield for cows receiving high concentrate diets (concentrate to forage ratio 75:25), but that it had no effect on cows receiving medium concentrate diets (concentrate to forage ratio 50:50). Buffers are thought to be most beneficial for cows consuming large amounts of energy in the form of concentrates (starch) to support high milk production.

The daily 4% FCM yield was also enhanced by buffer supplements in this experiment (Table4.3). Our results are in agreement with Cassida *et al.* (1989) who observed that the addition of dietary buffers was effective in improving 4% FCM. However, Moore *et al.* (1992) observed that buffer addition to the diet did not improve the FCM yield which was explained by the fact that the tendency for milk fat percentage was lower because of excess of propionate production in the rumen fluid, although the addition of buffers.

Milk fat percentage was not significantly different among the treatments; this may be due to the fact that, in this experiment, the acetate to propionate ratio did not differ among treatments (Chapter three). However, there was a strong tendency (P = 0.057) for the buffered diets to result in a higher milk fat percentage compared to control diet. This, together with milk yield differences, resulted in a higher fat yield for cows in the buffer treatments compared to the control treatment. Davis *et al.* (1964) reported that addition of buffers to high concentrate diets restored milk fat percentage to normal. Erdman (1988) demonstrated that the addition of a buffer to an alfalfa-based diet was not effective in improving milk composition and concluded that alfalfa hay was efficient in stimulating rumination and chewing activity; hence the high intrinsic buffering capacity of alfalfa. Positive correlations have been observed in high grain/low forage diets and ground or pelleted roughages where the reduction in milk fat corresponded to a decrease in rumen pH and an alteration in the molar ratio of VFA (Van Soest, 1963). It is therefore clear that the diet composition and physical form would affect the results of buffer supplementation and that the advantages would be more apparent in high concentrate diets or in diets that lack physical effective NDF.

Buffer supplement did not have an impact on milk protein content. These results are in line with Moore *et al.* (1992) who found that the addition of buffers to concentrate diets did not influence the percentage of milk protein. Xu *et al.* (1994) also demonstrated that, although the milk protein content of buffer supplemented diets was higher than for the control diet, the differences were not significant. In general, it appears that dietary buffers do not consistently alter milk protein percentage and yield (Cassida *et al.*, 1988; Harrison *et al.*, 1989). Milk lactose, total solids and SNF contents were not altered by the addition of the dietary buffers, but their daily yields were higher for cows receiving Acid Buf supplements compared to cows on the control diet. This can be explained by the effect of Acid Buf on milk yield. The lactose content of milk is usually quite constant.

The calcium concentration of the milk was found to be similar for all treatments (Table. 4.3) However, the Acid Buf treatment resulted in an increase in milk output compared to the other

two treatments, both of which contained limestone as principal source of calcium. Limestone in the control diet and Acid Buf in the experimental diet provided virtually the same amount of calcium, viz. 27 and 28 g/day, respectively. Calcium output in the milk was increased by almost 4 g/day as a result of the Acid Buf inclusion in the basal diet compared to control treatment. This result suggests that there is a marginal improvement in the utilization of calcium from Acid Buf compared with limestone for milk production.

4.5. Conclusion

This study demonstrated that the inclusion of Acid Buf at a level of 0.4% DM to a high concentrate TMR had beneficial effects on milk yield compared to sodium bicarbonate supplemented diets. The addition of either Acid Buf or sodium bicarbonate, in this experiment, improved the milk composition compared to the control diet, but there was no significant difference in terms of milk composition between buffer treated diets. Acid Buf supplemented at a rate of 90 g/cow per day appeared to be more effective than sodium bicarbonate supplemented at a rate of 180 g/cow per day.

4.6. Acknoledgements

Celtic Sea Mineral (Cork, Ireland) is acknowledged for partially financing the study.

4.7. References

AOAC (Association of Official Analytical Chemists), 2000. Official Methods of Analysis, 17 ed. AOAC, Gaithersburg, MD, USA.

Belibasakis, N. G & Triantos, A., 1991. Effects of Sodium bicarbonate on milk yield, milk composition, and blood components of dairy cows in early lactation. J. Dairy Sci. 74:467-472.

Boisclair, Y., Grieve, D. G., Allen, O. B. & Curtis, R. A., 1987. Effect of prepartum energy, body condition, and sodium bicarbonate on health and blood metabolites of Holstein coes in early lactation. J. Dairy Sci. 70:2280-2290.

Canale, C. J. & Stokes, M. R., 1988. Sodium bicarbonate for early lactation cows fed corn silage or hay crop silage-based diet. J. Dairy Sci. 71:373-380.

Cassida, K. A., Muller, L. D. & Sweeney, T. F., 1988. Sodium sesquicarbonate for early lactation dairy cows fed corn silage-based diets. J. Dairy Sci.71:381-387.

Coppock, C. E., Schelling, G. T., Byers, F. M., West, J. W. M. & Labore, J. M., 1986. A naturally occurring mineral as a buffer in the diet of lactating dairy cows. J. Dairy Sci. 69:111-123.

Cruywagen, C.W., Swiegers, J.P. & Taylor, S., 2004. The effect of Acid Buf in dairy cow diets on production response and rumen parameters. J. Dairy Sci. 87 (Suppl 1): 46.

Davis, C. L., Brown, R. E. & Beitz, D. C., 1964. Effect of feeding high grain restricted-roughage rations with and without bicarbonates on fat content of milk produced and proportions of volatile fat acids in the rumen. J. Dairy Sci. 47: 1217-1219.

Erdman, R. A., 1988. Dietary buffering Requirements of the lactating Dairy Cow: A Review: J. Dairy Sci. 71:3246-3266.

Erdman, R. A., Hemken, R. W. & Bull, L. S., 1982. Dietary sodium bicarbonate and maganesium oxide for early postpartum lactating dairy cow: effects on production, acid-base metabolism, and digestion. J. Dairy Sci. 65:712-731.

Escobosa, A., Coppock, C. E., Rowe, L. D., Jenkins, W. L. & Gates, C. E., 1984. Effects of dietary sodium bicarbonate and calcium chloride on physiological responses of lactating dairy cows in hot wheather. J. Dairy Sci. 67:574-584.

Harrison, J. H., Rily, R. E. & Loney, K. A., 1989. Effects of types and amount of buffer addition to grass silage-based total mixed ration on milk production and composition. J. Dairy Sci. 72:1824-1830.

Hogue, J. F., Tucker, W. B., Van Koevering, T. M., Vernon, R. K. & Adams., 1991. Controlled ruminal infusion of Sodium bicarbonate. 1. Influence of post feeding infusion interval on ruminal milieu. J. Dairy Sci. 74:1675-1683.

Kennelly, J. J., Robinson, B. & Khorasani, G. R., 1999. Influence of carbohydrate source and Buffer on Rumen Fermentation Characteristics, Milk Yield, and Milk Composition in Early-Lactating Holstein Cows. J. Dairy Sci. 82:2486-2496.

Krause, K. M. & Garret Oetzel, R., 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Anim. Feed Sci. Techn. 126: 215-236.

Mertens, D. R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Moore, J. A., Poore, Eck, T. P., Swingle, R. S., Huber, J. T. & Arana, M., 1992. Sorghum grain processing and buffer Addition for early lactation Cows. J. Dairy Sci. 75: 3465-3472.

Rogers, J. A., Davis, C. L. & Clark, J. H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrients utilization with minerals salts in dairy cows. J. Dairy Sci. 65:577-586.

Rogers, J. A., Muller, L. D., Davis, C. L., Chalupa, W., Kronfeld, D. S., Karcher, L. F. & Cummings, K. R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early laction. J. Dairy Sci. 68:646-660.

Russell, J. B. & Chow, J. M., 1993. Another theory for the action of ruminal buffers salts: decreased starch fermentation and propionate production. J. Dairy Sci.78:826-830.

Russell, J. B., 1998. The importance of pH in the regulation of ruminal acetate to propionate Ratio and Methane Production In Vitro. J. Dairy Sci. 81:3222-3230.

Sanschez,W. K., Beede, D. K. & Corenell, J. A., 1997. Dietary of sodium bicarbonate, sodium chloride mineral metabolism of Holestein cows. J. Dairy Sci. 80:1207-1216.

Solorzano, L. C., Armentano, L. E., Grummer, R.R. & Dentine, M. R., 1989. Effects of Sodium Bicarbonate and Sodium sesquicarbonate on lactating Holstein cows. J. Dairy Sci. 72:453-461.

Van Soest, P. J., 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. Dairy Sci. 46: 204-216.

Vicini, J. L., Cohick, W. S., Clark, J. H., McCutcheon, S. N. & Bauman, D. E., 1988. Effect of food intake and Sodium bicarbonate on milk production and concentration of hormones and metabolites in plasma of cows. J. Dairy Sci. 71:1232-1238.

Wales, W. J, Kolver, E. S. Thome P. L. & Egan, A. R., 2004. Diurnal variation in ruminal pH on the digestibility of highly digestible perennial Ryegrass during continuous culture fermentation. J. Dairy Sci. 87:1864-1871.

West, J. W., Coppock, C. E., Nave, D. H., Labore, J. M., Greene, L. W. & Odom, T. W., 1987. Effects of potassium carbonate on rumen function in lactating Holstein cows. J. Dairy Sci. 70:81- 90.

Xin, Z., Tucker, W. B. & Hemken, R. W., 1989. Effect of reactivity rate and particle size on magnesium availability, acid-base balance, mineral metabolism, and milking perfomance of dairy cows. J. Dairy Sci. 72:462-470.

Xu, S., Harrison, J. Riley, R. E & Loney, K. A., 1994. Effect of buffer addition to high grain total mixed rations on rumen pH, feed intake, milk production, and milk composition. J. Dairy Sci. 77:782-788.

Chapter five

General conclusion

In summary, the important goal of effective nutrition for high yielding dairy cows that require high energy density feeds, is to feed as much concentrate as possible in order to maximize energy intake and milk production without causing ruminal acidosis. However, the excessive intake of rapidly fermentable carbohydrate is the most obvious cause of ruminal acidosis. The inclusion of dietary buffers to diets with low effective fibre content is believed to compensate for a decrease in saliva output, thus counteracting the negative effects of high concentrate diets.

Addition of either Acid Buf or sodium bicarbonate as buffers to the basal diet in this study did not noticeably improve the mean rumen pH to what is normally accepted as an optimum pH for proper rumen function and fibre digestion. However, Acid Buf was proven to be effective compared to other treatments regarding rumen pH time spent below pH 5.5. The positive effect of Acid Buf inclusion at a level of 4 g/kg DM to the TMR appeared to be related to its buffering capacity in the rumen, thereby significantly reducing the time that ruminal pH was below 5.5. It is proposed that the slow release feature of Acid Buf prevented the pH from declining as fast as for the sodium bicarbonate supplemented or control diets. Some cows in the control treatment (no buffers) showed visible signs of acidosis. The results on volatile fatty acids, lactic acid and rumen ammonia concentration indicated that the supplementation of either buffer to the basal diet did not have a significant effect.

Acid Buf, included in the TMR at a level of 4 g/kg DM (90g/day), resulted in a 2 kg/day higher milk production than sodium bicarbonate included at 8 g/kg and a 4 kg/day higher production than the control diet. Milk fat content was higher for the Acid Buf and sodium bicarbonate treatments than for the control treatment.

The positive effects of Acid Buf was ascribed to its excellent buffering capacity (including slow release) allowing it to maintain ruminal pH above 5.5 for at least 20 hours per day. It was concluded that Acid Buf, supplemented at a level of 4 g/kg DM (allowing an intake of 90 g/cow per day), would counteract a decline in milk production and milk fat and would prevent ruminal acidosis in cows receiving high concentrate diets.