THE EFFECT OF ACID BUF AND COMBINATIONS OF ACID BUF AND SODIUM BICARBONATE IN DAIRY COW DIETS ON PRODUCTION RESPONSE AND RUMEN PARAMETERS

by

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The effect of Acid Buf and combinations of Acid Buf and Sodium bicarbonate in dairy cow diets on production response and rumen parameters

The objective of this study was to determine the effect of Acid Buf alone, or in combination with sodium bicarbonate, on milk production, milk composition and selected rumen parameters. A high concentrate TMR, formulated to be potentially acidotic, was used to construct four dietary treatments in which Acid Buf (AB), the skeletal remains of the seaweed *Lithothamnium calcareum*, was used alone or in combination with sodium bicarbonate (BC). The diets contained 3.5 g/kg of AB (Treatment 1) or 1.75 g/kg of AB and 1.75 g/kg of BC (Treatment 2) or 3.5 g/kg of AB and 3.5 g/kg of BC (Treatment 3) or 3.5 g/kg of AB and 5.2 g/kg of BC (Treatment 4). The response to treatment was determined using eight ruminally cannulated lactating Holstein cows randomly allocated to treatments according to a 4 x 4 (n=2) Latin square design, with four treatments and four periods. The total experimental period was 100 days in which every cow received each diet for a period of 18 days prior to a data collection period of 7 days. Rumen pH was monitored continuously over 2 days using a portable data logging system and in-dwelling electrodes. Samples of rumen fluid were collected for volatile fatty acid (VFA) analyses. During each data collection period, milk production was recorded twice daily for 7 days, whereas milk was sampled twice daily for five consecutive days for component analysis. Cows were fed *ad libitum* and dry matter intake was recorded individually. Treatment had no significant effect on milk production, milk composition or feed intake. Ruminal pH profiles of all the treatments indicated that the diets were well buffered. Average pH over 24 hours was 6.0, 6.1, 6.1 and 6.2 for Treatments 1, 2, 3 and 4, respectively. The pH did not go below 5.8 for any of the treatments and increasing levels of sodium bicarbonate increased the diurnal profile such that at the highest level (Treatment 4), the pH profile ranged from 6.1 to 6.5. Although not significant, Treatment 1 (Acid Buf alone) numerically resulted in the highest milk output without compromising milk quality. It is proposed that high rumen pH may impact negatively on milk output by increasing the acetate:propionate ratio to the detriment of rumen efficiency. Treatment had no significant effect on total VFA concentration, but there seemed to be a tendency for increased total VFA concentration as the level of sodium bicarbonate increased. The acetate:propionate ratio of Treatment 1 (2.91) was significantly lower than Treatment 4 (3.65) ($P<0.05$). The use of buffers which react to increasing acidity in the rumen may therefore provide an efficient, safe solution to rumen acidosis. This study confirmed previous results indicating that a daily intake of 80 g of Acid Buf by cows receiving high concentrate diets would support high milk production without compromising milk solids contents.

OPSOMMING

Die effek van Acid Buf en kombinasies van Acid Buf en Natriumbikarbonaat in melkkoeidiëte op melkproduksie, melksamestelling en rumenparameters

Die doel van die studie was om die invloed van Acid Buf alleen, of in kombinasie met natriumbikarbonaat, in melkbeesdiëte op melkproduksie en melksamestelling, asook op bepaalde rumenparameters, te bepaal. ‘n Volledige dieet met ‘n hoë kragvoerinhoud, wat geformuleer is om potensieel asidoties te wees, is gebruik om vier dieetbehandelings saam te stel waarin Acid Buf (AB), die skeletoorblyfsels van die seegras *Lithothamnium calcareum*, alleen of in kombinasie met natriumbikarbonaat (BK) gebruik is. Een basale diet is dus geformuleer en die behandelingsdiëte het onderskeidelik 3.5 g/kg AB (Behandeling 1) of 1.75 g/kg AB en 1.75 g/kg BK (Behandeling 2) of 3.5 g/kg AB en 3.5 g/kg BK (Behandeling 3) of 3.5 g/kg AB en 5.2 g/kg BK (Behandeling 4) bevat. Agt rumen-gekannuleerde lakterende Holsteinkoeie is ewekansig aan die behandelings toegeken in ‘n 4 x 4 (n=2) Latynse vierkantontwerp met vier behandelings en vier periodes. Die totale eksperimentele periode was 100 dae, waartydens elke koei elke behandeling vir 18 dae ontvang het voor die datakolleksieperiode van 7 dae. Rumen pH is voortdurend, elke vier minute, oor ‘n twee-dae periode gemeet met behulp van draagbare dataloggers en pH elektrodes wat binne-in die rumen gesetel was. Rumenvloeistof is versamel vir die bepaling van vlugtige vetsuurkonsentrasies. Gedurende elk van die datakolleksieperiodes is die melkproduksie twee maal per dag geneem vir sewe agtereenvolgende dae en melkmonsters is tweemaal per dag versamel vir vyf agtereenvolgende dae vir komponent–analise (samestelling?). Koeie is *ad libitum* gevoer en voerinnames is individueel bepaal. Die behandelings het geen betekenisvolle invloed op melkproduksie, melksamestelling of voerinnames gehad nie. Rumen pH profiele van al die behandelings het aangedui dat die diëte goed gebuffer was. Die gemiddelde pH oor 24 uur was 6.0, 6.1, 6.1 en 6.2 vir Behandeling 1, 2, 3 en 4, onderskeidelik. Die pH het nie onder 5.8 gedaal vir enige van die behandelings nie en die toenemende vlakke van natriumbikarbonaat het ‘n verhoging in die daaglikse pH-profiele tot gevolg gehad. By die hoogste buffervlakke (Behandeling 4) het die pH-profiel tussen 6.1 en 6.5 gevarieer. Hoewel nie betekenisvol nie, het Behandeling 1 (slegs Acid Buf) numeries die hoogste melkproduksie getoon, sonder dat melkkwaliteit beïnvloed is. Dit is voorgestel dat te hoë rumen pH ‘n nadelige impak op melkproduksie kan hê, as gevolg van ‘n verhoogde asetaat:propionaat verhouding wat rumen doeltreffendheid benadeel. Behandelings het geen betekenisvolle invloed op totale vlugtige vetsuurkonsentrasies gehad nie, maar daar was ‘n neiging vir hoër totale vlugtige vetsuurkonsentrasies namate die vlakke van natriumbikarbonaat verhoog het. Die asetaat:propionaat verhouding van Behandeling 1 (2.91) was betekenisvol laer as dié van Behandeling 4 (3.65). Die gebruik van buffers wat reageer op toenemende suurheidsgraad kan dus aangewend word as ‘n doeltreffende en veilige oplossing vir rumen asidose. Die studie het bevestig dat ‘n daaglikse inname van 80 g Acid Buf deur koeie wat ‘n hoë kragvoerdieet ontvang, hoë melkproduksie ondersteun sonder dat melksamestelling nadelig beïnvloed word.

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Notes

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has been unavoidable.

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CHAPTER 1
General introduction

Dairy cattle play an important role in human nutrition by providing a source of protein through the production of milk. The modern dairy cow is a result of many years of selection to produce cows with a genetic potential for high milk production with adequate milk composition. However, nutrition of dairy cattle is the key in enhancing performance and productivity of these animals.

Satisfying the nutrient requirements of dairy cows to ensure optimal productivity has resulted in the inclusion of high amounts of fermentable and starchy feeds to ensure increased energy intake, especially in early lactation where the energy requirement for lactation exceeds the energy supply (NRC, 2001). Large changes in the rumen environment are the inevitable consequence of this type of diet and the eating behaviour of the cow, which consumes large quantities of fresh feed when it's available. Rumen acidosis results when a sustained drop in rumen pH occur due to significant increases in fermentation producing high levels of volatile fatty acids and lactic acid. Excessive build up of acid has a detrimental impact on the bacterial population of the rumen and its ability to function efficiently and effectively (Krause & Oetzel, 2006), resulting in a variation in feed intake and a loss in production performance.

Diets high in starch usually lack sufficient amounts of effective fibre which contributes to the increased rumen acidity as these diets fail to sufficiently stimulate rumination resulting in reduced saliva production (Zebeli *et al*., 2006). Saliva contributes 30 to 40% to the buffering capacity of volatile fatty acids produced in the rumen during fermentation (Allen, 1997) and therefore maintaining optimal conditions for rumen microbial growth (Mertens, 1997). Maintaining the rumen ecosystem and satisfying the nutritional demands of the cow is a challenge as it is routinely upset by intakes of feed, especially when in large amounts.

Feeding large amounts of starchy concentrates with small amounts of forage, especially forage which has been fermented or finely chopped, may require the presence of buffers in the diet to compensate for the decreased saliva output in an attempt to maintain rumen function. Buffers function by maintaining the hydrogen ion concentration in the rumen and body fluids (Erdman, 1988) by increasing the rate of passage of liquid and volatile fatty acids from the rumen (Rogers *et al*., 1985). Sodium bicarbonate is a mineral buffer commonly added to high concentrate diets to increase and stabilize rumen pH (Solorzano *et al*., 1989). However, sodium bicarbonate is short lived in the rumen (Van Soest, 1994) and cannot effectively buffer an ongoing production of acids in the rumen (Russell & Chow, 1993).

Acid Buf is the skeletal remains of the seaweed *Lithothamnium calcareum*, harvested off the Irish Coast. Chemically it is almost 95% ash, mainly from calcium carbonate and magnesium carbonate but also contains

a variety of trace minerals. The product has been shown to possess a honeycomb structure, providing over ten times the surface area of that from an equivalent weight of limestone. The reactivity of the product is also increased by its molecular lattice structure, the calcium carbonate existing as a mixture of calcite, aragonite and vaterite compared to the stable calcite form only found in limestone (S.J. Taylor., 2009, Personal communication). It has been used as a buffer in ruminant nutrition. Cruywagen et al. (2004), reported Acid Buf to greatly impact rumen acidity and maintain high productivity in dairy cows without compromising milk quality. However, the extent of its effects on rumen metabolism and production response in dairy cows has not been rigorously evaluated. The objectives of this study were to determine the buffering capacity of Acid Buf. The effect Acid Buf alone, or in combination with sodium bicarbonate, has on rumen parameters and production response in dairy cows fed high concentrate diets was also determined.

References

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

Cruywagen, C.W., Swiegers, J.P., Taylor, S.J. and Coetzee, E., 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.

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

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

NRC, 2001. Nutrient requirements of dairy cattle (7th ed). Natl. Acad. Sci., Washington, DC, USA.

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

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

Solorzano, L.C., Armentano, L.E., Drummer, R.R. and 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., 1994. Nutritional ecology of the ruminant (2nd ed). In: Feeding strategies, taxonomy and evolution. Comstock Publishing Associates, London, UK.

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

CHAPTER 2

Literature review

2.1 Introduction

Animal production systems are of great importance to human society. Animals are produced and managed commercially under intensive conditions to provide many products which are valuable to humans, the most important one being food. In many parts of the world, there is a great shortage of food where millions of people are starving or suffering from malnutrition of which protein is the main component lacking in their diet.

Dairy cattle production systems are an important component of the food industry, providing a source of protein through meat and milk production. According to Hard (2002), the global population is expected to increase to more that 7.5 billion by the year 2020, requiring a doubling of animal protein production. Therefore, the demand for milk and milk products will also increase. Milk consumption trends will not decrease or fluctuate as meat consumption trends, as milk and milk products are subjected to fewer religious and social restrictions (McDonald *et al*., 2002).

Due to increasing demands for milk and milk products, dairy producers are under great pressure to increase their productivity and efficiency. For this reason, dairy farms are growing in size and utilizing more advanced technologies in an attempt to improve their productivity. Apart from the quantity of milk produced, more emphasis is placed on the quality of milk being produced, another factor contributing to the increased pressure put on dairy farmers. To ensure sustainability and profitability of dairy farms, farmers must produce products complying with market demands. In the past, dairy farmers were paid for their milk on its fat content (Cerbulis & Farrell, 1974). However, now more emphasis is placed on the milk protein content (Cerbulis & Farrell, 1974) as consumers demand more products of low fat content. Therefore, dairy farmers must focus on many factors to ensure high efficiency and productivity of their dairy herd to maintain profitability of their enterprise. Factors include management, use of improved and/or new technologies, breeding and most importantly, nutrition.

The quantity and quality of milk produced by dairy cows are determined by their genotype and environment (Chalupa *et al*., 1996). Cerbulis & Ferrell (1974) demonstrated how genetics result in variations in milk yield and composition by comparing different dairy cow breeds in South-eastern Pennsylvania. Through selection and biotechnology (e.g. artificial insemination, embryo transfer, gene transfer and cloning) it is possible to breed and/or produce cows with a high genetic potential for high milk yield of high quality

(Goddard & Wiggans, 1999). Improving the dairy herd genetically is an important component determining the herd's productivity, but it is a long term process.

Nutrition is a key factor influencing the performance, health and welfare of the cows (Poppi *et al*., 2000). However, milk composition (i.e. fat and protein content) can be changed by nutrition, thus providing producers means to adjust to the changing market demands on a short term basis (Chalupa *et al*., 1996). Therefore, cows with a high genetic potential for milk production receiving diets lacking in specific nutrients (e.g. energy, protein, vitamins or minerals) would result in suboptimal production responses. To prevent this from happening, producers provide dairy cows with highly digestible diets containing a high proportion of readily fermentable carbohydrates (Plazier *et al*., 2008), ensuring the cow's energy requirements are met. The high energy content of the provided diets will ensure maximum productivity, but can lead to severe consequences resulting in reduced productivity and eventually reduced profitability of the farm.

Over millions of years, ruminants have evolved to digest and metabolize predominantly forage diets (Van Soest, 1994; Krause *et al*., 2006; Dryden, 2008). Therefore, providing dairy cows with high concentrate diets (with limited amounts of effective fibre) often results in metabolic disorders, of which sub-acute rumen acidosis (SARA) is the most common and has substantial financial implications. SARA is a great concern to dairy farmers as it is associated with various undesirable disorders such as reduced dry matter intake and fibre digestion (Allen, 1997; Calsamiglia *et al*., 2008), milk fat depression, laminitis (Nocek, 1997), liver abscesses and even death can occur (Plazier *et al*., 2008).

In an attempt to manage and alleviate SARA, feed additives are added to dairy cow diets, of which buffers are the most common compounds used. Buffers can be provided through endogenous production (via saliva) and/or through dietary buffers of which sodium bicarbonate is the compound most commonly used in the industry (Chalupa *et al*., 1996). Though buffers have shown to be very effective in alleviating SARA, prevention is always better than cure. By focussing on various managerial practices (e.g. chemical and physical characteristics of feed, frequency of feeding, providing feed as a total mixed ration or feeding forage and concentrates separately, grouping of cows, number of cows per group, etc.) can reduce the risk of cows developing SARA. By ensuring adequate amounts of effective fibre in dairy cow diets, rumination will be stimulated sufficiently to ensure adequate saliva production which is very effective in buffering the acids that are produced during the fermentation of feeds (Carter *et al*., 1990; Mertens, 1997).

It is clear that SARA is of great concern to dairy farmers, not only in an economical sense, but also due to compromises in cow health and welfare. Understanding the complexity of the interactions between animal, rumen microbes and feed characteristics may enable the prevention of metabolic disorders, improvement of animal productivity and farm profitability.

2.2 The rumen

2.2.1 Rumen anatomy

The anatomy of the ruminant's gastrointestinal tract results in a different method of digestion than that in monogastric animals' gastrointestinal tracts. The ruminant's stomach is divided into four compartments; the reticulum, rumen, omasum and abomasum.

The rumen is a large muscular organ that comprises about 80% of the total stomach capacity (McDonald *et al*., 2002). The rumen is the first compartment to initiate digestion of ingested feed. However, digestion of nutrients in the rumen involves microbial digestion through fermentation. Microbial digestion prior to enzymatic digestion (as in the abomasum and small intestine) is a characteristic of the ruminant's digestive physiology and the result of thousands of years' evolution, which enable them to utilize forages and fibrous roughages as food sources (Van Soest, 1994).

The rumen wall is lined with papillae which increase the rumen's surface area allowing the absorption of the fermentation end products (mainly volatile fatty acids). Papillae distribution, size and number are closely related to the forage to concentrate ratio, feeding habits, forage availability and digestibility (Ishler *et al*., 1996). This is associated with the rate at which volatile fatty acids are produced. As the energy content of a diet increases, the rate of volatile fatty acid production increases, requiring an increased absorption rate in order to maintain an optimal rumen pH and tonicity. This is achieved by increasing the rumen surface area via increased papillae size and/or papillae numbers.

2.2.2 Rumen function

The rumen plays an important role in the digestion of fibrous feeds through microbial fermentation. Apart from serving as a fermentation vat, the rumen has other functions as well, contributing to the digestion of ingested feed.

Rumen motility (i.e. muscular contractions) is an important aspect of the rumen's function as the rumen is a large muscular organ. Strong contractions of the rumen allow coarse forage particles to be regurgitated for cud chewing. This result in feed particle size reduction, increasing microbial accessibility to potentially digestible nutrients as well as increasing the amount of nutrients that can be absorbed through the rumen epithelia. Cud chewing (rumination) in turn, stimulates saliva production which contributes to the rumen contents' buffering capacity. These ruminal contractions also cause digesta to be mixed with saliva (produced during eating and rumination) which ensures sufficient buffering of fermentation acids, as well as

promoting nutrient contact with rumen microbes and rumen epithelia for digestion and absorption of nutrients, respectively. During microbial fermentation, gases are produced of which carbon dioxide and methane is most abundant. Ruminal contractions result in these gases to be expelled via eructation which is important in the prevention of bloat (Ishler *et al*., 1996). Liquids, small feed particles and microbes are removed from the rumen to enter the abomasum and small intestines for enzymatic digestion as a result of ruminal contractions.

In order to maintain optimal rumen functioning and ensuring microbial survival, certain conditions within the rumen must be maintained. These conditions include: (1) maintaining a pH range of 5.8 to 6.4, (2) maintaining the rumen osmolarity near that of blood, (3) maintaining an anoxic environment and (4) maintaining the rumen liquor temperature between 38 to 42˚C (McDonald *et al*., 2002).

2.3 Rumen microorganisms

The rumen environment provides adequate conditions for various microbes to proliferate. The microbial consortium includes various bacteria, protozoa and fungi. From an ecological view point, the great diversity of microbes is considered to be a positive attribute for more stable and resilient microbial communities (Firkens & Yu, 2006).

Maintaining an optimal environment is important for ensuring microbial survival and functionality. Alterations in specific ruminal parameters can markedly effect microbial proliferation. Factors which can influence the rumen environment and microbial consortium include the diet composition, rumen pH and osmolarity.

Feed characteristics (e.g. chemical and physical characteristics) influence the microbial populations in the rumen. Microbes have certain preferences for specific substrates. Therefore, diet composition is an important component determining the composition of the microbial population. The degradability of cell walls, as determined by their chemical and physical characteristics, vary between plant species, cultivars and stage of plant growth, which influence the microbes' ability to adhere to feed particles as well as accessibility of cell wall polysaccharides to digestive enzymes (Flint, 1994). A diet high in roughage may result in a microbial population with increased numbers of cellulolytic microbes, whereas a diet high in grain may result in increased numbers of amylolytic microbes (Tajima *et al*., 2000). Fonty & Gouet (1994) reported increased numbers of fungi in the rumen when the diet is high in fibre. In addition, specific nutrients in the diet provided to ruminants, may stimulate growth of certain microbes. Russell & Sniffen (1984) reported improved cellulose digestion when the dairy cow diet was supplemented with certain C4 and C5 volatile fatty acids such as isobutyrate, valerate, isovalerate and 2-methyl butyrate. They attributed this

increased cellulose digestion to a stimulatory effect these volatile fatty acids have on cellulolytic microbes which resulted in enhancement of their growth. Sniffen & Robinson (1987) also reported the cellulolytic bacteria's requirement for isovalerate for growth. Microbes also need a source of nitrogen for microbial protein synthesis. Microbes require ammonia, peptides as well as amino acids for growth (Sniffen & Robinson, 1987).

Maintaining ruminal tonicity is another component influencing microbial survival, as there is a limit to which rumen microbes can tolerate increases in tonicity. Ruminal osmolarity normally ranges from 240 to 265 mOsm for animals on roughage diets and 280 to 300 mOsm on concentrate diets (Garza *et al*., 1989). The rate and extent of a post-prandial rise in ruminal fluid osmolarity depends on the diet, the amount of feed consumed in a given time, the activity of the rumen microbes and water intake as a result of the dissolution of minerals from ingested feed and the production of volatile fatty acids from microbial fermentation (Dijkstra *et al*., 1993). Gram-negative bacteria are more sensitive to high ruminal osmolarity than are gram-positive bacteria and protozoa are more sensitive to elevated tonicity than are bacteria, but ruminal microbes seem to be resilient to the normal short-term changes in ruminal tonicity (Carter & Grovum, 1990). After meal consumption, rumen osmolarity increases until a level is reached that inhibits microbial activity, allows time for the absorption of electrolytes and volatile fatty acids and influx of water into the rumen (Dijkstra *et al*., 1993). These mechanisms result in decreased rumen osmolarity in order to maintain homeostasis. High rumen osmolarity inhibits feed intake which also helps to restore tonicity values of ruminal fluid (Carter & Grovum, 1990).

McDonald *et al*. (2002) reported an optimal pH range between 5.8 and 6.4 for microbial survival. However, microbes differ in their optimal pH range where they are most active. Cellulolytic bacteria are most active in a pH range of 6.2 to 6.8, whereas amylolytic bacteria are most active in a pH range of 5.2 to 6.0 (Ishler *et al*., 1996). This is in agreement with Calsamiglia *et al*. (1999) and Mouriño *et al*. (2001) who reported reduced fibrolytic activity of microbial fermentation at a low pH of 5.8. This is attributed to a reduction in the ability of fibrolytic bacteria to adhere to feed particles or to the slow replication rate of fibrolytic bacteria at low pH, or to both (Mouriño *et al*., 2001; Calsamiglia *et al*., 2008). In addition, lactate-utilizing bacteria (e.g. *Megasphaera elsdenii*) decrease and lactate-producing bacteria (e.g. *Streptococcus bovis* and *Lactobacillus vitilinus*) increase in numbers at low pH levels (Mouriño *et al*., 2001). The efficiency with which microbes synthesize microbial protein is also influenced by rumen pH, as Calsamiglia *et al*. (2008) reported reduced efficiency when ruminal pH is below 5.5. Therefore, the microbial population shifts in accordance to pH fluctuations.

2.3.1 Rumen bacteria

The majority of the microbial population is represented by bacteria which can amount to 10^{10}-10^{11} cells per millilitre of rumen contents (McDonald *et al*., 2002; Madigan *et al*., 2003). Diversity within the bacterial population is extensive and the complexity great (Russell & Hespell, 1981) with a wide range of substrate affinities (Sniffen & Robinson, 1987) including cellulose, hemicellulose, starch, sugars, intermediate acids, protein and lipids. Table 2.1 provides a summary of the different bacterial species grouped according to their affinity for a preferred substrate.

Table 2.1 Grouping of rumen bacterial species according to the type of substrates fermented (Ishler *et al*., 1996; McDonald *et al*., 2002; Madigan *et al*.,2003)

Group	**Bacterial species**
Cellulolytic Species	*Fibrobacter succinogenes*
	Ruminococcus flavefaciens
	Ruminococcus albus
	Butyrivibrio fibrisolvens
Hemicellulolytic Species	*Butyrivibrio fibrisolvens*
	Bacteroides ruminicola
	Ruminococcus species
Amylolytic species	*Streptococcus bovis*
	Succinomonas amylolytica
	Ruminobacter amylophilus
	Prevotella ruminicola
	Selenomonas ruminantium
Sugar-utilizing species	*Treponema bryantii*
	Lactobacillus bitulinus
	Lactobacillus ruminus
Lactate-utilizing species	*Megasphaera elsdenii*
	Selenomonas lactilytica

2.3.2 Rumen protozoa

Protozoa can amount to 10^6 cells per millilitre of rumen contents (McDonald *et al*., 2002). Protozoa are quite diverse and have a high preference for starches and sugars (Sniffen & Robinson, 1987). According to Madigan *et al*. (2003), protozoa are not essential for rumen fermentation, although they do contribute to the overall process. However, Jouany & Ushida (1994) estimated that one-quarter to one-third of fibre

breakdown in the rumen is of protozoal origin, as they observed increased cellulose and hemicellulose digestion when they added protozoa to defaunated sheep. They attributed the increased cellulose and hemicellulose digestion to the ability of protozoa to hydrolyse hemicellulose by separating lignin from some of the potential degradable carbohydrates, making it more readily available to microbial enzymes. They also observed further increases in ligno-cellulose digestion when the diet was supplemented with starch, suggesting an interaction between dietary starch, the presence of protozoa and digestion of structural carbohydrates.

Many authors have reported predation on rumen bacteria by protozoa (Russell & Hespell, 1981; Sniffen & Robinson, 1987; Stewart, 1994; Ishler *et al*., 1996). Protozoal predation on bacteria is non-specific and plays an important role in controlling bacterial numbers in the rumen, especially during concentrate feeding (Sniffen & Robinson, 1987; Stewart, 1994).

2.3.3 Rumen fungi

Fungi are not as diverse as the bacterial species and can amount to 10^5 cells per millilitre of rumen contents (Sniffen & Robinson, 1987). Fungi play an important role in ruminal digestion of feeds. Fungi have been reported to have the most active cellulase-complex when compared to other cellulolytic bacteria (Stewart, 1994). Fonty & Gouet (1994) reported *Neocallimastix frontalis*, *Piromyces communis* and *Orpinomyces joyonii* to degrade cellulose more efficiently than certain cellulolytic bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*. Fungi show no lignolytic activity, but *Neocallimastix frontalis* has been reported to solubilize small amounts of lignin from plant cell walls, increasing accessibility to cellulose and hemicellulose, facilitating colonization and attack of plant particles by bacteria which are unable to adhere to lignified cell walls (Fonty & Gouet, 1994).

2.4 Microbial interactions

Realising the complexity of the various microbial interactions with the host animal, as well as between and within microbial species is of great importance. Understanding how microbes interact synergistically and antagonistically and how they are influenced by factors of the host animal may result in increased efficiency and productivity of the animals (Firkens & Yu, 2006). Unfortunately, the vast complexity of these interactions makes it difficult to predict the production response of animals when fed a particular diet.

2.4.1 Ruminant – Microbe interactions

The interactions between the microbial consortium and the host animal are important and can be regarded as a symbiotic one under normal conditions. The rumen of the host animal provides an adequate environment for various microbes to proliferate through maintaining anaerobiosis, optimal pH, temperature, osmolarity of rumen contents and the provision of nutrients. In return, the microbes ferment the ingested feed to produce volatile fatty acids such as acetic-, propionic- and butyric acid. The presence of a microbial consortium is what enables the ruminant to utilize forages and fibrous roughages as nutrient sources. Volatile fatty acids produced during microbial fermentation are absorbed through the rumen wall and can provide up to 80% of the ruminant's energy needs (Ishler *et al*., 1996; Hutjens, 2003). The microbes also synthesize amino acids and vitamins which provides the host animal with the main source of these nutrients. Twenty percent of the nutrients absorbed by the host is provided by the microbial mass (Madigan *et al*., 2003) of which 40 to 80% of the ruminant's daily amino acid requirement can be supplied from microbial protein flowing to the small intestine (Sniffen *et al*., 1987).

2.4.2 Microbe – microbe interactions

Interactions between the various rumen microbes are very important. However, the interactions between and within microbial species are far more complex than one might think. Important microbial interactions include predation, interspecies cross-feeding, amensalism and commensalism (Stewart, 1994).

Protozoal predation on bacteria is important to control bacterial numbers (as previously mentioned). In addition, the engulfed bacterial cells serve as a nitrogen source for protozoal protein synthesis, hence their growth (Ishler *et al*., 1996). The engulfed bacterial cells also serve as a source of precursors for the synthesis of protozoal nucleic acids (Russell & Hespell, 1981).

Interspecies cross-feeding occurs when one microbe produce end-products which are then utilized by another microbe. *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* hydrolyse hemicellulose and release soluble sugars, which are then utilized by *Butyrivibrio fibrisolvens* (Stewart, 1994).

Amensalism occurs when certain microbes are inhibited by factors produced by other microbes (Stewart, 1994). One would expect microbes that occupy the same ecological niche to act synergistically to achieve optimal digestion of that specific compound. This is not always the case. *Ruminococcus albus* and *R. flavefaciens* are well known cellulolytic bacteria. Fungi are also well known for their cellulolytic activity. However, when certain strains of *R. albus* and *R. flavefaciens* are co-cultured with fungi, fungal cellulolysis are inhibited through soluble factors produced by the bacteria (Stewart, 1994). Fonty & Gouet (1994)

reported that polypeptides are released by these bacteria which either inhibit fungal cellulolysis or limit their adhesion to cellulose.

Commensalism occurs when the presence of one microorganism stimulates the activity and/or growth of the second microorganism without the first one being influenced by the second microorganism (Russell & Hespell, 1981). Fonty & Gouet (1994) reported an increased rate of cellulolysis when *Selenomonas ruminantium* was co-cultured with fungi. Hemicellulose hydrolysis by *Neocallimastix frontalis* PNK2 increased by 40% when it was co-cultured with *Succinovibrio dextrinosolvens*, a saccharolytic bacteria (Fonty & Gouet, 1994).

2.5 Dairy cow diets

Dairy cow diets are formulated in an attempt to meet all their nutrient requirements for maintenance, ensuring adequate provision of nutrients for microbial sustainability and survival, as well as achieving maximal productivity.

2.5.1 Energy

Carbohydrates can contribute 60 to 80% of the dietary dry matter, while protein, fat and minerals make up the remaining portion (Hutjens, 2003). Carbohydrates are divided into two categories: structural carbohydrates (e.g. cellulose and hemicellulose) and non-structural carbohydrates (e.g. starch and sugars). Carbohydrates are digested in the rumen via microbial fermentation to produce organic acids. The primary volatile fatty acids in descending order of abundance are acetic-, propionic-, butyric-, isobutyric-, valeric- and isovaleric acids (Ishler *et al*., 1996). However, diet composition greatly determines the proportions of volatile fatty acids produced in the rumen. High forage diets result in higher acetate concentrations, whereas high grain diets result in higher propionate concentrations. Under optimal conditions, the acetate:propionate ratio should not be greater than 2.2 to 1 (Hutjens, 2003), therefore the forage to concentrate ratio is an important dietary factor to take into account when formulating dairy cow diets.

Volatile fatty acids, produced by microbial fermentation, are absorbed through the rumen wall and can provide up to 80% of the animal's energy requirements (Ishler *et al*., 1996), therefore representing the primary energy source for ruminants. Other sources of energy for ruminants include fatty acids which are mobilized from adipose tissue and are of great importance during early lactation of dairy cows, as well as dietary fats.

Apart from the animal's energy requirements, the rumen microorganisms' energy requirements are just as important to consider. Rumen microbes obtain their energy primarily from dietary carbohydrates and products of carbohydrate hydrolysis in the rumen. Microbes require energy for maintenance and growth, especially when microbes use non-protein nitrogen (NPN) sources for microbial protein synthesis. Unlike the host animal, microbes have a limited ability to utilize fats as energy source. Large amounts of dietary fat can reduce microbial growth and result in reduced organic matter digestibility (Chalupa *et al*., 1996).

2.5.2 Nitrogen

The ruminant animal's requirement for amino acids (primary nitrogen source) for maintenance, growth and production are supplied by microbial crude protein synthesized in the rumen, and dietary protein which escapes digestion in the rumen. After feed ingestion, protein is first exposed to microbial digestion in the rumen. Microbes degrade dietary protein to amino acids, ammonia and various non-nitrogen containing fragments which are then resynthesized to microbial protein (Shirley, 1986). The extent of protein degradability in the rumen varies (Russell & Hespell, 1981; Chalupa *et al*., 1996) and is an important factor to consider when determining the protein requirements of ruminants (Ørskov & McDonald, 1970; McDonald, 1981). However, the rate at which the different proteins can be hydrolyzed controls the extent of their degradation before they pass out of the rumen (Mackie & White, 1990).

Rumen microbes also require nitrogen sources for maintenance and growth. Most of the rumen microbes (especially fibrolytic bacteria) use ammonia as their primary nitrogen source for growth (Ishler *et al*., 1996). Hespell & Bryant (1979) reported that 50 to 70% of bacterial cell nitrogen to come from ammonia. However, Russell & Hespell (1981) reported the necessity for peptides and amino acids for bacterial growth when low quality diets (high fibre, low protein) are fed as up to 40% of the bacterial nitrogen did not come from ammonia. In addition, rumen microbes are able to utilize NPN-sources (e.g. urea) as a source of nitrogen. This is attributed to the hydrolysis of NPN-sources to ammonia, which is then incorporated into microbial protein (Shirley, 1986).

Chalupa *et al*. (1996) reported that one-third of the feed protein consumed is lost through faecal- and urinary excretions. By limiting these nitrogen losses, maximum growth and productivity of the animals can be achieved. Reduced nitrogen loss can be achieved if dietary protein bypass the rumen after the microbes' protein requirements are met (Shirley, 1986). Although microbial protein is of high biological value to ruminants, microbes are unable to produce all the essential amino acids required for animal growth and high levels of milk production (Ishler *et al*., 1996). Therefore, these amino acids must be supplemented in the diet. To ensure that these amino acids are absorbed by the animal, they must be protected from microbial digestion, thus by-passing the rumen, to be digested and absorbed in the small intestine.

2.6 Factors influencing dry matter intake by dairy cows

The production response of an animal is greatly determined by the total quantity of nutrients absorbed from the animal's gastrointestinal tract (Poppi *et al*., 2000). In turn, the amount of nutrients absorbed is then dependant on the quantity of feed ingested and the digestibility of that feed. If an excellent diet is formulated, providing all the essential nutrients and more, but the animal's feed intake is suboptimal, the animal will not be nearly as productive as was expected. There are various factors influencing feed intake which must be taken into consideration when formulating a diet. These factors can be categorized as feed-, animal-, environmental- and managerial factors.

2.6.1 Feed characteristics influencing feed intake

The chemical composition of feed determines the extent to which the feed can be digested, as well as the rate at which it is digested. Feeds with high structural carbohydrate contents are slowly digested by rumen microbes, therefore increasing feed retention time within the rumen and contributing to rumen fill (Mertens, 1997). Tension receptors are present in the reticulo-rumen wall and when the rumen's capacity becomes limiting, the receptors send signals to the animal's brain to cease eating (Van Soest, 1994). Therefore, feed high in fibre would result in reduced levels of feed intake. This is also true when a feed is highly lignified as rumen microbes are unable to digest lignin.

The physical characteristics of feed determine the rate of feed ingestion and rate of digestion. Large (fibrous) feed particles are ingested at slower rates compared to small (starchy) feed particles due to reduced ease of prehension. Large feed particles are digested at slower rates due to a reduced surface area to volume ratio when compared to small particles, in addition, contributing to rumen fill (as previously discussed). Large feed particles stimulate rumination in an attempt to reduce feed particle size mechanically. Therefore, large feed particles reduce ease of prehension, contribute to rumen fill, increase the time spent ruminating and therefore reducing time spent eating, resulting in reduced feed intake (Dado & Allen, 1995). For these reasons, care should be taken when processing feed, as the method and extent of processing greatly influence the feed's physical characteristics.

2.6.2 Animal factors influencing feed intake

The physiological state of an animal determines its metabolic requirement, in turn, influencing feed intake. Lactating and pregnant cows have higher nutritional requirements than dry cows, therefore they need to have higher levels of feed intake to meet their nutritional needs. In reality, cows tend to have low levels of feed

intake in early lactation and reach peak levels of feed intake in mid-lactation (Dado & Allen, 1995). Therefore, feed intake is associated with the animal's physiological state.

The health status of animals also influence feed intake. Healthy animals have good appetites and show adequate levels of feed intake in order to meet their nutritional needs. Reduced feed intake is usually the very first indication of ailments. In addition, many metabolic disorders result in reduced feed intake (Nocek, 1997). Therefore, reduced feed intake can be used as a diagnostic tool to identify disorders and/or diseases.

Individual animal behaviour also affects feed intake. It is well known that cows select specific feed ingredients from their ration which results in nutrients ingested to differ from that offered in the feed (Chalupa *et al*, 1996). This is why providing cows with a total mixed ration have proved to be beneficial in limiting cows' sorting ability and ensure optimal feed intake.

2.6.3 Environmental factors influencing feed intake

The most important environmental factor influencing feed intake is the climate to which the animals are exposed to. Heat stress resulting from high temperatures and humidity can result in severe feed intake depressions by up to 35% (Rhoads *et al*., 2009). Therefore, management failing to protect cows from these elements would result in severe feed intake depression and low herd productivity.

2.6.4 Managerial factors influencing feed intake

Water intake is highly associated with feed intake (Shirley, 1986). Availability of water greatly influences the amount of water consumed, in turn, influencing the amount of feed consumed. Water quality is also important, as water with a high salt content will reduce water intake and hence, also reduce feed intake. In addition, water that is contaminated with bacteria or metals or high in mineral concentrations (e.g. chloride) can have an effect on rumen microorganisms, resulting in altered microbial functioning and rumen fermentation which may influence animal performance and health (Ishler *et al*., 1996).

Grouping cows according to their stage of production is a common practice employed on dairy farms. However, care should be taken when grouping cows in order to limit competition (Chalupa *et al*., 1996). Dominant cows will eat greater amounts of feed and select for higher quality nutrients, leaving feed of low quality and quantity to the inferior cows. Therefore, these cows' feed intake will be limited in quantity as well as quality.

The number of cows per group is also important to consider. High densities in stalls can limit bunk space and access to feed as well as increasing the stress level of these cows (De Vries *et al*., 2004). These circumstances may result in reduced feed intake by individual cows.

Keeping cows in the milking parlour for longer periods than are necessary interferes with their normal eating pattern and therefore their feed intake. Time away from their stalls also reduces the time that they can eat during the day. Periods of feed withdrawal can result in rapid feed consumption once feed is available again. This in turn may result in metabolic disorders which increase morbidity and as a result, increase feed intake depression.

2.7 Sub-acute ruminal acidosis (SARA)

It is difficult to give a precise definition of SARA by reviewing various articles published, due to lack of overall agreement between authors for the criteria used to diagnose SARA. This may be due to the difficulty in itself to diagnose SARA, due to variable and subtle clinical signs associated with the syndrome (O'Grady *et al*., 2008). Many signs may appear months after the initial occurrence of a sustained rumen pH reduction or may be confused with other common ailments in dairy cattle (Enemark, 2008; O'Grady *et al*., 2008). However, all the literature reviewed confirms that SARA is caused as a result of feeding high concentrate, low fibre diets. SARA is the most common and important nutritional disease in the dairy production industry.

According to Enemark (2008), the factors considered for diagnosing SARA, include the depth of the pH reduction as well as the duration of the episode where pH is below a physiologically acceptable value. An acceptable physiological pH range for maintaining a well balanced microbial population is between 5.8 and 6.4 (Ishler *et al*., 1996) which also result in equilibrium between producers and utilizers of lactic acid (Nocek, 1997). Therefore, the majority of authors define the incidence of SARA as a condition caused when the rumen pH is below 5.5 (Nocek, 1997; Cottee *et al*., 2004; Gozho *et al*., 2005). Cerrato-Sánchez *et al*. (2008), O'Grady *et al*. (2008) and Plaizier *et al*. (2008) characterize SARA with a pH drop below 5, 5 for periods more than 174 minutes per day.

2.7.1 Cause

High concentrate diets may result in a reduction in rumen pH due to the rapid fermentation of readily fermentable carbohydrates and the increased production of volatile fatty acids, especially propionic acid (Calsamiglia *et al.*, 2008). In addition, the low roughage content of the diet results in inadequate stimulation of rumination, and thus saliva production, which results in reduced ability of feed to buffer the acids

produced (Mertens, 1997; Plaizier *et al*., 2008). During the reduction in rumen pH, the rumen microbial profile also changes; *Streptococcus bovis* and lactic acid producing bacteria both increase in numbers while there is a decrease in *Megasphaera elsdenii* and *Selenomonas ruminantium*, resulting in lactic acid production to exceed lactic acid utilization (Nocek, 1997). Changes in rumen pH significantly influence the microbial population in the rumen, resulting in altered fermentation characteristics (Calsamiglia *et al*., 1999; Cerrato-Sánchez *et al*., 2008).

Nocek (1997) reported that the production of excess lactic acid or reduced lactic acid utilization as a result of reduced rumen pH, may further contribute to reduced rumen pH, increasing the risk of SARA. Oetzel *et al*. (1999) reported that the lactate concentrations generally remained normal during SARA and concluded that the deleterious health effects due to SARA may be caused by elevated total or specific volatile fatty acid concentrations in ruminal fluid rather than elevated ruminal lactate concentrations.

2.7.2 Occurrence

SARA is a very common metabolic disorder in dairy cattle. Calsamiglia *et al*. (2008) reported that in intensive production systems, 14-40% of the animals in the herd suffer from acidosis, while Krause & Oetzel (2005) reported it to be 20%. Enemark (2008) reported that 19% of early lactation and 26% of mid-lactation cows in the United States are diagnosed to have SARA, whereas 11% of early lactation and 18% of mid-lactation cows in Germany have SARA. This supports Plaizier *et al*.'s (2008) findings that early lactation and mid-lactation cows are at greatest risk for developing SARA. This susceptibility can be explained due to the sudden change in the diet composition of early lactation cows. After parturition, the cows are suddenly given diets high in concentrate, thus neither the rumen epithelium nor the microbes are adapted to this high concentrate diet. The rapid volatile fatty acid production results in reduced rumen pH. In addition, the low fibre content of the diet fails to stimulate rumination and salivation which is a key component in buffering the fermentation acids (Mertens, 1997). According to Cerrato-Sánchez *et al*. (2008) high concentrate diets (with low fibre content) results in increased total volatile fatty acids as well as the molar percentage of propionic acid to increase and the molar percentage of acetic- and butyric acid to decrease, decreasing the acetate:propionate ratio. This is in agreement with Beitz & Davis (1964). On the other hand, mid-lactation cows reach a period of peak dry matter intake, resulting in high amounts of concentrate being consumed and overloading the homeostatic mechanisms to maintain the rumen pH within acceptable limits (Plazier *et al*., 2008).

O'Grady *et al*. (2008) reported the incidence of SARA in pasture fed dairy cattle in Ireland. The incidence of SARA is related to the high amounts of readily fermentable carbohydrate and low fibre content of the lush pastures.

2.7.3 Diagnosis

It is difficult to diagnose SARA, due to the lack of accurate diagnostic test strategies, combined with the variable nature of rumen pH in healthy cows (O'Grady *et al*., 2008). Milk fat depression is one of the most important consequences of SARA (Krause & Oetzel, 2005), but milk fat percentage alone cannot be used as a diagnostic tool as there is a poor correlation between milk fat percentage and the presence of SARA. However, Enemark (2008) suggests that when 10% of the cows' individual milk fat percentages are 2.5 or less, SARA can be suspected.

Enemark (2008) reported that SARA can be suspected when the incidence of rumenitis, metabolic acidosis, reduced dry matter intake, abomasal displacement and abomasal ulcers, laminitis and rumen tympany increase. Laminitis and lameness have been proved to be closely associated with the incidence of SARA (Nocek, 1997; Plaizier *et al*., 2008). Diarrhoea can also be used to diagnose SARA, as the faeces will be runny and yellow and a distinct sour smell and gas bubbles can be observed (Nocek, 1997). Nocek (1997) also reported a reduction in dry matter intake as being the most important clinical sign to diagnose SARA. According to Plaizier *et al*. (2008), reduced fibre digestion was observed due to the acid sensitivity of the fibrolytic rumen bacteria which cannot tolerate a rumen pH below 6.0. This is supported by the findings of Rotger *et al*. (2006) who reported reduced fibre digestion due to low rumen pH.

2.7.4 Consequence

The economic consequence of SARA is one of great importance and concern. Enemark (2008) reported an annual cost varying from US $500 million up to US $1 billion due to the incidence of SARA. The cost per affected cow per day amounts to US $1,12 when considering production losses, such as decreased milk production, decreased efficiency of milk production and pre-mature culling (Krause & Oetzel, 2005). Plaizier *et al*. (2008) reported that SARA reduced milk yield by 2.7 kg/day, milk fat production by 0.3 percentage points and milk protein production by 0.12 percentage points.

Apart from the production losses associated with SARA, another possible concern is toward the safety of meat consumption by humans and the risk of food borne diseases. Low rumen pH caused by high concentrate diets may result in more acidic conditions in the lower intestinal tract which ultimately also alters the microbial population at that location. This may result in acid-tolerant enteropathogenic strains of *Escherichia coli* (e.g. *E.coli* strain O157:H7) which could be introduced into meat products, especially ground meat products, posing a risk for human health (Madigan *et al*., 2003).

2. 8 Treatment and prevention of SARA

SARA is known to result from certain feeding conditions associated with the diets and/or feed management. Formulating a diet with a high energy content, management must be such that a proper period for adaptation to the diet is allowed. During this adaptation period, the ruminal mucosa and the microorganisms can adapt to ensure that continuous feeding of this high energy diet will not result in altered rumen fermentation. Also, introducing physical effective fibre to the diet will stimulate sufficient rumination and saliva production which prevents negative consequences associated with low rumen pH as saliva has the ability to buffer the acids produced during fermentation (Mertens, 1997).

Additional factors which may affect the incidence of SARA may include processing methods of feedstuffs, chemical treatment of feedstuffs, feeding time and frequency, feed additives (e.g. buffers, ionophores, antibiotics), access to clean water, between animal competition and environmental conditions. All of the above mentioned factors influence the feeding behaviour and thus dry matter intake of the individual animal (as previously discussed). It is what and how the animal eats that determines the physiological consequences.

Feed additives (e.g. buffers, direct-fed microbials and ionophores) are routinely added to high concentrate dairy diets in an attempt to alleviate and/or prevent metabolic disorders associated with low rumen pH and will be discussed individually.

2.9 Buffers

Mineral buffers are regularly added to diets in an attempt to prevent acidosis, especially in diets where the fibre content is too low. Buffers may prevent an overgrowth of acid tolerant lactobacilli, preventing potential reduction in rumen pH (Enemark, 2008). However, buffers should not be used on a routine basis to compensate for suboptimal feeding management.

2.9.1 Sodium bicarbonate

Sodium bicarbonate is the most common mineral buffer used in the dairy industry. It is a weak base that buffers the hydrogen ions of organic acids produced in the rumen (Keunen *et al*., 2003). Enemark (2008) reported that the addition of 150 g of sodium bicarbonate to the lactation diet per day had a positive effect on the milk yield, feed intake and milk fat percentage. An increase in dry matter intake and stabilization of the rumen pH has been reported by various authors (Hutjens, 2003; Keunen *et al*., 2003). Keunen *et al*. (2003) reported that the increase in rumen pH caused by sodium bicarbonate contributes to the prevention of rumen

epithelium damage and ruminal stasis associated with SARA. Davis *et al*. (1964) reported the addition of sodium bicarbonate to a high concentrate diet to result in a decreased molar percentage of propionate and an increased percentage of acetate and butyrate. Erdman *et al*. (1982) reported the addition of sodium bicarbonate to potentially acidotic diets to increase the acetate:propionate ratio. Although sodium bicarbonate is very popular, its buffering capacity is limited. According to Russell (1998), the pK_a of sodium bicarbonate is approximately 6.7, which means that its buffering capacity will become limited and it may be unable to prevent further pH declines when the pH is less than 6.0.

2.9.2 Acid Buf

Acid Buf is a natural product produced from calcified red seaweed (*Lithothamnium calcareum*) and is claimed to have more than twice the buffering capacity of sodium bicarbonate, resulting in increased milk yield and feed conversion (Enemark, 2008). The seaweed is harvested from the seabed off the Irish coast, washed, dried and milled to manufacture the grey to off white powder end product. Acid Buf contains high levels of calcium and magnesium as well as a number of essential trace minerals. The mineral composition of Acid Buf is presented in Table 2.2. Cruywagen *et al*. (2004) reported a positive effect of Acid Buf on rumen pH as it increased the rumen pH when high concentrate diets were fed to the cows. Cruywagen *et al*. (2004) also reported Acid Buf included at a level of 0.3% of dietary DM (or 80 g/cow per day) to be the optimal dose to ensure maintenance of high productivity and milk composition. In 2007, Cruywagen *et al*. again demonstrated the efficiency of Acid Buf, compared to sodium bicarbonate, and reported Acid Buf to have a great impact on rumen acidity and has the potential to prevent SARA from occurring. Beya (2007) confirmed that Acid Buf is more effective in its buffering capacity when compared to sodium bicarbonate, probably due to its slow release feature.

Table 2.2 The mineral composition of Acid Buf (CelticSea Minerals, Cork, Ireland)

Mineral ingredient	**Amount**	**Mineral ingredient**	**Amount**
Ash	950 g/kg	Iodine (I)	160 mg/kg
Calcium (Ca)	300 g/kg	Fluorine (F)	120 mg/kg
Magnesium (Mg)	55 g/kg	Zinc (Z)	25 mg/kg
Sodium (Na)	15 g/kg	Copper (Cu)	16 mg/kg
Sulphur (Na)	5 g/kg	Boron (B)	9.5 mg/kg
Iron (Fe)	1825 mg/kg	Cobalt (Co)	4 mg/kg
Potassium (K)	650 mg/kg	Molybdenum (Mo)	2.25 mg/kg
Phosphorus (P)	575 mg/kg	Selenium (Se)	1 mg/kg

2.9.3 Magnesium oxide

Magnesium oxide is another mineral buffer that is used commercially. Thomas *et al*. (1984) compared the effects of magnesium oxide to sodium bicarbonate when acidotic diets were fed to lactating dairy cows. They found that magnesium oxide increased the milk fat percentage more than did sodium bicarbonate, but found that sodium bicarbonate resulted in increased dry matter intake and produced a greater volume of milk. Hutjens (2003) suggested that the optimal way to apply magnesium oxide in the diet is with sodium-based buffers with a ratio of two to three parts sodium bicarbonate to one part magnesium oxide.

2.10 Direct fed microbials

2.10.1 *Saccharomyces cerevisiae*

Enemark (2008) reported a reduction in rumen acidity, improved digestion of corn silage, enhanced digestion of forage dry matter, increased milk production and increased dry matter consumption when direct fed microbials (*Enterococcus faecium* and *Saccharomyces cerevisiae*) were fed to early lactating dairy cows.
Hutjens (2003) reported the utilization of lactic acid by yeast cultures which may contribute to the increased rumen pH when yeast cultures are fed. Firkens (2002) reported that yeast cultures would increase feed intake, milk yield and milk fat percentage when included with 21% forage NDF diets. Early lactation appears to be the optimum time to feed yeast cultures to stabilize the rumen environment as cows shift from low to high energy diets.

2.10.2 *Megasphaera elsdenii*

Nocek (1997) reported that *Megasphaera elsdenii* prevented acute acidosis and increased feed intake when an animal follows a dietary switch, indicating the potential of *M. elsdenii* to accelerate ruminal adaptation from forage to grain without fermentation disturbances or metabolic diseases. In practice, *M. elsdenii* cultures should be dosed at least twice to fresh cows to be effective.

2.10.3 *Aspergillus oryzae*

Aspergillus oryzae is a fermentation extract produced form a selected strain of enzyme-producing *Aspergillus oryzae*. The fungal additive has its main effect in the rumen increasing cellulolytic bacteria numbers, shifting volatile fatty acid fermentation patterns and stabilizing ruminal pH. According to Hutjens (2003), *Aspergillus oryzae* (at a level of 3 g/day) would stimulate fibre-digesting bacteria and stabilize rumen pH when high grain diets are fed.

2.11 Ionophores

Monensin and lasalocid are antibiotics that can change rumen fermentation patterns. These products have shown to increase growth in dairy animals from 6 to 14% with no negative effects on reproduction, calving ease, or calf size. This is because these ionophores are coccidiostats, removing undesirable bacteria and stimulate the increase in desirable bacteria which improve fermentation. Erickson *et al*. (2003) reported that monensin has a positive effect on controlling sub-acute acidosis by controlling the daily fluctuations in ruminal pH in beef cattle fed high concentrate diets.

2.12 Conclusion

Dairy cattle play an important role in the food industry. Numerous research results have been employed to increase the productivity of dairy cows in order to increase profitability. Nutrition is the key factor determining the animal's productivity, health and welfare. High concentrate diets are commonly fed to lactating cows in an attempt to increase their productivity. Although increased productivity is obtained, the high concentrate diets may result in severe consequences, the most important one being sub-acute ruminal acidosis (SARA). SARA has a severe impact on the health of dairy cattle, but it is also of great economic concern due to reduced milk production, milk fat depression and its association with laminitis and lameness.

By employing adequate feeding strategies and optimizing management practices, conditions such as SARA can be limited. High energy diets need to contain enough effective fibre to stimulate rumination, in order to increase salivation which is an important buffer to the organic acids produced as a result of fermentation. By providing feed with the correct chemical and physical composition, the risk of SARA to develop may be reduced, which may reduce financial losses, resulting in a profitable business.

The use of dietary buffers has shown to be of great benefit in preventing pH drops below optimal limits which could have severe consequences. Many buffers are commercially available, sodium bicarbonate being the most popular. However, Acid Buf (a relatively new product) has shown to be very effective and holds great potential for future commercial application. The current study has been designed to determine the effect of Acid Buf alone, or in combination with sodium bicarbonate, on milk production, milk composition and rumen metabolism.

2.13 References

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

Beitz, D.C. and Davis, C.L., 1964. Relationship of certain milk fat depressing diets to changes in the proportions of the volatile fatty acids produced in the rumen. J. Dairy Sci. 47: 1213-1216.

Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism. MSc (Agric) thesis, University of Stellenbosch, South Africa.

Calsamiglia, S., Cardozo, P.W., Ferret, A. and Bach, A., 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. J. Anim. Sci. 86: 702-711.

Calsamiglia, S., Ferret, A., Plaixats, J. and Devant, M., 1999. Effect of pH and pH fluctuations on microbial fermentation in a continuous culture system. J. Dairy Sci. 82 (Suppl.1): 38.

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

Cerbulis, J. & Farrell, H.M., 1974. Composition of milk of dairy cattle: Protein, lactose and fat contents and distribution of protein fraction. J. Dairy Sci. 58: 817-827.

Cerrato-Sánchez, M., Calsamiglia, S. and Ferret, A., 2008. Effect of the magnitude of the decrease of rumen pH on rumen fermentation in a dual-flow continuous culture system. J. Anim. Sci. 86: 378-383.

Chalupa, W., Galligan, D.T. and Ferguson, J.D., 1996. Animal nutrition and management in the 21 st century: dairy cattle. Anim. Feed Sci. Tech. 58: 1-18.

Cottee, G., Kyriazakis, I., Widowski, T.M., Lindinger, M.I., Cant, J.P., Duffield, T.F., Osborne, V.R. and McBride, B.W., 2004. The effects of sub-acute ruminal acidosis on sodium bicarbonate supplemented water intake for lactating dairy cows. J. Dairy Sci. 87: 2248-2253.

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

Cruywagen, C.W., Taylor, S.J. and Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate + limestone on production response and rumen parameters. J. Dairy Sci. 90 (Suppl.1): 561.

Dado, R.G. & Allen, M.S., 1995. Intake limitations, feeding behaviour, and rumen function of cows challenged with rumen fill from dietary fibre or inert bulk. J. Dairy Sci. 78: 118-133.

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

DeVries,T.J., Von Keyserlingk, M.A.G. and Weary, D.M., 2004. Effect of feeding space on the inter-cow distance, aggression, and feeding behaviour of free-stall housed lactating dairy cows. J. Dairy Sci. 87: 1432-1438.

Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. and Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. Brit. J. Nutr. 69: 385-396.

Dryden, G.McL., 2008. Nutritional ecology. In: Animal Nutrition Science. Ed. Dryden, G.McL., CAB International, Oxfordshire, UK. pp. 1-15.

Enemark, J.M.D., 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. Vet. J. 176: 32-43.

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

Erickson, G.E., Milton, C.T., Fanning, K.C., Cooper, R.J., Swingle, R.S., Parrott, J.C., Vogel, G. and Klopfenstein, T.J., 2003. Interaction between bunk management and monensin concentration on finishing performance, feeding behaviour, and ruminal metabolism during an acidosis challenge with feedlot cattle. J. Anim. Sci. 81: 2869-2879.

Firkins, J. L. 2002. Optimizing rumen fermentation. In: Tri-State Dairy Nutr. Conf., Fort Wayne, IN. pp. 39–53.

Firkins, J.L. & Yu, Z., 2006. Characterisation and quantification of the microbial populations of the rumen. In: Ruminant physiology: Digestion, metabolism and impact of nutrition on gene expression, immunology and stress. Eds. Sejrsen, K., Hvelplund, T. and Nielsen, M.O., Wageningen Academic Publishers, Wageningen, Netherlands. pp.19-54.

Flint, H.J., 1994. Degradation of plant cell wall polysaccharides by rumen bacteria. In: Micro-organisms in ruminant nutrition. Eds. Prins, R.A. & Stewart, C.S., Nottingham University Press, Thrumpton, Nottingham. pp. 49-68.

Fonty, G. & Gouet, P.H., 1994. Plant cell wall degradation by anaerobic fungi. In: Micro-organisms in ruminant nutrition. Eds. Prins, R.A. & Stewart, C.S., Nottingham University Press, Thrumpton, Nottingham. pp. 97-112.

Garza, G.D., Owens, F.N. and Breazile, J.E., 1989. Effects of diet on ruminal fluid and on blood serum osmolality and hematocrit in feedlot heifers. Oklahoma Agricultural Experiment Station. Animal Science Research Report 127: 68-76.

Goddard, M.E. & Wiggans, G.R., 1999. Genetic improvement of dairy cattle. In: The genetics of cattle. Eds. Fries, R. & Ruvinsky, A., CAB International, Oxon, UK. pp. 512-533.

Gozho, G.N., Plazier, J.C., Krause, D.O., Kennedy, A.D. and Wittenberg, K.M., 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. J. Dairy Sci. 88: 1399-1403.

Hard, D.L., 2002. Innovative developments in the production and delivery of alternative protein sources for animal feeds with emphasis on nutritionally enhanced crops. FAO Expert Consultation and Workshop on alternative Protein for the Animal Feeding Industry. Illinois, USA.

Hespell, R.B. & Bryant, M.P., 1979. Efficiency of rumen microbial growth: Influence of some theoretical and experimental factors on Y^{ATP}. J. Anim. Sci. 49: 1640-1659.

Hutjens, M., 2003. Feeding a ruminant. In: Feeding Guide (2nd ed.). Eds. Hutjens, M., W.D. Hoards & Sons Company, Fort Atkinson, USA. pp. 6-12.

Ishler, V., Heinrichs, A.J. and Varga, G., 1996. From feed to milk: Understanding rumen function. Penn State University Extension Circular 422, University Park, PA. pp. 1-26.

Jouany, J.P. & Ushida, K., 1994. Plant cell-wall degradation by rumen protozoa. In: Micro-organisms in ruminant nutrition. Eds. Prins, R.A. & Stewart, C.S., Nottingham University Press, Thrumpton, Nottingham. pp. 69-78.

Keunen, J.E., Plazier, J.C., Kyriazakis, I., Duffield, T.F., Widowski, T.M., Lindinger, M.I. and McBride, B.W., 2003. Short Communication: Effects of subacute ruminal acidosis on free-choice intake of sodium bicarbonate in lactating dairy cows. Journal of Dairy Science 86: 954-957.

Krause, K.M. & Oetzel, G.R., 2005. Inducing subacute ruminal acidosis in lactating dairy cows. J. Dairy Sci. 88: 3633-3639.

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

Mackie, R.I. & White, B.A., 1990. Recent advances in rumen microbial ecology and metabolism: Potential impact on nutrient output. J. Dairy Sci. 73: 2971-2995.

Madigan, M.T., Martinko, J.M. and Parker, J., 2003. Microbial Growth. In: Brock Biology of Microorganisms (10th ed.). Ed. Carlson, G., Pearson Education, Inc., Upper Saddle River, NJ. pp. 138-166.

McDonald, I., 1981. Short note: A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96: 251-252.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A., 2002. Animal nutrition (6th ed). Pearson Education Inc., Harlow, UK. pp. 163-243.

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

Mouriño, F., Akkarawongsa, R. and Weimer, P.J., 2001. Initial pH as a determinant of cellulose digestion rate by mixed ruminal microorganisms in vitro. J. Dairy Sci. 84: 848-859.

Nocek, J.E., 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80: 1005-1028.

Oetzel, G.R., Norlund, K.V. and 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.

O'Gady, L., Doherty, M.L. and Mulligan, F.J., 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. Vet. J. 176: 44-49.

Ørskov, E.R. & McDonald, I., 1970. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92: 499-503.

Plazier, J.C., Krause, D.O., Gozho, G.N. and McBride, B.W., 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176: 21-31.

Poppi, D.P., France, J. and McLennan, S.R., 2000. Intake, passage and digestibility. In: Feeding systems and feed evaluation models. Eds. Theodorou, M.K. & France, J., CAB International Publishing, Oxon, U.K. pp. 35-52.

Rhoads, M.L., Rhoads, R.P., Van Baale, M.J., Collier, R.J., Sanders, S.R., Weber, W.J., Crooker, B.A. and Baumgard, L.H., 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: Production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92: 1986-1997.

Rotger, A., Ferret, A., Calsamiglia, S. and Manteca, X., 2006. Effects of non-structural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism in vivo and in vitro with high-concentrate beef cattle diets. J. Anim. Sci. 84: 1188-1196.

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.

Russell, J.B.& Hespell, R.B., 1981. Microbial rumen fermentation. J. Dairy Sci. 64: 1153-1169.

Russell, J.B. & Sniffen, C.J., 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci. 67:987-994.

Shirley, R.L., 1986. Activity of Rumen Microbes. In: Nitrogen and energy nutrition of ruminants. Ed. Cunha, T.J., Academic Press, Inc., Orlando, Florida. pp. 9-75.

Sniffen, C.J. & Robinson, P.H., 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci. 70: 425-441.

Stewart, C.S., 1994. Plant-animal and microbial interactions in ruminant fibre digestion. In: Micro-organisms in ruminant nutrition. Eds. Prins, R.A. & Stewart, C.S., Nottingham University Press, Thrumpton, Nottingham. pp. 13-28.

Tajima, K., Arai, S., Ogata, K., Nagamine, T., Matsui, H., Nakamura, M., Aminov, R.I. and Benno, Y., 2000. Rumen bacterial community transition during adaptation to high-grain diet. Anaerobe 6: 273-284.

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

Van Soest, P.J., 1994. Nutritional ecology of the ruminant (2nd ed). In: Feeding strategies, taxonomy and evolution. Comstock Publishing Associates, London, UK.

CHAPTER 3
General materials and methods

Methods pertaining to the different experiments in this study are explained in the relevant chapters following the style of scientific articles. It was decided, however, to expand on some methods in this chapter in order to provide the reader with more background, especially in the case of referenced procedures.

The protocol for the entire study was approved by the Stellenbosch University's Animal Ethics Committee (Reference # 2005B03001).

3.1 Animals and housing

The study was conducted at the Welgevallen Experimental Farm of the Stellenbosch University. Eight ruminally cannulated lactating Holstein cows, 114 ± 9.3 (SE) days in milk (DIM) and producing 34.4 ± 2.9 (SE) kg milk/day, were used in the trial. The cows were housed individually in semi-open pens (3 x 5 m) with cement floors and sand beds provided for resting and sleeping. The pens were cleaned twice daily throughout the duration of the trial by hosing and sweeping away accumulated manure while the cows were at the milking parlour to prevent interference with their normal eating behaviour and to limit stress.

3.2 Experimental design and treatments

Cows were randomly allocated to treatments according to a 4 x 4 Latin square design (n=2) with four treatments and four periods. All cows received all four treatments during the course of the trial. The trial duration was 100 days. Each period (25 days) consisted of an 18-day adaptation period and a 7-day data collection period.

The four treatments had the same basal diet. The difference between the treatments was attributed to the different inclusion levels of Acid Buf (AB) and sodium bicarbonate (BC). Table 3.1 presents the ingredient composition and chemical composition of the experimental diets. The treatments were as follows:

Treatment 1: Basal diet plus AB, included at 3.5 g/kg to provide a daily intake of 80 g of AB/cow
Treatment 2: Basal diet plus AB and BC, both included at 1.75 g/kg to provide a daily intake of 40 g of AB and 40 g of BC/cow
Treatment 3: Basal diet plus AB and BC, both included at 3.5 g/kg to provide a daily intake of 80 g of AB and 80 g of BC/cow

Treatment 4: Basal diet plus AB and BC, included at 3.5 g/kg and 5.2 g/kg, respectively, to provide a daily intake of 80 g of AB and 120 g of BC/cow

Table 3.1 Ingredient and chemical composition of the experimental diets

Ingredient (g/kg)	Treatments[1]			
	AB (80g/d)	40 AB + 40 BC (40 + 40 g/d)	80 AB + 80 BC (80 +80 g/d)	80 AB + 120 BC (80 + 120 g/d)
Oat hay	176	176	176	176
Lucerne hay	176	176	176	176
Wheat bran	38	35	34	33
Soybean meal	74	74	74	74
Sunflower meal	37	38	37	37
Fishmeal	26	26	26	26
Ground corn	404	404	404	404
Urea	4.3	4.3	4.3	4.3
Molasses	30	30	30	30
Megalac	25	25	25	25
MinVit	2.2	2.2	2.2	2.2
Limestone	-	1.6	-	-
Salt	3	3	3	3
Acid Buf	3.5	1.7	3.5	3.5
Sodium bicarbonate	-	1.7	3.5	5.2
Chemical composition (DM):				
Forage (g/kg)	345	345	345	345
CP (g/kg)	172	171	170	170
NDF (g/kg)	262	262	262	262
NFC (g/kg)	471	471	471	471
Ca (g/kg)	8.6	8.6	8.6	8.6
P (g/kg)	4.5	4.5	4.5	4.5
Diet ME (MJ/kg)	12.1	12.1	12.1	12.1

[1]AB = Acid Buf; BC = sodium bicarbonate.

3.3 Feeding and milking program

Cows were individually fed ad libitum. Feed was provided twice daily at 07h00 and again at 16h00. Feed troughs were thoroughly cleaned before each allocation of fresh feed by sweeping up all the refusals from the previous feeding period. Clean water was available at all times.

Cows were milked twice daily at 05h30 and again at 16h00. Proper milking procedures were employed during each milking session to maintain udder health.

3.4 Data collection

3.4.1 Feed samples

Feed samples were collected daily from all treatments during each collection period. The samples from each treatment were pooled after the collection period and thoroughly mixed in order to take a good representative sample. The representative feed samples of each treatment were used for physical and chemical analyses. Physical analysis included the determination of the feed particle size distribution with the aid of a Penn State particle separator. Chemical analyses were done to determine DM, CP, NDF, NFC and ash content of the feed. Feed particle size was reduced as samples were ground to pass through a 2mm screen prior to chemical analysis. Dry matter, ash and CP were determined as described by AOAC (2002). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined as described by Robertson & Van Soest (1981) and Goering & Van Soest (1970), respectively.

3.4.2 Milk samples

Daily milk yields were recorded by hand at every milking during each data collection period for each cow.

Milk samples were collected twice a day for five consecutive days during each data collection period and composited in amounts proportional to yield (10 ml/L milk produced) per cow per day. Milk samples were immediately preserved with potassium dichromate ($K_2Cr_2O_3$) after collection. Milk samples were analyzed for fat, protein, lactose, total solids and MUN with the aid of a Milk-O Scan 605 analyzer (Foss Electric, Hillerod, Denmark) at the Dairy Laboratory of the Agricultural Research Council at Elsenburg, Stellenbosch.

3.4.3 Rumen pH profiles

During each data collection period, rumen pH was measured continuously every four minutes for two consecutive days with the aid of TruTrack data loggers (Model pH-HR, Intech Instruments LTD, NZ). The pH meters were calibrated using Omnilog Data Management Program Version 1.68 with buffer solutions of pH 4 and 9 before insertion into the rumens of the cannulated cows.

Stainless steel capsules were specially designed to house the pH data loggers and electrodes in a manner so as to protect the data loggers in the rumen, yet allowing the electrodes to protrude from the capsule via water-tight fittings, thus enabling exposure to rumen liquor and measuring the rumen pH. The capsules housing the data loggers and probes were inserted into the rumens of the cannulated cows at 06h00 on day two and removed at 06h30 on day four of the data collection period. The recorded pH measurements were downloaded from the data loggers onto a computer using the Omnilog Data Management Program, version 1.68. All pH data were reduced to average hourly values in order to construct the graphs and for statistical analyses.

3.4.4 Rumen liquor samples

On the third day of each data collection period, rumen liquor samples were collected via the cannulae, squeezed through two layers of cheesecloth (removing particulate matter). Then, 20 ml aliquots were transferred to airtight containers containing 4 ml 25% H_3PO_4 (4.47 M) which served as preservative. The samples were stored frozen at -10˚C pending analysis. The rumen liquor samples were collected before morning feeding, as well as 2, 4 and 6 hours after feeding. The samples were analyzed for volatile fatty acid concentrations. Volatile fatty acid concentrations were determined by gas-liquid chromatography. Sample preparations prior to gas-liquid chromatography involved neutralization of the acid preservative (H_3PO_4) with NaOH, followed by a "clean-up" procedure adapted from Siegfried *et al*. (1984) (discussed later in more detail).

3.5 Statistical analysis

The data from this study was subjected to a general linear model (GLM) analysis of variance (ANOVA) using Statistica 8.1 (2009) according to a 4 x 4 Latin square design. Main effects were cow, treatment and period. Means were separated with a Bonferoni test. Differences were declared significant at $P<0.05$, whereas tendencies were considered at $P<0.10$.

3.6 Analytical Methodologies

3.6.1 Feed sample analyses

3.6.1.1 Moisture

AOAC (2002) Official Method 934.041: Determination of moisture contents of feed.

Labelled porcelain crucibles were cleaned and dried for two hours in an oven at 100˚C. The crucibles were allowed to cool down in a desiccator for 30 minutes after which the weight of each crucible was recorded. The crucible was then placed onto a scale and zeroed. Two grams of a feed test sample was weighed into the crucible, placed in an oven at 100˚C and dried for 24 hours. The crucibles were then removed from the oven and cooled for 30 minutes in a desiccator after which the weight of the dried samples were recorded.

% Moisture = [(dry crucible mass + sample mass – mass of dry sample in crucible) / (sample mass)] x 100

% DM = 100 - % moisture

3.6.1.2 Ash

AOAC (2002) Official Method 942.05: Ash

Two grams of moisture-free feed test sample was weighed into a moisture-free porcelain crucible and placed into a temperature-controlled furnace set at 500˚C for 6 hours. After 6 hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight). The samples were then transferred directly into a desiccator, allowed to cool down for 30 minutes and weighed (g).

% Ash = [(mass of crucible and ash – empty dry crucible) / sample weight] x 100

% Organic matter = 100 - % Ash

3.6.1.3 Crude Protein

AOAC (2002) Method 990.03: Crude Protein in Animal Feed

Materials:

Apparatus:	LECO FP-528, Protein/Nitrogen Determinator (Leco Corporation, St. Joseph, USA)

Accessories:	502-186 Tin Foil Cups
Sample Weight:	~ 0.150 g
Calibration Standard:	Alfalfa
Furnace Temperature:	850˚C
Protein Factor:	6.25

Procedure:

1. The LECO FP-528 was operated in accordance with manufacturer instructions (i.e. gas supply checked, necessary maintenance of crucibles, filters, reduction heater tubes, etc.).
2. Blanks (gas) were analyzed until a plateau was reached. Three to five additional blanks were analyzed and the blank standard was set using the data sets obtained.
3. Five alfalfa standards (using the Tin Foil Cups) at 0.10g were analyzed and the drift was corrected as was necessary.
4. A feed sample (0.15 g) was weighed to the nearest 0.01 g into an empty tin foil cup. All weights were recorded. The foil cup was then twisted shut and placed into the carousel sample tray. The sample was combusted and the % N was obtained.
5. % Crude Protein = % N x 6.25

3.6.1.4 Neutral detergent fibre (NDF)

Neutral detergent fibre was determined as described by Robertson & Van Soest (1981) with the aid of the Fibertech System M, 1020 Hot extractor (SMM Instruments Pty Ltd., Cape Town, South Africa).

Reagent:

Neutral detergent solution (NDS)

- Thirty grams of sodium-lauryl-sulphate were dissolved in 500 ml distilled water after which 10 ml 2-Ethoxyethanol was added.
- Then 18.61 g of EDTA ($Na_2EDTA.2H_20$) and 6.81 g of Sodium-borate decahydrate ($Na_2B_4O_7.10H_2O$) were weighed out into a 1 L Erlenmeyer-flask after which 200 ml distilled water was added. The solution was heated and stirred until everything was dissolved. The sodium-lauryl-sulphate solution was then added and stirred.
- 4.56 g Di-sodium-hydrogen-phosphate (Na_2HPO_4) was dissolved in 100 ml distilled water and was added to the Erlenmeyer-flask containing the mixed solution.
- The Erlenmeyer-flask was then filled to volume (1 L) with distilled water.

Procedure:

1. An empty, clean 2-pore glass crucible was dried for 2 hours in an oven set at 100˚C.
2. The crucible was removed from the oven, transferred directly into a desiccator and was allowed to cool down for 30 minutes. The weight of the dried crucible was then recorded.
3. The crucible was placed onto a scale and ~1 g of the feed sample was weighed into the glass crucible. The sample weight (WS) was then recorded.
4. The crucible (with sample) was placed in the heating unit of the Fibertech apparatus.
5. The valves were closed and the water-tap opened for cooling purposes of the apparatus.
6. One hundred millilitres of cool NDS reagent was added into the crucible.
7. The heat was increased to 100˚C until the solution reached boiling point.
8. Then 0.1 ml heat stable α-Amylase (Sigma nr. A3306) was added into the crucible.
9. The temperature was reduced to 65˚C and left to boil for 1 hour.
10. The heating unit was turned off and the liquid was filtered from the crucible with the aid of the apparatus' vacuum-system.
11. The sample was washed three times with warm distilled water and then rinsed one time with a little acetone.
12. The crucible containing the feed sample was removed from the apparatus and placed into a drying-oven for 24 hours at 100˚C.
13. The crucible was removed from the oven, transferred immediately into a desiccator and allowed to cool down for 30 minutes. The weight of the crucible containing the dried feed sample (W1) was then recorded.
14. The crucible was placed into a temperature-controlled furnace at 500˚C for 6 hours. After 6 hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight).
15. The sample was then directly transferred into a desiccator, allowed to cool down for 30 minutes, after which the weight of the crucible with the ash (W2) was recorded.
16. The %NDF was calculated as follow: %NDF = (W1 – W2) / WS

NOTE: It is imperative that the crucibles are allowed to cool down for at least two hours after incineration, to prevent the crucible from damage due to sudden changes in temperature.

3.6.1.5 Acid detergent fibre (ADF)

Acid detergent fibre was determined as described by Goering & Van Soest (1970) with the aid of the Fibertech System M, 1020 Hot extractor (SMM Instruments Pty Ltd., Cape Town, South Africa).

Reagent:

Acid detergent solution (ADS)

- Twenty grams of N-Cetyl-N,N,N-Trimethyl Ammonium Bromide (CTAB) was added to 1 L standardized H_2SO_4 (28 ml 98% H_2SO_4 filled to volume (1 L) with distilled water)

Procedure:

1. An empty, clean 2-pore glass crucible was dried for 2 hours in an oven set at 100˚C
2. The crucible was removed from the oven, transferred directly into a desiccator and was allowed to cool down for 30 minutes. The weight of the dried crucible was then recorded.
3. The crucible was placed onto a scale and ~1 g of the feed sample was weighed into the glass crucible. The sample weight (WS) was then recorded.
4. The crucible (with sample) was placed in the heating unit of the Fibertech apparatus.
5. The valves were closed and the water-tap opened for cooling purposes of the apparatus.
6. One hundred millilitres of cool ADS reagent was added into the crucible.
7. The heat was increased to 100˚C until the solution reached boiling point.
8. The temperature was reduced to 65˚C and left to boil for 1 hour.
9. The heating unit was turned off and the liquid was filtered from the crucible with the aid of the apparatus' vacuum-system.
10. The sample was washed three times with warm distilled water and then rinsed one time with a little acetone.
11. The crucible containing the feed sample was removed from the apparatus and placed into a drying-oven for 24 hours at 100˚C.
12. The crucible was removed from the oven, transferred immediately into a desiccator and allowed to cool down for 30 minutes. The weight of the crucible containing the dried feed sample (W1) was then recorded.
13. The crucible was placed into a temperature-controlled furnace at 500˚C for 6 hours. After 6 hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight).
14. The sample was then directly transferred into a desiccator, allowed to cool down for 30 minutes, after which the weight of the crucible with the ash (W2) was recorded.
15. The %ADF was calculated as follow: %ADF = (W1 – W2) / WS

NOTE: It is imperative that the crucibles are allowed to cool down for at least two hours after incineration, to prevent the crucible from damage due to sudden changes in temperature.

3.6.2 Milk sample analysis

3.6.2.1 Milk composition

Milk samples were preserved with potassium dichromate ($K_2Cr_2O_3$) after collection. Milk samples were analyzed for fat, protein, lactose, total solids and MUN with the aid of a Milk-O Scan 605 analyzer (Foss Electric, Hillerod, Denmark) at the Dairy Laboratory of the Agricultural Research Council at Elsenburg, Stellenbosch.

3.6.3 Rumen liquor samples

3.6.3.1 Volatile fatty acid concentrations

Rumen liquor samples (20 ml) were preserved with 4 ml 25% H_3PO_4 (4.47 M) and then stored frozen at minus 10˚C pending analysis.

Sample preparation:

A. Neutralization of acid preservative

The acid preservative H_3PO_4 (4.47 M) was neutralized by adding NaOH of the same volume (4 ml) to the rumen sample. However, the molarity of NaOH had to be three times that of the acid in order to obtain neutralization. This is based on the following equation:

$$H_3PO_4 + 3\ NaOH \rightarrow Na_3PO_4 + 3\ H_2O.$$

B. 'Clean-up' procedure of rumen liquor (modified from Siegfried *et al*., 1984)

The 'clean-up' procedure deproteinizes the rumen liquor samples as well as removing the sugars. This results in a fairly clean solution of fermentation products to be analyzed for volatile fatty acids via gas-liquid chromatography.

Reagents:

1. Calcium hydroxide solution (CHS) – 52.9 g of $Ca(OH)_2$ was weighed into a 250 ml Erlenmeyer flask. A large stir bar and 200 ml of ultra-pure water were then added. The stoppered flask was then inverted several times to suspend the solution. When the reagent was dispensed, a stir plate was used in order to maintain a homogenous slurry during pipetting.
2. Cupric sulphate solution (CSR) – 50.0 g of $CuSO_4.5H_20$ was weighed into a 500 ml volumetric flask which was then dissolved in ~400 ml of ultra-pure water. Two grams of crotonic acid (2-butenoic

acid, Sigma nr. C4630) was then added to the solution. The solution was then mixed and diluted to volume (500 ml) with distilled water.

Procedure:

1. A 1.5 ml sample of rumen fluid was transferred into a 1.7 ml centrifuge tube and centrifuged at ~12000 x g for 10 minutes.
2. Then 600 µl of supernatant was transferred to duplicate empty 1.7 ml centrifuge tubes. After centrifugation, 600 µl of CHS and 300 µl of CSR were added to the tubes. The tubes were then capped, vortexed and frozen.
3. The tubes were then thawed and centrifuged for 10 minutes.
4. Then, 1000 µl of supernatant was transferred to clean 1.7 ml centrifuge tubes containing 28 µl of concentrated H_2SO_4. The tubes were then capped and frozen again.
5. The tubes were then allowed to thaw after which it was frozen again.
6. The tubes were then thawed and centrifuged for 10 minutes.
7. The supernatant was transferred to the vials used for gas chromatography. The vials were capped and stored at room temperature or in a refrigerator.

Notes on the method:

1. The method was originally developed for silages, but works well on rumen liquor as the CHS and CSR solutions deproteinize the samples and remove sugars, leaving a fairly clean solution of fermentation products, including non-volatile and volatile fatty acids.
2. Use of 1.7 ml centrifuge tubes prevents loss of sample due to the popping of caps that may result from freezing 1.5 ml samples in 1.5 ml tubes.
3. Sulphuric acid should be pipetted into the clean 1.7 ml tubes (step 4) while the tubes are being centrifuged (step 3), as prolonged exposure of the plastic to concentrated sulphuric acid will damage the tubes.
4. The amount of supernatant transferred in steps 4 and 7 is not critical. It is more important to get a clean supernatant, free of pelleted solids, than it is to get quantitative transfer. Use of the internal standard crotonic acid (added with the CSR) allows adjustment of concentrations from samples of varying volumes transferred at steps 4 and 7.

Gas-liquid chromatography

A. Volatile fatty acid standard solution

Procedure:

1. ~80 ml ultra-pure water was added to a 100 ml volumetric flask, after which the following was added:

Acetic acid	57.3 µl
Propionic acid	74.6 µl
Iso-butyric acid	92.8 µl
Butyric acid	91.8 µl
Iso-valeric acid	54.5 µl
Valeric acid	108.4 µl
Concentrated Sulphuric acid	41.5 µl

2. The solution was then diluted to a total volume of 100 ml with ultra-pure water

B. Gas-liquid chromatography

Apparatus: Focus GC Model (Thermo Finnigan, Austin, Texas, USA)

Conditions:

Internal standard:	Crotonic acid
Column:	BPX21, 30m x 0.25mm ID, 0.25µm film
Initial temperature:	60 ^{0}C, 5 min
Rate 1:	7 ^{0}C/min
Final temperature:	160 ^{0}C
Detector:	FID, 260 ^{0}C
Injector:	220 ^{0}C
Split flow:	20:80
Split ratio:	80
Carrier gas:	Hydrogen, 1ml/min
Injection volume:	1µL
Run time:	35 min

3.7 References

AOAC International, 2002. Official methods of analysis (17th ed). Association of Official Analytical Chemists, Arlington, Virginia, USA.

Goering, H.K. & van Soest, P.J., 1970. Forage Fibre Analyses: Apparatus, reagents, procedures and some applications. Agricultural Handbook No. 379. USDA-ARS, U.S. Dept. of Agric. Washington, DC.

Robertson, J.B. & van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: Basic and clinical nutrition vol 3. The analysis of dietary fibre in food. Eds. James, W.P.T. and Theander, O. Dekker, NY. pp. 158-276.

Siegfried, V. R., Ruckermann, H. and Stumpf, G., 1984. Method for the determination of organic acids in silage by high performance liquid chromatography. Landwirtsch. Forsch. 37: 298–304.

Statistica 8.1., 2009. StatSoft Inc., USA.

CHAPTER 4

Determining the buffering capacity of Acid Buf alone and in combinations with sodium bicarbonate in vitro

Abstract

The aim of the study was to determine the *in vitro* buffering capacity of Acid Buf (AB), sodium bicarbonate (BC) and combinations of Acid Buf and sodium bicarbonate in rumen fluid. Buffers were analyzed for titratable acidity (milliequivalents of acid required to reduce pH to 4.5) and acid buffering capacity (milliequivalents of acid required to decrease pH by one unit). Buffers were suspended in 100 ml rumen fluid obtained from one ruminally cannulated Holstein cow and incubated in a water bath maintained at 39ºC. Buffer treatments were 80 mg of AB (Treatment 1) or 40 mg of AB plus 40 mg of BC (Treatment 2) or 80 mg of AB plus 80 mg of BC (Treatment 3) or 80 mg AB plus 120 mg of BC (Treatment 4) or 80 mg of BC (Treatment 5) or no buffer (Treatment 6). Treatment 4 had the highest buffering capacity amongst all treatments, although not significant compared to Treatments 1 and 3. This study proved that Acid Buf alone (5.35) had a significantly higher buffering capacity compared to sodium bicarbonate (4.87) in terms of the milliequivalents of acid required to reduce the pH by one unit. The additional bicarbonate inclusions in Treatments 3 and 4 did not improve their overall buffering capacities as there were no significant differences when compared to Treatment 1 which contained only Acid Buf. Therefore, Acid Buf alone can be very effective in preventing severe pH reductions when included in high concentrate diets and more effective when compared to sodium bicarbonate.

4.1 Introduction

In order to obtain and maintain maximum productivity, dairy cows are fed high concentrate diets, often with inadequate amounts of effective fibre. High energy diets are rapidly fermented by rumen microorganisms, producing volatile fatty acids at a rapid rate. This results in the accumulation of volatile fatty acids and hydrogen ions causing the rumen pH to decline. Low rumen pH (below 5.8) results in altered rumen fermentation due to a population shift in the rumen consortium towards increased numbers of amylolytic and lactate producing microbes (Counotte *et al*., 1979; Calsamiglia *et al*., 1999). This may result in metabolic disorders with severe consequences of which reduced productivity is the most important in terms of financial and animal health implications. To maintain physiologically sound pH levels in the rumen, the volatile fatty acids and hydrogen ions must be removed. This is achieved by alkalization and buffering by saliva, feed, feed degradation products and dietary buffers (Allen, 1997). Buffering capacity refers to the number of moles of hydrogen ions that must be added to one litre of solution to decrease the pH by one unit (Counotte *et al*., 1979; Kohn & Dunlap, 1998).

The inclusion of effective fibre in dairy cow diets can prevent the rumen pH to decrease below optimal levels. Coarse fibre particles stimulate rumination, therefore saliva production. Saliva entering the rumen is able to buffer the acids produced during fermentation, resulting in increased rumen pH and reduced occurrences of metabolic disorders (Carter & Grovum, 1990). The ability of saliva to increase rumen pH is due to the presence of bicarbonate and hydrogen phosphate ions (Carter & Grovum, 1990; Sauvant *et al*., 1999; Maekawa *et al*., 2002). Saliva composition has been reported to be relatively constant with the bicarbonate and phosphate concentration being approximately 120 meq/L and 25 meq/L, respectively (Sauvant *et al*., 1999). At a pH of 6.0, about 94% of the potential buffering capacity of hydrogen phosphate (pKa = 7.2) is used as the associated hydrogen ions flow from the rumen as dihydrogen phosphate, whereas more than 50% of the potential buffering capacity of bicarbonate (pKa = 6.1) is used at this pH by dehydration of carbonic acid to water and carbon dioxide (Allen, 1997). The total buffering capacity of saliva can be estimated as the sum of bicarbonate and hydrogen phosphate concentrations (± 152 meq/L), but the actual buffering capacity of saliva ranges from 50% of this value at neutral pH to nearly 100% at pH 5.5 (Allen, 1997).

It is clear that the dietary composition (with special reference to the fibre content) is an important component contributing to the buffering of fermentation acids by stimulating rumination and salivation. Apart from this, feedstuffs have an inherent buffering capacity, although it is considerably variable (Griger-Reverdin *et al*., 2002). Jasaitis *et al*. (1987) reported cereal grains to have low buffering capacities, low protein and grass forages to have intermediate buffering capacities and high protein feeds to have high buffering capacities. The protein content of feeds contributes to its buffering capacity due to the buffering ability of the amino

groups (Jasaitis *et al*., 1987). Total ash content (Jasaitis *et al*., 1987; Wohlt *et al*., 1987) and cation exchange capacity (Carter & Grovum, 1989) of feedstuffs have been reported to give a good indication of the feedstuff's buffering capacity. Jasaitis *et al*. (1987) have also reported feed particle size to contribute to the feed's buffering capacity. Although feedstuffs have inherent buffering capacities, Allen (1997) reported its contribution to be small in healthy lactating cows (within the pH range 5.5 to 6.8) as most of the buffering by feeds occurs below pH 5.

Dietary buffers are added to high concentrate diets in an attempt to maintain physiologically sound pH levels in the rumen by neutralizing the fermentation acids. Various dietary buffers are commercially available and vary considerably in their buffering capacity. To ensure effective and efficient use of dietary buffers it is important to consider the inherent buffering capacities of the feedstuffs in the diet (Jasaitis *et al*., 1987), although the contribution of feed buffering capacities is small (Allen, 1997). The acid buffering capacities of dietary buffers have been determined by titration, as well as through analyses of rumen fluid obtained from animals receiving defined diets (Wohlt *et al*., 1987). Jasaitis *et al*. (1987) reported the buffering capacities of mineral buffers to be highest when compared to all feedstuffs of which carbonate buffers generally have higher buffering capacities than phosphate buffers. This attributes to sodium bicarbonate being the most preferred buffer as it has proved to be beneficial in preventing post-prandial decreases in rumen pH (Russell & Chow, 1993). Wohlt *et al*. (1987) reported that much of the buffering capacity of sodium bicarbonate occurs between pH 4 to 6, which is in disagreement with Russell (1998) who reported sodium bicarbonate's buffering capacity to become limited when the pH is below 6.0.

Acid Buf is the calcified remains of the seaweed *Lithothamnium calcareum* which has been shown to be a very good dietary buffer. Cruywagen *et al*. (2007) reported Acid Buf to be very efficient in buffering rumen pH when a potentially acidotic diet was fed to lactating Holstein cows and they also compared it with sodium bicarbonate. They reported the Acid Buf treatment to maintain a higher minimum rumen pH (5.42) when compared to the sodium bicarbonate treatment (pH = 5.37) although the difference was not significant. However, the time that pH was below 5.5 was shorter for the Acid Buf treatment (4 h) than for the other treatments, *viz.* 7.7 h and 13 h for the sodium bicarbonate and unbuffered control treatment, respectively. Beya (2007) reported a greater impact of Acid Buf inclusion (at 90 g/day) to potentially acidotic diets on rumen acidity and milk production compared to a sodium bicarbonate inclusion of 180 g/day.

Sodium bicarbonate is generally regarded as the most effective buffer on the market and therefore the most common one to be used in high concentrate dairy cow diets. Because Beya (2007) reported a greater effect of Acid Buf on rumen pH compared to sodium bicarbonate, the aim of this study was to determine the *in vitro* buffering capacities of Acid Buf, sodium bicarbonate and different combinations of these two buffers in rumen fluid with the aid of a titration experiment.

4.2 Materials and methods

4.2.1 Collection and preparation of rumen liquor

Rumen liquor (~1 L) was collected before the morning feeding (07h00) from the rumen of a single cannulated Holstein cow housed at the Welgevallen Experimental Farm of the Stellenbosch University. The cow had free access to oat hay and received 15 kg/day of a commercial semi-complete lactation diet. The rumen liquor was collected in a thermos flask, filled to the brim to prevent aeration. In the laboratory, it was filtered through a 2 mm mesh sieve to remove course particulate matter and transferred to a 2 L Erlenmeyer flask, kept in a water bath at 39º C and gassed with CO_2 to provide a gas layer in order to keep it anaerobic. One hundred millilitres of the rumen fluid was dispensed into each of six 250-ml Erlenmeyer flasks. Each flask received either 80 mg of Acid Buf alone (Treatment 1), 40 mg of Acid Buf plus 40 mg of $NaHCO_3$ (Treatment 2), 80 mg of Acid Buf plus 80 mg of $NaHCO_3$ (Treatment 3), 80 mg Acid Buf plus 120 mg of $NaHCO_3$ (Treatment 4), 80 mg of $NaHCO_3$ alone (Treatment 5), or no buffer addition as control (Treatment 6). These buffer inclusion levels were chosen to represent the treatments that were used in the milk production and rumen metabolism studies reported later. However, in those trials, $NaHCO_3$ was not used alone and there was also not a negative control treatment. The flasks were incubated in a water bath maintained at 39˚C and were stirred and gassed with CO_2 at ten minute intervals. The experiment was repeated five times on five different days.

4.2.2 Laboratory analysis

All pH measurements were recorded with the aid of WTW 340i pH data loggers fitted with WTW Sentinx 41 electrodes. The pH meters were adjusted to room temperature and calibrated with WTW certified buffer solutions of pH 4.01 and 7.00. Each Erlenmeyer flask was provided with a separate pH meter to avoid contamination between samples and to ensure pH readings were taken at the same time intervals.

Titrations were performed by the addition of 2 mL aliquots of 0.1N HCl until the pH was decreased to 4.5. The initial pH and all pH measurements taken during titration were recorded after five minutes of equilibration.

The titratable acidity was calculated by recording the total volume of acid added to each sample and then multiplying the value by the acid normality (0.1N), therefore representing the milliequivalents of acid required to lower the sample pH to 4.5 (Jasaitis *et al*., 1987). The acid buffering capacity of the respective buffer treatments was then calculated by dividing titratable acidity by the total change in pH units (from the initial pH to pH of 4.5).

4.2.3 Statistical analysis

The experiment was repeated five times on five different days. The pH meters were allocated randomly to the different treatments at each repetition (block). Data was normalized to mean initial pH. Mean buffering capacity values were subjected to a Main Effects ANOVA with Treatment and Block as main effects with the aid of Statistica 8.1 (2009). Treatment means were separated with a Tukey HSD test. Statistical differences were declared at $P<0.05$. A repeated measurements analysis (Statistica 8.1) was done on values over time to obtain the error bars indicated in Figure 4.1.

4.3 Results

The titration curves of the different buffer treatments are shown in Figure 4.1, while Table 4.1 presents the results of the treatment buffering capacities.

Table 4.1 Buffering capacities of the treatments, expressed as the amount of acid required (meq 0.1N HCl) to produce a single unit change in pH.

Item	Treatment[1]						
	1	2	3	4	5	6	P
Buffering capacity	5.35^{ab}	5.01^{bc}	5.38^{a}	5.46^{a}	4.87^{c}	4.39^{d}	<0.01

[1]Treatments (amount of buffer added to 100 mL rumen liquor): Treatment 1 = 80 mg Acid Buf (AB) alone; Treatment 2 = 40 mg AB plus 40 mg sodium bicarbonate (BC); Treatment 3 = 80 mg AB plus 80 mg BC; Treatment 4 = 80 mg AB plus 120 mg BC; Treatment 5 = 80 mg BC alone; Treatment 6 = No buffer added.

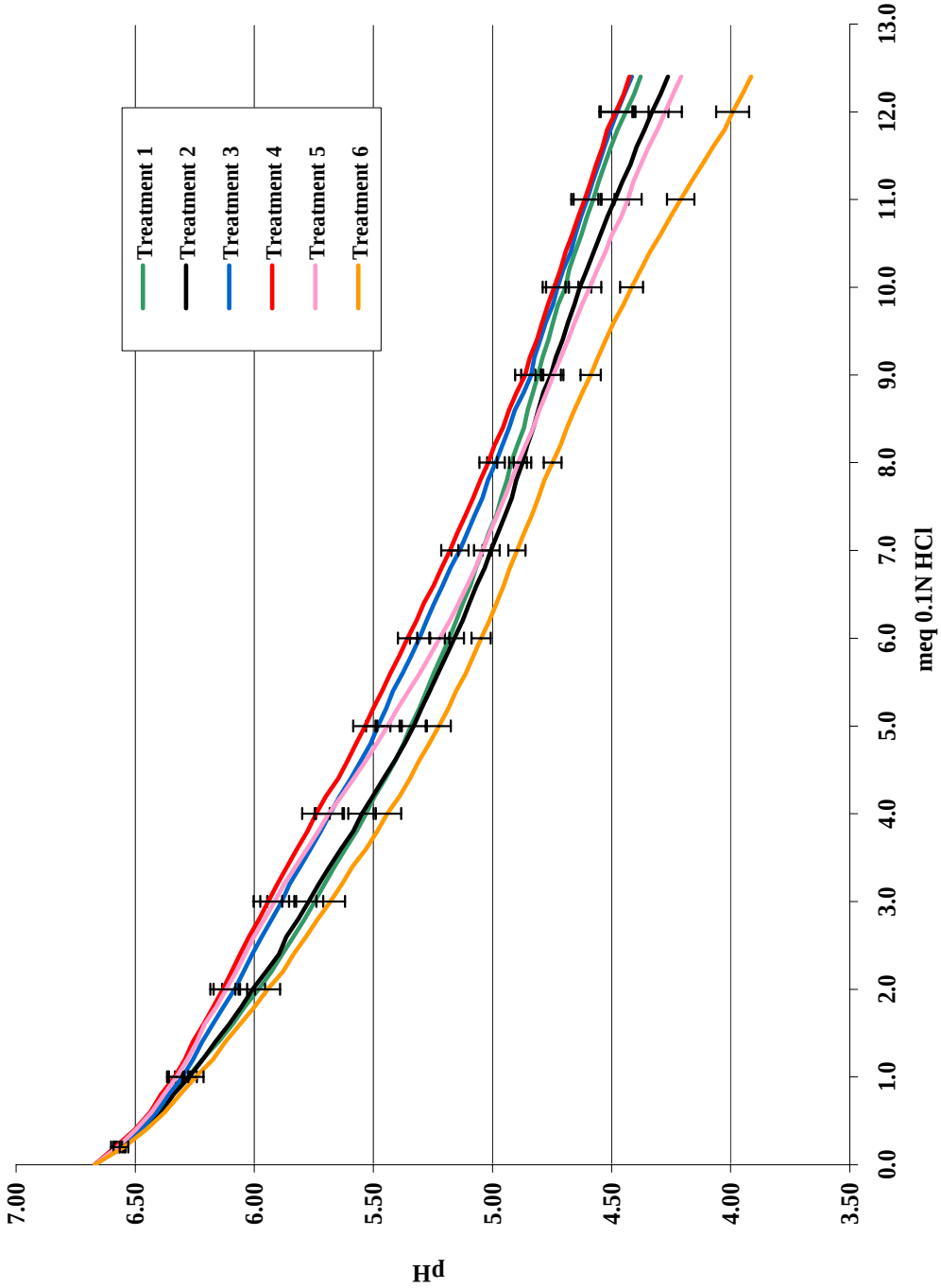

Figure 4.1 Milliequivalents of acid (0.1N HCl) added to rumen liquor buffered with Acid Buff or $NaHCO_3$ alone, or in different combinations, to decrease pH to 4.5.

Treatments (amount of buffer added to 100 mL rumen liquor): Treatment 1 = 80 mg Acid Buf (AB) alone; Treatment 2 = 40 mg AB plus 40 mg sodium bicarbonate (BC); Treatment 3 = 80 mg AB plus 80 mg BC; Treatment 4 = 80 mg AB plus 120 mg BC; Treatment 5 = 80 mg BC alone; Treatment 6 = No buffer added.

4.4 Discussion

From Figure 4.1 it can be seen that the pH of the unbuffered control treatment (Treatment 6) dropped much faster than any of the buffered treatments. It also appears that Acid Buf is a slower release buffer (Treatment 1) than $NaHCO_3$ (Treatment 5). The pH of the BC treatment (Treatment 5) stayed higher with acid addition than that of the Acid Buf treatment (Treatment 1), but they arrived at the same pH (5.14) with the addition of 6.4 meq 0.1N HCl. Thereafter, pH declined faster for the BC treatment. For the other treatments, pH declined slower as more buffer was added to the rumen liquor. At pH 4.5, Treatment 1 had approximately the same buffering capacity as Treatment 4 (80 mg AB + 120 mg BC).

It is clear from Table 4.1 that all the buffer combinations improved the buffering capacity of rumen liquor. Treatment 4 had the highest buffering capacity amongst all treatments, although not significantly so compared to Treatments 1 and 3. This could be attributed to the high inclusion levels of both Acid Buf and $NaHCO_3$. Treatment 6 represented the control treatment in which no buffer was added to rumen fluid and therefore had the lowest buffering capacity amongst all treatments. However, this indicates the rumen fluid's inert ability to buffer the increasing acidity. This can be attributed to the large amount of saliva present in the rumen fluid which contains bicarbonate and phosphate buffers as 70 to 90% of all fluid entering the rumen is saliva (Carter & Grovum, 1990). Ammonia produced by protein degradation in the rumen also contributes to the rumen fluid's buffering ability as hydrogen ions associate with ammonia to form ammonium ions which passes through the omasal orifice with liquid flow (Allen, 1997). In addition, the presence of volatile fatty acids in the rumen fluid also contributes to the buffering of acids. According to Russell & Chow (1993) volatile fatty acids contribute little to buffering capacity at pH 6.43, but accounts for nearly all buffering at pH 5.5 when bicarbonate was removed from rumen fluid.

Research on the buffering capacity of Acid Buf is limited whereas sodium bicarbonate has proved to have a high buffering capacity (Le Ruyer & Tucker, 1992). Beya (2007) suggested that Acid Buf included at 90 g/day in high concentrate diets to be more efficient in preventing rumen pH reductions compared to sodium bicarbonate included at 180 g/day. This study proves that Acid Buf (5.35) has a significantly higher buffering capacity compared to sodium bicarbonate (4.87) in terms of the amount of acid required (meq 0.1N HCl) to reduce the pH by one unit. The additional bicarbonate inclusions in Treatments 3 and 4 did not improve their overall buffering capacities as there were no significant differences when compared to Treatment 1 which contained only Acid Buf. Therefore, it could be postulated that the addition of Acid Buf alone can be very effective in preventing severe pH reductions when included in high concentrate diets and even more so compared to sodium bicarbonate as one unit of Acid Buf alone had a higher buffering capacity than one unit of $NaHCO_3$ alone.

4.5 Conclusion

The addition of sodium bicarbonate to high concentrate diets is a common practise employed in the dairy industry to prevent the occurrence of metabolic disorders associated with low rumen pH. The popularity of sodium bicarbonate is attributed to its effectiveness in increasing rumen pH and maintaining (or increasing) productivity of the cows when high concentrate diets are being fed. However, recent research has shown Acid Buf to be very effective in its ability to prevent rumen pH reductions and maintain dairy cows' productivity. Results from the buffering capacity experiment indicated that Acid Buf appears to be an effective slow release buffer that could be used in high concentrate dairy cow diets. It would be of interest to compare different combinations of Acid Buf and $NaHCO_3$ in practical diets to investigate the effect of such combinations on milk production, milk composition, rumen pH characteristics and volatile fatty acid yields.

4.6 References

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

Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism. MSc (Agric) thesis, University of Stellenbosch, South Africa.

Calsamiglia, S., Ferret, A., Plaixats, J. and Devant, M., 1999. Effect of pH and pH fluctuations on microbial fermentation in a continuous culture system. J. Dairy Sci. 82 (Suppl.1): 38.

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

Counotte, G.H.M., van't Klooster, A.T., van der Kuilen, J. and Prins, R.A., 1979. An analysis of the buffer system in the rumen of dairy cattle. J. Anim. Sci. 49: 1536-1544.

Cruywagen, C.W., Taylor, S.J. and Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate + limestone on production response and rumen parameters. J. Dairy Sci. 90 (Suppl.1): 561.

Giger-Reverdin, S., Duvaux-Ponter, C., Sauvant, D., Martin, O., do Prado, I.N. and Muller, R., 2002. Intrinsic buffering capacity of feedstuffs. Anim. Feed Sci. Tech. 86: 83-102.

Jasaitis, D.K., Wohlt, J.E. and Evans, J.L., 1987. Influence of feed ion content on buffering capacity of ruminant feedstuffs in vitro. J. Dairy Sci. 70: 1391-1403.

Kohn, R.A. & Dunlap, T.F., 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. J. Anim. Sci. 76: 1702-1709.

Maekawa, M., Beauchemin, K.A. and Christensen, D.A., Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85: 1165-1175.

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.

Russell, J.B. & Chow, J.M., 1993. Another theory for the action of ruminal buffer salts: Decreased starch fermentation and propionate production. J. Dairy Sci. 76: 826-830.

Sauvant, D., Meschy, F. and Mertens, D.R., 1999. Empirical modelling of buffer recycling in cattle. J. Dairy Sci. 82: 39.

Statistica 8.1., 2009. StatSoft Inc., USA.

Wohlt, J.E., Jasaitis, D.K. and Evans, J.L., 1987. Use of acid and base titrations to evaluate the buffering capacity of ruminant feedstuffs in vitro. J. Dairy Sci. 70: 1465-1470.

Chapter 5

The effect of Acid Buf and combinations of Acid Buf with sodium bicarbonate on rumen metabolism in dairy cows

Abstract

The objective of this study was to determine the effect of Acid Buf alone, or in combination with sodium bicarbonate, on selected rumen parameters. A high concentrate TMR, formulated to be potentially acidotic, was used to construct four dietary treatments in which Acid Buf (AB), the skeletal remains of the seaweed *Lithothamnium calcareum*, was used alone or in combination with sodium bicarbonate (BC). The diets contained 3.5 g/kg of AB (Treatment 1) or 1.75 g/kg of AB and 1.75 g/kg of BC (Treatment 2) or 3.5 g/kg of AB and 3.5 g/kg of BC (Treatment 3) or 3.5 g/kg of AB and 5.2 g/kg of BC (Treatment 4). The response to treatment was determined using eight ruminally cannulated lactating Holstein cows randomly allocated to treatments according to a 4 x 4 (n=2) Latin square design, with four treatments and four periods. The total experimental period was 100 days in which every cow received each diet for a period of 18 days prior to a data collection period of 7 days. Rumen pH was monitored continuously over 2 days using a portable data logging system and in-dwelling electrodes. Samples of rumen fluid were collected for volatile fatty acid (VFA) analyses. Ruminal pH profiles of all the treatments indicated that the diets were well buffered. Average pH over 24 hours was 6.0, 6.1, 6.1 and 6.2 for Treatments 1, 2, 3 and 4, respectively. The pH did not go below 5.8 for any of the treatments and increasing levels of sodium bicarbonate increased the diurnal profile such that at the highest level (Treatment 4), the pH profile ranged from 6.1 to 6.5. It is proposed that high rumen pH may impact negatively on animal productivity by increased the acetate:propionate ratio to the detriment of rumen efficiency as a result of increased energy loss due to methanogenesis. Treatment had no significant effect on total VFA concentration, but there seemed to be a tendency for increased total VFA concentration as the level of sodium bicarbonate increased. The acetate:propionate ratio of Treatment 1 (2.91) was significantly lower compared to Treatment 4. This study confirmed daily intake of 80 g of Acid Buf by cows receiving high concentrate diets to counteract increasing acidity in the rumen and therefore provide an efficient, safe solution to rumen acidosis and maintaining animal productivity.

5.1 Introduction

The rumen pH of healthy lactating cows ranges from 5.8 to 6.5 (Cerrato-Sánchez *et al*., 2008). When the rumen pH is within this range, the rumen microbes will proliferate, rumen fermentation will be optimal, rumen motility will be efficient, rumen epithelia will be well developed and maintain integrity and absorption will be adequate (Carter & Grovum, 1990 and Allen, 1997). This will result in efficient dry matter intake, sufficient digestibility of feeds and adequate absorption of essential nutrients resulting in optimal animal productivity (Poppi *et al*., 2000). In turn, this would ensure profitability of the dairy farm.

In the rumen, carbohydrates are fermented by rumen microbes to volatile fatty acids, predominantly acetic-, propionic- and butyric acids. The proportions of volatile fatty acids produced are determined by the diet composition (Russell, 1998) and its physical characteristics (French *et al*., 1999 and Yang & Beauchemin, 2007), where acetic- and propionic acids predominate when forage-based or grain-based diets are fed, respectively (Ishler *et al*., 1996). The volatile fatty acids are rapidly absorbed through the rumen wall in order to maintain optimal pH levels and rumen osmolarity to ensure microbial survival (Carter & Grovum, 1990). In addition, the structural carbohydrates stimulate rumination and salivation which buffers the fermentation acids produced and contributing to maintain optimal rumen pH levels (Mertens, 1997; Yang & Beauchemin, 2007).

To comply with the high nutritional requirements of lactating dairy cows for maximal productivity, dairy rations are formulated to contain large amounts of concentrates. As a result, the amounts of effective fibre included in these diets are often suboptimal. This results in rapid fermentation of feed to volatile fatty acids, volatile fatty acid accumulation and the rumen pH to decline. This is what commonly happens after meal consumption. However, if the rate of volatile fatty acid production exceeds the rate of volatile fatty acid absorption, the pH may decline to critical levels below 5.8. Corley *et al*. (1999) estimated the fractional production rate of volatile fatty acids (as a fraction of total volatile fatty acids) to be 0.46 and 0.35/h for concentrate and forage-based diets, respectively. The extent of the pH reduction and the time it remains below 5.8 has devastating results of which sub-acute rumen acidosis (SARA) is the most common and severe consequence. Sub-acute rumen acidosis is also associated with various other production disorders including reduced dry matter intake (Allen, 1997; Maekawa *et al*., 2002), reduced fibre digestion (Grant & Mertens, 1992; Rotger *et al*., 2006), reduced protein digestion (Calsamiglia *et al*., 1999), milk fat depression (Van Soest, 1963; Russell & Chow, 1993), diarrhoea (Maekawa *et al*., 2002), laminitis (Nocek, 1997), rumenitis and liver abscesses (Allen, 1997) which further contribute to the vast implications caused by pH levels below 5.8.

The inclusion of mineral buffers to high concentrate dairy cow diets is a common practice employed in the feed industry in an attempt to alleviate the incidences of metabolic disorders associated with low ruminal pH. Sodium bicarbonate is the most common buffer added to dairy cow diets (Xu *et al*., 1994). Many experiments have been conducted in order to determine the effects of sodium bicarbonate on rumen parameters. Most researchers agree that sodium bicarbonate effectively results in increased rumen pH (Rogers *et al*., 1982; Solorzano *et al*., 1989) and increased acetate to propionate ratios (Davis *et al*., 1964; Erdman, 1988; Kennelly *et al*., 1999) which resulted in increased dry matter intake (Donker & Marx, 1980; Rogers *et al*., 1985), increased fibre digestion (Vicini *et al*., 1988), alleviating milk fat depression (Thomas *et al*., 1984) and increased milk yields (Erdman *et al*., 1980; Belibasakis & Triantos, 1991).

Acid Buf is the calcified remains of the seaweed *Lithothamnium calcareum* and has potential to be included as a buffer in high concentrate dairy cow diets. Cruywagen *et al*. (2004) reported the optimum dose of Acid Buf inclusion in high concentrate dairy cow diets to be 0.3% of dietary dry matter (or 80 g/cow per day) in order to optimize milk output and efficiency of feed into milk. In another study, Cruywagen *et al*. (2007) compared the effects of Acid Buf and sodium bicarbonate on rumen pH. They reported the Acid Buf treatment to maintain a significantly higher minimum rumen pH (5.42) when compared to a control treatment (5.19) and also found the time that ruminal pH was below 5.5 to be shorter for the Acid Buf treatment (4h) when compared to the other treatments, *viz.* 7.7h for the sodium bicarbonate treatment and 13 h for the control treatment. Beya (2007) reported that Acid Buf ingested at 90 g/cow per day had a greater effect on rumen pH and on the prevention of sub-acute rumen acidosis than sodium bicarbonate ingested at a level of 180 g/cow per day.

Literature regarding the effects of Acid Buf on dairy cow rumen parameters is rather limiting in comparison with sodium bicarbonate. Therefore, the aim of this study was to determine the effects of Acid Buf and combinations of Acid Buf and sodium bicarbonate on rumen metabolism with regards to pH profiles and volatile fatty acid concentrations.

5.2 Materials and Methods

5.2.1 Animals and housing

Eight ruminally cannulated lactating Holstein cows were used in the trial which was done at the Welgevallen Experimental farm of the Stellenbosch University. Animals were housed individually in 3 x 5 m pens in a well ventilated, semi-open barn with a cement floor. Each cow had access to a sand-bedded sleeping crate, a feeding trough and fresh water via a ball valve controlled water bowl. The trial protocol was approved by the Stellenbosch University's Animal Ethics Committee (Reference: 2008B03003).

5.2.2 Experimental design and treatments

Cows were randomly allocated to treatments according to a 4 x 4 (n=2) Latin square design with four treatments and four periods. All treatments included a basal diet, formulated to be potentially acidotic (Table 3.1, Chapter 3). The only difference between treatments pertained to the amount of Acid Buf (AB) and sodium bicarbonate (BC) inclusion levels. The experimental diets contained either 3.5g/kg of AB (Treatment 1), or 1.75 g/kg of AB and 1.75 g/kg of BC (Treatment 2), or 3.5 g/kg of AB and 3.5 g/kg of BC (Treatment 3), or 3.5 g/kg of AB and 5.2 g/kg of BC (Treatment 4). The total experimental period was 100 days during which all cows received each diet for a period of 18 days prior to a data collection period of 7 days.

5.2.3 Data collection and chemical analysis

During each data collection period, rumen pH was measured continuously every four minutes for two consecutive days with the aid of TruTrack data loggers (Model pH-HR, Intech Instruments LTD, NZ). The pH meters were calibrated using Omnilog Data Management Program, version 1.68 with buffer solutions of pH 4 and 9 before insertion into the rumens of the cannulated cows.

Stainless steel capsules were specially designed to house the pH data loggers and electrodes in a manner so as to protect the data loggers in the rumen, yet allowing the electrodes to protrude from the capsule via water-tight fittings, thus enabling exposure to rumen liquor and measuring the rumen pH. The capsules housing the data loggers and probes were inserted into the rumens of the cannulated cows at 06h00 on day two and removed at 06h30 on day four of the data collection period. The recorded pH measurements were downloaded from the data loggers onto a computer using the Omnilog Data Management Program, version 1.68. All pH data were reduced to average hourly values in order to construct the graphs and for statistical analyses.

On the third day of each data collection period, rumen liquor samples were collected via the cannulae, squeezed through two layers of cheesecloth (removing particulate matter). Then, 20 ml aliquots were transferred to airtight containers containing 4 ml 25% H_3PO_4 (4.47 M) which served as preservative. The samples were stored frozen at -10˚C pending analysis. The rumen liquor samples were collected before morning feeding, as well as 2, 4 and 6 hours after feeding. The samples were analyzed for volatile fatty acid concentrations. Volatile fatty acid concentrations were determined by gas-liquid chromatography. Sample preparations prior to gas-liquid chromatography involved neutralization of the acid preservative (H_3PO_4) with NaOH, followed by a "clean-up" procedure adapted from Siegfried *et al*. (1984). The 'clean-up'

procedure deproteinizes the rumen liquor samples as well as removing the sugars. This results in a fairly clean solution of fermentation products to be analyzed for volatile fatty acids via gas-liquid chromatography.

Feed samples were collected daily from all treatments during each collection period. The samples from each treatment were pooled after the collection period and thoroughly mixed in order to take a good representative sample. The representative feed samples of each treatment were used to determine the feed particle size distribution with the aid of a Penn State particle separator.

5.2.4 Statistical analysis

The data from this study was subjected to a general linear model (GLM) analysis of variance (ANOVA) using Statistica 8.1 (2009) according to a 4 x 4 Latin square design. Main effects were cow, treatment and period. Means were separated with a Bonferoni test. Differences were declared significant at $P<0.05$, whereas tendencies were considered at $P<0.10$.

5.3 Results

5.3.1 Rumen pH

Rumen pH results are presented in Table 5.1, while Figure 5.1 illustrates the pH fluctuations over a period of 24 hours. The basal diet was formulated to be potentially acidotic in an attempt to reduce rumen pH to 5.5 or lower, to determine the effects of dietary buffer supplementation on rumen metabolism. The mean pH of Treatment 1 (pH = 6.03) was significantly lower than that of Treatment 4 (pH = 6.28). The maximum pH value tended to be higher for Treatment 4 than for Treatment 1, while the minimum values for Treatments 1 to 3 were all lower than that of Treatment 4.

Looking at Figure 5.1, it would seem that the maximum and minimum pH values indicated in Table 5.1 do not correspond with the lines in the figure. However, it should be kept in mind that the minimum and maximum values in Table 5.1 are the minimum and maximum observed pH values of individual cows in each treatment, while the lines in Figure 5.1 represent average values of all the cows per treatment. Therefore, high and low spikes are smoothed out in the graph.

Table 5.1 The effect of Acid Buf (AB) alone and in combination with sodium bicarbonate on mean, minimum and maximum ruminal pH

Item	Treatment 1	Treatment 2	Treatment 3	Treatment 4	P
Mean pH	6.03[a]	6.11[ab]	6.15[ab]	6.28[b]	0.013[b]
Maximum pH	6.47	6.53	6.53	6.65	0.083
Minimum pH	5.59[a]	5.66[a]	5.70[a]	5.91[b]	0.360

[a, b] Row means with common superscripts do not differ (P < 0.05)

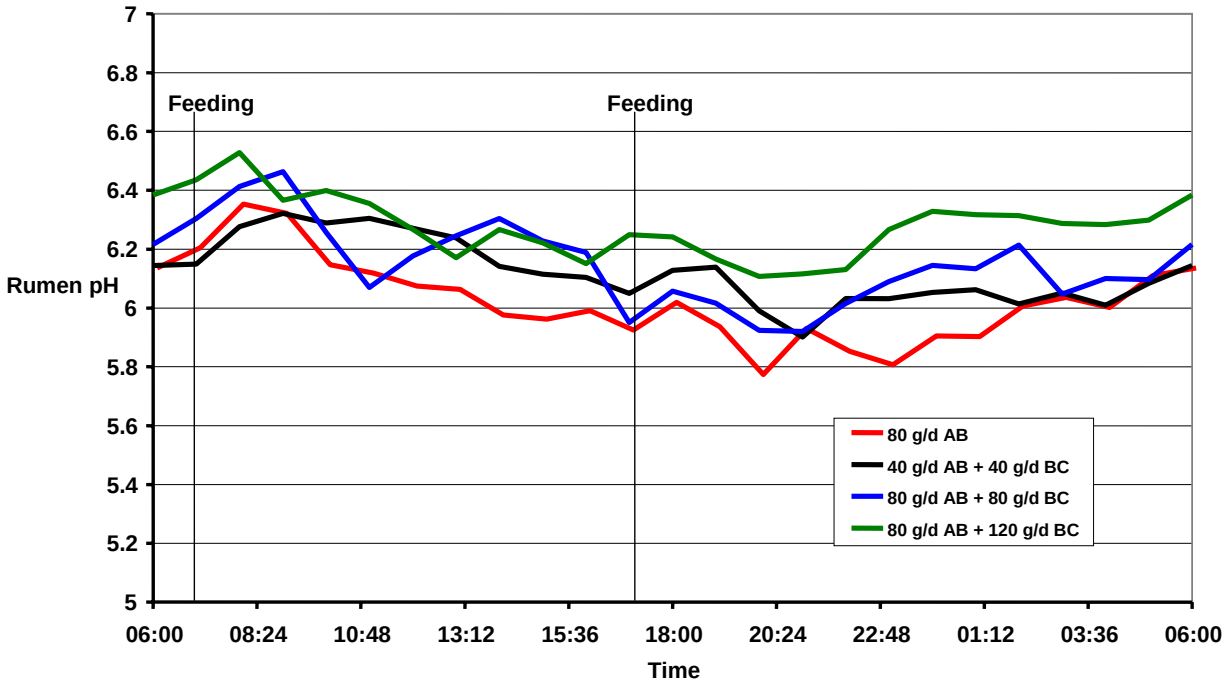

Figure 5.1 The effect of Acid Buf (AB) alone and in combination with sodium bicarbonate (BC) on rumen pH profiles of lactating Holstein cows

5.3.2 Rumen volatile fatty acid concentrations

The results of the rumen volatile fatty acid concentrations are presented in Table 5.2. There was no significant difference in total volatile fatty acid (VFA) concentration between treatments, except for Treatment 4 (80 g/day of AB plus 120 g/day of BC) which had a significantly higher VFA concentration when compared to Treatment 2 (40 g/day of AB plus 40 g/day of BC). There was also a tendency (P < 0.10) for Treatment 4 to result in higher VFA concentrations than Treatment 1 (80 g/day of AB).

Acetate concentration, as well as propionate concentration, was affected by treatment. The acetate concentration of Treatment 4 was significantly higher when compared to Treatments 1 and 2. The

propionate concentration of Treatment 1, on the other hand, was significantly higher than that of Treatment 2. There was also a tendency ($P < 0.10$) for the propionate concentration of Treatment 1 to be higher when compared to Treatments 3 and 4. The acetate to propionate ratios were also affected by treatment, where that of Treatment 1 was significantly lower compared to Treatments 2 and 4.

Table 5.2 The effect Acid Buf and combinations of Acid Buf and sodium bicarbonate on rumen volatile fatty acid concentrations

Item	Treatment				P
	80AB	40AB + 40 BC	80AB + 80BC	80AB + 120BC	
Acetate (mM/L)	90.5[a]	89.3[a]	98.6[ab]	104.8[b]	0.025
Propionate (mM/L)	31.6[a*]	26.0[b]	30.2[ab]	29.6[ab]	0.104
Butyrate (mM/L)	0.559	0.632	0.579	0.524	0.296
Iso-butyrate (mM/L)	10.52	9.83	10.98	10.51	0.869
Valerate (mM/L	0.885	1.024	0.926	0.799	0.200
Iso-valerate (mM/L)	1.170	1.038	1.151	1.136	0.587
Total VFA (mM/L)	135.2[ab*]	127.9[a]	142.5[ab]	147.4[b]	0.083
Acetate:propionate	2.91[a]	3.67[b]	3.28[ab]	3.65[b]	0.018

[a, b] Row means with common superscripts do not differ ($P < 0.05$)

* Row means tendency to differ ($P < 0.10$)

5.3.3 Feed particle size

The feed particle size data of each treatment is presented in Table 5.3.

Table 5.3 Feed particle size distribution of all treatment diets. Mean values are percentages of the sieved feed samples.

Screen	TMR[a]	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Upper (%)	2 - 8	6.16 ± 3.37 (SD)[b]	5.81 ± 2.87 (SD)	7.33 ± 2.92 (SD)	5.54 ± 2.19 (SD)
Middle (%)	30 – 50	14.88 ± 4.21 (SD)	13.54 ± 3.55 (SD)	15.40 ± 3.97 (SD)	15.98 ± 1.42 (SD)
Lower (%)	30 – 50	31.48 ± 2.62 (SD)	28.00 ± 3.16 (SD)	31.37 ± 2.44 (SD)	28.97 ± 3.62 (SD)
Pan (%)	≤ 20	47.48 ± 5.68 (SD)	52.65 ± 6.08 (SD)	45.89 ± 7.44 (SD)	49.51 ± 1.44 (SD)

[a]Recommendation of feed particle size for a total mixed ration (TMR) (Heinrichs & Kononoff, 2002).

[b]SD = Standard deviation

From Table 5.3 it is clear that the particle size distribution of the different treatments were not as recommended. The diets consisted of predominantly small feed particles as the large forage particles were limited. Therefore these diets have the potential to result in great rumen pH reductions as rumination and salivation will be limited.

5.4 Discussion

5.4.1 Rumen pH

Feeding high concentrate diets to dairy cows result in reduced rumen pH levels due to increased volatile fatty acid production as a result of microbial fermentation. Rumination and therefore also salivation is reduced when feeding high concentrate diets to cows, due to a lack of effective fibre (Mertens, 1997). In this trial, the basal diets of the treatments were formulated to be potentially acidotic and rumen pH levels below 5.5 were expected, as have been observed by Cruywagen *et al*. (2007) with a similar diet. From the above mentioned feed particle size results, it can be noted that the physical properties of the treatment diets should result in reduced rumen pH values. However, this was not the case in this trial as all minimum pH values were higher than 5.6, with the mean pH of all treatments being higher than 6.0, indicating that all treatments were effective in buffering the acids produced during fermentation and preventing the rumen pH to drop to critical levels which could lead to metabolic disorders.

Cruywagen *et al*. (2004) observed increased rumen pH as the dose of Acid Buf increased and concluded that the optimal dose of Acid Buf to be 80 g/day. In 2007, Cruywagen *et al*. reported Acid Buf (at 90g/day) to have a greater impact on rumen acidity than sodium bicarbonate (at 180g/day). In the current study, Acid Buf (at 80g/day) yet again proved to be very effective in buffering rumen pH as the Acid Buf treatment prevented rumen pH to decrease to critical levels. The additional sodium bicarbonate inclusion with Acid Buf contributed to maintain high rumen pH levels and many authors (Rogers *et al*., 1982; Erdman, 1988; Solorzano *et al*., 1989) reported sodium bicarbonate to be very effective in increasing rumen pH when included in high concentrate diets.

Van Kessel & Russell (1996) reported methane production to become limited when the rumen pH is below 6.0. This is in agreement with Lana *et al*. (1998) who reported lower methanogenesis at low pH levels. In this study, Treatment 1 (80g/day of AB) maintained a mean pH value of 6.03, whereas the other treatments resulted in mean pH values greater than 6.0. Therefore, one can suggest that the Acid Buf treatment would result in lower methanogenesis compared to the other treatments, which could result in greater performances of animals due to reduced energy losses from methane.

5.4.2 Rumen volatile fatty acid (VFA) concentrations

The propionate concentration was affected by treatment, as there was a tendency for Treatment 1 (80g of AB/day) to be higher than Treatment 3 (80g of AB + 80g of BC/day) and Treatment 4 (80g of AB/day + 120g of BC/day). Beya (2007), however, reported treatments to have no effect on the propionate concentration. Russell & Chow (1993) reported sodium bicarbonate to increase the animal's water intake which increases the rate of dilution and flow of starch from the rumen resulting in reduced propionate production. This is in agreement with Erdman *et al*. (1982) who reported sodium bicarbonate to decrease propionate concentration in the rumen. This would explain why Treatment 4 had a lower propionate concentration than Treatments 1 and 3, as Treatment 4 had a higher level of sodium bicarbonate, although the differences were not significant.

The acetate concentration was also affected by treatment as the acetate concentration of Treatment 4 was significantly higher than Treatment 1. Erdman *et al*. (1982) reported the addition of sodium bicarbonate to high concentrate diets to have no effect on acetate concentration but rather only on the propionate concentration. Our results showed that the acetate concentration increased as the sodium bicarbonate inclusion level increased. There was also a tendency for total VFA concentration to increase with increasing buffer addition. However, there are other factors that also play a role. Dijkstra et al. (1992 and 1993) observed a non-linear increase in VFA absorption from the rumen with a decrease in rumen pH. They ascribe this phenomenon to the fact that, as pH values approach the p*K* values of VFA, more VFA will be in the undissociated form which diffuses through epithelial membranes more readily than the anionic form which exists at higher pH. The p*K* values of acetic, propionic and butyric acids are 4.75, 4.87 and 4.81, respectively. Furthermore, fractional absorption rates will increase in the order of acetic, propionic and butyric acid. One could thus expect that total VFA and, especially acetic acid, would disappear from the rumen at a faster rate at lower pH levels. The higher total VFA and acetic acid concentrations observed in the current study as the mean rumen pH values increased, can thus be explained.

An important aspect regarding acetate concentrations in the current study pertains to methane production. According to Moss *et al*. (2000), acetate promotes methane production and the increased acetate concentrations observed in the current study with increased buffer inclusion levels, could thus affect methane production. Methane production results in energy loss to the animal, which in turn could result in reduced animal productivity (Lana *et al*., 1998). Johnson & Johnson (1995) reported cattle to lose 6% of their ingested energy as methane, whereas McDonald et al. (2002) suggests an even higher value of 8%. Apart from the energy loss to the animal as a result of methane production, methane also contributes to the "greenhouse" effect. Tamminga (1992) reported the total worldwide methane production of domestic and wild animals to be close to 60-100 million metric tonnes per annum, of which cattle are estimated to produce 74%.

The acetate to propionate ratios were also affected by treatments. The acetate to propionate ratio of Treatment 1 was significantly lower when compared to Treatments 2 and 4. Beya (2007) also reported the Acid Buf treatment to have lower acetate to propionate ratios. Many authors (Davis *et al*., 1964; Erdman *et al*., 1980; Rogers *et al*., 1982; Erdman, 1988; Kennelly *et al*., 1999;) reported increased acetate to propionate ratios when sodium bicarbonate was included in high concentrate diets and attributed this increase to a reduction in propionate production (Erdman *et al*., 1982). This is in agreement with the results we obtained from this study as we have observed increased acetate concentrations as the inclusion levels of sodium bicarbonate increased, impacting on acetate:propionate ratios. Van Kessel & Russel (1996) reported that the acetate:propionate ratio should not be greater than 3.0 in order to maintain optimal rumen functioning. From our results, Treatment 1 (80 g of AB/day) resulted in an acetate:propionate ratio (2.9) below to that suggested by Van Kessel & Russel (1996) with the other treatments resulting in ratios greater than 3.

5.5 Conclusion

Sodium bicarbonate is well known for its beneficial effects on rumen pH and VFA concentrations and is also the most preferred buffer used in the dairy industry. Acid Buf has proved to maintain pH levels within physiological limits and maintained more favourable acetate:propionate ratios. Theoretically, less methane would be produced when Acid Buf is included in high energy dairy rations, resulting in less energy loss for the animal and resulting in less methane emission which contributes to the global "greenhouse" effect. However, future research focussing on the effect Acid Buf has on rumen metabolism with emphasis on the ability to reduce methane production, will surely be beneficial if this proves to be true.

5.6 References

Allen, M.S., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fibre. Journal of Dairy Science 80: 1447-1462.

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

Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism. MSc (Agric) thesis, University of Stellenbosch, South Africa.

Calsamiglia, S., Ferret, A., Plaixats, J. and Devant, M., 1999. Effect of pH and pH fluctuations on microbial fermentation in a continuous culture system. Journal of Dairy Science 82 (Suppl.1): 38.

Carter, R.R. & Grovum, W.L., 1990. A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: Salivation, motility and microbes. Journal of Animal Science 68: 2811-2832.

Cerrato-Sánchez, M., Calsamiglia, S. and Ferret, A., 2008. Effect of the magnitude of the decrease of rumen pH on rumen fermentation in a dual-flow continuous culture system. Journal of Animal Science 86: 378-383.

Corley, R.N., Murphy, M.R., Hackley, K.C. and Liu, C.-L., 1999. Estimation of the production rate of volatile fatty acids in the rumen under steady state conditions. J. Dairy Sci. 82 (Suppl. 1): 43.

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

Cruywagen, C.W., Taylor, S.J. and Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate + limestone on production response and rumen parameters. J. Dairy Sci. 90 (Suppl.1): 561.

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

Dijkstra, J., Neal, H.D.StC., Beever, D.E., and France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. J. Nutr. 122: 2239-2256.

Dijkstra, J., Boer, H., Van Bruchem, J., Bruining, M. and Tamminga, S., 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. Brit. J. Nutr. 69: 385-396.

Donker, J.D. & Marx, G.D., 1980. Sodium bicarbonate in diets for milking Holstein cows. J. Dairy Sci. 63: 931-935.

Erdman, R.A., 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71: 3246-3266.

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

French, P.D., Stallings, C.C, James, R.E. and Miller, C.N., 1999. Effect of kernel processing corn silage on milk yield and composition in Holstein and Jersey cattle. J. Dairy Sci. 82 (Suppl.1): 43.

Grant, R.J. & Mertens, D.R., 1992. Development of buffer systems for pH control and evaluation of pH effects on fibre digestion in vitro. J. Dairy Sci. 75: 1581-1587.

Heinrichs, J.A. and Kononoff, P., 2002. Evaluating particle size of forages and TMRs using the new Penn State forage particle separator. Cooperative Extension, College Agric. Sci. 42: 1-15.

Hutjens, M., 2003. Feeding a ruminant. In: Feeding Guide (2nd ed.). Eds. Hutjens, M., W.D. Hoards & Sons Company, Fort Atkinson, USA. pp. 6-12.

Ishler, V., Heinrichs, V. and Varga, G., 1996. From feed to milk: Understanding rumen function. Penn State University Extension Circular 422, University Park, PA. pp. 1-26.

Johnson, K.A. & Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73: 2483-2492.

Kennelly, J.J., Robinson, B. and 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.

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

Maekawa, M., Beauchemin, K.A. and Christensen, D.A., Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. J. Dairy Sci. 85: 1165-1175.

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

Moss, A.R., Jouany, J.-P. and Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49: 231-253.

Nocek, J.E., 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80: 1005-1028.

Poppi, D.P., France, J. and McLennan, S.R., 2000. Intake, passage and digestibility. In: Feeding systems and feed evaluation models. Eds. Theodorou, M.K. & France, J., CAB International Publishing, Oxon, U.K. pp. 35-52.

Rogers, J.A., Davis, C.L. and Clark, J.H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral 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. and Cummings, K.R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early lactation. J. Dairy Sci. 68: 646-660.

Rotger, A., Ferret, A., Calsamiglia, S. and Manteca, X., 2006. Effects of non-structural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism in vivo and in vitro with high-concentrate beef cattle diets. J. Anim. Sci. 84: 1188-1196.

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.

Russell, J.B. & Chow, J.M., 1993. Another theory for the action of ruminal buffer salts: Decreased starch fermentation and propionate production. J. Dairy Sci. 76: 826-830.

Solorzano, L.C., Armentano, L.E., Grummer, R.R. and Dentine, M.R., 1989. Effects of sodium bicarbonate or sodium sesquicarbonate on lactating Holsteins fed a high grain diet. J. Dairy Sci. 72: 453-461.

Statistica 8.1., 2009. StatSoft Inc., USA.

Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75: 345-357.

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

Van Kessel, J.A.S. & Russell, J.B., 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. 20: 205-210.

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. and Bauman, D.E., 1988. Effects of feed intake and sodium bicarbonate on milk production and concentrations of hormones and metabolites in plasma of cows. J. Dairy Sci. 71: 1232-1238.

Xu, S., Harrison, J.H., Riley, R.E. and 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.

Yang, W.Z. & Beauchemin, K.A., 2007. Altering physically effective fibre intake through forage proportion and particle length: Chewing and ruminal pH. J. Dairy Sci. 90: 2826-2838.

CHAPTER 6

The effect of Acid Buf and combinations of Acid Buf with sodium bicarbonate on milk yield and milk composition of dairy cows

Abstract

The objective of this study was to determine the effect of Acid Buf alone, or in combination with sodium bicarbonate, on milk production and milk composition. A high concentrate TMR, formulated to be potentially acidotic, was used to construct four dietary treatments in which Acid Buf (AB), the skeletal remains of the seaweed *Lithothamnium calcareum*, was used alone or in combination with sodium bicarbonate (BC). The diets contained 3.5 g/kg of AB (Treatment 1) or 1.75 g/kg of AB and 1.75 g/kg of BC (Treatment 2) or 3.5 g/kg of AB and 3.5 g/kg of BC (Treatment 3) or 3.5 g/kg of AB and 5.2 g/kg of BC (Treatment 4). The response to treatment was determined using eight ruminally cannulated lactating Holstein cows randomly allocated to treatments according to a 4 x 4 (n=2) Latin square design, with four treatments and four periods. The total experimental period was 100 days in which every cow received each diet for a period of 18 days prior to a data collection period of 7 days. During each data collection period, milk production was recorded twice daily for 7 days, whereas milk was sampled twice daily for five consecutive days for component analysis. Cows were fed ad libitum and dry matter intake was recorded individually. Treatment had no significant effect on milk production, milk composition or feed intake. Although not significant, Treatment 1 (Acid Buf alone) numerically resulted in the highest milk output without compromising milk quality. It is proposed that high rumen pH may impact negatively on milk output by increasing acetate: propionate ratio to the detriment of rumen efficiency. This study confirmed previous results indicating that a daily intake of 80 g of Acid Buf by cows receiving high concentrate diets would support high milk production without compromising milk solids contents.

6.1 Introduction

Dairy cows are provided with high concentrate diets which ensure their nutritional requirements are met and ensuring maximum productivity. However, these high energy diets could result in reduced productivity as a result of metabolic disturbances. Sub-acute rumen acidosis is a very common disorder associated with high concentrate diet feeding and may result in other metabolic disorders which further reduce animal productivity and animal health. Milk fat depression (MFD) is another important consequence of high concentrate diets which greatly influence milk quality and therefore farm profitability.

Milk fatty acids are predominately the result of *de novo* synthesis in the mammary gland from acetate and 3-hydroxybutyrate, produced by ruminal fermentation of carbohydrates and by rumen epithelium from absorbed butyrate, respectively (Ishler *et al*., 1996). The remaining milk fatty acids (less than 10%) are supplied by mobilization of body fat, but this fraction increases in ruminants that are in a negative energy balance (e.g. early lactation) in direct proportion to the extent of the energy deficit (Bernard *et al*., 2006).

The physiological mechanisms by which high energy diets result in MFD is not clearly understood. Two theories evolved in an attempt to explain the possible mechanisms resulting in MFD. The first theory, known as the glucogenic-insulin theory, attributes MFD to a reduced lipid precursor supply to the mammary gland (Griinari & Bauman, 2006), therefore *de novo* fatty acid synthesis in the mammary gland is limited, resulting in reduced fat secretion in milk.

Microbial fermentation of high energy, low fibre diets will result in increased concentrations of propionic acid compared to acetic acid. Propionic acid is absorbed through the rumen wall, transported to the liver and undergoes gluconeogenesis to produce glucose, thus resulting in increased blood glucose concentrations. Increased blood glucose concentration stimulates the release of insulin, which is known to inhibit mobilization of lipids and stimulate lipid synthesis in adipose tissue and therefore limiting the amount of fatty acids transported to the mammary gland and resulting in reduced milk fat content (Griinari *et al*., 1998). However, Bernard *et al*. (2006) reported that less than 10% of milk fatty acids come from body fat mobilization if the cow is not in a negative energy balance. Therefore, one may conclude that MFD is more severe in early lactation cows, as they are usually in a negative energy balance and have suboptimal levels of feed intake, of which the energy content is high and effective fibre usually limiting.

The glucogenic-insulin theory mainly focuses on the fatty acid precursors provided from mobilized body fat. From another view point, limiting fatty acid precursors from dietary origin could also reduce *de novo* fatty acid synthesis in the mammary gland. High concentrate diets with limited amounts of effective fibre result in reduced acetate to propionate ratios (Beitz & Davis, 1964). Erdman (1988) reported the greatest reduction

in milk fat percentage to occur when the acetate to propionate ratio is less than 2.0. Therefore, acetate becomes limiting, reducing the fatty acid precursors delivered to the mammary gland and result in reduced milk fatty acid synthesis and therefore reducing the fat content of milk.

The second theory, known as the biohydrogenation theory, attributes MFD to inhibitors of milk fat synthesis that form during ruminal biohydrogenation of 18-carbon polyunsaturated fatty acids (Griinari & Bauman, 2006). During ruminal digestion, *trans*-fatty acids are formed as intermediates during the biohydrogenation of polyunsaturated fatty acids (Griinari *et al*., 1998). Figure 6.1 presents the pathways of linoleic acid (C18:2) biohydrogenation in the rumen. Griinari & Bauman (2006) reported that under certain dietary conditions, rumen biohydrogenation pathways are altered, producing unique fatty acid intermediates of which some are inhibitors of milk fat synthesis. Feeding low fibre diets, *trans*-10,*cis*-12 CLA (conjugated linoleic acid) has been reported to act as the inhibitor of milk fat synthesis (Griinari *et al*., 1998; Griinari & Bauman, 2006). For these reasons, Griinari *et al*. (1998) suggested two dietary conditions to result in MFD. These are (1) alteration in rumen microbial processes (which occur when feeding high energy diets with limited effective fibre and (2) the presence of sufficient amounts of 18-carbon polyunsaturated fatty acids (that result from supplementing dairy rations with plant- and/or marine oils). Beitz & Davis (1964) reported MFD to occur when a diet was supplemented with cod liver oil but they did not explain the mechanism by which the added oil resulted in reduced milk fat.

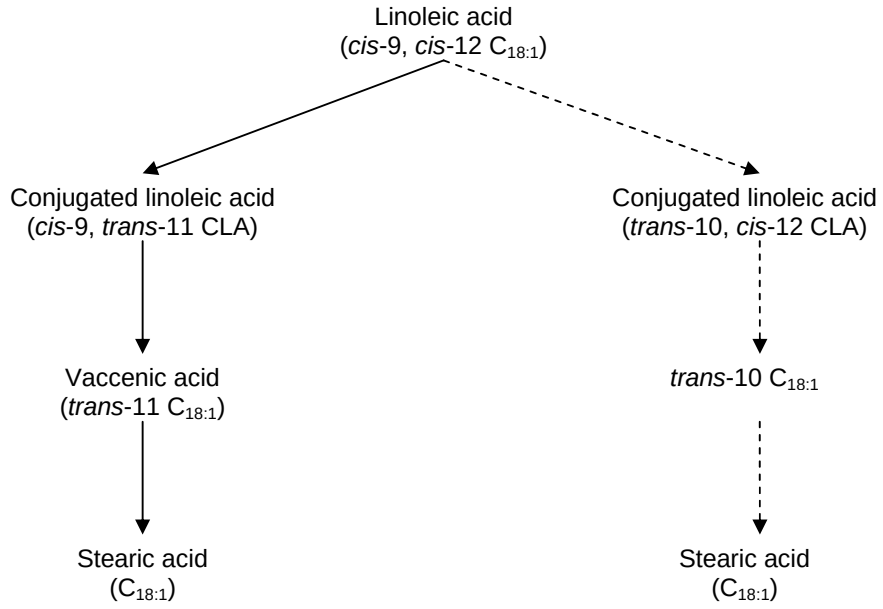

Figure 6.1 Pathways of linoleic acid biohydrogenation in the rumen (Griinari & Bauman, 2006)

Buffers are routinely added to high concentrate dairy rations in an attempt to alleviate MFD and increase milk yield. Sodium bicarbonate has proved to be very effective in alleviating MFD by maintaining the rumen pH within the physiological range of 5.8 to 6.5 when high concentrate diets were fed to lactating cows (Rogers *et al*., 1982; Solorzano *et al*., 1989). Many authors have reported increased milk yields when supplementing dairy rations with sodium bicarbonate and attributed these increases in production to increased dry matter intake (Donker & Marx, 1980; Erdman *et al*., 1980; Thomas *et al*., 1984; Rogers *et al*., 1985; Vicini *et al*., 1988). However, Griinari & Bauman (2006) attributed the increased milk fat to the reduced concentration of *trans*-10,*cis*-12 CLA when sodium bicarbonate was included in high energy rations with limited amounts of effective fibre.

Acid Buf (the skeletal remains of the seaweed *Lithothamnium calcareum*) is another buffer proven to be effective when supplemented to potentially acidotic dairy rations. Cruywagen *et al*. (2004) reported an inclusion of Acid Buf at 0.3% of the dietary dry matter (or 80g/day) to be sufficient in order to optimize milk output and efficiency of feed into milk. In another study, Cruywagen *at al*. (2007) compared Acid Buf with sodium bicarbonate in terms of their effects on milk yield and composition. They reported the Acid Buf treatment to have resulted in significantly higher daily milk yields of 31.6 L/cow, compared to 27.6 and 29.1 L/cow for the control and sodium bicarbonate treatments, respectively. Cruywagen *et al*. (2007) also reported higher milk fat content for the Acid Buf treatment (42.1 g/kg) when compared to the control (38.6 g/kg) and sodium bicarbonate (41.8 g/kg) treatments, although the difference was only significant when comparing the Acid Buf treatment with the control treatment. Beya (2007) suggested Acid Buf (at 90 g/cow per day) to be a more effective buffer compared to sodium bicarbonate (at 180 g/per cow per day). Literature on the efficiency of Acid Buf is rather limited, but thus far it has proved to be effective in potentially acidotic dairy rations.

The aim of this study was to determine the effects of Acid Buf and combinations of Acid Buf and sodium bicarbonate on dry matter intake (DMI), milk yield and milk composition of dairy cows.

6.2 Materials and methods

6.2.1 Animals and housing

Eight ruminally cannulated lactating Holstein cows were used in the trial. The same animals were used in this trial as in the rumen metabolism trial. Their housing and care were described in detail in Chapter 5.

6.2.2 Experimental design and treatments

Cows were randomly allocated to treatments according to a 4 x 4 (n = 2) repeated Latin square design with four treatments and four periods. Details pertaining to the experimental design were described in Chapter 5.

6.2.3 Data collection and chemical analysis

Individual feed intake was recorded daily during the seven day collection period by monitoring the amount of feed supplied and refusals weighed back. Representative feed samples from each treatment were analysed for dry matter (DM) as described by AOAC (2002) in order to determine individual cow dry matter intake (DMI).

Milk yields were recorded by hand at every milking during each data collection period for each cow. Milk samples were collected twice daily for five consecutive days during each data collection period and were pooled in amounts proportional to yield (10 ml/L milk produced) per cow per day. Milk samples were immediately preserved with potassium dichromate ($K_2Cr_2O_3$) after collection. Milk samples were analyzed for fat, protein, lactose, total solids and MUN with the aid of a Milk-O Scan 605 analyzer (Foss Electric, Hillerod, Denmark) by the Dairy Laboratory of the Agricultural Research Council at Elsenburg, Stellenbosch.

6.2.4 Statistical analysis

Data were analysed with the General Linear Model (GLM) procedure of Statistica 8.1 (2009) according to a balanced 4 x 4 Latin square design. Fixed effects were cow, treatment and period. Bonferoni tests were done to compare treatment means and differences were considered significant at $P < 0.05$.

6.3 Results

The results on daily milk production, milk composition and daily DMI are presented in Table 6.1. Dry matter intake averaged 24.4 kg/cow/day and was not affected by treatment. Treatment had no effect on daily milk production. Milk composition (fat, protein, lactose, total solids, SNF) was also not affected by treatment.

Table 6.1 The effect of Acid Buf (AB) and combinations of Acid Buf and sodium bicarbonate on milk production, milk composition and daily dry matter intake

Item	Treatment 1 80AB	Treatment 2 40AB+40BC	Treatment 3 80AB+80BC	Treatment 4 80AB+120BC	Significance P
Milk production (kg/day)	34.1	32.5	33.5	33.4	0.78
4% Fat corrected milk (kg/day)	32.1	31.6	31.6	31.5	0.96
Milk fat (%)	3.70	3.86	3.74	3.66	0.41
Milk fat (kg/day)	1.26	1.25	1.25	1.22	0.91
Milk protein (%)	3.34	3.31	3.37	3.32	0.90
Milk protein (kg/day)	1.14	1.08	1.13	1.11	0.82
Milk lactose (%)	4.77	4.74	4.79	4.73	0.71
Milk lactose (kg/day)	1.63	1.54	1.60	1.58	0.73
Total solids (%)	12.53	12.63	12.62	12.43	0.46
Total solids (kg/day)	4.27	4.10	4.23	4.15	0.88
SNF (%)	8.83	8.77	8.88	8.77	0.49
SNF (kg/day)	3.01	2.85	2.97	2.93	0.85
Daily DM intake (kg)	24.6	23.5	24.8	24.5	0.44
Milk/kg DM	1.39	1.38	1.35	1.36	0.88
DM/kg milk	0.72	0.72	0.74	0.73	0.91

SNF = Solids not fat

6.4 Discussion

Daily dry matter intake (DMI) was not affected when the basal diet was supplemented with Acid Buf at 3.5 g/kg (or 80 g/cow per day). Additional sodium bicarbonate inclusions did not affect dry matter intake. Erdman (1988) reported sodium bicarbonate to have little effect on DMI when added to diets where the forage content is higher than 30%. This is in agreement with our study as the forage content of the diet was ~35%. Rogers *et al*. (1982), Erdman & Sharma (1989) and Kennelly *et al*. (1999) also found no effect of sodium bicarbonate on DMI. However, Erdman *et al*. (1980), Thomas *et al*. (1984), Rogers *et al*. (1985), Vicini *et al*. (1988) and Solorzano *et al.* (1989) reported increased DMI when high concentrate diets were supplemented with sodium bicarbonate.

Milk yield in our study was not affected by treatment. This is in contrast to the observations of Cruywagen *et al*. (2007) who reported increased milk yields when 80 g/day Acid Buf was supplemented to potentially acidotic diets. The difference is that the current study did not include an unbuffered control treatment as in the case of Cruywagen *et al.* (2007). Some other authors (Erdman *et al*., 1980; Thomas *et al*., 1984; Rogers

et al., 1985; Vicini *et al*., 1988 and Kennelly *et al*., 1999) also reported increased milk yields when high concentrate diets were supplemented with sodium bicarbonate. However, according to Erdman (1988), sodium bicarbonate had little effect on milk yield when the diet contained more than 30% forage. Kennelly *et al*. (1999) reported sodium bicarbonate to have no effect on milk yield when cows were fed a medium concentrate diet (concentrate to forage ratio 50:50). In agreement with our study, Solorzano *et al*. (1989) reported no effect on milk yield when sodium bicarbonate was added to a high concentrate dairy ration.

Although the total volatile fatty acid concentration in this study increased as the sodium bicarbonate inclusion levels increased in the treatment diets (Chapter 5), it did not affect milk production or milk composition. It should be noted that VFA concentrations do not necessarily reflect total VFA yields, because ruminal VFA concentration values are affected by VFA turn-over rates. Dijkstra et al. (1992) observed a non-linear increase in VFA absorption from the rumen with a decrease in rumen pH. In the current study, mean rumen pH values increased with increased buffer supplementation and it is thus postulated that total VFA production did not differ between treatments, therefore no VFA concentration effects were observed in milk production and composition.

It is well documented that potentially acidotic diets, reducing rumen pH below 5.8, result in milk fat depression (MFD) (Beitz & Davis, 1964). As previously discussed, there is some controversy on the exact mechanism resulting in reduced milk fat production. However, many authors (Van Soest, 1963; Beitz & Davis, 1964) attribute MFD to reduced acetate production as acetate is the main precursor for *de novo* milk fat synthesis in the mammary gland (Ishler *et al*., 1996). Cruywagen *et al*. (2007) reported a significantly higher milk fat content when Acid Buf (included at 4 g/kg or 90 g/day) was compared to a control (unbuffered) treatment formulated to be potentially acidotic. It is also well known that the addition of sodium bicarbonate to high concentrate diets increase milk fat content (Davis *et al*., 1964; Donker & Marx, 1980; Thomas *et al*., 1984; Rogers *et al*., 1985; Solorzano *et al*., 1989; Belibasakis & Triantos, 1991) after MFD has been identified. In our study, milk fat content was not affected by treatment. This can be explained by referring to the fact that all treatments maintained an average rumen pH above 6.0 and all acetate to propionate ratios were greater than 2.2 (refer to Chapter 5). Therefore, milk fat depression has not occurred in order for the buffered treatments to alleviate the depression. The acetate and total volatile fatty acid concentrations increased as the amount of sodium bicarbonate included with Acid Buf increased (as discussed in Chapter 5), but this was not reflected in the milk fat content nor overall milk quality. In addition, feeding excess sodium bicarbonate can have adverse effects upon magnesium homeostasis, as Rogers *et al*. (1985) reported sodium bicarbonate supplementation to depress serum concentrations of magnesium in lactating cows. It should be emphasized that buffers are included in high concentrate diets to prevent and/or alleviate milk fat depression and should not be used as a dietary tool to increase milk fat

content, i.e. if milk fat depression has already occurred, buffers can result in increased milk fat, but if the milk fat content is relatively normal, the addition of buffers will not result in further milk fat increases.

Milk protein content was not affected by treatment. This is in agreement with Cruywagen *et al*. (2007) who reported Acid Buf to have no effect on milk protein content. This is also in agreement with Rogers *et al*. (1985), Vicini *et al*. (1988), Erdman & Sharma (1989), Solorzano *et al*. (1989) and Moore *et al*. (1992) who reported sodium bicarbonate to have no effect on milk protein content when included in high concentrate dairy rations. Thomas *et al*. (1984) reported cows fed a high concentrate diet containing no buffer (control treatment) to have had significantly greater milk protein content than cows fed a high concentrate diet supplemented with sodium bicarbonate. However, they admitted to have no explanation as to why the milk protein content was higher for cows in the control treatment. It is well known that nutrition affects milk composition, but the amount of change possible in the protein content of milk is very small compared to the amount of change possible in milk fat content (Emery, 1978; Erasmus *et al*., 1985; Sutton, 1989; Ng-Kwai-Hang *et al*., 1993). For this reason, dietary buffers do not consistently alter milk protein content (Cassida *et al*., 1988).

Milk lactose, solids-not-fat (SNF) and total milk solids were not affected by treatment. This is in agreement with Rogers *et al*. (1982) and Beya (2007) who reported no effects on these milk components by sodium bicarbonate and Acid Buf, respectively. Milk lactose content is usually quite constant and is very difficult to change via dietary means (Sutton, 1989).

6.5 Conclusion

This study confirmed previous results for the optimal inclusion level of Acid Buf to potentially acidotic diets to be 80 g/day (or 3.5 g/kg) in order to support high milk production without compromising the milk solid contents. This study also showed that adding Acid Buf alone to high concentrate diets are very effective, as additional sodium bicarbonate did not improve milk yield or milk composition which could result in unnecessary expenditures to the farmer.

6.6 References

AOAC International, 2002. Official methods of analysis (17th ed). Association of Official Analytical Chemists, Arlington, Virginia, USA.

Beitz, D.C. & Davis, C.L., 1964. Relationship of certain milk fat depressing diets to changes in the proportions of the volatile fatty acids produced in the rumen. J. Dairy Sci. 47: 1213-1216.

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

Bernard, L., Leroux, C. and Chilliard, Y., 2006. Characterisation and nutritional regulation of the main lipogenic genes in the ruminant lactating mammary gland. In: Ruminant physiology: Digestion, metabolism and impact of nutrition on gene expression, immunology and stress. Eds. Sejrsen, K., Hvelplund, T. and Nielsen, M.O., Wageningen Academic Publishers, Wageningen, Netherlands. pp.295-326.

Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate on production response and rumen metabolism. MSc (Agric) thesis, University of Stellenbosch, South Africa.

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

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

Cruywagen, C.W., Taylor, S.J. and Beya, M.M., 2007. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate + limestone on production response and rumen parameters. J. Dairy Sci. 90 (Suppl.1): 561.

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

Dijkstra, J., Neal, H.D.StC., Beever, D.E., and France, J., 1992. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. J. Nutr. 122: 2239-2256.

Donker, J.D. & Marx, G.D., 1980. Sodium bicarbonate in diets for milking Holstein cows. J. Dairy Sci. 63: 931-935.

Erasmus, L.J., Hermansen, J.E. and Rulquin, H., 1985. Nutritional and management factors affecting milk protein content and composition. In: Trends in milk composition: Influence of feed on major components of milk. Eds. Itabashi, H. & Baevre, L., International Dairy Federation, Brussels, Belgique.

Erdman, R.A., 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71: 3246-3266.

Erdman, R.A., Botts, R.L., Hemken, R.W. and 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. & Sharma, B.K., 1989. Effect of yeast culture and sodium bicarbonate on milk yield and composition in dairy cows. J. Dairy Sci. 72: 1929-1932.

Emery, R.S., 1978. Feeding for increased milk protein. J. Dairy Sci. 61: 825-828.

Grinarii, J.M. & Bauman, D.E., 2006. Milk fat depression: concepts, mechanisms and management applications. In: Ruminant physiology: Digestion, metabolism and impact of nutrition on gene expression, immunology and stress. Eds. Sejrsen, K., Hvelplund, T. and Nielsen, M.O., Wageningen Academic Publishers, Wageningen, Netherlands. pp.389-409.

Griinari, J.M., Dwyer, D.A., McGuire, M.A., Bauman, D.E., Palmquist, D.L. and Nurmela, K.V.V., 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

Ishler, V., Heinrichs, A.J. and Varga, G., 1996. From feed to milk: Understanding rumen function. Penn State University Extension Circular 422, University Park, PA. pp. 1-26.

Kennelly, J.J., Robinson, B. and 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.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan, C.A., 2002. Animal nutrition (6th ed). Pearson Education Inc., Harlow, UK. pp. 163-243.

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

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E. and Monardes, H.G., 1982. Environmental influences on protein content and composition of bovine milk. J. Dairy Sci. 65: 1993-1998.

Rogers, J.A., Davis, C.L. and Clark, J.H., 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral 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. and Cummings, K.R., 1985. Response of dairy cows to sodium bicarbonate and limestone in early lactation. J. Dairy Sci. 68: 646-660.

Solorzano, L.C., Armentano, L.E., Grummer, R.R. and Dentine, M.R., 1989. Effects of sodium bicarbonate or sodium sesquicarbonate on lactating Holsteins fed a high grain diet. J. Dairy Sci. 72: 453-461.

Statistica 8.1., 2009. StatSoft Inc., USA.

Sutton, J.D., 1989. Altering milk composition by feeding. J. Dairy Sci. 72: 2801-2814.

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

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. and Bauman, D.E., 1988. Effects of feed intake and sodium bicarbonate on milk production and concentrations of hormones and metabolites in plasma of cows. J. Dairy Sci. 71: 1232-1238.

CHAPTER 7

General conclusion

The high nutritional requirements of dairy cattle results to feeding high concentrate diets in order to maximize energy intake and productivity without altering rumen function and predisposing animals to metabolic disorders of which ruminal acidosis is the most important in terms of animal health, production and financial losses. The addition of buffers to high concentrate diets with limited fibre can counteract the negative effects of high concentrate diets.

Acid Buf proved to have a significantly higher *in vitro* buffering capacity (milliequivalents of acid required to reduce pH by one unit) than sodium bicarbonate. Acid Buf with additional sodium bicarbonate appeared to result in higher buffering capacities, although not significantly so, proving that Acid Buf alone is efficient to prevent severe pH reductions.

Mean pH for all treatments were above 6.0 which indicate that all treatment diets were well buffered. Treatment 1 (Acid Buf included at 3.5 g/kg DM) resulted in the lowest mean pH whereas Treatment 4 (3.5 g/kg of Acid Buf plus 5.2 g/kg of sodium bicarbonate) was significantly higher.

Treatment had no effect on total volatile fatty acid concentrations. However, there was a tendency towards increased total volatile fatty acid concentrations as the level of sodium bicarbonate increased. In addition, as the sodium bicarbonate inclusion levels increased, the acetate concentrations increased, propionate concentrations decreased, resulting in a higher acetate:propionate ratio in Treatment 4 compared to Treatment 1. The effect of Treatment 4 on rumen metabolism may negatively affect animal productivity, as it was proposed that fairly high rumen pH levels (above 6.0), in association with high acetate: propionate ratios above 3:1, may negatively impact rumen fermentation, increase methanogenesis and result in reduced animal productivity due to energy loss via methane production.

Treatment had no effect on milk production, milk composition or dry matter intake. Although not significant, Treatment 1 numerically resulted in the highest milk output without compromising milk quality. The higher acetate concentrations of treatments with additional sodium bicarbonate did not increase milk fat concentration.

This study confirmed previous results that, with high concentrate diets, a daily Acid Buf intake of 80 g/cow would support high milk production without compromising milk solids content. This level of Acid Buf would keep the rumen healthy by preventing a pH nadir in the acidosis danger area. Furthermore, Acid

Buf's buffering action prevents rumen pH to rise too high, which could lead to excess acetate production, therefore Acid Buf should be effective in limiting methane production and improving rumen efficiency. Additional sodium bicarbonate feeding did not improve milk yield or milk composition further, but resulted in acetate: propionate ratios that might increase methane emissions in cows.