Buffer supplementation in concentrates for Jersey cows grazing spring ryegrass pasture

by

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Declaration

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Abstract

Title:	Buffer supplementation in concentrates for Jersey cows grazing spring ryegrass pasture
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Pasture is the cheapest available source of nutrients and in the Southern part of the Western Cape of South Africa the most common used pasture system is kikuyu grass, over-sown with ryegrass. For this reason, it is important to optimally utilise the pasture and to ever try to improve pasture based feeding systems. High quality ryegrass creates a risk for subclinical rumen acidosis (SARA) for dairy cows. Supplementing concentrates, which is inevitable as energy is the first limiting nutrient for dairy cows, increases the risk of incidence. The addition of buffers to total mixed ration feeding systems has achieved great success in diets containing high levels of concentrates. Information on buffers regarding pasture based systems is, however, lacking, especially pertaining to SARA. The cost of adding buffers to concentrates fed to grazing dairy cows is a concern. If, however, there is a challenge on the rumen, buffer addition has proved to increase the milk fat content and therefore the increased income might justify the expense. The purpose of this study was to determine whether the addition of buffers to concentrates supplemented to grazing dairy cows could utilise pasture optimally, whilst increasing milk yield and improving milk composition, and maintaining rumen functioning.

Fifty four high producing Jersey cows were blocked according to milk yield, days in milk and lactation number. Cows within blocks were then randomly allocated to one of three treatments. Treatments included no buffer inclusion (CON), Acid Buf (AB) at a level of 10 g/kg and sodium bicarbonate (SB) at a level of 20 g/kg of the concentrate DM. Cows received 6.6 kg “as is” concentrate per day, consisting of 62% maize, 15% hominy chop, 11% bran, 4% soybean oilcake, 4% molasses, minerals and vitamins. Buffers were mixed into the concentrates beforehand to ensure intakes of 120 g of sodium bicarbonate or 60 g of Acid Buf per cow/day. Cows grazed high quality ryegrass during spring and were allocated 10 kg DM pasture per cow/day with *ad libitum*

access to fresh water. Milk production was recorded daily and milk composition fortnightly, after an adaptation period of 14 days. Six ruminally cannulated Jersey cows grazed with the production study cows, to be used for a separate rumen study. These cows were divided into three groups of two and were allocated to each treatment. Cows were crossed-over through-out the duration of the trial to ensure that all cannulated cows received each treatment. An *in sacco* digestibility trial was done and rumen pH and volatile fatty acid (VFA) concentrations were also determined.

Milk production (kg/day) was 20.2, 20.3 and 20.5, whereas 4% fat corrected milk production (kg/day) was 20.8^{d}, 21.8^{cd}, 21.9^{c} for the CON, SB and AB treatments, respectively. Milk fat content did not differ among treatments and was 42.4, 45.0 and 45.1 g/kg, whereas milk protein tended to be different at 34.1^{d}, 35.6^{c} and 35.1^{cd} g/kg for CON, SB and AB, respectively. Milk lactose differed among treatments and was 44.9^{b}, 47.6^{a} and 47.6^{a} g/kg, whereas milk urea nitrogen was 10.5^{a}, 9.7^{ab}, 9.6^{b} for CON, SB and AB, respectively. Total VFA and proportions of individual VFA's did not differ among treatments. Treatment also had no effect on mean ruminal pH and time spent below critical pH values. Pasture DM and NDF digestibility did not differ among treatments.

The results indicated that milk production and rumen functioning can be maintained with the addition of buffers to grazing cows, even though no differences were found between control and buffered treatments. The milk composition was, however, favourably affected by buffers and it could be economically viable for farmers using similar production systems.

Uittreksel

Titel:	Buffer supplementering in kragvoere vir Jerseykoeie op lente-raaigrasweiding
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Weiding is die goedkoopste voedingsbron vir melkbeeste en in die Suidelike deel van die Wes-Kaap waar kikoejoe gewoonlik oorgesaai word met raaigras, word daar altyd gepoog om beter benutting van weiding te bewerkstellig. Hoë kwaliteit weiding kan egter ‘n risiko vir subkliniese rumenasidose (SARA) inhou. Die byvoeding van kragvoere is onvermydelik, siende dat energie die eerste beperkende nutrient vir melkbeeste is en dit verhoog die risiko nog verder. Totaal gemengde rantsoene het al groot sukses behaal met die invoeging van buffers. Inligting aangaande die gebruik van buffers vir weidende melkbeeste is egter beperk, veral met betrekking tot die voorkoms van SARA. Die ekstra koste vir buffers kan ‘n rede tot kommer wees. Die insluiting van buffers is egter al bewys om die impak op die rumen te verlaag en hoër melkvet tot gevolg te hê en daarom mag die verhoogde inkomste moontlik die koste rondom buffers regverdig. Die doel van die studie was om te bepaal of bufferinsluiting in kragvoere vir weidende diere die weidingsbenutting sodanig kan optimaliseer dat melkproduksie en melk samestelling verbeter en goeie rumengesondheid terselfdertyd gehandhaaf kan word.

Vier en vyftig hoë produserende Jerseykoeie is volgens melkproduksie, dae in melk en laktasienommer geblok. Koeie is vervolgens ewekansig aan een van drie behandelings toegeken. Behandelings het die volgende ingesluit: geen buffers (KON), Acid Buf (AB) teen 10 g/kg DM en natriumbikarbonaat (SB) teen 20 g/kg kragvoer. Elke koei het 6.6 kg (natuurlike vogbasis) konsentraat per dag ontvang, waarvan die samestelling as volg was: 62 % mielies, 15 % hominy chop, 11 % semels, 4 % soja-oliekoek, 4 % melasse, minerale en vitamiene. Die buffers is sodanig in die onderskeie kragvoere ingemeng om te verseker dat koeie 120 g koeksoda of 60 g Acid Buf per dag inneem. Koeie is van 10 kg DM hoë-gehalte weiding per koei/dag voorsien en koeie het

vrye toegang tot skoon drinkwater gehad. Melkproduksie is daagliks aangeteken en melkmonsters is twee- weekliks geneem om melksamestelling te bepaal. Ses rumen-gekannuleerde Jerseykoeie het saam met die res gewei. Hierdie koeie is in ‘n aparte rumenstudie gebruik. Die koeie is verdeel in drie groepe van twee en deur die loop van die studie is koeie oorgeplaas op ander behandelings soadat elke koei elke behandeling ontvang het. ‘n *In sacco*-verteringstudie is gedoen en rumenparameters wat bepaal is, sluit in die bepaling van rumen pH en vlugtige vetsuur (VVS) konsentrasies. Melkproduksie (kg/dag) was 20.2, 20.3 en 20.5, terwyl die 4% vet-gekorrigeerde melkproduksie (kg/dag) 20.8^{d}, 21.8^{cd} en 21.9^{c} kg/dag was vir die KON, SB en AB behandelings, onderskeidelik. Melkvetinhoud het nie tussen behandelings verskil nie en was 42.4, 45.0 en 45.1 g/kg, terwyl die melkproteïeninhoud, waarvan die waardes 34.1^{d}, 35.6^{c} en 35.1^{cd} was vir die KON, SB en AB behandelings, onderskeidelik, geneig het om te verskil. Die laktose-inhoud het verskil tussen behandelings en was 44.9^{b}, 47.6^{a} en 47.6^{a} g/kg, terwyl en melk-ureumstikstof 10.5^{a}, 9.7^{ab}, 9.6^{b} was vir KON, SB en AB, onderskeidelik. Totale vlugtige vetsure (VVS) en proporsies van individuele VVS het nie tussen behandelings verskil nie. Behandeling het ook geen invloed op gemiddelde rumen pH of tyd wat pH onder kritiese waardes was, gehad nie. Weidingverteerbaarheid (DM en NDF) het nie verskil tussen die drie behandelings nie.

Hierdie resultate dui daarop dat melkproduksie en rumengesondheid onderhou kan word deur die insluiting van buffers in die kragvoer vir weidende koeie, al is geen verskil tussen die kontrole en gebufferde behandelings gevind nie. Die melksamestelling is egter gunstig deur buffers beïnvloed en buffers kan ekonomies geregverdig word vir boere wat soortgelyke produksie stelsels toepas.

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Abbreviations

ADF	Acid detergent fibre
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
BCS	Body condition score
BW	Body weight
cm	Centimeter
CP	Crude protein
DIM	Days in milk
dL	Deciliter
° C	Degree Celsius
DMI	Dry matter intake
DM	Dry matter
EE	Ether extract
eNDF	Effective neutral detergent fibre
FCM	Fat corrected milk
g	Gram
GE	Gross energy
ha	Hectare
IVDMD	In vitro dry matter digestibility
IVOMD	In vitro organic matter digestibility
kg	Kilogram
LAN	Limestone Ammonium Nitrate
ME	Metabolisable energy
MJ	Mega Joules
mMol	Milli-mol
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NDICP	Neutral detergent insoluble crude protein
NH3-N	Ammonia nitrogen
NPN	Non protein nitrogen
NSC	Non-structural carbohydrates
NRC	National Research Council
OM	Organic matter
peNDF	Physically effective neutral detergent fibre

%	Percentage
SARA	Subacute ruminal acidosis
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of the mean
TMR	Total mixed ration
R	South African Rand
RPM	Rising plate meter
VFA	Volatile fatty acids

Chapter 1: Introduction

In January 2007, there were 3 899 milk producers in South Africa and this number has decreased to only 2 123 in January 2013 (Coetzee, 2013). Farmers are presently leaving the industrial due to low gross margins and to try to relieve this pressure, milk production should be increased while keeping cost to a minimum. The milk price has a significant effect on the dairy feeding system used, and according to (Penno *et al*., 1996) pasture-based feeding systems can reduce milk production cost, resulting in a high cost-effective milk output per hectare of land. Pasture based systems are widely used in the Western Cape Province of South Africa, especially in the Southern Cape area of the province. The Western Cape has the greatest contribution to the total milk production in South Africa (Coetzee, 2013). Coetzee (2013) also mentioned the trend for a higher production in pasture-based areas. Considering the facts the ideal is, to better utilise the pasture available.

To optimally utilise pasture, however, the rumen conditions must be favourable. In De Veth & Kolver (2001a) it has been noted that the ruminal pH of cows on high quality pasture can often be below the pH value for optimum digestion. A depression of pH may negatively influence the rumen digestion and hence the production of milk. The lowered pH in the rumen has been reported to decrease milk fat content (Staples & Lough, 1989) and therefore the milk income would be decreased. Using dietary buffers could improve animal performance under the above mentioned conditions. Research on the effect of buffer inclusion preventing milk fat depression is extensive; research regarding the use of buffers on high quality pasture is, however, limited in comparison. The cost of supplementing buffers would, however, be the deciding factor. Supplement usage proves valuable when the income from the extra milk exceeds the cost of the supplement. Farmers are striving for better results without increasing the input cost. The cheapest source of nutrients is, however, pasture and adding supplements to better utilise what is available is more viable than obtaining additional resources.

A study was thus planned to investigate the effect of a slow release calcareous marine algae buffer and a conventional, widely used buffer on rumen metabolism and milk production responses of Jersey cows grazing ryegrass pasture. The slow release buffer has the added benefit of being high in minerals, especially Ca. When the rumen is functioning under ideal conditions the milk composition would likely be favourably influenced. The aim of the study was to determine the effect of buffer addition on the milk yield, milk composition, BW and BCS, and ruminal parameters of cows grazing ryegrass pasture during spring.

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Chapter 2: Literature review

2.1 Introduction

Pasture is continuously looked upon with greater interest because of a reduction in expenses for feed, equipment, and buildings. The improved animal health and reproduction reported, as well as the growing pressure to reduce or manage cattle waste in a better way are all reasons why pasture seems to be a more suitable production system (Staples *et al*., 1994). Pasture is known as the cheapest source of nutrients (Clark & Kanneganti, 1998) and thus using pasture would result in lower cost feeding systems. However when producing milk from pasture, metabolisable energy is the first limiting factor (Bargo *et al*., 2003; Kolver, 2003) and therefore pasture diets should be supplemented with concentrate feeds. Diets based on pasture plus concentrate supplement are characterised by depressed rumen pH (<6.0) (Holden *et al*., 1995). Lush pastures itself have been noted to depress milk fat percentage, possibly because of a reduced rumen pH (Huber *et al*., 1964; Polan *et al*., 1978; Kesler & Stringer, 1981). Some milk payment schemes are mainly based on milk composition, specifically milk fat and protein content. To ensure high profitability, pasture usage must be optimised without affecting the composition of milk produced. Adding buffering agents to concentrate supplement could be a safety measure for optimal milk composition.

2.2 Pasture-based feeding systems

2.2.1 Kikuyu over-sown with ryegrass

Kikuyu (*Pennisetum clandestinum*) is a perennial pasture species well adapted and dominant in summer and autumn (Dickinson *et al*., 2004) in the milking region of the Southern Cape of South Africa (Botha *et al*., 2008b). However, this grass species is dormant in this region during late winter and early spring (Botha, 2003). Kikuyu can withstand intensive grazing due to the robustness and creeping nature of the pasture (Dickinson *et al*., 2004). Kikuyu develops thick rhizomes below the soil and stolons on top of the soil (Dickinson *et al*., 2004) by which the grass spreads over the soil surface. Reeves (1997) suggested that kikuyu, when managed appropriately, can sustain high stocking rates and milk production per hectare. However, Marais (2001) stated that it has a relatively low nutrient value when compared to temperate pasture species and as such causes a low milk production. The main nutrients that are limiting are the digestible energy content and the digestibility of structural carbohydrates. Energy is known as the first limiting factor for milk production (Marais, 2001). Botha *et al.* (2008a) proposed the establishment of legumes and other grasses into kikuyu to improve the seasonal dry matter (DM) production and the quality of the

pasture. Clark & Kanneganti (1998) in agreement with this mentioned using a combination of forage species to ensure year round good quality forage.

Cherney & Allen (1995) stated that pasture used for dairy cows are mainly temperate species. Annual ryegrass (*Lolium multiflorum*) is a temperate grass species that is prevalent in areas with winter rainfall or where it is cultivated under irrigation to supply quality fodder during late autumn and in spring (Dickinson *et al*., 2004). This makes the Southern Cape region an ideal growing environment for this species (Tainton, 2000). Dickinson *et al*. (2004) indicated that annual ryegrass established in March will grow up to mid-November and that the production potential peak for spring (mid-October) can be up to 120 kg DM/ha/day. Fulkerson *et al*. (2006) indicated that annual ryegrass has a higher metabolisable energy (ME) than kikuyu. Kikuyu and annual ryegrass can be classified as warm season (C4) and cool season (C3) grasses, respectively, with ideal growing conditions at 30 to 40 ºC for kikuyu and 15 to 25 ºC for annual ryegrass (Nelson, 1996). This makes ryegrass an ideal grass species to combine with kikuyu.

Botha *et al.* (2008b) found an increase in grazing capacity and total milk production during spring when cows grazed kikuyu over-sown with annual ryegrass. The kikuyu-ryegrass combined system had a high ($P \leq 0.05$) ME during spring but it decreased in summer and autumn, because of the more dominant kikuyu (Botha *et al.,* 2008a). This indicates the value of this combined system as pasture for dairy cows during spring. Upon analysis of the mineral composition of pasture Botha *et al*., (2008a) found that the Ca content of the kikuyu-ryegrass pasture was relatively low when considering the requirement of dairy cows, and stated that Ca should be supplemented on these systems. The conclusion was made that the kikuyu-ryegrass system is favourable because of the high seasonal DM production, the ease of execution and management, and the fact that kikuyu production potential is maintained during summer and autumn (Botha *et al*., 2008a).

2.2.2 Pasture management

Ryegrass can be classified as a short-growing temperate grass species which according to Clark & Kanneganti (1998) can be grazed to a lower residue, providing there is ample water and nutrients available for regrowth. Reeves *et al*. (1996) stated that optimally ryegrass should be grazed at the three-leaf stage of growth. Voisin (1959) strongly advocated using rotational grazing to ensure sufficient time for grazing as well as pasture recovery. Dickinson *et al*. (2004), in agreement with this, recommended rotational grazing for dairy cows with strip grazing as the preferred option. Strip grazing is similar to rotational grazing but animals are moved to different strips within a paddock each day, instead of grazing the entire paddock for a few days and being moved to the next paddock (Clark & Kanneganti, 1998). Soiling of fresh pasture is minimised when

strip grazing is applied as urination and defecation are mostly limited to previously grazed pasture (Tainton, 2000). Strip grazing is rather intensive and a way to ensure maximum production from pasture is achieved. An extensive rest period should however be applied if most of the leaf area is removed under grazing, during this time the canopy can recover and storage reserves can be replenished (Duell, 1985). Fulkerson & Slack (1994) recommended residual pasture height to be 5 cm after defoliation as an effective compromise for optimal pasture growth, quality and botanical composition. Over-utilisation of pasture may affect pasture production adversely, while under-utilisation of pasture may detriment species composition and nutritive value (Stockdale, 2000). Cooper & Saeed (1949) recommended a grazing interval of 28 to 30 days to maintain carbohydrate storage reserves in the stubble and root.

2.2.3 Pasture intake

A restricted pasture intake can limit the milk production of high yielding dairy cows (McGilloway & Mayne, 1996; Kolver & Muller, 1998). On the other hand, unrestricted pasture allowance could result in a high post grazing pasture height. This increase in residual pasture height could lead to the deterioration of pasture as season progresses (Peyraud & Delaby, 2001). It is thus important to accurately assess forage mass available to apply proper forage budgeting. The recommendation made to limit the deterioration of pasture, as a result of low pasture utilisation, is to allocate two times the expected pasture DMI when cows are also fed supplements (Bargo *et al.,* 2002a). The problem with pasture based feeding systems is however, that pasture dry matter intake (DMI) can only be estimated as a group and not individually (Kolver & Muller, 1998).

Pasture intake can be determined via direct or indirect methods. Direct methods are costly and invasive to the animal, whereas indirect methods tend to be less accurate. The mode of action for indirect methods is to determine pasture yield before and after grazing, namely the pasture intake (Kellaway *et al*., 1993). The rising plate meter (RPM) is an indirect pasture estimate method that integrates sward height and density into one measure (Sanderson *et al*., 2001). The RPM is an instrument for pasture measurement that is based on the original model, Ellinbank pasture meter, developed by Earle & MacGowan (1979). This ruler method is reliant on a linear relationship between pasture canopy height and the yield of forage (Sanderson *et al*., 2001). The determined level of error for pasture yields estimated using a RPM is 10 % (Rayburn & Rayburn, 1998). Sanderson *et al*. (2001) however calculated an error of 26 %, which tended to be lowest for all indirect pasture measurement methods. To increase the accuracy of the RPM short grazing cycles should be applied (Smith *et al*., 2005) and recalibrating the RPM for the specific region and pasture species (Sanderson *et al*., 2001).

2.2.4 Pasture composition

Kolver *et al*. (1998) states that intensive grazing systems are successful when based on high quality pasture. Characteristics of high quality pasture includes an *in vitro* DM digestibility of 70% and higher (Kolver *et al*., 1998); DM, crude protein and NDF of 18 – 24%, 18 – 25%, and 40 – 50%, respectively (Bargo *et al.*, 2003); quick fibre digestion rate, of 10 to 16 %/h (Kolver, 1997); and a NDF digestibility of 70 to 80 % (Kolver *et al.,* 1998; Van Vuuren *et al.,* 1992). According to Bargo *et al.* (2003) the low DM content of high quality ryegrass may, however, reduce the intake of pasture by dairy cows. Furthermore, the ruminal pH for cows grazing high quality pasture could reach a mean pH of 5.8 to 6.2 (Carruthers *et al*., 1997; Kolver *et al*., 1998; Van Vuuren *et al*., 1992). This is below the value (pH 6.2) identified as critical for fibre digestion by the CNCPS (Pitt *et al.,* 1996). Energy is, however, the first limiting factor for milk production from pasture (Kolver, 2003; Kolver & Muller 1998) and supplemental feeding of concentrates might be needed to fulfil requirements for milk production. The pasture composition from various studies is depicted in Table 2.1.

Table 2.1 Quality of annual ryegrass in spring (September to November) obtained from previous studies

Authors	**Nutrient composition (g/kg) DM unless otherwise stated)**[1]			
	CP	ME[2]	NDF	ADF
Meeske *et al*. (2006)	180	10.9	490	280
Fulkerson *et al*. (2007)	252	10.1	513	270
Van der Colf (2011)	241	11.7	454	-
Lingnau (2011)	259	11.4	541	261
Van Wyngaard (2013)	215	11.5	494	302

[1] – CP: crude protein; ME: metabolisable energy; NDF: neutral detergent fibre; ADF: acid detergent fibre
[2] – MJ/kg DM

2.2.5 Concentrate supplementation

Dairy cows that are known for high milk yield need energy supplements when grazing pasture, to be sure they will reach their genetic potential for intake and milk production (Bargo *et al.*, 2002b; Mertens, 1994). Therefore, the main objectives for supplementing pasture with concentrate is to increase the total DMI and the energy intake compared to a pasture only feeding system (Peyraud & Delaby, 2001; Stockdale, 2000), and to increase the profit per cow as well as per unit of land (Kellaway & Porta, 1993; Fales *et al*., 1995). Other objectives include, increased milk production per cow, higher stocking rate and milk production per unit of land (Stockdale, 1999;

Bargo *et al*., 2003), improved pasture usage because of the higher stocking rate, maintained or improved BCS (Bargo *et al*., 2003), and improve overall dairy farm profitability.

The aim when feeding supplemental concentrates is to increase the supplemental effect without increasing the substitution effect (Clark & Kanneganti, 1998). The definition of substitution according to Kellaway & Porta (1993) is the decrease in pasture intake noted per kilogram of supplemental feed given. Some of the factors known to influence the substitution rate include pasture allowance (PA), level of concentrate fed, pasture digestibility, chemical and physical properties of the concentrate fed, and the stage of lactation (Kellaway & Porta, 1993). The quality of pasture and allowance thereof, together with the nutritional value of the concentrate fed and the level at which the concentrate is fed will affect the milk response of cows grazing pasture with supplemented concentrate (Bargo *et al*., 2003). Meeske *et al.* (2006) found that the addition of concentrates to the diet of grazing dairy cows increased the yield of milk, milk fat, protein per lactation and body condition score.

Concentrates provides additional energy, protein or minerals especially when grazed forage cannot provide in the animals' nutrient requirements. Concentrate supplements should be fed at a rate between two and six kg DM/d (Delaby *et al.*, 2001). An increase above this range will lead to a substitution of pasture intake (Delaby *et al.*, 2001). When concentrate is expressed as a ratio of milk produced the recommendation is to feed concentrate at a rate of 1 kg per 3 – 5 kg milk produced (Dhiman *et al.,* 1997). Feeding supplemental concentrate may prove economically viable when reasonably priced feedstuffs are available (Holden *et al.*, 1995).

2.3 Ruminal pH

Ruminal pH is represented by the consortium of relative concentrations of bases, acids, and buffers (Plaizier et al., 2009). There is a fine pH balance to optimally utilise substrates and produce products. The optimal pH for lactate utilisation is between pH 5.9 and 6.2 (Counette & Prins, 1979) and a drastic decrease in pH would thus inhibit lactate usage and cause an even greater pH decrease. An accumulation of the products of ruminal fermentation leads to a reduced ruminal pH if not buffered (Plaizier et al., 2009) or absorbed. Extended periods of low ruminal pH can adversely affect feed intake, microbial metabolism, and nutrient degradation (Stone, 2004; Krause & Oetzel, 2006; Enemark ,2008). This could also lead to the incidence of laminitis, inflammation, diarrhoea, and milk fat depression. Among animal variation regarding the susceptibility to low rumen pH, is influenced by feed intake level, diet selection, salivation and rumination, rumen microbial population, incidences of acidosis in the past, and the fractional passage rate of digesta (Schwartzkopf-Genswein *et al*., 2003).

Each cow has an inherent capacity to buffer and absorb acid. The capability to do this will determine how much the ruminal pH will decrease after the consumption of a meal (Krause & Oetzel, 2006). The ruminal pH varies a great deal between 5.5 and 7.0 because of diet and time of feeding; ruminants however possess a highly developed system to help keep the pH as constant as possible.

Ruminal pH will vary greatly when considered within 24 h; the pH is however mostly maintained within the physiological range by the intricate developed system within the cow (Krause & Oetzel, 2006). De Veth & Kolver (2001b) confirmed this variation in diurnal pH for dairy cows on pasture. If more acid, as a product of fermentation, is however produced, the system cannot buffer it and ruminal pH may drop considerably (Krause & Oetzel, 2006). Kolver & de Veth (2002) reported that a variation in mean pH from 5.8 to 6.2 were associated with high milk yields and microbial N yields, which indicates that the variation in diurnal pH ranges are affecting cow performance only minimally. This phenomenon is confirmed by the continuous culture study by De Veth & Kolver (2001b) who noted high pasture digestion (67% digestibility of NDF) and microbial growth even when the pH has been suboptimal for extended periods of time (pH 5.4 for 12h). The explanation for the fact that ruminal fermentation could be maintained despite the variation in ruminal pH is that the ruminal pH is optimal for a sufficient amount of time during the day to allow microbial attachment and digestion.

2.3.1 Physiology

It is common for ruminal pH to exhibit shifts of 0.5 – 1.0 pH units within a 24 h period (Dado & Allen, 1993; Nocek *et al*., 2002). A shift of that magnitude represents a 5 to 10-fold change in hydrogen ion concentration. Ruminal pH varies regularly after eating; this poses quite a problem to evaluate ruminal pH and in these circumstances, the best option is to acquire pH data continuously by indwelling electrodes (Krause & Oetzel, 2006) or otherwise at fixed times after feeding.

Woodford & Murphy (1988) stated that diets of different composition might have the same mean ruminal pH but the amount of time spent below a specific pH value will differ. Krause *et al.* (2002) further found that dietary factors rather than mean ruminal pH affected the area on the graph below pH 5.8. The dietary factors as mentioned by Krause *et al.* (2002) included forage particle size and inclusion of ruminally fermentable carbohydrates. When the pH is continuously monitored, a better idea of whether the mean ruminal pH, the lowest pH value, or the amount of time that the pH is below a threshold value, is significant regarding subacute ruminal acidosis (SARA; Krause & Oetzel, 2006). Feed intake may be inhibited by low ruminal pH because of the increased osmolality of the ruminal contents (Carter & Grovum, 1990). Inflammation of the ruminal epithelium may result when ruminal acidosis occurs, this aids in further depressing feed intake

(Krause & Oetzel, 2006). Ruminants will regulate their feed intake as well as trying to regulate their ruminal pH. They will attempt to stabilise the ruminal pH by buffering the products of fermentation and although the effect will be rather small, it will still aid in preventing disease in dairy cows on highly fermentable diets (Firkins, 1997).

Diet composition does not affect the mean ruminal pH as drastically as it does the lowest ruminal pH (Krause & Oetzel, 2006). An example of this would be Kennelly *et al*. (1999) who indicated that mean ruminal pH of 6.31 and 6.15 ($P<0.05$) for cows on diets containing concentrate levels of 0.50 and 0.75, respectively. In that same study the lowest pH readings recorded were 5.9 and 5.5, respectively.

Kolver & de Veth (2002) reported that microbial N flow from the rumen, milk yield, milk protein yield, and the concentrations of acetate, propionate and butyrate in the rumen, as well as the total volatile fatty acids (VFA) in the rumen were negatively related to ruminal pH. When acetate was expressed as a proportion of total VFA, it was positively related to ruminal pH. The acetate:propionate ratio, milk fat percentage and fat:protein ratio was also positively related to ruminal pH. This is when the data was however analysed within study. Kolver & de Veth (2002) stated that a low mean ruminal pH (5.6 to 6.2) in dairy cows on diets high in fresh pasture was related to increased microbial N flow from the rumen, higher VFA concentration, increased DMI, and increased yields of milk, milk protein and milk fat. The increased flow of microbial N from the rumen as associated with pH that is considered to be low (Pitt *et al.*, 1996), is in accordance with in vitro studies done by De Veth & Kolver (2001a).

2.3.2 Causes of depression

The anaerobic microbes in the rumen and cecum involved in the process of carbohydrate fermentation produce VFA's and lactate. These organic acids are absorbed and used for tissue metabolism. A decrease in rumen pH is observed when VFA's and lactic acid accumulate in the rumen and the buffering agents present cannot keep up with this change (Plaizier *et al*., 2009). Fermentation acid production rate in the rumen is nearly twice that of salivation (Allen, 1997). A sudden increase of carbohydrate supply is one of the main causes of pH depression. This results in an increase in the prevalence of lactate as well as the total acid in the digesta. The abrupt increase in carbohydrates in the diet can cause lactate to accumulate exceeding the very low concentrations that is normally present in the digestive tract. Ruminal lactate concentrations will rarely reach 100mM (Owens *et al.*, 1998). VFA accumulation in the rumen is usually not sufficient to reduce pH significantly. Occasionally, when the acid production exceeds the acid absorption, an occurrence because of either rapid production, inhibited absorption, or reduced dilution, the VFA

concentration increases drastically. In some studies the pH was said to decrease below even pH 5.0 without the presence of lactate. These studies suggest that total VFA load rather than solely lactate is responsible for acidosis (Britton & Stock 1987; Oetzel *et al*., 1999), especially in the case of subacute acidosis. The decrease of ruminal pH below pH 5.0 have also been mentioned to be because of the presence of lactate that is responsible for the increased hydrogen ion concentration. The reason for the greater effect of lactate on the pH when compared to similar amounts of other ruminal acids is the lower pK value. Usually acid accumulation is prevented by absorption from the rumen; the greater osmolality of the rumen content however inhibits the rate of absorption (Tabaru *et al*., 1990).Krause & Oetzel (2006) summarised the causes of ruminal pH depression leading to sub-acute ruminal acidosis as the lack of ruminal buffering because of the lack of dietary fibre and/or physical effective fibre, excessive intake of highly fermentable carbohydrates, as well as a rumen that is not adapted to a highly fermentable diet. Energy intake and microbial protein production can be maximised when ruminal degradation is increased. The increase in fermentation acids as product of degradation however needs to be compensated for by either increasing NDF or peNDF as a means to maintain ruminal pH by salivary buffering (Allen, 1997). Increased peNDF may be more effective in this as it increases ruminal fermentation as well as microbial protein production (Allen, 1997). The drop in pH causing acidosis is difficult to reverse or even control. O'Grady *et al.* (2006) reported the incidence of SARA in pasture fed dairy cows and stated the possible reason for this as the high rumen digestibility of pasture. Westwood *et al.* (2003) also raised concern regarding pasture stating that lush pastures has high concentrations of rapidly fermentable carbohydrates and low levels of physical effective fibre which could place dairy cows at risk.

2.3.3 Volatile fatty acid and ruminal pH relationship

Volatile fatty acids are weak acids that establish equilibrium between the acid and a conjugate base (Kohn & Dunlap, 1998). The volatile fatty acids produced, as a product of fermentation, is acetic acid, propionic acid and butyric acid. These VFA provide up to 70% of the energy supplied to the ruminant (France & Dijkstra, 2005). Different VFA have distinct functions to fulfil in the metabolic processes. Propionic acid for example is a substrate for gluconeogenesis and is the main source of glucose in the rumen. A study done by Orskov *et al*. (1969) investigated the effects of iso-caloric infusion of the rumen with acetic compared to propionic acid and the difference in partitioning in energy. Propionic acid infusion seemed to favour the body tissue deposition whereas acetic favoured milk fat content. Whereas butyric acid is the most important of the three VFA concerning source of energy for the rumen epithelium (Kristensen, 2005). Mentschel *et al*. (2001) also mentioned the important mitotic effects of butyric acid that could possibly help stimulation of acid removal through the rumen wall. Conditions that tend to favour butyric over

acetic or propionic acid production would cause a decrease in acid formation and may prevent a low ruminal pH. When pH is below pH 6.0 it has been found that VFA production shifts from acetic and butyric, to a lesser extent, to propionic acid production (Bannink *et al*., 2008). Because propionic acid affects insulin secretion and body fat deposition in the same way, a shift in VFA production towards propionic acid could thus be express in decreased milk fat concentration.

Rumen pH is directly and negatively related to VFA production (Allen, 1997) by rumen bacteria, absorption of VFA across the ruminal wall, the flow of saliva and its buffering constituents into the rumen, feed acidity, and also water outflow to the lower part of the digestive tract (Erdman, 1988). Kolver & de Veth (2002) noted the decrease in pH associated with the increased VFA concentration as a product of fermentation, which confirms the concept that ruminal production of VFA is responsible for the reduction in pH (Allen, 1997). Ruminal pH is often influenced by eating and chewing behaviour. As expected a decrease can be seen after meals and an increase during rumination. The ruminal pH decline is faster after eating as meal size increase and NDF content decrease (Allen, 1997). Allen (1997) however found that this negative relationship between ruminal pH and VFA production is a weak one. It is possible that this weak relationship is the result of differences between diets in the removal, buffering and neutralisation of acids that affects this relationship between VFA's and pH (Dijkstra *et al.*, 2012). In a number of studies there proved to be a significant difference among diets in the linear regression coefficient which is representative of the relationship between these two parameters (Briggs *et al*., 1957). These studies also showed a replacement of roughage with higher levels of grain increased the value of the regression coefficient significantly, whereas increased protein content in the diet had low regression coefficients. Buffers included in the diet depicted decreased slopes in the linear regression equations (Emmanuel *et al.*, 1970), clearly indicating the lower sensitivity of the pH to VFA concentration.

The removal of VFA's from the rumen is via passage out of the rumen in the liquid phase as well as via absorption (Allen, 1997; Aschenbach *et al*., 2009). When VFA absorption is facilitated by the removal of un-ionised acid and the exchange of ionised VFA's for bicarbonate (Stevens, 1970), the pH can be maintained close to neutrality. When less VFA is absorbed, a drop in pH occurs because of accumulation of VFA in the rumen as well as a decrease in bicarbonate input from the blood. Macleod *et al*. (1984) mentioned that a decrease in ruminal pH could affect the absorption of VFA by increasing the absorption of butyrate and propionate and reducing the absorption of acetate. VFA not absorbed is dependent on the rate of absorption, which is increased at lower ruminal pH (Dijkstra *et al.*, 1993). An increased feed intake could lead to a decreased pH and thus increase the absorption rate, which may counteract the increased fractional passage rate.

These acids present in the rumen can also be buffered by dietary features such as the capacity of cell walls to exchange cations, and the addition of buffers in the ration (Allen, 1997).

There are three mechanisms for VFA absorption. The first is by the absorption of undissociated VFA via passive lipophilic diffusion, this directly affects the pH in the rumen seeing as the passive transfer of undissociated VFA into the blood eliminates the protons together with the anion. Here the chain length of the VFA affects the rate of absorption, where butyric is the longest followed by propionic and finally acetic acid which is the shortest (Walter & Gutknecht, 1986). This process is, however, also affected by the pH because it is proven (Dijkstra *et al*., 1993; Lopez *et al*., 2003) that at lower pH a larger proportion of the acids is presented in the undissociated form. The observed increase did however not reach the level as predicted by Henderson-Hasselbalch equilibrium of undissociated and dissociated VFA. Dissociated VFA are absorbed with the aid of carrier proteins and it costs energy. An anion gap has to be maintained across the cell wall of the ruminal epithelial cell. To achieve this, the VFA anion is accompanied by either anion secretion from or cation absorption to the cell to compensate for the charge on the VFA anion. The main pathway for non-diffusional absorption of the anion is an exchange of VFA anion for bicarbonate (Gabel *et al.*, 2002). This is the second mechanism of VFA absorption. Studies done on sheep have shown that up to 50% of VFA can be absorbed with the bicarbonate dependent mechanism (Ash & Dobson, 1963; Penner *et al*., 2009). Bicarbonate is sourced from the ruminal wall; this can be obtained from the blood or from *de novo* synthesis within the epithelial cell (Gabel *et al.*, 2002). The last mechanism of action is a recent finding of an active protein mediated, bicarbonate independent absorption of dissociated VFA (Penner *et al*., 2009). The downside of this mechanism is the fact that protons are left within the rumen and a weak base removed, and thus it adversely affects pH (Dijkstra *et al.*, 2012). Ruminal VFA have an average pKa of 4.9 and will therefore shift to the undissociated form when the pH decreases to pH 5.5. This will release a hydrogen ion into the ruminal fluid and therefore facilitate VFA absorption, which is only absorbed over the ruminal wall in the undissociated form (Krause & Oetzel, 2006). The size and density of rumen papillae determines the rate at which fermentation acids are removed from the rumen (Van Soest, 1994). Inflammation of the ruminal wall because of reduced pH can lead to impaired absoprtion of these acids and therefore the risk for SARA is increased (Plaizier et al., 2009).

2.3.4 Preventing depression

Preventing SARA is not only important for economic reasons but also for animal welfare issues (Krause & Oetzel, 2006). Management is an important tool to prevent the onset of acidosis. Two common methods would be to dilute the diet with roughage or controlling the starch intake. Increased roughage will decrease the eating rate and meal size. An increased chewing time

because of the greater roughage concentration will lead to more saliva production, which will act as a buffering agent. The smaller particle size of the roughage as caused by the greater mastication will increase the rate of fermentation, these ruminal acids are then neutralized by the saliva (Owens *et al.*, 1998). Increasing the peNDF content of the diet by increasing the NDF content or increasing the chop length of forage could prevent SARA. This is dual purpose seeing as it increases chewing and salivation as well as diluting the starch concentration of the diet (Beauchemin & Penner, 2009). The amount of fibre and the particle length of forages, collectively known as physically effective NDF (peNDF), in the diet has a great impact on the rumination abilities and thus ruminal pH via the salivary buffer provided (Yang & Beauchemin, 2007). The effect peNDF has on the pH is because of the mastication and ruminating abilities, meal size, and rumen motility (Allen, 1997). A decrease in ruminal pH can be prohibited by increasing the ruminal input of bases or buffers or feeds that yield these (Owens *et al.*, 1998).

2.3.5 Effect on digestibility

A high level of digestibility can be maintained at low pH on pasture-only diets, possibly because that the need for effective fibre are lower on these diets than for cows on mixed forage-concentrate diets. The quantity effective fibre may be 40 – 50% in pasture of good quality (Kolver *et al*., 1998). Kaufman (1976) predicted a direct relationship between fibre content of the diet and pH in the rumen. This linear function entailed that a 1% decrease in fibre resulted in a 0.066 pH unit decline. According to CNCPS the fibre digestibility rate is reduced at pH lower than 6.2 and will ultimately cease at pH below 5.7 (De Veth & Kolver, 2001a). Fibre digestion is likely to be impaired by SARA seeing as fibrolytic rumen bacteria is acid sensitive and will reduce in numbers below pH 6.0 (Shi & Weimer, 2002). It is possible that fibre digestion will be depressed at lower pH because of the influence of pH on cellulolytic bacteria (Grant & Mertens, 1992). A change in pH has been found to influence the digestibility of DM, De Veth & Kolver (2001a) found that with an increase in pH from 5.4 to 6.6 the true digestibility quadratically increased from 54.8% to 68.5%. The same was noted for OM digestibility, which increased from 57.6% to 70.3% at the same pH values. True digestibility of both OM and DM was greatly reduced below pH 5.8 and the optimum pH was determined to be pH 6.38 and 6.35 for OM and DM, respectively. The relationship between apparent digestibility and pH was in accordance with this (De Veth & Kolver, 2001a). De Veth & Kolver (2001a) found pH 6.35 to be the optimum pH value for DM digestion of pasture-only diets which is in broad agreement with Hutjens *et al*. (1996) and Pitt *et al*. (1996) who concluded a pH range of 6.0 – 6.3 for forage-concentrate combination feeds. The pH range to optimise digestion could thus be stated as pH 5.8 – 6.6. This is in agreement with the high levels of digestion found in studies conducted in New Zealand where up to 80% OM digestion was obtained within the pH range 5.8 to 6.2, for dairy cows grazing fresh high quality pasture (Carruthers *et al*., 1997; Kolver *et*

al., 1998). In various studies it has been found that cellulose digestion was reduced at pH below 6.0 to 6.2 (Orskov & Fraser, 1975; Terry *et al.*, 1969). The effect of pH on cellulose and DM digestion has been proved many times (Erdman, 1988). The optimal range for cellulose digestion is pH 6.4 to 6.8 (Mould *et al*., 1984; Terry, 1969). Studies have proved the importance of adding buffers to systems, which are lacking in fibre to achieve a more favourable pH (Kilmer *et al*., 1981; Rogers *et al*., 1982; West *et al*., 1987).

2.3.6 Bacterial population

The ability of forage digesting microbes to grow and produce acetate is reduced substantially (Russell & Dombrowski, 1980) under low pH conditions whereas the ability of the starch digesting organisms to survive and keep producing propionate is affected less. An increase in fermentable carbohydrate in the diet will cause an increase in bacteria numbers. The fall in pH, as consequence, favours the growth of *Streptococcus bovis* (Russel & Hino, 1985) which in turn produces lactate as product of fermentation. The general idea is that if pH is maintained > 6.2 for most of the day then an optimal rumen environment for cellulolytics to flourish can be obtained (Mertens, 1979). Cellulolytic bacteria have been noted to be more sensitive to pH changes than amylolytic bacteria (Therion *et al.*, 1982). Cellulolytic bacteria struggle to survive when pH falls below pH 6.2 (Calsimiglia *et al.,* 1999). De Veth & Kolver (2001b) found that microbial protein synthesis is most efficient at pH 5.95. Russell *et al.* (1979) further stated that rumen microbes prefer a pH range of 6.5 to 6.8. Ruminal pH definitely plays an important role in competition among bacteria. Bacteria diversity would decrease as forage to concentrate ratio decrease and acidosis occur (Petri *et al*., 2013). During clinical acidosis cellulolytic bacteria decline and acid resistant bacteria increase, i.e. *Streptococcus* and *Lactobacillus*. It is however possible that there is a core microbiome in the rumen that remains stable regardless of the diet or host genetics (Petri *et al*., 2013). Deviations from this microbiome may be indicative of disease (Petri *et al*., 2013).

Anaerobic microbes thrive when free glucose is available. This is however not the case under acidosis circumstances and as of yet this cannot be explained. It is possible that glycolysis is partially blocked during acidosis which would lead to a high free glucose concentration in the rumen (Owens *et al.*, 1998). A decrease in pH have been indicated to change the microbial population (Mould & Orskov, 1983) or the microbes are forced to change their metabolic pathway (Esdale & Satter, 1972). It has been observed that bacteria change their pathway in reaction to a change in pH (Dijkstra *et al., 2012*).

Streptococcus bovis has high growth rates when high levels of starch and sugars are present in the rumen. At these higher growth rates this organism begins to ferment glucose to lactate instead of VFA, this further decreases pH and creates the ideal environment for lactobacilli to

produce even more lactate (Russell & Hino, 1985). Lactate has a lower pKa than VFA of 3.9. This creates a downward spiral of ruminal pH seeing as the lactate will not dissociate at pH 5.0 like VFA and thus remains in the rumen, to decrease the pH even further (Krause & Oetzel, 2006). When this production of lactate begins, the lactate-utilising bacteria (*Megashaera elsdenii* and *Selenomonas ruminantium*) start to metabolise the lactate and then proliferate (Goad *et al*., 1998). These bacteria can be seen as beneficial and will change the lactate into VFA, which can be protonated and absorbed. The problem is however that the growth of these bacteria is inhibited at pH below 5.0 and the production of lactate will in many cases exceed the utilisation (Russell & Allen, 1983). It is possible that the conversion of lactate in the rumen will not be achieved fast enough to stabilise the pH in the rumen.

Protozoa is also quite sensitive to pH and they will not survive extended periods of pH below 5.5 (Quinn *et al*., 1962). When the presence of bacteria and protozoa are decreased the ruminal microflora are not as stable and not able to maintain normal ruminal pH during periods of sudden change in diet (Garry, 2002).

2.3.6.1 Bacteria

Rumen fibre digestion by the fibrolytic bacteria are affected by low pH, a decrease in pH below critical values cause a rapid decrease in fibre digestion in the rumen (Erdman, 1988). In contrast to this, amylolytic bacteria are stimulated in growth and activity at low pH (Mackie *et al*., 1978). Calsamiglia *et al*. (2008) found in a study that was conducted that pH rather than type of diet affected fibre degradation. The effect of diet composition on the pH can however not be ignored. A diet consisting of predominantly concentrate would for instance facilitate a pH decline leading to a suppression of the fibrolytic bacteria that would consequently decrease fibre digestion. The critical pH for fibre digestion as reported by Erdman (1988) and Mourino *et al.* (2001) is 6.0 – 6.3. Cellulolytic bacteria are reported (Weimer, 1996) to be affected at pH drastically below pH 6.0. More recent work by Palmonari *et al*. (2010) have however reported the presence of normal cellulolytic bacteria populations even at very low pH. These findings are supposedly explained by the dynamic changes and fluctuations in pH along with the cross-feeding of cellodextrins (Dijkstra *et al*., 2012). Ruminal pH is depressed due to diet transition, adaptation and recovery and the effect this has on diversity and density of bacteria is an important indication of how the rumen changes in an advantageous way for stabilisation of rumen environment and animal health (Hook *et al*., 2011). The low ruminal pH during SARA reduces the number of species of bacteria present in the rumen on any given time. The bacteria that remain have high metabolic activity (Garry, 2002).

2.3.7 Ruminal pH on pasture based feeding systems

Average values for daily ruminal pH have been reported as between 5.6 and 6.4 for dairy cows grazing high quality pasture (Van Vuuren *et al*., 1992; Stockdale, 1994; Carruthers *et al*., 1997; Kolver *et al.,* 1998). Rumen pH values of 5.5 to 6.6 have been reported for diets containing forage with concentrate (Allen, 1997; Mertens, 1997). In studies where forage plus concentrate were fed, lower ruminal pH was associated with an increased concentration of VFA in the rumen, more ruminally degradable OM, and higher OM intake, as well as decreased milk fat percentage, forage NDF, and particle length index (Allen, 1997; Mertens, 1997). De Veth & Kolver (2001a) suggested that cow performance will not be affected as severely when the pH decreases below pH 6.0 on high quality pasture as suggested by Pitt *et al*., (1999).

The reported incidence of ruminal acidosis on pasture are very few, even though Stockdale (1994) reported a pH value as low as 5.6 in dairy cows fed pasture. O'Grady *et al.* (2008) found that cows grazing predominantly ryegrass pastures have the potential to develop SARA. Rearte *et al.* (1984) reported a normal pH for cows grazing high quality pasture with added concentrate. In that case, the average pH remained above 6.9 throughout the trial. Kolver & de Veth (2002) found that even though ruminal pH ranged between 5.8 and 6.2, dairy cow performance was not negatively impacted. It was further concluded that if depressed DMI and milk production are indicators of SARA then above-mentioned pH range is not related to SARA for cows fed highly digestible pasture. De Veth & Kolver (2001a) proposed that high quality pasture is highly digestible and the lactic acid concentration in the rumen associated with it, is low. De Veth & Kolver (2001a) confirmed that when cows are fed highly digestible pasture the product of fermentation responsible for the low pH is more likely VFA than lactate. Furthermore the preferred degradation of starch instead of fibre by microbes on high concentrate diets, are not present in pasture diets. Mould *et al*. (1984) found that pasture digestibility is less affected by a reduced pH than feeds of low quality. Bramley *et al*. (2008) stated that ryegrass and other forages high in NFC may increase the risk for acidosis. Extensive surveys on pasture are however lacking.

2.4 Sub-acute ruminal acidosis (SARA)

2.4.1 General information

Acidosis can be defined as the decrease in the alkali component in body fluids relative to the acid content (Stedman, 1982). Acidosis can be acute or subclinical. The incidence of acute acidosis exhibits as an illness when the animal consumed great amounts of readily fermentable carbohydrates and the ingesta pH is reduced. Subacute ruminal acidosis is a digestive disorder that is difficult to diagnose because it is subtle, nonexclusive, and often delayed from time of

incidence (Enemark, 2008). Subclinical acidosis is expressed in that feed intake and animal performance is reduced but animals may not appear sick. Ruminal pH is the only reliable tool to diagnose SARA (Keunen *et al*., 2002). Ruminal pH is measured to differentiate between acute and subclinical acidosis, with pH 5.2 and 5.6 used as yardsticks respectively (Cooper & Klopfenstein, 1996). There is however disagreement to the precise threshold indicative of SARA. Plaizier (2004) used pH 6.0 as the threshold that is indicative of SARA because below this value fibrolytic bacteria growth is impaired (Shi & Weimer, 2002). Duffield *et al*. (2004) however indicated that SARA occurs when ruminal pH is depressed below 5.8 (Nordlund & Garrett, 1994). Krause & Oetzel (2006) stated that SARA could be defined as a moderate depression in ruminal pH, where pH ranges from about 5.5 to 5.0. Enemark *et al.* (2002) agrees with this pH range as indicator of SARA. The best indicator for SARA seems to be with continuous pH monitoring where the time below a certain pH is used as diagnostic measure (Keunen *et al*., 2002). These episodes are usually between chronic and acute in duration (Garrett *et al*., 1999; Nordlund *et al*., 1995). According to Kleen *et al.* (2003), Stone (2004) and Gozho *et al*. (2005) SARA occurs when pH is depressed (< 5.6) for prolonged periods daily (> 3 h/day). With SARA the pH recovers after every bout of low pH which is unlike the situation with acidosis (Beauchemin & Penner, 2009). When bouts of low ruminal pH last for more than 3 – 4 h, impaired fibre digestion (Russell & Wilson, 1996), decreased absorptive capacity (Harmon *et al*., 1985) and damage to rumen epithelium may occur (Beauchemin & Penner, 2009).

Bicarbonate, the natural occurring buffer in the body, is responsible for buffering the pH of body fluids. The degree of bicarbonate buffering possible, will determine whether the body fluid pH will be depressed during acidosis. A decrease in bicarbonate can affect the functioning of the central nervous system even if the blood pH is not adversely affected (Owens *et al.*, 1998). SARA affects between 10% and 40% of dairy cattle in a herd, which could result in large financial losses as well as concern for animal welfare (Garrett *et al*., 1999). A problem posed is that for energetic efficiency to be maximised a high extent of fermentation is desired whereas for acidosis prevention a slow rate is preferred (Owens *et al.*, 1998).

2.4.2 Effect on ruminal parameters

2.4.2.1 Volatile fatty acid composition

A decrease in pH during starch fermentation by mixed bacteria caused a reduced acetic acid molar proportion compared to a rise in propionic and butyric acid proportions (Marounek *et al*., 1985). Strobel & Russell (1986) in comparison reported an increase in butyric and lactic acid and a decrease in propionic acid molar proportions during a decrease in pH when starch was fermented by mixed bacteria. In the same experiment, other substrates increased the proportions of butyric,

lactic and propionic acid at a decreased pH. Results are thus quite contradicting but it can be concluded that there is a definite pH-substrate interaction. Russell (1998) concluded in a study done to investigate the effects of pH and feedstuff on VFA profile that in the pH rage of 5.8 – 6.5, pH explained about 25% of the decrease in acetate to propionate ratio. In a study by Calsamiglia *et al*. (2008) it was found that acetate as well as butyrate were reduced by the effects of pH but were not affected by the substrate present. This study was done in the pH range 4.9 – 7.0 and the concentrate levels were 400 g or 900 g concentrate/kg. This same study indicated that propionic acid concentration increased with a decrease in pH and was higher with the higher concentrate content diet. These results indicate the effects of pH on VFA profile;the in vitro and in vivo results differ rather significantly however (Dijkstra *et al*., 2005) and care should be taken when making decisions based on in vitro studies. According to Harmon *et al*. (1985) repeated exposure to low ruminal pH will also adversely affect the absorption potential of the ruminal epithelium.

In the study by De Veth & Kolver (2001a) it was found that total VFA production increased ($P<0.05$) quadratically with an increase in pH. It seemed that VFA and specifically acetate was lowest at pH 5.4 and only small differences were found between pH 5.8 and 6.6 (De Veth & Kolver, 2001a). Propionate followed the same pattern as acetate and total VFA. For butyrate, iso-butyrate, valerate, and iso-valerate there was no clear connection with the pH fluctuations (De Veth & Kolver, 2001a). The linear increase between VFA and pH is consistent with results found in continuous culture studies (Hoover *et al*., 1984; Shriver *et al*., 1986). VFA production is stated as being a function of microbial growth, which is why a relationship would probably be noted between the VFA production and microbial activity. Russell (1998) found that the greatest reduction in microbial growth occurs below pH 5.7 which is closely related to the critical value for VFA production as well.

2.4.2.2 Rumen Ammonia

The removal of VFA from the rumen by absorption increases the inflow of urea into the rumen (Thorlacius *et al*., 1971). Urea is converted to ammonia as soon as it enters the rumen. The high pK value of ammonia causes it to easily bind protons and it is then mostly present as NH_4^+. When NH_4^+ is then removed from the rumen, the proton is removed as well. A study by Abdoun *et al*. (2007) indicates that at a pH of 6.5 or lower, the affinity for NH_4^+ absorption is higher than for NH_3 absorption. Up to 90% of urea in the body can be recycled to the gut with the greatest amount being brought back to the rumen. Data about the mechanism and amount of urea transferred to the rumen and ammonia absorbed are limiting, it may however be that the secretion of urea and the absorption of ammonia are important in ruminal pH regulation. Feeds with higher degradable protein content diet could thus inhibit a decrease in pH as caused by rapidly fermentable carbohydrates, by removing the increased H^+ ions in the form of NH_4^+. At a low ruminal pH the

protein synthesis by microbes are inhibited and ammonia not captured, causing an increased excretion of N via the urine (Dijkstra *et al*., 2012).

In the study by De Veth & Kolver (2001a) it was found that the ammonia-N concentration was lowest at pH 5.8 and accordingly highest at pH 6.6. It was however surprising in this study that an increase in ammonia was found when pH decreased from 5.8 to 5.4. The explanation given for this phenomenon was that the utilisation of ammonia by the microbes possibly decreased, which could be indicative that proteolytic bacteria are still active at lower pH. Wallace *et al*. (1997) proposed quite a wide range for proteolytic bacteria from 5.5 to 7.0, which corresponds with the finding that proteolytic bacteria are still active at low pH.

2.4.3 Effect on production parameters

Low ruminal pH directly impairs energy intake and protein absorption which are key factors limiting production potential of high producing dairy cows (Allen, 1997). According to Sutton (1989) and Kennelly & Glimm (1998), milk fat respond well to dietary manipulation (change up to 3 %) compared to milk protein that only shows minor changes of up to 0.6 percentage units. A study on a New York state dairy farm indicated the detrimental effect of SARA on production parameters. Milk yield was reduced by 2.7 kg/day, milk fat by 0.3 percentage units and milk protein by 0.12 percentage units (Stone, 1999).

2.4.3.1 Milk fat

Milk fat percentage is known as a diagnostic measure for SARA (Allen, 1997; Mertens, 1997), apparently it reflects the adequacy of ruminal pH and eNDF. The lower NDF content of fresh forage as well as less mastication needed because fresh forage are not as mature, have been noted to cause a lower milk fat percentage (Eastridge, 2006). Vazquez & Smith (2000) found that FCM production is highly associated to DMI, pasture dry matter intake (PDMI), and NDF of pasture selected. According to Allen (1997) there is a positive relationship between ruminal pH and milk fat percentage, and it was found that a decrease in ruminal pH from pH 6.5 to 5.8 will cause a decrease in milk fat from 4.5 % to 3.0%. Kolver & de Veth (2002) also found a decrease in milk fat percentage (from 4.7 % to 4.0 %) over the pH range 6.5 to 5.8, the decrease in milk fat percentage was however about half of what Allen (1997) predicted it to be. It is also possible that the decrease in milk fat content that was found in Kolver & de Veth (2002) study is attributable to the decreased milk fat percentage that is commonly found when milk yield increases.

Results regarding the relationship between ruminal pH and milk fat is variable. Allen (1997) found a relationship when summarising data from various trials, whereas Garrett (1996) indicated the relationship to be poor and the study by O'Grady *et al.* (2008) was in agreement with this.

Experimentally induced SARA are also inconsistent as to its effect on milk fat. The reason for this is proposed to be the duration of SARA exposure. Where short bouts of ruminal pH depression was found to not impair milk fat content (Krause & Oetzel, 2006).

Changes in milk fat content are related to the VFA changes in the rumen (Meijs, 1986). The ratio of acetate:propionate produced determines the milk fat content. This ratio is determined by the feed consumed (Meijs, 1986; Kennelly & Glimm, 1998; Bargo *et al.*, 2003; Sairanen *et al.*, 2006). Starch rich concentrate supplements are known to produce more propionate which in turn depresses the milk fat content (Meijs, 1986; Carruthers & Neil, 1997; Bargo *et al.*, 2003). The relationship between milk fat and rumen acetate:propionate ratio is curvilinear and milk fat percentage declined the most when the acetate:propionate ratio was less than 2.0 (Erdman, 1988). Drops in pH associated with high levels of concentrate fed, inhibits cellulolytic bacteria activity. This results in a decrease in acetate production which negatively affects milk fat content (Van Soest *et al.*, 1991; Bargo *et al.*, 2002b; Baumann & Griinari, 2003). Milk fat is the most sensitive to dietary manipulation of all the milk solids (Stockdale *et al*., 2003).

2.4.3.2 Milk protein

The milk protein content does not vary greatly and shows little response to protein levels in supplements (Bargo *et al.*, 2003; Sairanen *et al.*, 2006). With high levels of protein supplementation the milk protein did not vary whereas the milk production showed a 6 – 18 % increase (Bargo *et al.*, 2003). Microbial protein synthesis is optimised when a carbohydrate source is supplied to the rumen microbes (Bargo *et al.*, 2002b; Sayers *et al.*, 2003; Schwab *et al.*, 2008). Tas *et al*. (2005) mentioned stimulating milk protein by increasing propionate production in the rumen. From Meijs (1986) it is clear that starch rich concentrates stimulate propionate production and is thus associated with a possible depressed pH.

2.4.3.3 Milk lactose and somatic cell count

Milk lactose content is the most stable of all the milk components and nutritional manipulation is not common (Sutton, 1989; Kennelly & Glimm, 1998; Schwab *et al*., 2008). Variations are likely to occur with breed or protein content (NRC, 2001). Lactose are known to decrease in response to high SCC (Kitchen, 1981) because of increased osmotic pressure in the mammary gland (Welper & Freeman, 1992). Gibson (1989) reported 4.7 % as the average lactose content for Jersey cows. A lactose range of 4.61 – 5.04 % was determined across six dairy breeds (Welper & Freeman, 1992), and the NRC (2001) reported 4.85 % as the average lactose content of milk. Changes in milk lactose can be seen as unimportant because it is only likely to occur under severe feeding conditions (Jenkins & McGuire, 2006).

Somatic cells are generally consistent of udder epithelial cells and leukocytes (De Villiers *et al.*, 2000). The number of these cells present in the milk is subject to lactation number and udder irritation or injury (De Villiers *et al*., 2000). The leukocyte concentration corresponds to udder health and could impair milk production (Kitchen, 1981; De Villiers *et al*., 2000). Somatic cell count is used as an udder health indicator, where a count above 300 000 cells per mL of milk is indicative of subclinical mastitis and abnormal (De Villiers *et al.*, 2000). The SCC has to be maintained below 500 000 cells per mL milk to be used for human consumption (De Villiers *et al.*, 2000).

2.4.3.4 Milk urea nitrogen

Milk urea nitrogen can be used as a nutritional health indicator for lactating dairy cows (Kohn, 2007). Protein enters the rumen where is it hydrolysed by micro-organisms to NH_3 (Parker *et al*., 1995; Bucholtz & Johnson, 2007). Approximately 10 % flows through to the small intestine (Parker *et al*., 1995; Huntington & Archibeque, 2000) where ammonia is absorbed from the gastrointestinal tract, transported to the liver and converted to urea (Parker *et al.,* 1995; Huntington & Archibeque, 2000; Bucholtz & Johnson, 2007). This urea filters back to the blood and is eventually recycled back to the rumen or is excreted via urine or milk. The blood urea concentration is directly proportional to the excreted urea in the urine or milk (Jonker *et al.,* 1998). Milk urea nitrogen and blood urea nitrogen have extensively been used to indicate the protein status of the diet (Jonker *et al.*, 1999). Pasture based dairy cows supplemented with concentrate supplement has an improved microbial activity which enhances nitrogen utilisation and decreases MUN excretion (Carruthers & Neil, 1997; Sairanen *et al*., 2006). Variation in MUN corresponds to protein to energy ratio (Roseler *et al.*, 1993).

The recommended MUN differ as influenced by milk yield, stage of lactation and live weight changes (Kohn, 2007). The recommended acceptable range is between 8 and 12 mg/dL, as sampled from a bulk tank (Kohn, 2007). Individual sampling however varies greatly (8 – 25 mg/dL) and values should therefore be averaged to be useful (De Villiers *et al.*, 2000).

2.5 Buffers

A buffer can be described as a salt of a weak acid, oxide or hydroxide which neutralises acids in the feedstuffs or, acids produced during the digestion and metabolism of nutrients (Chalupa & Schneider, 1985). A true buffer is said to lessen a decrease in pH but not cause an increase in pH. Erdman (1988) stated that a buffer is known by the fact that in aqueous solution it will resist change in pH when a strong acid or base is added to the solution. For a substance to be a buffer, it has to meet the following requirements: it has to be water-soluble, it should be a weak acid or base or salt thereof, and its pKa has to be near the physiological pH of the substance to be

buffered (Erdman, 1988). Buffers present itself in three ways, in saliva, naturally occurring in the ingested feed, and added dietary buffers (Erdman, 1988).

2.5.1 Natural buffering systems

The two main buffers responsible for the neutral conditions in the rumen are bicarbonate and phosphate (Counotte *et al*., 1979). Saliva contains inorganic buffers (Church, 1988) and other substances such as sodium, potassium, bicarbonates and phosphates (Van Soest, 1994). Buffering from saliva can also be known as endogenous buffers (Krause & Oetzel, 2006). Factors that are important in determining saliva flow are feed DM, forage intake, and forage particle size (Erdman, 1988). The role of dietary fibre in saliva production is not clear but saliva production has been related to feed intake level (Dijkstra *et al*., 2012). An increased time spent chewing, i.e. eating and ruminating, is assumed to cause greater saliva production (Church, 1988). Smaller forage size generally lowers the saliva production because of the decreased chewing time (Woodford *et al*., 1986). Roughage consumption is associated with an increased salivation rate when compared to concentrate consumption. Maekawa *et al.* (2002) however found that resultant saliva production is unchanged because even though peNDF stimulates chewing and salivation, resting saliva production is unchanged. Staples & Lough (1989) states that there is no relation between VFA content increase and saliva production.

The bicarbonate present in the saliva is the predominant buffer in the body of the ruminant. Half of this buffer originates from the saliva and the other half enters in exchange for ionised acids absorbed (Owens *et al.,* 1998). Bicarbonate concentration in the saliva remains constant at about 120mM, and the secretion rate can be approximately 250 L/d (Erdman, 1988; Cassida & Stokes, 1986). Dijkstra *et al*. (2012) noted that the amount of bicarbonate present in the saliva is typically higher than the amount included in the diet. When the assumption is made that 90% of bicarbonate is converted to CO_2, the total amount of bicarbonate in the rumen can bind 60% of protons released on a high roughage diet and 50% when on a high concentrate diet (Dijkstra *et al*., 2012). The downside of salivation is however that it is not triggered by decreased ruminal pH but by the time the cows spends eating, ruminating and resting (Maekawa *et al.,* 2002), diet composition is therefore important in stimulating buffering.

The dietary cation-anion difference (DCAD) largely explains the natural buffering capacity of the diet. It has been found by Block & Sanchez (2000) and Sanchez *et al*. (1994) that higher DCAD are present in diets with higher Na and K relative to Cl and S, these diets tend to support higher ruminal pH, and increase DM intake as well as milk production. DCAD can be defined as milliequivalents of Na^+ K – Cl per unit of DM. Studies conducted over the time period 1984 to 1997 were investigated by Hu & Murphy (2004), where it was found that an increase in DCAD caused a

quadratic increase in milk yield and DM intake. Further increases were in blood pH and HCO_3 concentration, which could indicate an improved acid-base balance in the cows (Hu & Murphy, 2004). Diets formulated to supply a high DCAD typically require inclusion of buffers in the formulation (Krause & Oetzel, 2006). Diets with a high concentrate content have low or negative DCAD which adds to the risk of acidosis, which is already present because of high fermentable carbohydrates in the diet (Krause & Oetzel, 2006).

2.5.2 Exogenous buffering

It is possible that beyond the fact that forages create buffering by stimulating saliva production it has inherent buffering capacity (Erdman, 1988). Exogenous buffering explains this phenomenon of buffering by the diet itself. This has an important effect on ruminal pH. The buffering capacity of feeds are dependent on the existing relationship between strong cations and anions in the feedstuff. The capacity for cation exchange is influenced by the concentration of charged groups such as proteins and lignins, these will exchange cations for protons (McBurney *et al*., 1983). The buffering capacity differs considerably between feedstuffs. Studies done by Jasaitis *et al*. (1987) proved that cereal grains have a low buffering capacity (Erdman, 1988), grasses and feeds with a low protein content have an intermediate buffering capacity (Allen, 1997), and legumes and other feeds with a high protein content have a high buffering capacity. Protein sources and hays are very effective in buffering from pH 4 to 9 (Erdman, 1988). The buffering capacity of forages also tend to increase with increased maturity (Jasaitis *et al*., 1987). Allen (1997) compared buffering capacity of feeds with the effects of saliva and found that the effect of saliva was still far greater. The theory of physical effective fibre was developed by Mertens (1997) this is indicative of the ability of a feed to stimulate chewing and therefore saliva production and buffering in the rumen. In this regard Pitt *et al.* (1996) reported that NDF itself is not an adequate measure to determine effectiveness of fibre in buffering through increased salivation. Pitt *et al.* (1996) indicated that NDF can explain 30 % of ruminal pH variation.

The particle size method to determine eNDF or peNDF is not applicable to fresh pasture, which makes the determination thereof quite difficult (NRC 2001).

2.5.3 Inclusion of buffers

Buffers added in the diet are usually most effective 4 – 8 hrs after feeding when the pH are proved to be at its lowest (Erdman, 1988). Erdman (1988) even states that the main effect of dietary buffers is to reduce the pH depression occurring 2 to 8 hrs after feed consumption.

2.5.3.1 Sodium Bicarbonate

The supplementation of sodium bicarbonate (SB) in the diet of high producing dairy cows is standard practice in many parts of the world. Sodium bicarbonate inclusion in diets should be

limited to prevent reduced intake because of decreased palatability (Rauch *et al*., 2012). Results considering the effects of SB on rumen pH are varying in different studies. The typical response expected is an increase in pH, but there is however reports of no effects (Hu & Murphy, 2005) or even a decrease in pH (Rogers *et al*., 1985).

Hu & Murphy (2005) after looking at results from 30 different studies, concluded that effects of SB is dependent on the type of forage in the diet of lactating dairy cows when fed total mixed rations (TMR's). In that specific study advantages were restricted to corn silage based diets. The difference in response to SB relative to the main forage source in the diet could partly be due to the fibre content of the forage source. In a study reported by Rauch *et al.* (2012) a likely increase in pH is proposed based on the tendency found in the SB diet in an in vitro study. Khorasani & Kennelly (2001) found that when rumen pH ranged from 5.7 to 6.8 buffer addition had no effect. Erdman (1988) indicated that SB has the capacity to increase the milk fat percentage by 0.4 percentage units. SB further increased rumen pH by up to 0.26 percentage units and the rumen acetate:propionate ratio by 0.52 percentage units. This was however in low forage diets. Diets containing > 30 % forage had smaller responses in milk fat when buffer was added. This is also true for the increase in acetate:propionate ratio and pH as caused by SB inclusion, 0.15 and 0.05 respectively. Little effect was found for milk production when SB was added to the diet. When SB was fed on pasture based systems Erdman (1988) noted little response for intake, milk yield and milk composition.

Recent advances in the effects of Na on the environment have caused greater concern and regulations in California (California Regional water control board, 2007). Boundaries are already set to limit the discharge of fixed solids (FS), Na being one of these. High Na levels have been found to adversely affect ground and surface water for consumption by humans or livestock, or for irrigation purposes (Berg *et al*., 2010). It contributes to the soil degradation, which causes reduced biomass yield (Mengel & Kirkby, 2001). The use of SB has shown to increase the excretion of Na, and as SB is widely used it might be viable to consider a different buffer for inclusion in lactating dairy cow rations.

2.5.3.2 Acid Buf

According to Enemark (2008) Acid Buf has twice the buffering capacity of sodium bicarbonate increasing milk yield and feed conversion. It is made from calcified red seaweed (*Lithothamnium calcareum*) harvested off the Irish coast. In the manufacturing process it is washed, dried and milled to obtain a grey to off-white powder as final product. The supplementation of Acid Buf in high concentrate dairy cattle diets showed an increased ruminal pH (Cruywagen *et al.*, 2004). Cruywagen *et al.* (2004) furthermore determined 0.3 % of DMI (80

g/cow/day) as the optimal inclusion level of Acid Buf for ensuring high productivity and milk composition. Cruywagen *et al.* (2007) reported that Acid Buf had a great influence on ruminal pH and could possibly prevent SARA, especially when compared to the effect of sodium bicarbonate. Beya (2007) stated the greater buffering capacity of Acid Buf when compared to sodim bicarbonate was probably due to slow release.

2.5.4 Response of ruminal parameters to buffer inclusion

Research regarding this topic is variable and limited. Most information included refers to total mixed ration diets.

Kennelly *et al.* (1999) found in a study with TMR fed cows that the treatments with added buffer did not exhibit a change in average rumen pH. In the study by Khorasani & Kennelly (2001) total VFA concentration was increased by the addition of SB, in the diet. These results were on 50:50 or 75:25 concentrate to forage based diets. Rearte *et al.* (1984) stated that proportions of VFA were not affected by buffer when supplemented in concentrates fed to cows grazing high quality pasture. Kennelly *et al.* (1999) found that buffer inclusion in the diet did exhibit an increase in total VFA production. The diets containing buffer in the TMR caused an increase in the average concentrations of rumen acetate, butyrate, iso-butyrate, valerate, iso-valerate, and the acetate:propionate ratio; propionate being the only VFA to decrease because of buffer inclusion.

Khorasani & Kennelly (2001) also found that rumen ammonia concentration was not affected by the addition of buffers, especifically SB, in the diet. These results were from cows fed 50:50 or 75:25 concentrate to forage based diets. In a TMR study done by Kennelly *et al.* (1999) it was found that the treatments with added buffer had higher rumen NH_3-N.

2.5.5 Response of production parameters

2.5.5.1 Milk Production

Milk yield increased when buffer was added to a TMR diet with a 75:25 concentrate to forage ratio, no effect on milk yield was however found when a diet containing 50:50 concentrate to forage was fed (Kennelly *et al.*, 1999). In the trial conducted by Rearte *et al.* (1984) it was found that milk production, milk fat content and milk protein content was not affected when buffer was added in the concentrate supplemented to pasture. Khorasani & Kennelly (2001) reported that the addition of buffer to the diet fed to lactating dairy cows did not affect milk yield; milk fat depression was however prevented upon buffer addition to the diet. This was also reported in other studies by Kalscheur *et al*. (1997) and Kennelly *et al*. (1999). The key factor involved here is probably the stabilisation of the rumen pH environment for sensitive bacteria (Khorasani & Kennelly, 2001). Wenping & Murphy (2005) found that milk production and the protein proportion as well as yield

was unaffected by the inclusion of buffers in the concentrate regardless of the type of forage they were fed.

2.5.5.2 Milk composition

Erdman (1988) found in a study that buffers help to improve milk fat depression, this is said to be because of the decrease in pH that is prohibited by the buffers. Kennelly *et al.* (1999) found that 4% FCM and milk fat yield were increased when adding buffer to TMR diets. Rearte *et al.* (1984) found that added buffer in the concentrate did not alleviate the slight depression in milk fat when cows grazed pasture with supplemented concentrate. Prior to the trial the milk fat was recorded as 3.6% and it remained this low throughout. This is in accordance with previous studies (Fisher, 1979; McClymont, 1950; Muller *et al*., 1979; Waite *et al*., 1959).

An increase was found for the 4% FCM and the milk fat yield, whereas milk protein and lactose were unaffected by the addition of buffer in the diet (Khorasani & Kennelly, 2001). Kennelly *et al.* (1999) found that only the treatment groups with TMR diets containing buffer was affected considering the milk protein yield. The diet containing 75:25 concentrate to roughage ratio showed an increase in milk protein yield whereas the treatment with a 50:50 ratio had a lower yield. It has however been accepted according to literature that dietary buffer do not consistently change milk protein content (Cassida *et al.*,1988; Harrison *et al.*, 1989, Xu *et al.*, 1994). Buffer addition to TMR diets had a moderate increasing effect on the milk lactose concentration (Kennelly *et al.*, 1999).

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Chapter 3: Pasture management

3.1 Introduction

In this chapter the pasture management concerning establishment, yield determination, and grazing management of kikuyu over-sown with ryegrass pasture is represented. The nutritive value of the kikuyu/ryegrass pasture grazed by the experimental group of cows from both the production (Chapter 4) and the rumen (Chapter 5) studies, is also presented. For ease of reference all future accounts of pasture will refer to ryegrass pasture.

3.2 Materials and Methods

3.2.1 Location and environment

The study was conducted at the Outeniqua Research Farm near George in the Western Cape Province of South Africa. The coordinates for the farm is 22° 25' 222" E and 33° 58' 702" S at an altitude of 204 m above sea-level. George is known for its temperate climate and higher than average rainfall. The total average rainfall per annum for the past 47 years (1967 – 2012) was approximately 740 mm. The monthly average rainfall from September to November, the period over which the trial was conducted, over the last 47 years' was recorded as 69.6 mm. Minimum and maximum temperatures for this area was 10 ºC and 20 ºC respectively, as calculated over September to November from 2006 to 2012. Daily and cumulative monthly rainfall as well as the average minimum and maximum, and daily temperatures were recorded and obtained from the Agro-Climatology database (ARC, 2012). The soil type of the paddock used for the trial is locally referred to as a Witfontein soil form (Soil Classification Working Group, 1991).

3.2.2 Paddock design

The 8.55 ha paddock used consisted of kikuyu pasture, over-sown with Italian ryegrass. Italian ryegrass, cultivar Jeanne, was sown in March 2012 at 20 kg per ha using an Aitchison seeder (2.4 m Aitchison 3116 C Seedmatic with 16 rows). During the trial period the botanical composition consisted mainly of Italian ryegrass pasture, and since it was during spring, it provided ideal growing conditions for Italian ryegrass (Van der Colf, 2011). The paddock was under permanent irrigation and divided into 39 strips by putting up poly wire with an electrical current. On average, the strips were measured to be 150 m in length and 15 m in width, the exception was strips 35 to 39 where the paddock made a slight angle which resulted in shorter strips (see Figure 3.1). The paddock was divided into strips to easily allocate specific amounts of pasture to dairy cows. Strips were divided by electrical fencing along the 9 sprinklers of the irrigation system, with equal distances of 15 m apart. The distance between the sprinkler lines was 15 m resulting in an

area of 225 m^2 for each sprinkler block. These areas were used to simplify pasture allocation. Cows on the paddock had *ad libitum* access to fresh water at all times.

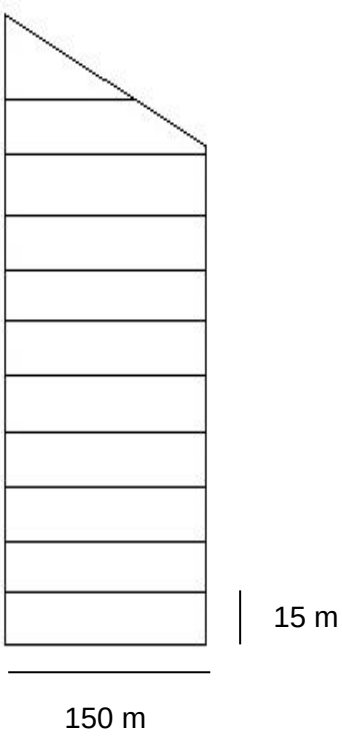

Figure 3.1 Structure of the paddock used for 60 Jersey cows grazing ryegrass during spring

3.2.3 Pasture management

3.2.3.1 Establishment

Kikuyu (*Pennisetum clandestinum*) is being used as a permanent pasture base on the paddock. Annual Italian ryegrass (*Lolium multiflorum Lam. var. italicum*) is over-sown once a year to increase pasture productivity. For the study, Italian ryegrass was over-sown in March of 2012 to ensure optimal establishment by spring 2012, i.e. September. Prior to over-sowing, kikuyu was grazed to a height of 50 mm above ground, after which kikuyu was mulched to ground level and the seeds were drilled into the mulch layer using an Aitchison Seedmatic-3116C seeder (Botha, 2003; Van der Colf, 2011). The seeding rate applied was 25 kg/ha (Van der Colf, 2011).

3.2.3.2 Pasture grazing, fertilisation and irrigation

A total of 60 Jersey cows strip grazed the 8.55 ha paddock as a group between milking times. The cows started grazing at the eastern side of the paddock and moved through to the western side. Pasture was grazed to a height of 50 mm and a grazing cycle of approximately 28 days was maintained, which is consistent with the recommendation of Van der Colf (2011). The

strips were fertilised with limestone ammonium nitrate (LAN, 28% N) at 42 kg N/ha after each grazing, which amounted to three bags of fertiliser per ha. Irrigation was scheduled as needed after fertilisation to reduce N losses. Irrigation was scheduled to ensure tensiometers were maintained at a reading of -25 kPa.

3.2.4 Pasture measurements

Pasture height was measured using the RPM with a disk area of 0.098 m^2. The mean height was determined by taking 100 RPM readings in a zigzag pattern over the entire strip the day before grazing and also after each grazing. The total height was then divided by the amount of readings (100) to determine the average height of the pasture on the strip. To determine the amount of pasture consumed by the grazing cows the difference in before and after grazing height was inserted into a regression equation that estimates the pasture yield in kg DM/ha. The grass DM intake per cow per grazing was determined using the regressin equation. To get the regression equation samples were cut weekly, three at each sward height (low, medium and high sward growth). Samples were cut using a metal ring designed to measure 30 mm from the ground and to fit around a RPM. The samples were cut by placing the metal ring at random sites on each specific strip. These samples were dried at 60°C for 72 h (Botha, 2003) to determine the DM content and thus the DM yield of the pasture. The seasonal regression used is a linear model that correlates the pasture height (RPM) to DM pasture yield (Earle & McGowan, 1979), in the following structure: Y = (a x H) + b, where 'Y' is dry matter yield in kg DM/ha, 'a' = gradient, 'H' = recorded RPM height, and 'b' = intercept value. The sward height as measured pre-grazing was placed in the regression equation to estimate the yield on the specific strip. Thereafter an equation was used to determine what the amount of sprinkler blocks had to be allocated to provide in the dairy cows daily requirement. This seasonal regression is a good way to estimate the pasture yield and allocate pasture accordingly.

3.2.5 Pasture allocation

Pasture was allocated to grazing cows at approximately 10 kg DM/cow/day above 30 mm, to ensure that ample pasture is supplied and to leave a range for error. Previous experience has shown the use of the seasonal regression equation is the best way to allocate pasture. Pasture allocation was done based on the DM herbage yield on the specific strip above 30 mm (or a RPM reading of 6), which was determined by using a general seasonal regression. The equation used for pasture allocation was Y = (H x 119.94) – 897.71, where 'Y' = herbage DM yield (kg DM/ha) and 'H' = recorded height of RPM. Van Wyngaard (2013) determined this equation by cumulating regressions. The regression was specifically determined on kikuyu over-sown with Italian ryegrass during spring and it was used in this study to estimate pre- and post-grazing pasture measures. Pasture allocation was continually altered to keep the post-grazing height at 50 mm (a RPM

reading of 10). This was done by adjusting the allocated kilogram DM pasture per cow as based on the DM yield per ha determined by the seasonal regression equation used. Pasture allowance is usually estimated at ground level or 50 mm above ground level because it is accepted that everything below that level is not available for grazing (Dillon, 2006). Pasture availability at 30 mm aboveground is thus an overestimate to ensure ample pasture is available for the grazing cows and to create a range for error rather than to over-graze pasture. Pasture consumed was calculated as the pasture removed (the difference between pasture yield before and after grazing) per cow for the specific allocated area per day (irrigation head spaces converted into ha).

3.2.6 Pasture sampling

Pasture sampling was done every week on one specific strip the day before the strip was grazed; samples were cut at 30 mm (RPM reading of 6). Three randomly chosen areas were sampled and the samples were dried for 72 h at 60°C (Botha, 2003). Samples were weighed before and after drying to determine the DM content. The three samples per week were pooled and then samples from every two weeks were pooled to create a representative sample consisting of six original samples, this amounted to four samples over eight weeks that were analysed. These samples were milled (SCW Hammer mill, 1 mm screen) and preserved in a 0 – 4 °C cold room at Stellenbosch University laboratory while awaiting analysis.

3.2.7 Analytical methods

All four composite pasture samples were analysed in duplicate for parameters as mentioned in Table 3.1. The nitrogen (N) fraction of pasture was used to calculate the crude protein (CP) content using the formula CP = N x 6.25 (NRC, 2001). Samples were analysed for NDICP (neutral detergent insoluble crude protein) and ADICP (acid detergent insoluble crude protein) by analysing for CP on the residues of NDF and ADF, respectively. The *in vitro* organic matter digestibility (*IV*OMD) was done using sheep ruminal fluid obtained from ruminally fistulated sheep at Elsenburg experimental farm, Stellenbosch, South Africa. Gross energy and *IV*OMD is used to calculate the metabolisable energy content of the pasture, ME = 0.81 x GE x *IV*OMD for forages (ARC, 1984; MAFF, 1992). Pasture minerals determined were Ca, P and K. To estimate the non fibre carbohydrate (NFC) portion of the pasture the following formula could be used: NFC = [100 – (% NDF + % ash + % CP + % EE)] (NRC, 2001).

Table 3.1 Different analytical methods applied to determine pasture quality

Parameter[1]	Reference	Procedure
DM	AOAC, 2002	934.01
OM	AOAC, 2002	942.05
N for CP	AOAC, 2002	990.03, LECO FP-528
EE	AOAC, 2002	920.39
NDF	Robertson & Van Soest, 1981	ANKOM fibre analyser
ADF	Robertson & Van Soest, 1981	ANKOM fibre analyser
ADL	Robertson & Van Soest, 1981	-
***IV*OMD**	Buys *et al*., 1996/Tilley & Terry, 1963	Two stage procedure
GE	MC 1000 Modular Calorimeter	-

[1] DM - Dry Matter; OM - Organic Matter; N - Nitrogen; CP - Crude Protein; EE - Ether Extract; NDF - Neutral Detergent Fibre; ADF - Acid Detergent Fibre; ADL - Acid Detergent Lignin; *IV*OMD - *In Vitro* Organic Matter Digestibility; GE - Gross Energy

3.3 Results

3.3.1 Climate

The on-farm weather station was used to gather information on the weather conditions during the trial period; data was collected from the ARC (2012). In Figure 3.2 the total monthly rainfall for the duration of the trial, September 2012 to November 2012, is compared to the average total monthly rainfall from June 2006 to April 2013. The mean maximum and minimum temperatures for each month of the study were compared to the data of the same period over the last 7 years.

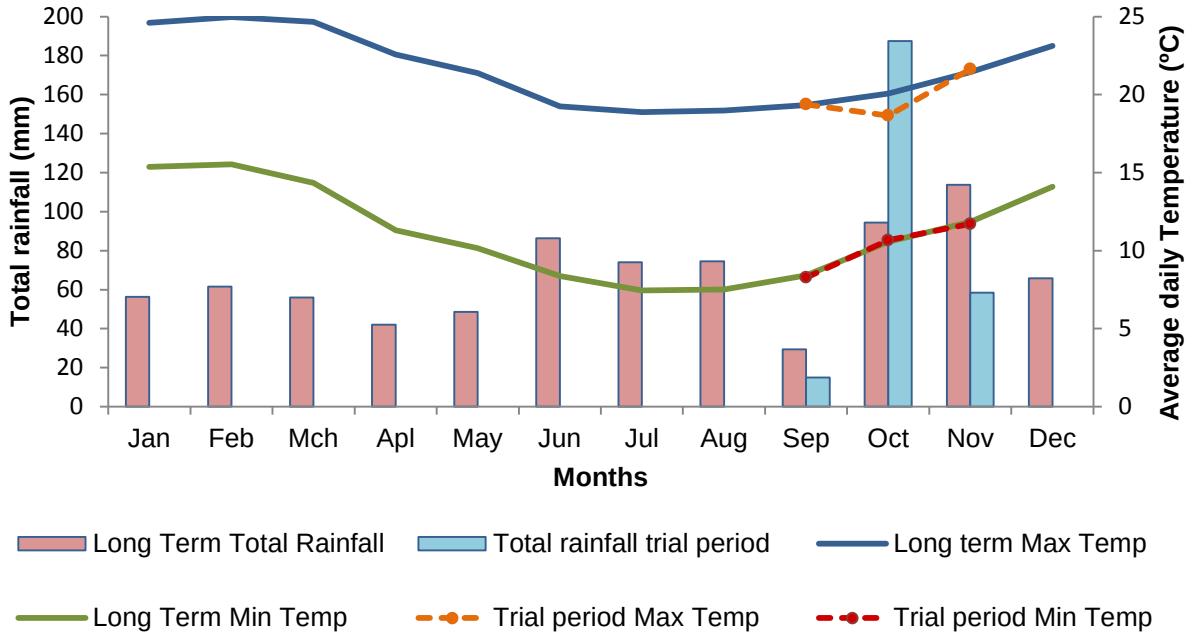

Figure 3.2 Long term (2006 – 2013) average monthly rainfall and maximum and minimum temperatures compared to monthly rainfall and maximum and minimum temperatures over trial period

3.3.2 Pasture management

The seasonal regression calculated from samples of ryegrass pasture cut throughout the duration of the trial is depicted in Figure 3.3. The equation generated is Y = 50.204*H – 76.046, where Y is the DM herbage available and H represents the RPM reading. This regression was constructed after the completion of the trial. The original regression equation (Section 3.2.5) was only used as an aid to allocate pasture.

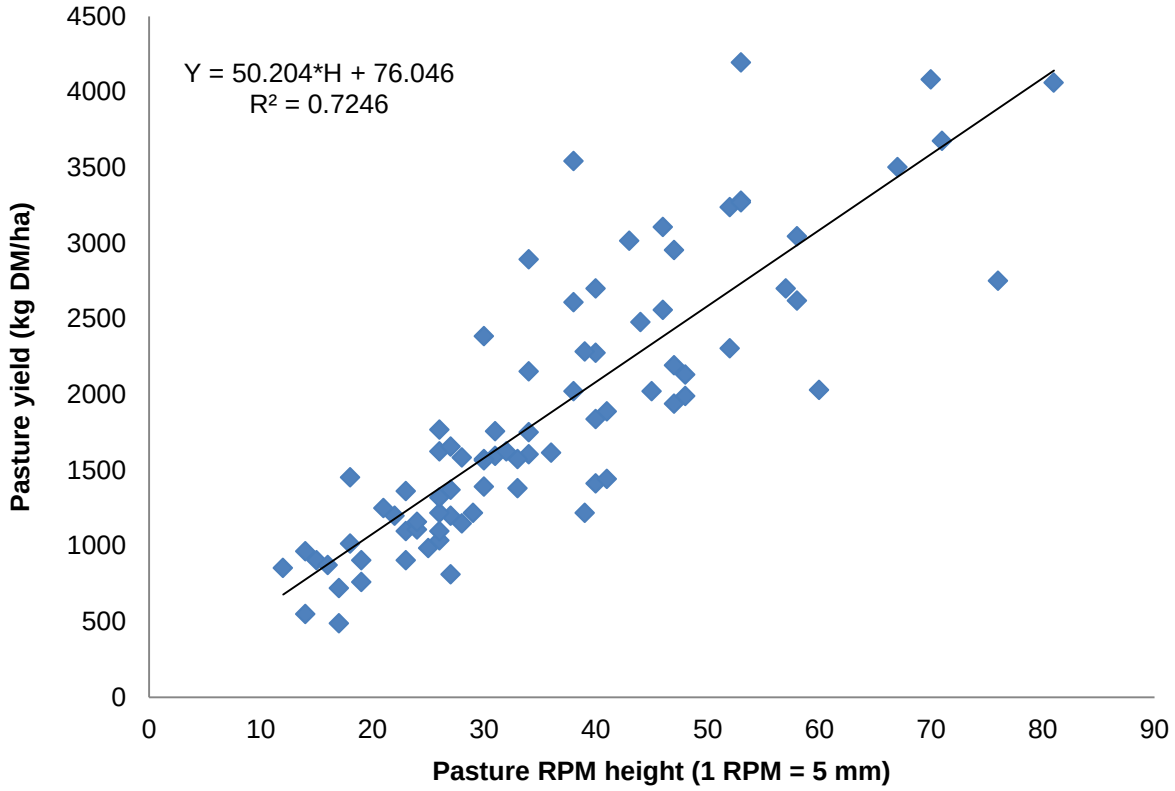

Figure 3.3 Pasture yield as affected by pasture rising plate meter (RPM) based on pasture samples cut during the trial period

In Table 3.2 the pre- and post-grazing average values as collected over the duration of the trial are depicted. The pasture allocated was lower than the 10 kg DM that was inserted into the regression equation (Section 3.2.5) to estimate the pasture allocation.

Table 3.2 Mean rising plate meter (RPM) readings, pasture dry matter (DM) yield and pasture allocation of ryegrass pasture before and after each grazing, determined by seasonal regression Y = 50.204 x H + 76.046

Parameter[1]	Pasture values
Before grazing	
RPM height	27.4 ± 7.49
Pasture yield (kg DM/ha)	1740 ± 276.2
Pasture allocated (kg DM/cow/day)	8.56 ± 1.495
After grazing	
RPM height	10.5 ± 1.41
Residual pasture after grazing (kg DM/ha)	604 ± 70.9
Pasture removed (kg DM/ha)	1136 ± 262.1
Pasture consumed (kg DM/cow/day)	4.9 ± 1.30

[1] RPM – Rising Plate Meter; DM – Dry matter

3.3.3 Pasture quality

The mean nutrient composition of ryegrass pasture for the duration of the trial during the spring of 2012 is presented in Table 3.3.

Table 3.3 Mean (± SD) pasture quality determined from pasture samples collected over eight weeks (n=4)

Nutrient (g/kg DM, or as stated)	
Dry matter	165 ± 18.9
Organic matter	901 ± 13.2
IVOMD (%)	81.8 ± 2.62
ME (MJ/kg)	12.2 ± 0.43
CP	170 ± 17.7
CP:ME ratio	1.39 ± 0.176
NDF	475 ± 44.7
ADF	246 ± 14.2
ADL	18.4 ± 3.91
NFC	22.0 ± 6.69
ADICP	59.0 ± 5.91
EE	36.5 ± 2.46
Ca	3.90 ± 0.424
P	3.90 ± 0.571
Ca:P ratio	1.02 ± 0.220

The effect of the progression of spring over the 8 week sample collection period is illustrated in Figure 3.4. A definitive increase in NDF, ADF and ADL can be seen as the season progresses. The DM, IVOMD and ME however decreased over the period of the trial.

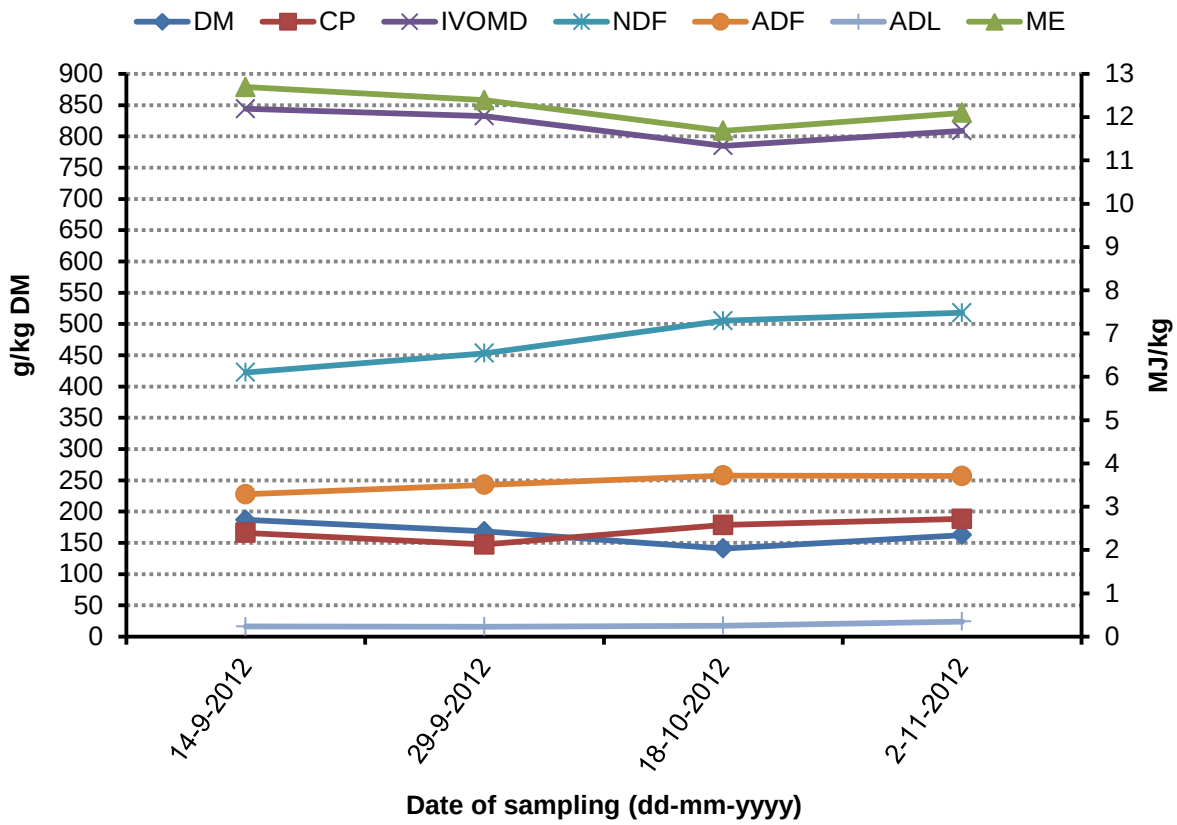

Figure 3.4 Effect of changing season (spring to summer) on nutrient composition of pasture from samples collected over eight weeks (DM – Dry matter; CP – Crude protein; IVOMD – In vitro organic matter digestibility; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; ADL – Acid detergent lignin; ME – Metabolisable energy)

3.4 Conclusion

Pasture quality was mostly as anticipated for spring but some nutrients proved to be lower than expected. In essence the effect of pasture plus concentrate fed, on different parameters need to be considered to make a definitive conclusion. The same for pasture intake, where the estimated intake can be considered very low but various factors need to be considered before any distinct conclusion can be drawn.

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Chapter 4: Milk production study

4.1 Introduction

The research consisted of two separate studies, a production study and a rumen study. Both were conducted at the same time and thus under identical conditions. This chapter will focus on the production study and the rumen study will be presented in Chapter 5. The production study was conducted to determine the effect of the supplementation of buffers in the concentrate fed to dairy cows grazing ryegrass pasture on the milk production and milk composition. The cows used for this study were grouped with the cows used in the rumen study, these cows grazed together and were milked together according to their treatment groups. The lactation data of the ruminally cannulated cows used in the rumen study was not included in the production study.

4.2 Materials and Methods

4.2.1 Animal welfare

The project was approved by the Animal Ethics Committee (DECRA no: R12/67) of the Western Cape Department of Agriculture. Care was taken of animals according to the Standard Operating Procedures for the Outeniqua Dairy Herd.

4.2.2 Duration of study

The study was conducted between the 13th of September 2012 and the 15th of November 2012. An adaptation period of 15 days was allowed, followed by a data collection period of 48 days for the production study.

4.2.3 Allocation of cows

Fifty four high producing Jersey cows from the Outeniqua Research farm dairy herd were used in the study. Multiparous cows were selected that were less than 250 DIM. To limit variation, first lactation cows were not included as they are smaller and produce less milk than multiparous cows. The cows were stratified according to milk production, days in milk (DIM), and lactation number, and then randomly allocated to three treatment groups in a randomized block design. The mean milk production, DIM and lactation number of the cows in each treatment at the beginning of the study is shown in Table 4.1. The values for milk production, DIM and lactation number at the beginning of the trial was taken from the average value for August of 2012. Cows within blocks were randomly allocated to one of three treatments (Control, Acid Buf, and Sodium Bicarbonate) to reach a total of 18 cows per treatment. Six ruminally cannulated cows were added for the rumen study (more information Chapter 5) and two cows were randomly assigned to each treatment and

rotated to have all cows on each treatment once. This amounted to three groups of 20 cows to ensure ease of feeding and milking in the parlour.

Table 4.1 Mean (± s.d.) milk production, milk parameters, live weight and body condition score of Jersey cows receiving one of three treatments at the start of the trial (n=18 per group)

Parameters[1]	Treatments		
	Control	Acid Buf	Sodium Bicarbonate
Milk yield (kg/cow/d)	17.9 ± 1.96	18.1 ± 2.10	18.1 ± 2.13
Milk fat (%)	5.00 ± 0.523	5.07 ± 0.487	4.99 ± 0.605
4% FCM (kg/cow/d)	20.6 ± 1.91	21.0 ± 2.34	20.7 ± 2.36
DIM (d)	83.5 ± 74.76	84.4 ± 80.03	73.2 ± 68.47
Lactation nr	4.11 ± 1.967	3.89 ± 1.875	3.94 ± 2.235
BW (kg)	371 ± 40.2	378 ± 41.9	373 ± 37.2
BCS (scale 1 to 5)	2.04 ± 0.129	2.13 ± 0.177	2.11 ± 0.246

[1] FCM – Fat corrected milk; DIM – Days in milk; BW – Live weight; BCS – Body condition score

4.2.4 Feed and pasture allocation

For more information regarding pasture allocation see 3.2.5. Cows grazed Italian ryegrass pasture as a group of 60 cows. Grazing time was 24 h each day except for the time spent in the milking parlour where concentrate was supplied. Cows were collected from pasture about half an hour before milking and they returned as soon as possible after milking. Cows were handled in a very subdued and calm manner at all times. Before each milking the cows had to be divided into their three respective groups, as per their treatment. Cows were identified according to their treatment group by means of a coloured and numbered tag attached to a light metal chain around their necks. Cows receiving the control treatment had red tags, blue tags for the Acid Buf treatment and yellow tags were used for the Sodium Bicarbonate treatment group. The three different groups were all separated into different holding pens and entered the milking parlour in the same order each milking session. Cows were fed concentrates individually in the milking parlour at an “as is” rate of 3.3 kg/cow/milking, which amounts to 6.6 kg/cow/day concentrate fed “as is”. Cows were milked at 05:30 and 14:00 every day. The inclusion of Acid Buf and Sodium Bicarbonate in the respective treatments was 1% and 2%, amounting to approximately 60 g/cow/day and 120 g/cow/day on a DM basis. The composition of the concentrates for the different treatments is

presented in Table 4.2. Acid Buf has a high Ca and Mg content (see Table 4.3), and therefore lower levels of MgO and feed lime were included in the Acid Buf treatment. This was to ensure similar concentrates over all treatments (see composition of treatments in Table 4.2). The three concentrate treatments were formulated to be iso-nitrogenous, and iso-energetic.

Table 4.2 The ingredients and chemical composition (g/kg) of different concentrate supplements fed to three different treatment groups

Ingredient (g/kg)	Treatments		
	Control	**Acid Buf**	**Sodium Bicarbonate**
Maize	620	620	620
Hominy chop	150	150	150
Wheat bran	114	112	89
Soybean Oilcake	40	40	45
Molasses	40	40	40
Feed lime	22	15	22
Salt	10	10	10
MgO	3	2	3
Premix	1	1	1
Sodium Bicarbonate	0	0	20
Acid Buf	0	10	0
Nutrients (g/kg DM basis or as stated)[1]			
Dry matter	864	864	867
Organic matter	944	952	925
Crude protein	114	113	112
NDF	137	136	127
ME (MJ/kg)	13.1	13.0	12.8
Ca	10.4	11.0	10.4
P	3.60	3.70	3.40
Mg	4.20	4.00	4.10
K	5.60	5.70	5.50
Na	4.70	4.90	11.00

Feeds were formulated, mixed, pelleted and bagged by a commercial feed company. A batch of 10 ton was mixed for each treatment, and bagged in 50 kg bags for ease of transporting and storage. Concentrates were pelleted to increase consumption. The concentrate for each of the

treatments were bagged in a different colour bag, orange for Sodium Bicarbonate, white for Acid Buf, and pink for the control. This is similar to the colour tags used for the cows (Section 4.2.4) in order to prevent error with feed allocation. To create ease of feeding 3.3 kg ("as is") of concentrate were weighed by hand into plastic bags, these bags represented one cows' feed for a single milking. Twenty of these plastic bags were placed in a 50 kg feedbag to move to the parlour and decant in the feeding troughs before the cows of the specific treatments group entered the milking parlour.

Table 4.3 Chemical composition of Acid Buf buffer as provided by feed company

Mineral (g/kg, as is)	Acid Buf
Ca	300.0
Mg	50.0
P	5.7
K	6.5
Na	12.0

4.2.5 Data collection

4.2.5.1 Feed sampling

Grab samples of each concentrate of the individual treatments were taken three times per week and pooled for a composite weekly representative sample. Thereafter weekly samples were pooled again to have a two-weekly representative sample. This amounted to four samples of each treatment concentrate feed, and thus a total of 12 feed samples across the three treatments. Samples were milled (SCW Hammer mill, 1 mm screen) and stored in clearly marked sealed honey jars. The jars were placed in a 0 – 4 ºC freezer pending analysis at SU Animal Science Laboratory (Department of Animal Science, Stellenbosch University, Stellenbosch).

4.2.5.2 Milk yield and sampling

Cows walked from the paddock where they were kept to the milking parlour before and after each milking, covering a distance of approximately 800 m from the milking parlour to the paddock. Cows grazed and walked to and from the milking parlour as one group. At the parlour, cows were divided into their treatment groups according to the coloured tags, prior to entering the milking parlour. Previously weighed out feed was manually fed out into the feeding troughs, 20 bags separate for each treatment and weighed out for each cow. Cows were milked at 05:30 and 14:00, with a twenty point Dairy Master swing over milking system with weigh-all electronic milk meters

(Total Pipeline Industries, 33 Van Riebeeck Street, Heidelberg, 6665). Udder health was maintained by applying standard protocols as prevention methods. Methods included washing the udder and disinfecting teats before milking and using a teat dip/spray after milking.

Milk yield of each individual cow was recorded by the milking system for each milking time. Milk yield is determined electronically and thus care should be taken to make sure that the system identifies the cows correctly and identifies all the cows. Milk production per cow per day was then determined by combining the recorded yield for each milking time; the average milk yield per cow over the duration of the study was determined. The 4% fat corrected milk (FCM) yield was determined using the following formula: (0.4 x kg milk yield) + (15 x kg milk fat).

Milk samples were taken every two weeks for each cow, with a representative sample for the morning and the afternoon milking. Because of the difference in time between milking intervals, morning and afternoon milk sample sizes were determined by the respective milking intervals. Thus, for each hour between milkings one millilitre of milk was sampled, resulting in sample sizes of 16 ml in the morning and 8 ml in the afternoon. This amounted to a representative sample of 24 ml to be sent for analysis. To ensure that the samples taken were representative of the milk produced, the sampling bottles (which were connected to the milking system) were tilted three times to evenly distribute the milk components before a measured volume was transferred into the final sample bottle. The milk samples were preserved by potassium dichromate ($K_2Cr_2O_3$) and transported to the analytical laboratory. Four composite milk samples were taken in total for each cow over the duration of the trial.

4.2.5.3 Live weight and body condition scoring

Cows were weighed at the beginning of the trial on two consecutive days and the average of this was taken as the initial live body weight (BW). The reason for using the average of two consecutive days is to reduce the cow variation as caused by pasture intake, water intake, defecation and urination. Cows were weighed in the afternoon after they were milked to ensure that the extra weight of a full udder was not added. The Tru-Test EziWeigh version 1.0 scale (0.5 kg accuracy, Auckland, New Zealand) was used. Body condition scoring (BCS) was also done at the beginning of the trial in order to calculate the change in BCS over the trial period. The scoring system used was the five point scale as described by Wildman *et al.* (1982). Body condition scoring was done subjectively by observing the cows' outer appearance and by palpating the back and hind quarters. At the end of the trial period the body weight was also determined in the same way as in the beginning, and the same for the BCS. This was done to get an average change in BW and BCS after the study period as caused by the treatment applied. Pieter Cronje (Animal production department Technician at the Outeniqua Research farm) performed the BCS in both

cases. The BW and BCS were expressed as the mean for each treatment group at the beginning and end and the change over the study period (as can be seen in Table 4.1).

4.2.6 Analytical methods

4.2.6.1 Feed

Concentrate samples were subjected to the same analysis and analytical procedures as the pasture samples as mentioned in 3.2.7. The only difference was that the equation used to calculate the ME of feed samples was: ME = 0.84 x GE x OMD (ARC, 1984; MAFF, 1992).

4.2.6.2 Milk samples

The preserved milk samples were transported over-night to Lactolab (Irene, Pretoria, 0062) where each milk sample was analysed for somatic cell count (SCC), milk urea nitrogen (MUN), and milk components (milk fat, milk protein, and milk lactose). The analysis for milk components was done using Fourier Transform Spectrometer technology by means of the Bentley FTS (Bentley Instruments Inc., Minnesota, USA, 55318), MUN analysis by using a ChemSpec 150 (Bentley Instruments Inc., Minnesota, USA, 55318) which utilises a Berthelot reaction, and the SCC analysis was done using flow cytometry with the Somacount FCM (Bentley Instruments Inc., Minnesota, USA, 55318).

4.2.7 Statistical analysis

All data were analysed using the mixed procedure of SAS (2012). The Milk production, body weight and body condition score values were analysed as a randomised complete block design with fixed effects of treatments and cows within treatments as random effects. Differences between means were tested using Tukey's test with level of significance at $P \leq 0.05$ and tendencies for treatment differences at $P \leq 0.10$ (Ott & Longnecker, 2001).

4.3 *Results*

4.3.1 Concentrate supplement nutrient composition

The estimated chemical composition of the concentrate feed was supplied by NOVA Feeds (Table 4.2). The actual composition as determined by lab analysis from feed samples taken over the trial period is presented in Table 4.4.

Table 4.4 Mean (±SD) chemical composition of concentrate supplements fed to Jersey cows grazing ryegrass pasture during spring (n=4)

Nutrient (g/kg DM or as stated)	Treatment		
	Control	Acid Buf	Sodium Bicarbonate
Dry matter	887 ± 1.7	886 ± 1.0	885 ± 1.2
Organic matter	943 ± 2.9	947 ± 3.9	941 ± 2.6
*IV*OMD (%)	91.2 ± 0.69	91.7 ± 0.60	91.6 ± 0.72
ME (MJ/kg)	14.0 ± 0.05	14.1 ± 0.19	13.9 ± 0.11
NFC	640 ± 8.1	647 ± 15.2	642 ± 11.9
CP	106 ± 2.3	104 ± 1.9	106 ± 0.9
CP:ME ratio	0.76 ± 0.018	0.74 ± 0.015	0.76 ±0.013
NDF	159 ± 5.9	160 ± 10.3	157 ± 9.9
ADF	44.9 ± 0.59	44.6 ± 1.31	43.4 ± 2.76
ADL	4.7 ± 2.79	4.6 ± 0.61	4.4 ± 1.18
EE	37.6 ± 1.91	35.8 ± 3.85	36.1 ± 2.14
Ca	9.9 ± 0.19	9.5 ± 0.22	9.3 ± 0.24
P	4.2 ± 0.13	4.0 ± 0.05	4.0 ± 0.08
Ca:P ratio	2.4 ± 0.08	2.4 ± 0.05	2.3 ± 0.06
K	7.1 ± 0.18	6.9 ± 0.05	7.0 ± 0.10

4.3.2 Milk production

Mean milk production, 4% FCM and milk composition of cows fed the three different treatments is shown in Table 4.5. The milk yield did not differ between the three treatments (P = 0.82).

The 4% FCM showed that there was a tendency for the treatments to differ (P = 0.08) and in Table 4.6 it is clear that Acid Buf tended to differ from the control treatment (P = 0.10); no other tendencies were observed (P > 0.10).

Table 4.5 Milk production parameters of Jersey cows on cultivated ryegrass pasture supplemented with concentrates containing different buffering supplements

Parameter	Treatments			SEM	P-value
	Control	Acid Buf	Sodium Bicarbonate		
Milk yield (kg/cow/d)	20.2	20.5	20.3	0.35	0.82
4% FCM (kg/cow/d)	20.8[c]	21.9[d]	21.8[d]	0.35	0.08
Milk composition					
Milk fat (g/kg)	42.4	45.1	45.0	0.12	0.21
Milk protein (g/kg)	34.1[c]	35.0[d]	35.6[d]	0.05	0.09
Milk lactose (g/kg)	44.9[a]	47.6[b]	47.6[b]	0.03	< 0.0001
MUN (mg/dL)	10.5[b]	9.59[a]	9.7[a]	0.28	0.05
SCC (x 10^3/ml)	107	146	132	24.5	0.52

[a, b] means in the same row with different superscripts differ (P < 0.05)

[c, d] means in the same row with different superscripts tend to differ (P < 0.10)

4.3.3 Milk composition

The mean milk solids for each treatment are presented in Table 4.5.

4.3.3.1 Milk fat

The milk fat content did not differ between the different treatments (P = 0.21).

4.3.3.2 Milk protein

A tendency for a difference in the milk protein content was found between the treatments (P = 0.09) and the differences of least square means (Table 4.6) indicates that the Sodium Bicarbonate treatment had a tendency to have a higher milk protein content than the control treatment (P = 0.08). The other comparisons proved no difference (P > 0.1).

Table 4.6 Fixed effects comparison of the milk components of different treatments when treatments tended to differ at P ≤ 0.10

Parameter tested	P-value			
	4% FCM	Milk protein	Milk lactose	MUN
Acid Buf vs. Sodium Bicarbonate	0.98	0.71	0.93	0.94
Acid Buf vs. Control	0.10	0.32	<0.0001	0.06
Sodium Bicarbonate vs. Control	0.15	0.08	<0.0001	0.12

4.3.3.3 Milk lactose

The milk lactose differed between the different treatments (P < 0.0001). Table 4.6 indicated that both the buffer treatments (Acid Buf and Sodium Bicarbonate) had higher milk lactose content than the control treatment (P < 0.0001).

4.3.3.4 Milk urea nitrogen (MUN) and somatic cell count

A difference in MUN was found among the treatments at a P = 0.05 level. The differences of least square means indicated that the Acid Buf had a tendency to have a lower MUN (P = 0.06) than the control treatment. There were no differences in SCC between the treatments (P = 0.52).

4.3.4 Live weight and body condition scoring

Body weight, BCS and change in BW and BCS is presented in Table 4.7. There were no changes in any of the measured parameters (P > 0.05).

Table 4.7 Mean BW and BCS before and after the trial of cows receiving concentrate supplement with or without buffer inclusion

Parameter	Treatment			SEM	P-value
	Control	Acid Buf	Sodium Bicarbonate		
Body Weight					
BW before (kg)	371	378	373	8.04	0.83
BW after (kg)	393	403	396	7.76	0.64
BW change (kg)	21.9	25.3	23.2	2.33	0.58
Body condition score					
BCS before (1 to 5)	2.04	2.13	2.11	0.04	0.33
BCS after (1 to 5)	2.19	2.25	2.17	0.06	0.60
BCS change (1 to 5)	0.15	0.12	0.06	0.05	0.44

4.4 Conclusion

The addition of buffer supplements in the concentrate fed to grazing Jersey cows did not affect (P>0.05) the milk yield, protein and fat concentrations, or SCC. Milk lactose differed between the control treatment and the buffered treatments and MUN differed between the Acid Buf and control treatment. Neither BW nor BCS was affected by the addition of buffers to the concentrate supplement. The economy of the change in milk composition should be considered to fully comprehend the effect of adding buffers to concentrate supplements. The economic weight of changes such as these will determine the viability of adding the cost to a normal dairy feed.

4.5 References

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Chapter 5: Rumen study

5.1 Introduction

The rumen study was conducted to determine the effect of the addition of buffers in the concentrate supplement fed to dairy cows grazing ryegrass pasture on rumen fermentation parameters. The cows used for this study grazed and were milked together with the cows of the milk production study according to their treatment groups. The milk production data of the ruminally cannulated cows was not included in the production study results. The cows used in the production study were not affected in any way by the rumen study.

5.2 Materials and Methods

5.2.1 Location and environment

For more information regarding the environment, climate and topography of the site of this study, refer to 3.2.1.

5.2.2 Animal welfare

Ruminally cannulated cows were available at the Outeniqua Research farm. Cows had been previously fitted with rumen cannulae according to the standard operating procedure approved by the ethical committee of the Department of Agriculture.

5.2.3 Duration of study

Cows were introduced to the concentrate feed 14 days prior to data collection to adapt them to the feed. Three days before the start of each adaptation period, cows were placed in the allocated paddocks (4.2.3). Each data collection period lasted for 7 days, after which the following adaptation period to a different treatment concentrate started. This 21-day cycle was repeated three times to result in three periods of data collection. The trial was thus conducted over 63 days.

5.2.4 Allocation of cows

Six ruminally cannulated Jersey cows were used in the trial. Cows were randomly (random function in Microsoft Excel, 2010) allocated to either the control treatment (no buffer), or the Acid Buf (1%) or Sodium Bicarbonate (2%) treatments, resulting in two cows per treatment. Cows were rotated after each period (two weeks adaptation and one week data collection), so that by the end of the experimental period each cow had been on each treatment in a three by three cross-over design (three periods and three treatments). The six cannulated cows were in a pasture based system and grazed with the cows from the production study on the same kikuyu-ryegrass pasture. The cannulated cows were tagged with differently coloured tags to identify them with their

appropriate treatments. As mentioned in Section 4.2.3 of Chapter 4, the coloured tags facilitated the separation of treatment groups for specific concentrate feeding, prior to milking.

5.2.5 Feed and pasture allocation

For more information regarding the feeding of treatment concentrates refer to 4.2.4 and 3.2.5 for more detail on pasture allocation.

5.2.6 Collection of rumen data

During each seven day data collection period, different analytical data collection procedures were applied. The sequence of collection usually commenced with rumen pH data being recorded, then samples of rumen digesta were taken, and lastly *in sacco* DM and NDF digestibility of ryegrass at 12 hrs and 30 hrs, was determined. This was done for each one of the three periods. Analytical methods of data collection are described more extensively in each subsequent sub-division.

5.2.6.1 Ruminal pH logging system

Ruminal pH was measured and recorded using an indwelling pH data logger. The logger used was a TruTrack pH Data Logger (Model pH-HR mark 4, Intech Intruments LTD, New Zealand). The logger and electrode were inserted into protective capsules that were watertight, flexible and not discomforting to cows. The capsules allow maximal function of the electrodes and optimal movement to access the rumen content. A cannula plug was attached to each capsule preventing air from entering the rumen to maintain anaerobic conditions. Before inserting the logger systems into the rumen of cows, the loggers were calibrated at pH 4 and 9. The program used to read the logger system for calibration before insertion of the logger and data downloading afterwards, was Omnilog Data Management Program (Version 1.64).

Approximately half an hour prior to insertion, the loggers were started in the Omnilog Program, and immediately before insertion loggers were rinsed with distilled water. Cannula plugs were removed and loggers were inserted at approximately 6:30 AM or directly after milking on a Friday and removed on the Monday morning after milking at the same time. Cows were secured in a crush to facilitate placement of the loggers. The only exception to this was for the third data collection period where data loggers were inserted at 15:00 PM on the Friday and removed at 15:00 PM on the Monday. This was after the afternoon milking in both cases. The electrode of the data logger was submerged in a (KCl) solution at all times, except while it was calibrating or inserted in the rumen for data collection. Ruminal pH was recorded at 10-minute intervals over 72 h. Loggers were removed after 72 h of data collection and the original cannula plug was inserted once again. Logging of pH data was stopped in the laboratory and the pH data was downloaded onto an Excel file for later processing. Loggers were rinsed after each data collection period.

The pH data loggers were recalibrated prior to each data collection period. The calibration procedure was done in the same way every time and the same logger was used for each cow for all three data collection periods. This resulted in less variation due to a specific logger to be used for each cow on all treatments. Data sets were condensed by combining the 10-minute intervals into 30-minute intervals. This was done by taking the average of the pH reading at the time before, at and after the specified time, the mean for each time was then calculated over the three days of data capturing. This was done for each cow on each treatment. Eventually, all the data for each of the six cows were combined to give a representative pH value for each treatment.

5.2.6.2 Rumen liquor sampling

Rumen liquor was sampled to determine the ruminal VFA and the NH_3-N concentrations. Samples were extracted on the same day of the data capturing period at 6:30 AM, 13:30 PM and 20:00 PM. Cows were secured in a crush for extraction of samples. Samples were extracted from the rumen with a customised hand drain pump, which consisted of a 500 mm aluminium rod (5 mm in diameter), an airtight sampling container, and a hand-operated suction pump. The full length of the aluminium rod was inserted into the rumen via a 5 mm hole in the cannula plug, the rod was then moved up and down in the rumen cavity while extracting the rumen fluid into the sampling bottle. Suction was created by the negative vacuum that forms in the sampling bottle with each draw of the pump; this allows the rumen liquor to freely flow into the sampling bottle. A 100 ml sample was taken from each cow on each one of the three treatments. Rumen liquor of each cow was collected separately in a sampling bottle marked with the name of the cow. Immediately after sample collection the pH of that sample was measured with a handheld pH meter (WTW pH 340i pH meter/data logger attached with a WTW Sentix 41 pH electrode; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) and recorded. Samples were kept in the shade at all times to minimise further fermentation and to avoid volatilisation of the contents. After the pH measurement, sampling bottles were sealed airtight and transported to the on-site laboratory to be filtered.

Rumen liquor samples were filtered through two double layers of cheesecloth to obtain a liquid only sample (solid particles removed). The original sample was divided into two sampling bottles 20 ml in volume, for VFA and ruminal NH_3-N analyses, respectively. No preservative was added for either sample. Samples were clearly labelled with the cow name and a key code, which was indicative of the cow, treatment and time at which the sample was taken. Sample bottles were sealed airtight and stored in a -20° C freezer, awaiting future analysis. In total 108 samples were collected, of which 54 for VFA analysis and 54 for rumen NH_3-N analysis.

5.2.6.3 *In sacco* study

An *in sacco* Dacron bag study was conducted, according to the technique described by Cruywagen (2006), to determine DM_d and NDF_d (12 h and 30 h timepoints) of the ryegrass sample as affected by the different treatments. A representative ryegrass sample was cut at a height of 30 mm, placed in a brown paper bag and dried at 60° C for 72 hours. The dried herbage was pooled and cut into 5 mm segments to use in the Dacron bags (Taweel *et al*., 2004). Dacron bags were clearly marked and dried at 60° C for 72 hours, after which they were weighed directly from the oven (Sartorius L420P scale, with 0.001 g accuracy) to determine the weight of the bags. After the scale was tared, approximately 5 g of the cut ryegrass sample was weighed accurately (± 0.001g) into the bag. The scale was tared again, after which the bag was closed securely with a cable tie and weighed once more. This resulted in a recorded weight for the bag, DM pasture sample, and cable tie for each of the prepared bags. Three extra bags were also weighed to be used as the 0 hour or control bags. These bags were prepared in exactly the same manner except they were not ruminally incubated.

Six ruminally cannulated cows (two cows per treatment) were used for the rumen incubations of the Dacron bags. Six bags were prepared for each cow of which three were incubated for 12 hours and the other three for 30 hours. Ladies stockings, 40 decitex and of the non-“antimicrobial” type, were used to incubate the Dacron bags in the rumen. As described by Cruywagen (2006) the bags for each incubation time were placed in one leg of the stocking and knots were made to separate the bags from one another. A large glass marble was placed in the toe of the stocking, to act as a weight that would help ensure that the stocking stayed submerged in rumen liquor at all times. A knot was also made between the weight and the first bag. The stocking was fastened to the cannula plug by an embedded metal ring.

After morning milking, the cows were secured in a crush to remove the cannula plug and replace it with the plug that had the stocking attached to it. At the end of the first incubation time the cannula plug was removed and the one leg of the stocking was freed from the rumen content and cut off. Care was taken to not expose the other leg of the stocking to air and replace the cannulae plug as quick as possible. After removal of the stockings from the rumen, the bags were extracted from the stocking, rinsed with tap water to remove all rumen particulate matter still attached, and then frozen at -20° C. At the end of the final incubation time (30 hours) the cannula plug was removed completely and replaced with the original cannula plug. These bags were also rinsed to remove rumen particulate matter and frozen. After all data collection periods were concluded, bags were placed in water in a twin tub washing machine. Dacron bags were washed three times on the five-minute cycle to remove all rumen fluid still present. After the washing cycles, bags were spun for three minutes to remove all excess water. The same procedure was

applied to the 0 hour bags. After washing and spinning the bags, the bags were dried at 60° C for 72 hours and weighed directly out of the oven. Bags were sealed in plastic bags and stored for NDF analysis at a later stage.

5.2.7 Analytical methods

5.2.7.1 Rumen liquor samples

Samples were kept frozen from time of collection until analysis. Before analysis, samples were defrosted at room temperature to prevent volatising of rumen content. The procedure used to determine the NH_3-N content of the rumen liquor was as described by Broderick & Kang (1980). The rumen liquor was analysed for the VFA profile using the HPLC method, after undergoing a 'clean-up procedure'. Proteins and sugars were removed in this procedure to render a clean sample with only fermentation products remaining for analysis (Siegfried *et al*., 1984). It is important to note that 0.2 g of crotonic acid instead of 2.0 g was used in the preparation of the cupric sulphate reagent; this was done on recommendation of the biochemist responsible for VFA analysis (Fletcher Hiten, Stellenbosch University, personal communication). A Walter 717 auto-sampler equipped with a RI Detector and Biorad Aminex HPX 87H column was used in this method.

5.2.7.2 In sacco study

The Dacron bags were cut open and the residues were used for determination of NDF concentration. The contents of the three bags used for each specific incubation time per cow were pooled and uniformly mixed in a glass beaker; from there a representative sample was randomly selected for NDF analysis. The procedure for NDF determination is described in 3.2.7.

5.2.8 Statistical analysis

All data were analysed using the mixed procedure of SAS (2012). The pH, volatile fatty acid, and *in sacco* values were analysed as a cross-over experimental design in time (period) with fixed effects of treatments and cows within sequence as random effects. Differences between means were tested using Tukey's test and significance was declared at $P < 0.05$ and tendencies to differ at $P < 0.10$ (Ott & Longnecker, 2001).

5.3 Results

5.3.1 Ruminal pH Logging

The rumen pH recorded and averaged over the 24 h is presented in Figure 5.1. Throughout the day the ruminal pH did not differ between the different treatments. Standard error of the means

(SEM) are included in the graph as error bars; they are not indication of significance. Distinctive pH drops can be noted after 05:30 and 14:00, this is indicative of pH drops after concentrate feeding.

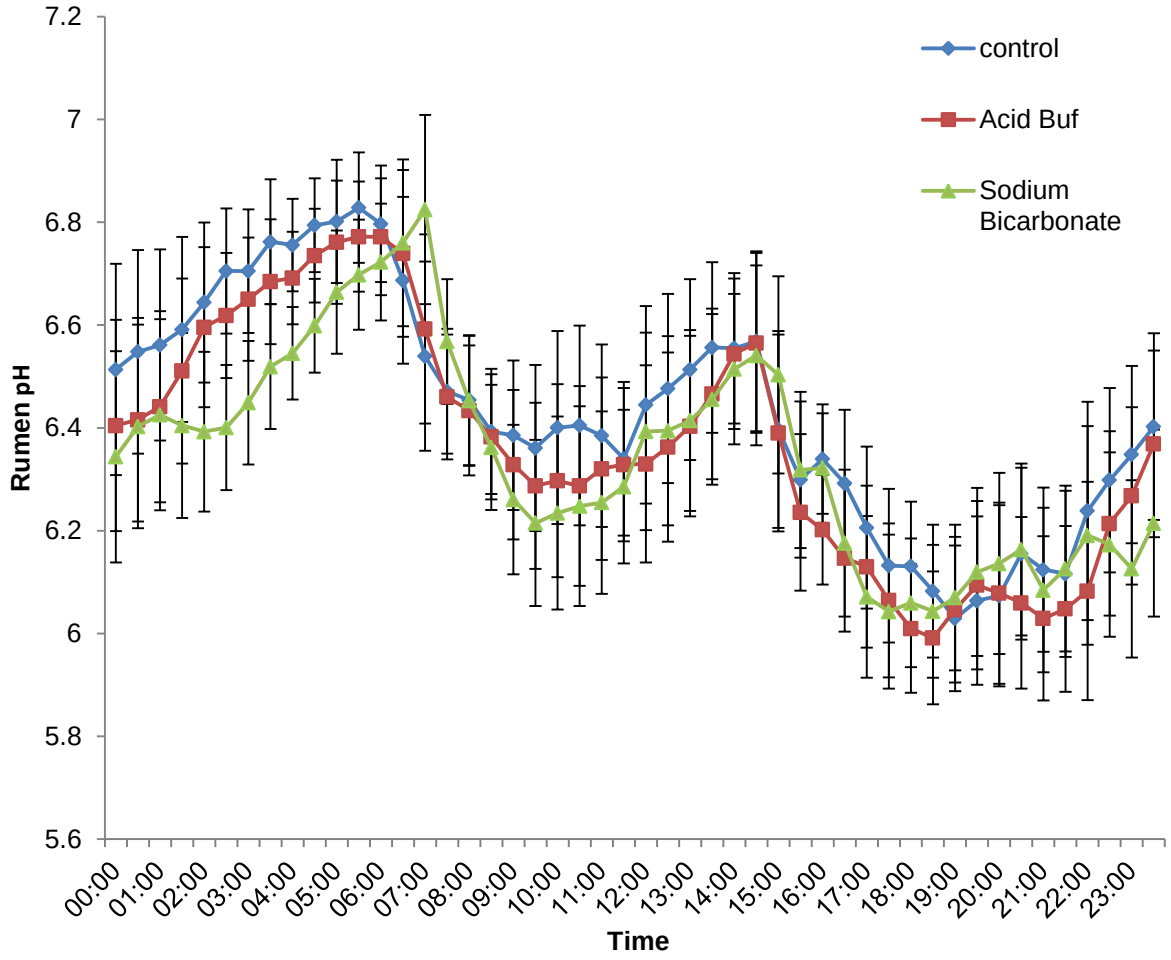

Figure 5.1 Diurnal fluctuations in ruminal pH of Jersey cows (n=6) grazing ryegrass pasture during spring supplemented with 6.6 kg (as is) concentrate per day, including either Acid buf (1%), Sodium Bicarbonate (2%), or no buffer (control), error bars indicate SEM

The average rumen pH for each treatment over 24 h is presented in Figure 5.1. For the time period of 02:00 to 04:30 AM a difference in pH was found between the treatments ($P < 0.05$), Sodium Bicarbonate was lower compared to Acid Buf and the control treatments. The pH ranges for this time period were 6.38 to 6.58, 6.58 to 6.71, and 6.59 to 6.78, for Sodium Bicarbonate, Acid Buf and the control, respectively. The highest and lowest pH value and the time of incidence for each treatment are presented in Table 5.1.

Table 5.1 Mean, highest and lowest pH (± s.d.) recorded by the pH logging system over 72h in Jersey cows (n=6) grazing ryegrass pasture supplemented with 6.6 kg (as is) concentrate including either Acid Buf (1%), Sodium Bicarbonate (2%) or no buffer (control)

pH Parameter	Treatments		
	Control	Acid Buf	Sodium Bicarbonate
Average ruminal pH	6.39 ± 0.291	6.35 ± 0.273	6.32 ± 0.263
Maximum ruminal pH[1]	6.79 ± 0.184, 05:30	6.76 ± 0.100, 05:30	6.75 ± 0.123, 06:30
Minimum ruminal pH[1]	6.00 ± 0.163, 19:00	5.99 ± 0.075, 18:30	6.01 ± 0.178, 18:30

[1]pH and specific time at which it occurred.

Table 5.2 indicates the number of hours spent below the specified pH values, 6.2, 6.0 and 5.8. No differences in time spent below these critical pH values were found between the treatments.

Table 5.2 Mean time (hours) spent below ruminal pH of 6.2, 6.0 or 5.8 of Jersey cows (n=6) grazing ryegrass pasture supplemented with 6.6 kg (as is) concentrate including either Acid Buf (1%), Sodium Bicarbonate (2%) or no buffer (control)

pH	Treatments			SEM	P-value
	Control	Acid Buf	Sodium Bicarbonate		
< 6.2	6.25	7.92	8.83	2.19	0.56
< 6.0	2.33	2.17	3.08	1.09	0.72
< 5.8	0.42	0.25	0.08	0.26	0.66

5.3.2 Rumen liquor samples

The mean ruminal concentration of total and specific volatile fatty acids, NH_3-N and handheld pH measurements for each treatment at three time intervals are represented in Table 5.3. *Iso*-Butyrate of buffered concentrates were higher at time 06:00 than the control treatment (P = 0.002).

The handheld pH value was higher for Sodium Bicarbonate treatment compared to the control (P = 0.03) at time 06:00.

Table 5.3 Concentrations of volatile fatty acids (VFA), ruminal ammonia nitrogen (NH_3-N) and handheld pH measurement of ruminal fluid collected at three time intervals from Jersey cows (n=6) grazing ryegrass pasture supplemented with 6.6 kg (as is) concentrate including either Acid buf (1%), Sodium Bicarbonate (2%) or no buffer (control)

Rumen parameter (mmol/L, or as stated)	Time	Treatments			SEM	P-Value
		Control	Acid Buf	Sodium Bicarbonate		
Total VFA	06:00	177	173	160	9.2	0.43
	13:00	166	165	154	10.3	0.57
	20:00	160	174	176	7.1	0.16
Acetic acid	06:00	116	111	102	6.4	0.33
	13:00	108	107	99	6.7	0.51
	20:00	105	113	114	4.4	0.17
Propionic acid	06:00	25.4	24.4	22.5	1.35	0.34
	13:00	22.7	23.1	20.7	1.43	0.361
	20:00	25.7	28.3	28	1.09	0.231
Acetic:Propionic acid ratio	06:00	4.54	4.61	4.52	0.15	0.853
	13:00	4.77	4.63	4.83	0.204	0.3588
	20:00	4.08	4.00	4.07	0.106	0.6095
Butyric acid	06:00	18.6	18.0	16.2	1.14	0.357
	13:00	17.0	16.9	16.1	1.25	0.759
	20:00	17.5	19.7	20.1	1.02	0.091
***Iso*-Butyric acid**	06:00	14.4^{a}	17.1^{b}	17.3^{b}	1.36	**<0.01**
	13:00	15.9	16.2	16.3	2.08	0.987
	20:00	9.79	11.02	10.92	0.939	0.6044
Valeric acid	06:00	2.10	1.94	1.81	0.114	0.2314
	13:00	1.65	1.37	1.29	0.154	0.2600
	20:00	1.51	1.69	1.75	0.107	0.0660
***Iso*-Valeric acid**	06:00	1.12	0.84	0.75	0.291	0.2503
	13:00	0.62	0.38	0.35	0.304	0.3259
	20:00	0.72	0.80	0.80	0.346	0.6888
NH_3-N (mg/dL)	06:00	25.0	24.0	24.5	0.71	0.624
	13:00	23.1	20.1	21.5	0.88	0.096
	20:00	26.4	25.8	26.4	0.26	0.232
Handheld pH	06:00	6.17^{a}	6.28^{ab}	6.41^{b}	0.055	0.03
	13:00	6.01	6.03	5.98	0.067	0.8303
	20:00	5.49	5.50	5.42	0.034	0.2265

^{a,b} means in the same row with different superscripts differ (P < 0.05)

The mean values obtained from the three sampling periods are summarised in Table 5.4. No differences were found for any of the parameters. The mean value of NH_3-N tended (P = 0.09) to differ between the treatments. The control treatment showed a tendency (P = 0.08) to be higher than the Acid Buf treatment.

Table 5.4 Mean daily volatile fatty acid (VFA) concentrations, ruminal ammonia nitrogen concentrations and handheld pH measurement in rumen fluid collected at three time intervals from Jersey cows grazing ryegrass pasture supplemented with 6.6 kg (as is) concentrate including either Acid Buf (1%), Sodium Bicarbonate (2%) or no buffer (control)

Rumen parameter	Treatments			SEM	P-value
	Control	Acid Buf	Sodium Bicarbonate		
Total VFA (mmol/L)	168	171	163	6.2	0.57
Acetic acid (mmol/L)	110	110	105	4.2	0.57
Propionic acid (mmol/L)	24.6	25.3	23.8	0.87	0.384
Acetic:Propionic acid ratio	4.46	4.41	4.47	0.14	0.773
Butyric acid (mmol/L)	17.7	18.2	17.5	0.86	0.667
***Iso*-Butyric acid (mmol/L)**	13.4	14.8	14.8	0.97	0.303
Valeric acid (mmol/L)	1.76	1.67	1.62	0.0821	0.4985
***Iso*-Valeric acid (mmol/L)**	0.82	0.67	0.63	0.301	0.2847
NH_3-N (mg/dL)	24.8^{a}	23.3^{b}	24.1^{ab}	0.44	0.090
Handheld pH	5.89	5.94	5.93	0.041	0.5532

^{a,b} means in the same row with different superscripts differ (P < 0.1)

5.3.3 In sacco dacron bag study

The DM and NDF digestibility of pasture samples that were incubated in the rumen of Jersey cows for 12 and 30 hours are represented in Table 5.5.

No differences were found amongst the three treatments at time 12 h as well as 30 h. Figure 5.2 is a graphical representation of the same data to exhibit Standard Error of the Mean by use of error bars.

Table 5.5 Mean % of dry matter (DM) disappearance and neutral detergent fibre (NDF) disappearance of pasture at 12 and 30 hours of incubation in the rumen of Jersey cows (n=6) grazing ryegrass pasture supplemented with 6.6 kg (as is) concentrate including either Acid Buf (1%), Sodium Bicarbonate (2%) or no buffer (control)

Parameter	Incubation time (h)	Treatments			SEM	P-value
		Control	Acid Buf	Sodium Bicarbonate		
DM_d (%)	12	62.6	62.2	61.2	1.31	0.753
	30	79.4	80.0	79.1	0.77	0.686
NDF_d (%)	12	33.2	33.0	31.3	2.30	0.805
	30	62.4	63.5	61.5	1.55	0.662

5.4 Conclusion

The inclusion of buffers in the dairy concentrate did not affect any of the rumen paramenters. There were no differences in ruminal pH, volatile fatty acids or NH_3-N between the three treatments ($P < 0.05$). The ruminal digestibility also did not differ between the different treatments ($P < 0.05$). Results suggest no clear benefit of adding any of the buffers to the concentrates, on rumen parameters of Jersey cows grazing ryegrass pasture.

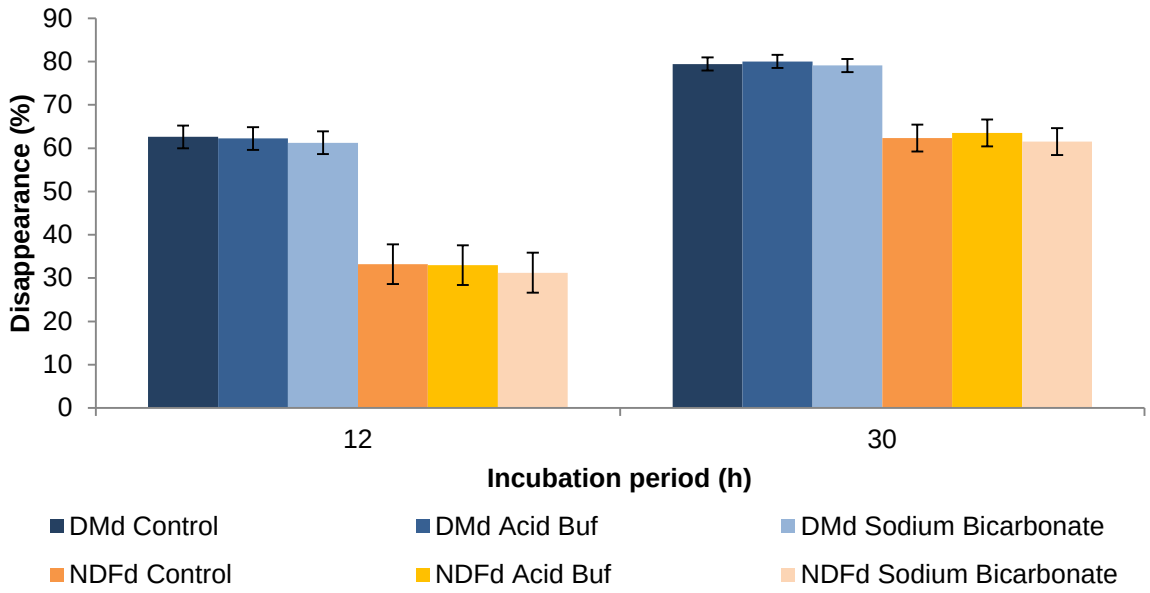

Figure 5.2 In sacco dry matter and NDF disappearance of ryegrass pasture in cows (n=6) fed 6.6 kg (as is) concentrate per day, including either Acid Buf (1%), Sodium Bicarbonate (2%), or no buffer (control), error bars indicate SEM (90%)

5.5 References

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Chapter 6: Discussion

6.1 Pasture

6.1.1 Climate

The rainfall for September and November in the trial period was below average (15.0 mm vs. 29.5 mm and 58.4 mm vs. 113.7 mm, respectively), whereas the rainfall in October greatly exceeded the average (187.5 mm vs. 94.4 mm) as shown in Figure 3.2. The lower rainfall in September and November did, however, not pose a problem seeing as ample reserved water was available for irrigation. On average the per annum rainfall as calculated over the last 47 years was 738.9 mm which is much lower than the 866.1 mm measured for 2012 (ARC, 2012). This indicates that water could not have been a limiting factor regarding the performance of the pasture. The average rainfall for September to November 2012 was 7.7 mm higher than the average in the same period over the last seven years. It can therefore be said that the rainfall was sufficient to optimise pasture performance for the duration of the trial.

The temperatures for the trial period were in close comparison to the long term values. This was as expected, considering George's temperate climate.

6.1.2 Pasture management

Cows strip-grazed the pasture to ensure that maximum production potential is achieved. By the time the trial had terminated the paddock was grazed three times. The average pasture yield before grazing was 1739.95 kg DM/ha which is just higher than the 1700 kg DM/ha recommended by McEvoy *et al*. (2009) and lower than the 2200 kg DM/ha indicative of deteriorating pasture quality. The RPM is used to determine the amount of pasture to be allocated per grazing. It is merely an estimation and cannot accurately determine the amount of pasture consumed. Rayburn & Rayburn (1998) found a 10 % error when using the RPM to estimate pasture yield. It is however important for the pasture management to determine the pre- and post-grazing heights of the pasture. Adequate pasture allowance is maintained to ensure good pasture utilisation (McGilloway & Mayne, 1996), an over-estimate of pasture yield could lead to an over- or under-utilisation of pasture which will negatively influence the pasture for the rest of the season. The ideal would be to determine individual pasture intake, which is difficult to do on pasture-based systems. It is not viable to use the RPM for this function (Reeves *et al*., 1996). A shorter time spent grazing each strip is known to enhance the reliability of the RPM reading (Smith *et al*., 2005). In this study cows grazed strips either 6 h or 14 h, and cow behaviour was monitored to ensure cows were ruminating; an indication that ample pasture was available.

The average post-grazing RPM height for the duration of the trial was 10.9. Post-grazing heights on the RPM should not be below 10 as is specified by Stockdale (2000) and Fulkerson & Donaghy (2001). This indicates that pasture was not over- utilised during this time and that regrowth and quality could be maintained. The reason for this post-grazing height is possibly because the regression equation used to allocate pasture was determined earlier in the same season of a previous year (Van Wyngaard, 2013). Variation occurred between data points, some greater than others, and this could have influenced the outcome of the specific regression. Data represented in Table 3.2 were obtained from the regression equation expressed in Figure 3.3. This equation was generated from cuts collected during this study. The regression was determined on the same paddock on the exact same farm and thus the variation is not as much as it would have been in another area, paddock and season (Sanderson *et al*., 2001). Ultimately more pasture could have been provided to increase the pasture allocated (pasture allocated 8.6 kg DM/cow/day instead of estimated 10 kg DM/cow/day) but there is no indication that the cows had a deficit regarding pasture availability.

6.1.3 Pasture quality

The quality of pasture in this study was determined in the laboratory and is depicted in Table 3.3. According to Bargo *et al.* (2003) high quality pasture has a DM, CP, and NDF of 18 – 24%, 18 – 25%, and 40 – 50%, respectively. These standards were not met in the current study for DM and CP but, NDF values were well within the range. The CP content, as recorded from the pasture samples taken over 8 weeks in the present study, was much lower than mentioned. Meeske *et al.* (2006) reported a CP value of 180 ± 45.9 g/kg for spring. The value obtained in the present study was, however, within the range indicated by Meeske *et al.* (2006) and Van Vuuren *et al.* (1991) (156 to 298 g/kg). Muller & Fales (1998) indicated that NDF for cool season grasses should be between 40.0 and 52.7 % which is consistent with our findings. A possible explanation for the low CP value is because not enough N was added to the pasture after grazing. A yellowish colour was noted on the pasture in the trial period which may be indicative of an N shortage. When the performance of the cows are considered (4.3.2) the protein supplied was not deficient. The ADF of the pasture was maintained well above the recommendation of the NRC (2001) which is 19 – 21 % to ensure that milk fat is not depressed.

The nutritive composition of the pasture (Figure 3.4) follows the expected trend as the season progressed, indicative of pasture quality deterioration. The ME and the *IV*OMD decreased as the season progress and the NDF and ADF increased. In this study, the CP content increased over time which is in agreement with Van Wyngaard (2013), although not as would be expected (Van Vuuren *et al.*, 1991). The fertiliser could not have had an effect on this as it was fixed at 42 kg N/ha for the entire trial period. The height of sampling could influence the CP content, where

samples taken at 30 mm could have a lower CP than samples cut at a height of 50 mm. Climatic factors (rainfall and temperature) could also have played a role in the volatility of N and leaching of fertiliser, and for that reason could have affected the CP content of the pasture. Carruthers & Neil (1997) reported on the influence of temperature and rainfall on the volatility and leaching of N in pasture based systems.

6.2 Milk production study

6.2.1 Concentrate supplement nutrient composition

When comparing the estimated feed composition to the actual composition as determined in the lab analyses, all values corresponded with only minor differences. This is confirmation of a thoroughly mixed batch of concentrate used for the trial. Concentrate supplements were formulated on an iso-nitrogenous basis, which is confirmed by the consistent CP content of all three treatment concentrates as illustrated in Table 4.4. The NDF content of the concentrates proved to be within spec.

Other parameters to consider are the *IV*OMD, ME, NDF and EE, which all proved to be the same for the treatment concentrates. This confirms that the concentrate supplement in itself could not affect the production performance displayed in this trial but rather the addition of the buffers would.

6.2.2 Milk production

Milk production of cows before the onset of the trial are presented in Table 4.1 and Table 4.5 presents milk production data as accumulated throughout the trial period. The change in milk production observed during the trial period (Table 4.1 vs. Table 4.5) cannot be explained by the composition of the concentrate as much as to the level of concentrate feeding or even the higher quality of pasture ingested during the trial. Cows were taken out of a large group of more than 200 cows and during the study only 60 cows were managed more intensively as a group on pasture. This may have resulted in more accurate pasture allocation, improved pasture availability and pasture intake. Treatment had no effect on milk yield; milk yield of the control treatment did not differ from buffered treatments. Low ruminal pH will impair energy intake and protein absorption causing a limitation on the production potential of dairy cows (Allen, 1997). Kolver & De Veth (2002) and Stone (1999) published data that was in agreement with this statement. In this study, however, milk production did not decrease in the control treatment which could be indicative of rumen health, regardless of buffer inclusion (See 6.3.1). Rearte *et al*. (1984) stated that milk production was not affected when buffers were added in the concentrate fed to grazing dairy cows.

6.2.3 Milk composition

6.2.3.1 Milk fat

The milk fat content expressed in Table 4.5 did not differ between the three different treatments. Total mixed ration studies report an increase in milk fat when adding buffer to the diets (Kennelly *et al*., 1999; Kolver & De Veth, 2002). Erdman (1988) noted that little response in milk fat content on pasture based systems upon the inclusion of buffer in the diet. Rearte *et al*. (1984) also stated that the addition of buffer in the concentrate did not alleviate the slight depression in milk fat noted on pasture based systems. According to Allen (1997), milk fat percentage can be a diagnostic measure for SARA, which in this case was apparently not prevalent. In this study milk fat was not depressed when considering the value for control and buffered treatments alike. Therefore it cannot be said that milk fat was not alleviated but rather that the circumstances were not such that milk fat content was compromised. Milk fat depression is said to be related to a reduced acetate:propionate ratio (lower than 2.2:1; Bauman & Griinari, 2003) which was also not the case in this study and therefore it can be said that the ruminal data support the findings in milk fat.

When examining the milk fat content before the onset of the trial (Table 4.1), it was higher than during the trial period (Table 4.5). Considering the rumen data and specifically the pH data (6.3.1) the decline in milk fat was probably not due to a depressed ruminal pH but rather the higher quality pasture provided during the trial and a higher milk yield. According to Huber *et al*. (1964) high quality pasture reduces milk fat percentage cows.

When the milk fat from this study is compared to other studies on pasture, it proved to be comparable when similar concentrate levels were fed (± 44.5 g/kg, Meeske *et al.*, 2006) and high when lower concentrate levels were fed (± 41.0 g/kg, Meeske *et al.*, 2009). It was, however, rather low when compared with studies where high fibre concentrate diets were supplemented (± 46.5 g/kg, ± 49.0 g/kg; Van Wyngaard 2013; Steyn, 2012). The average milk fat values obtained for all treatments during the trial are consistent with reports of others. Even when considering the control treatment, there is no evidence of compromised ruminal health exhibited through the milk fat content.

6.2.3.2 Milk protein

The milk protein content of the three treatments is presented in Table 4.5, which indicates that there were no differences between treatments. There was, however, a tendency for the Sodium Bicarbonate treatment to differ from the control treatment (P = 0.08, Table 4.6). According to Bargo *et al*. (2003) milk protein does not generally respond to dietary manipulation, which is in agreement with this study. Khorasani & Kennelly (2001) found that milk protein content did not

differ upon inclusion of buffer to the diet. Rearte *et al*. (1984) found that milk protein content of cows did not differ when fed control or buffered concentrate with pasture. Even though data regarding the effect of buffer inclusion on the milk protein content are variable, authors have concluded that dietary buffers do not consistently change the milk protein content (Cassida *et al*., 1988; Xu *et al*., 1994).

The milk protein content values obtained in the current study were lower than the average milk protein content of Jersey cows in South Africa (3.85 %, Logix Milk, Suretha Francis, suretha@studbook.co.za, 2012) and values reported by Erasmus (2009). It was, however, in agreement with Meeske *et al*. (2006), Steyn (2012) and Van Wyngaard (2013). A possible explanation for the lower protein content is because a lower level of concentrate was fed which directly influences the milk protein content because of the decreased ruminally available energy (Sayers, 1999; Reis & Combs, 2000; Bargo, 2002c; Bargo *et al*., 2003).

6.2.3.3 Milk lactose

Cows on the control treatment had lower lactose content than cows on either of the buffered treatments. This was unexpected, as Kennelly & Glimm (1998), Schwab *et al*. (2008), Sutton (1989) and NRC (2001) stated that lactose is the most stable of the milk components and dietary manipulation is not generally possible. The NRC (2001) stated that it does vary with breed and milk protein concentration. Udder health or SCC could also be responsible for a change in lactose content (Kitchen, 1981; Welper & Freeman, 1992). An increase in milk volume in the mammary gland, in response to the increased SCC, could cause a decrease in lactose content. This was not the case in this study as the SCC was low, especially in the control treatment which exhibited the lowest SCC, and no difference were found between treatments. The buffered treatments exhibited values that were similar to the 4.7 - 4.85 % of milk, as mentioned by Gibson (1989) and NRC (2001), the control value was, however, well below this value. In two studies on buffers included in TMR diets, it was found that the lactose content increased moderately in the buffered diets compared to the control (Kennelly *et al*., 1999) and alternatively no difference was found (Khorasani & Kennelly, 2001). At this stage the difference in lactose content was still unexplained as lactose content is known for low variability (Welper & Freeman, 1992; Sutton, 1989) and the measurements are high in accuracy.

6.2.3.4 Milk urea nitrogen

Acid Buf tended to result in a lower MUN than the control treatment. The MUN is said to increase when the ratio between ingested protein and energy increases (Hof *et al*., 1997). In the current study, the protein to energy ratio was the same for all treatments (Table 4.4) based on concentrate consumption; and cows in the different treatments grazed the same pasture and thus

pasture protein content could not differ. The MUN values were in the range indicated by Kohn (2007) as acceptable (8 – 12 mg/dL) indicating that dietary protein was supplied well within the recommended range. The difference in MUN in our study was biologically insignificant.

6.2.3.5 Somatic cell count

The SCC values did not differ between treatments and the level maintained was well below values indicative of subclinical mastitis (300 x 10^3 cells/mL milk, De Villers *et al*., 2000). Welper & Freeman (1992) indicated the range for SCC of different dairy breeds to be between 285 x 10^3 and 309 x 10^3 cells/mL of milk, with Jersey cows exhibiting the highest values. The values in this study were thus well below what is indicated as average values.

6.2.4 Live weight and body condition scoring

No differences in body weight or body condition score were found between treatments. This was expected since cows received the same feeding. Bargo *et al*. (2002b) stated that body weight is not subject to change in such a short time as it would compromise the feeding study. In this study the body weight gain was small and solely because of an increase in concentrate fed from before to during the trial. Body condition score indicated that cows were able to gain condition whilst producing more milk and maintaining milk components.

6.3 Rumen

6.3.1 Rumen pH profiles

The only differences in rumen pH was recorded between 02:00 and 04:30 AM (5.3.1) where sodium bicarbonate exhibited lower ruminal pH than Acid Buf and the control treatment (Figure 5.1). These differences, although significant, were minor and not alarming because the pH was still well above the threshold indicative of SARA (Nordlund & Garrett, 1994; Pitt *et al*., 1996; Plaizer, 2004; Krause & Oetzel, 2006). Different pH values have been reported as the threshold for SARA, however, pH should generally not fall below 6.0, or at least not below 5.5. It is possible that the small difference in pH in that time period is caused by cow variability or even logger differences.

When considering the shape of the curve in Figure 5.1, it appears that the extent of pH fluctuations were the same for all three treatments, averaged over 24 h. The two cyclic pH drops that are illustrated in the curve in Figure 5.1 (at times 09:00 AM and 18:00 PM) are characteristic of a normal diurnal pH fluctuation. According to De Veth & Kolver (2001b) it is normal for large diurnal variations in ruminal pH to occur in dairy cows grazing pasture. That is why it is advantageous the continuously monitor pH due to its high diurnal variation (Keunen *et al.,* 2002; Duffield *et al*., 2004). The drops in pH were 2.5 h and 3.5 h post-feeding, respectively.

There were no differences between average pH values of the three treatments as measured over 72 h with indwelling pH logging systems (Table 5.1, Section 5.3.1). These averages were within the range of pH 6.0 to 6.9 that is the optimal pH range for stimulating ruminal fibre digestion (Pitt *et al*., 1996; Kolver *et al*., 1998). These average values also indicate that even the control treatment (no buffer added to the concentrate) did not result in a pH indicative of SARA over 24h. According to Plaizier (2004) this is below pH 6.0 because that is when fibrolytic bacterial growth is impaired. It was found that the ruminal pH values were highest just prior to morning milking or around the time of morning milking (05:30 and 06:30). The lowest values were found 3.5 h to 4 h after the afternoon milking for all three treatments. This is in agreement with the statement by (Bargo *et al.,* 2002a) reporting that pH is highest just prior to milking and lowest 2 – 5 hrs after milking (Nordlund & Garrett, 1994; Nocek, 1997; Cajarville *et al*., 2006).

Mould *et al.* (1983) and Hoover (1986) stated that even though drops in pH will negatively influence fibre digestion (pH < 6.0, Shriver *et al.,* 1986) the duration determines the effects on microbial activity, and that drops of one to two hours at a time do not have long-term inhibitory effects. AlZahal *et al.* (2007) indicated that SARA occurs when ruminal pH is below pH 5.6 for 283 min or < 5.8 for 475 min. Gozho *et al.* (2005) indicated that a pH threshold of between 5.2 and 5.6 for > 174 min/day can be indicative of SARA. This was, however, not the case in the current study where time below pH 5.8 did not reach one hour for any of the three treatments. Although the ruminal pH decreased below pH 6.0 for more than two hours over the 72 hours data collection in the current study, it did not seem to have a detrimental effect on rumen health when considering the results presented in 5.3.2 and 5.3.3. Furthermore, there was no difference in time below critical pH values as would be expected for the control treatment, indicating that rumen fermentation and the ruminal environment were the same for all treatments regardless of buffer inclusion.

6.3.2 Rumen samples

6.3.2.1 Volatile fatty acids

There were no differences in total volatile fatty acid concentrations among the three treatments (Table 5.3 & Table 5.4). When considering the proportions of volatile fatty acids, no differences were found for the different times (Table 5.3) or for the daily proportions (Table 5.4). Rearte *et al.* (1984) found no change in total VFA or VFA proportions as a result of buffer inclusion, which is in agreement with the current study. Studies reported VFA concentrations of >130 mmol/L for dairy cows grazing pasture and supplemented with concentrate (Bargo *et al*., 2002a; Reis *et al.,* 2001). In the current study, the levels of total VFA were exceptionally high because of the high acetic acid levels. It was expected that this would accompany a depression in pH as it was reported by Seymour *et al.* (2005) that ruminal pH is negatively related to total VFA. It was,

however, not the case in this study (Section 6.3.1). The high levels of acetic acid, as well as the high acetate:propionate ratio, would also generally be expected to accompany an increase in milk fat content (Seymour *et al.*, 2005; Kennelly & Glim, 1998), which was not found to be true in the current study. In general, an increased NDF content would serve as an explanation for a high acetic acid:propionic acid ratio (Sairanen *et al.*, 2006). The NDF value for pasture was rather high (3.3.3) but not to such an extent to cause such a ratio. Erdman (1988) expressed a 2:1 ratio as the threshold for milk fat depression which, is in agreement with the results found in 4.3.2. De Veth & Kolver (2001a) stated that there is a positive relationship between pH and total VFA, as well as pH and acetic acid and propionic acid. The high acetic acid proportion could not be thoroughly explained by literature or Dr Paul Weimer (personal communication) and it does not correspond with other results found in this study and seeing as it does not differ between treatments it is possibly not caused by ruminal activity but rather laboratory error.

Values obtained for the other two of the three principle rumen VFA (Seymour *et al*., 2005) were in the same order as other published data (Bargo *et al*., 2003).

6.3.2.2 Ruminal ammonia nitrogen profile

No differences in ruminal ammonia nitrogen concentration were found among the three treatments at any of the three time intervals (Table 5.3), neither for the mean daily NH_3-N concentration (Table 5.4). The mean values recorded in this study were higher than found in a study by Bargo *et al.* (2002a) where the mean value for dairy cows on pasture plus concentrate was 19.9 mg/dL. Satter & Slyter (1974) reported that very high NH_3-N levels (up to 80 mg/dL) would not inhibit rumen microbial activity. On the other hand, the lowest levels where rumen microbes can function were reported as between 1 and 6 mg/dL (Satter & Slyter, 1974; Hoover, 1986; Khalili & Sairanen, 2000). The values reported in this study corresponded well to values obtained in other studies on pasture (Lingnau, 2011; Steyn, 2012; Bargo *et al.*, 2002a) which indicates that N was sufficiently utilised from pasture. These levels of NH_3-N proved sufficient to maintain rumen activity in the case of all three abovementioned studies. The study by Khorasani & Kennelly (2001) also found no difference in NH_3-N levels where buffers were included in the diet.

Samples were taken after morning milking and before afternoon milking, and according to Bargo *et al.* (2002a) NH_3-N tends to increase after morning milking and again after the afternoon milking in response to pasture ingested. This trend corresponds to observations in the present study. The pH data discussed in Section 6.3.2.1 is in agreement with the NH_3-N data, where an increase in NH_3-N after morning and afternoon milking coincides with the decrease in pH. The increase in NH_3-N arises because of the pH drop that inhibits micro-organisms to utilise NH_3-N for microbial protein synthesis.

6.3.2.3 Handheld pH values

The handheld pH values differed between the control and the sodium bicarbonate treatments at 06:00, where the control treatment had a lower pH (Table 5.3). There is no clear explanation for this phenomenon. No differences were found for any of the other time intervals (Table 5.3) or for the mean pH (Table 5.4). The highest pH was recorded at time 06:00 as this was just after concentrate consumption and rumen pH had not yet been affected by digestion of concentrate.

To shed light on the differences found between the handheld pH values and the logger pH values, Duffield *et al.* (2004) stated that measured pH values may differ, depending on the technique used to collect the ruminal fluid. Colman *et al.* (2010) reported that ruminal pH may vary greatly at different locations in the rumen and at different times during the day. It is also important to keep in mind that the handheld pH logger was merely used to measure the pH of the rumen samples as they were collected so a direct comparison between the pH and the other rumen parameters can be made at the specific times.

6.3.3 In sacco dacron bag study

Neither the dry matter degradability nor the NDF degradability differed among treatments at any of the time intervals (Table 5.5). The high DM_d values obtained corresponds to the high *IV*OMD reported (Table 3.3) for the ryegrass samples analysed. The degradability corresponds to the time spent below certain pH; according to De Veth & Kolver (2001a) digestibility is greatly impaired when as little as 4 hours is spent below specifically pH 5.8 (Table 5.2). A pH average of 6.35 (De Veth & Kolver, 2001a) or within the range of 6.0 to 6.3 (Hutjens *et al*., 1996; Pitt *et al.,* 1996) is suggested as the optimum pH for DM_d which is in close agreement with means obtained for all three treatments in this study (Table 5.1). The pH at which fibre digestion would be impaired is indicated as 6.2 (De Veth & Kolver, 2001a) and a drastic reduction in fibrolytic bacteria numbers will occur below pH 6.0 (Shi & Weimer, 2002). The amount of hours spent below these critical values, however, seemed to not have had an influence on the digestion, which is in agreement with the continuous culture study by De Veth & Kolver (2001a) where it was determined that pasture digestion and microbial growth could be maintained even though pH was suboptimal for extended periods of time. The reason for this seemed to be the fact that the pH was optimal for a sufficient amount of time during the day that would allow microbial attachment and digestion (average pH, Table 5.).

6.4 Conclusion

The climate observed during the trial period was as expected for this specific season and therefore did not affect the outcome of this study. The increase in rainfall was to such a point that quality of

pasture should be improved. The reason for the low CP content is unclear and there can only be speculated as to the cause. It is however reassuring that the MUN levels were comparable with other studies. The other nutrients were also comparable with other studies and no effect on milk production would be expected. The estimate obtained for pasture intake even though surprisingly low, did not seem to affect the expression in milk yield. Animal behaviour during the trial period never seemed to express lack of feed intake. The slight change in milk composition could be valuable to assess with regards to its economic impact. Rumen parameters didn't seem to be affected by the adaptation of the diet and the expression in the form of the milk production confirms this. Ruminal pH was not affected in the control treatment to the point where cows were approaching SARA but even small improvement in milk composition can prove to be financially advantageous.

6.5 References

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Chapter 7: Economic evaluation

7.1 Introduction

Milk price drives the dairy industry and the sharp decrease in 2009 placed farmers under a lot of pressure to increase efficiency of productionand in a cost effective way. The milk price recovered in 2010, followed by a decrease in mid- 2011 to mid- 2012. Since then prices have recovered and a new record was reached in March 2013. The price as recorded in January 2013 was R 3.60 as opposed to the R 2.97 in January 2011 (Coetzee, 2013). The market is, however, never stable and the volatility is a risk for dairy farmers. Even though the number of milk producers in the Western Cape has shown a 35 % decrease over the past 6 years (Coetzee, 2013), the annual milk production has been steady and even tended to grow over the same period. New ways to optimise production and facilitate an increase are continuously investigated. It is important to look at the economic impact of diet improvements, and the use of supplements are shown to be viable when the input cost exceeds the increase in income. It is thus important that we look at the economic impact of this study.

7.2 Economy

The figures for the economic evaluation were calculated on a herd size of 300 cows, which is the average herd size per producer in the Southern Cape. The milk production and composition, as obtained for each treatment in this study, is depicted in Table 7.1.

Even though no differences were found in milk production among treatments, the milk price was calculated for the specific values obtained for each treatment. This is true for the milk fat as well; the milk protein did, however, differ between treatments and the effect thereof on the milk price is evident. The feed price is calculated from raw material prices as supplied by NOVA Feeds in June 2013 and the pasture cost is obtained from the Outeniqua Research farm in 2013. NOVA Feeds also supplied the price per ton of the buffers (Acid Buf or Sodium Bicarbonate) that were included in the concentrate rations. The net daily profit only depicts the margin over feed cost and does not consider the cost of the labour, machinery or any other farm related running costs.

Table 7.1 Increased profit for buffer treatments compared to control as calculated for margin over feed cost for a dairy herd of 300 cows in milk

Parameter[1]	Treatments		
	Control	Acid Buf	Sodium Bicarbonate
Milk production (kg/cow/day)	20.2	20.5	20.3
Milk Production (kg/herd/day)	6060	6150	6090
Milk fat (%)	4.24	4.51	4.50
Milk protein (%)	3.41	3.50	3.56
Milk price (R/kg)	3.98	4.12	4.17
Milk income (R/herd/day)	24118.8	25338.0	25395.3
Concentrate price (R/t)	2490.6	2556.7	2552.3
Concentrate inclusion level (kg as is)	6.6	6.6	6.6
Concentrate price (R/cow/day)	16.44	16.87	16.85
Concentrate price (R/herd/day)	4932	5061	5055
Buffer price (R/t)	0.00	7770	4580
Buffer inclusion level (kg as is)	0.00	0.066	0.132
Buffer price (R/cow/day)	0.00	0.51	0.61
Buffer price (R/herd/day)	0.00	153	183
Pasture price (R/kg)	1.20	1.20	1.20
Pasture allowance (kg DM)	8.6	8.6	8.6
Pasture price (R/cow/day)	10.32	10.32	10.32
Pasture price (R/herd/day)	3096	3096	3096
Total feed cost (R/herd/d)	8028	8157	8151
Margin over feed cost (R/herd/day)	16090.8	17181.0	17244.3
Increased margin over feed cost compared to control (R/herd/day)	-	1090.2	1153.5

[1] R – South African currency, rand; DM – Dry matter; herd – Average herd size in Southern Cape of South Africa is 300 cows

The net daily profit achieved from the addition of buffers to the diet can be seen in the increased margin over feed cost compared to the control. This can primarily be ascribed to the

higher milk income and the higher milk price. The milk protein content was, however, the main factor affecting the milk price and milk fat to a lesser extent. Feed price did not differ greatly between treatments because of the low buffer inclusion rate (10 g/kg for Acid Buf and 20 g/kg for Sodium Bicarbonate). In conclusion, the economic evaluation indicates that it is viable to add buffer to the concentrate supplement if the composition of milk is altered to the point that the milk income will exceed the cost of the included buffer. A seemingly trivial increase in milk income does make a difference when looking at a herd of 300 cows. It is, however, important to consider the cost of the supplement and the variation in milk price as determined by milk buyers. This would ultimately influence the feasibility to use buffers in the concentrate supplement.

Chapter 8: General conclusion

The aim of this study was to determine whether it is necessary and/or economically viable to add a buffer to the concentrate fed to dairy cows on pasture. In this study, the milk composition showed tendencies to differ for milk protein, and a difference was found in milk lactose between treatments. Even the 4 % FCM showed a tendency to differ. In comparison, the rumen parameters did not differ among treatments. Rumen health and function seemed unanimously acceptable between all three treatments.

Considering all aspects of this study, the rumen health was expressed in the milk production but there were no notable improvements in rumen parameters after the addition of buffers. The fact that milk composition was altered to such an extent that the milk price was altered for each treatment could be the deciding factor. Maintaining the health of the cows whilst acting in a pre-cautionary manner for the possible incidence of SARA on high quality pasture, and reaping the benefits of added milk income, might justify the use of buffer in the dairy concentrate for cows grazing ryegrass pasture. Increased concentrate inclusion would change the outcome as well as pasture with an even lower NDF (40 %). Pasture intake in this study was probably underestimated since a ruminal challenge would be expected for diets containing 55:45 concentrate to forage ratio. The results for this study is however conclusive and the use of buffers are warranted even under these conditions.

Chapter 9: Critical evaluation

Pasture

The general inaccuracy of the pasture intake method is of concern. The RPM and seasonal regression has its flaws and the need for more accurate and animal specific methods is clear. This was, however, not the main focus of this study and is hence not detrimental to the study itself.

Rumen Study

The indwelling pH loggers used for this study presented a challenge. Hours were spent to calibrate loggers. There is, however, not a concern as to the reliability of the data and better equipment would solely be for ease of research.

Feed allocation

Further research to determine the effect of buffer inclusion on milk and rumen parameters on dairy cows grazing pasture is definitely needed. It would be valuable to determine whether feed allocation as well as concentrate to pasture ratio would have had an effect on the outcome. For this study and this purpose the conditions were aligned to the general practice in the surrounding farming area.