The effect of concentrate feeding strategies on rumen parameters, milk production and milk composition of Jersey cows grazing ryegrass (*Lolium multiflorum*) **or kikuyu** *(Pennisetum clandestinum)* **pasture**

by

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at

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Department of Animal Sciences

Faculty of AgriSciences

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Abstract

Title:	The effect of concentrate feeding strategies on rumen parameters, milk production and milk composition of Jersey cows grazing ryegrass (*Lolium multiflorum*) or kikuyu (*Pennisetum clandestinum*) grass pasture.
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Ruminal pH is the rumen condition that varies most. Studying the cause and effect of diurnal variation offers a means of improving ruminal conditions for rumen micro-organisms with subsequent improvements in fibre degradation, milk yields and milk composition. A research project was conducted to test the following hypothesis: feeding 6 kg (as is) concentrate per cow/day in different weight ratios, divided in a morning and an afternoon allocation, will result in an improved ruminal pH profile and a concurrent increase in fibre fermentation, milk yield and improved milk composition. The project was conducted at the Outeniqua Research Farm in the Southern Cape of South Africa. A randomized block design was used and two phases, *viz.* ryegrass phase and a kikuyu phase, were applied. Each phase was conducted during the growing season of the grass specie used as pasture. A rumen study and a production study were done in each phase. The rumen study used six cannulated multiparous cows per treatment, testing rumen pH, volatile fatty acid (VFA) concentration, *in sacco* degradation of neutral detergent fibre (NDF) and dry matter (DM). The production study used 42 multiparous cows, blocked according to days in milk (DIM), parity and milk yield, and cows were randomly divided into three treatment groups. The study tested milk yield, milk composition and changes in body weight (BW) and body condition score (BCS). Treatments consisted of a maize based concentrate supplement (6 kg per cow/day, as is) being fed to cows on pasture in different weight ratios between morning and afternoon allocation. Treatments used in the production study were:

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- Concentrate fed: 5 kg during morning; 1 kg during afternoon
- Concentrate fed: 4 kg during morning; 2 kg during afternoon
- Concentrate fed: 3 kg during morning; 3 kg during afternoon

The first and the last treatments mentioned above were used in the rumen studies. Results of the rumen study of the ryegrass phase showed no differences in ruminal pH between treatment means. The time (h) spent below the critical ruminal pH, *i.e.* 5.8, was of short duration (2.92 to 3.42 hours). The concentration of certain VFA's differed at times, but the differences were not reflected in graphs and data of the ruminal pH. No differences were observed in *in sacco* NDF and DM degradation between treatment means. Similar results were mostly obtained in the kikuyu phase. No differences were found in either daily maximum, minimum or mean ruminal pH between treatment means. Though time (h) spent below the critical ruminal pH was of a long duration (7.1 hours) it did not differ between treatments. No differences were observed between treatment means for *in sacco* NDF and DM degradation.

Results of the production study of the ryegrass phase showed no differences in milk yield or milk composition between treatment means. The same was observed in the kikuyu phase except that milk fat was higher in the treatment group that received the 3:3 kg ratio treatment. The fact that treatments did not differ in terms of milk yield and milk composition in the production studies can be attributed to no differences between treatments in terms of rumen parameters observed in the rumen studies.

Thus, feeding a higher proportion of concentrate in the morning relative to the afternoon for a total of 6 kg per cow/day does not affect ruminal pH, fibre digestion or milk production. Based on the results of both phases it can be concluded that the null hypothesis should be rejected.

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Uittreksel

Titel:	Die invloed van kragvoer voedingsstrategieë op rumen parameters, melkproduksie en melksamestelling van Jerseykoeie op raaigras- (*Lolium multiflorum*) of kikoejoe- (*Pennisetum clandestinum*) weidings.
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Rumen pH is die rumenparameter wat die meeste varieër. Bestudering van die oorsake en gevolge van daaglikse variasie bied geleenthede om rumentoestande vir rumenorganismes te verbeter. Gevolglik kan veseldegradering, melkopbrengs en melksamestelling ook verbeter. ‘n Studie is gedoen om die hipotese te toets dat rumen pH verbeter kan word deur ‘n groter proporsie van die daaglikse toekenning van kragvoer (6 kg) in die oggend as in die middag te voer. ‘n Verhoging in veselvertering, melkopbrengs en ‘n beter melksamestelling behoort waargeeneem te word met ‘n verhoogde rumen pH. Die projek is uitgevoer op die Outeniqua Navorsingsplaas in die Suid-Kaap in Suid-Afrika. ‘n Ewekansige blokontwerp is gebruik en twee fases is uitgevoer, nl. die raaigrasfase en kikoejoefase. Elke fase is gedurende die groeiseisoen van die betrokke grasspesie uitgevoer. ‘n Rumen- en produksiestudie is gedurende elke fase gedoen. Die rumenstudie het gebruik gemaak van ses gekannuleerde, meervoudige pariteit koeie, per behandeling. Rumen pH, vlugtige vetsuur (VVS) konsentrasie en *in sacco*-degradering van neutraal bestande vesel (NBV) en droëmateriaal (DM) is getoets. In die produksiestudie is 42 meervoudige pariteit koeie gebruik. Koeie is opgedeel volgens dae in melk, pariteit en melkopbrengs en ewekansig verdeel in drie groepe. Die produksiestudie het melkopbrengs, melksamestelling en veranderinge in liggaamsmassa en liggaamskondisie bestudeer.

Behandelings toegepas in die produksiestudie het bestaan uit ‘n kragvoeraanvulling met ‘n mieliebasis wat aan koeie op weidings gevoer is in verskillende gewigsverhoudings tussen oggend- en middagvoeding, as volg:

- Kragvoer gevoer: 5 kg gedurende die oggend; 1 kg gedurende die middag
- Kragvoer gevoer: 4 kg gedurende die oggend; 2 kg gedurende die middag
- Kragvoer gevoer: 3 kg gedurende die oggend; 3 kg gedurende die middag

Die eerste en laaste behandelings is aangewend in die rumenstudies. Resultate van die rumenstudie van die raaigrasfase het geen verskille tussen behandelings in terme van rumen pH opgelewer nie. Die tyd (ure) waartydens pH onder die kritiese vlak (pH 5.8) was, was van korte duur (2.92 tot 3.42 ure). Die VVS konsentrasie het by tye verskil, maar die verskille was nie duidelik waarneembaar uit die grafieke en data van rumen pH nie. Geen behandelingsverskille is waargeneem in terme van *in sacco* degradering van NBV of DM nie. Soortgelyke resultate is verkry in die rumenstudie van die kikoejoe-fase. Geen verskille is waargeneem t.o.v. maksimum, minimum of gemiddelde daaglikse pH tussen behandelingsgemiddeldes nie. Hoewel die tydsduur waartydens pH onder die kritiese vlak van 5.8 was, so lank as 7.1 ure was, het dit nie tussen behandelings verskil nie. Geen verskill is waargeneem tussen behandelings ten opsigte van *in sacco* NBV en DM degradering nie.

Resultate van die produksiestudie van die raaigrasfase het geen verskille getoon in melkopbrengs of melksamestelling tussen behandelings nie. Dieselfde is waargeneem vir die kikoejoefase, maar bottervet was wel hoër vir die behandelingsgroep wat die 3:3 kg verhouding ontvang het. Die gebrek aan respons in die produksiestudies kan toegeskryf word aan die nul-respons waargeneem in die rumenstudies i.t.v. rumen pH. Die voer van groter proporsies kragvoer in die oggend, relatief tot die middag, vir ‘n totale daaglikse hoeveelheid van 6 kg per koei/dag beïnvloed nie rumen pH, veselvertering of melkproduksie nie. Gegrond op die resultate van beide fases kan die gevolgtrekking gemaak word dat die nul-hipotese verwerp behoort te word.

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DEDICATION

Ad maiorem dei gloriam

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CHAPTER 1

General Introduction

Dairy production in the Republic of South Africa is shifting from interior areas like the central Highveld and Free State to the pasture based areas (Lactodata, 2010) mostly located along the coast of the Southern Cape, Eastern Cape and KwaZulu - Natal (Gertenbach, 2006). Production from planted pasture differs from production based on total mixed rations (TMR). The biggest difference perhaps is the lower milk yield as a result of lower total dry matter intake (DMI) observed for grazing cows receiving concentrate (White *et al*., 2001; Bargo *et al*., 2002). Supplementary feeding of energy dense concentrates is used to improve total DMI and milk production per cow compared to cows receiving pasture only diets (Peyraud & Delaby, 2001; Stockdale, 2000). Other reasons for supplementation include: improved utilization of pasture with increased stocking rates and longer lactation lengths during periods of pasture shortages *etc*. (Kellaway and Porta, 1993). Energy rich concentrates may, however not be used without constraint since high levels (>10 kg per cow/day) may be detrimental to metabolic health of cows (Bargo *et al*., 2003). Sayers (1999) has shown decreased ruminal pH with increasing levels of concentrate supplementation. Apart from this, the marginal milk response (MR) to concentrate has been described as having a curvilinear nature (Kellaway & Porta, 1993) with MR decreasing as concentrate DM intake increases (St - Pierre, 2001). Possible alternatives to increased concentrate supplementation might be to improve the feed value (quality) of the pasture by breeding and/or improving the utilization of current pasture species. Pasture species differ in feed value and suitability for animal production. Kikuyu (*Pennisetum clandestinum*) grass, for instance, is low in readily digestible non-structural carbohydrates, deficient in sodium and contains anti - nutritional compounds like oxalic acid (Marais, 2001).

Milk producers in the Southern Cape that use irrigated planted pastures mostly apply a grazing system with kikuyu grass oversown with either annual ryegrass (*Lolium multiflorum*) or perennial ryegrass (*Lolium perenne*) (personal communication, P.R. Botha, Outeniqua Research Farm, P.O. Box 249, George, 6530. July 2007). Nutrient analyses of these species from previous studies indicate that the major portion of the total dry matter is composed of NDF (Botha *et al*., 2008; Meeske *et al*., 2009). The logical focus area of improving utilization of these species would thus be to improve digestion and digestion rate of the NDF portion. Fibrolytic bacteria are important in this regard. Rumen bacteria represent the most numerous microbial group in the rumen ecosystem and the most

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predominant fibriolytic bacteria being *Fibrobacter succinogenes, Ruminococcus albus and Rominococcus flavefaciens* (Miron *et al*., 2001; Dehority, 2003). Creating the ideal rumen environment for the proliferation and function of these bacteria is important. This entails, among other rumen conditions, the ruminal pH. Rumen bacteria are sensitive to low pH (Stewart, 1977; Russel & Dombrowski, 1980). De Veth & Kolver (2001b), for instance, reported a negative linear relationship between time at suboptimal pH (5.4) and microbial N flow *in vitro.* The extent to which ruminal pH influences growth rate depends on the level of ruminal pH. At an external pH (pHe) of 5.6 growth rates are extremely low while at pHe 6 half of the maximum potential growth rates are realized (Miyazaki *et al.,* 1992). Other than the effect of pH on growth, low pHe also seem to influence the action of micro - organisms. Roger *et al*., (1990) reported that adhesion of *F. succinogenes* to feed particles differed at different pH levels *in vitro*. Increases in pH from 4.5 to 6 resulted in increased adhesion, while stable levels of adhesion were reached at between 6 - 7 (Roger *et al.,* 1990). Early studies, *in vitro* (Hiltner & Dehority, 1983) and *in vivo* (Mould *et al*., 1983), have shown that optimal pH for microbial digestion of fibre is in the range of pH 6.6 to 7.0. De Veth & Kolver, (2001b) has set the optimal at pH 6.3.

Obtaining and maintaining the ideal ruminal pH by means of feeding cows on pasture is a challenge. Ruminal pH of cows fed high quality pasture shows diurnal variation (Wales *et al.,* 2004) and often reach levels below that recommended to optimize digestion of pasture (De Veth & Kolver, 2001a). Studies by Malleson (2008) and Erasmus (2009) showed diurnal variation occurring as two periods of pronounced decline close to access to fresh pasture and/or time when concentrate were fed. Minimum daily pH was observed during the afternoon. Since pronounced diurnal fluctuations may necessitate continuous readjustments in the metabolism of micro-organisms, it may be detrimental to the fibrolytic bacteria (Mertens, 1979).

The current research project aimed to address the fluctuation in ruminal pH below the critical value (5.8) (De Veth & Kolver, 2001a) for fibre digestion occurring during the afternoon period. The hypothesis was that the fluctuation in ruminal pH during the afternoon (16:00) would be less by increasing the ratio of morning to afternoon concentrate allocation during milking. In order to test the hypothesis a detailed literature review was conducted on, among other things, rumen anatomy and function, rumen micro-organisms, factors influencing ruminal pH and the effect of ruminal pH on microbial growth and action as well as on milk yield, milk composition and fibre digestion. Two studies *i.e.* a rumen study and a production study were conducted per phase, in two phases *i.e*. a ryegrass and a kikuyu phase. The rumen studies tested changes in rumen pH, volatile fatty acid (VFA) concentration of rumen fluid and changes in neutral detergent fibre (NDF) degradation *in sacco*. The production study tested mean milk yield and milk composition.

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CHAPTER 2

Literature review

2.1 Introduction

Ruminants depend on a complex symbiotic relationship with ruminal micro-organisms for energy and protein. Central to this relationship is the rumen; the largest of the four stomach compartments. The rumen acts like a large fermentation vat and houses complex populations of micro-organisms. Physiologically it is well adapted for fibre fermentation. Orderly rumen contractions for instance ensure adequate mixing and buffering and assist with rumination. Ensuring the success of the symbiotic relationship requires maintaining ecological conditions in the rumen that will lead to microbial proliferation and function. Seven ecological conditions are important. These are: I) Ruminal pH, II) NH_3-N concentration, III) osmotic pressure, IV) temperature, V) anaerobiosis, VI) oxidation-reduction potential and VII) structure of digesta (Dehority, 2003). Various factors and interventions can affect conditions in the rumen of cows. In Figure 2.1, a schematic representation is given of factors and interventions that have an effect on the ecological conditions and the subsequent production of volatile fatty acids (VFA's) and microbial protein (MP). In this thesis, the focus will mostly be on the ruminal pH of cows that graze on irrigated pasture and receive concentrate supplements.

Human interventions (H, see Figure 2.1) consists of three pillars *i.e.*: H1) pasture management, H2) feeding management and H3) rumen manipulation. Pasture management (H1) entails pasture allowance (PA), fertilization levels, pasture specie selection, grazing systems *etc*. These aspects influence the ruminal environment in different ways, for instance legumes and ryegrass pasture has different effects on the ruminal pH due to different rates of fermentation. Different levels of PA influence ruminal pH differently.

Feeding management (H2) consists of managing quantity and quality of concentrate supplement provided to grazing cows as well as time of the day when concentrates are provided. Providing concentrate of high starch content result in increased rates of fermentation; influencing rumen pH. Feeding management (H2) and pasture management (H1) are sometimes not clearly set apart since PA can be categorized under both classes of human interventions. Rumen manipulation (H3, see

Figure 2.1) by means of supplementing diets with dietary buffers can offset decreases in ruminal pH and is necessary when high levels of starch is included in the diet or when the cows own buffer system can no longer cope.

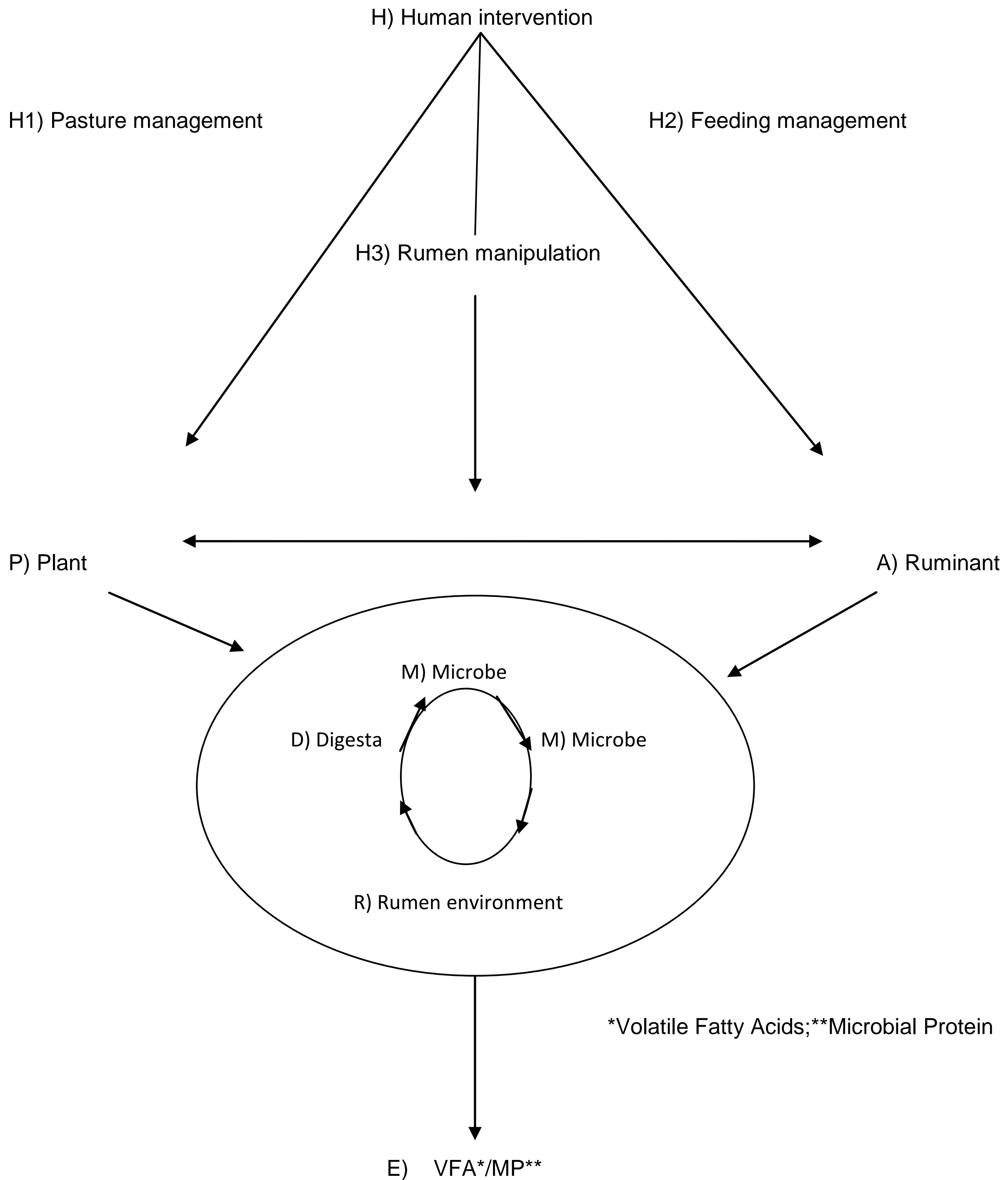

Figure 2.1 Factors and human interventions affecting rumen conditions

Factors that can affect the rumen environment include: P) plants, A) the ruminant and M) micro-organisms. The cell wall matrix of plants, for example, hinders access of micro-organisms to the inner, more digestible parts of plant cells and thus affects the rate of fermentation and ruminal pH. Plant material also has an inherent buffering capacity (BC) that influence ruminal pH in some instances. Saliva from the ruminant (A), secreted during eating and ruminating influences pH. Saliva contains sodium, potassium, phosphate and bicarbonate that serve as a buffer to prevent ruminal pH from decreasing below critical levels. Micro-organisms (M) affect ruminal pH, NH_3-N and the gas composition of the rumen. Ruminal pH is closely related to VFA concentration that is produced as a result of microbial fermentation. Ammonia N is utilized by the micro-organisms for synthesis of MP (McDonald *et al*., 2002).

2.2 Rumen anatomy and function

The bovine rumen varies in size from 35 – 100 L (Dehority, 2003) and surpasses the other three stomach compartments (reticulum, omasum, abomasum) in size. The rumen and reticulum are often considered and studied as a single organ (reticulo-rumen) because separation is only partial and because free exchange of contents is possible (Van Soest, 1994). The reliculo-ruminal fold and various pillars are responsible for the partial separation of the two compartments and aid in contraction necessary for mixing digesta and saliva. During contraction the rumen and reticulum become smaller and more liquid ingesta are circulated and generally forced upward through the floating matt of more solid digesta (Van Soest, 1994). Contractions are orderly and synchronized. The rumen is lined with non-mucus-producing, keratinized, stratified squamous epithelium that is the site of absorption, active transport of sodium and chloride and passive transport of VFA's, water and substances such as urea (Van Soest, 1994). Papillae, up to 1.5 cm in length protrude into the lumen of the rumen and are more pronounced in the ventral regions where nutrient concentration is more pronounced. Papillae increase the surface area for absorption and tend to be more pronounced in rumens of cattle receiving high levels of concentrate.

2.3 Rumen micro-organisms

Based on current knowledge of the rumen ecosystem, three species of micro-organisms represent populations within the ecosystem *i.e.* protozoa, bacteria and fungi. Colonization of the rumen starts immediately after birth and follows a typical ecological succession (Cheng *et al.,* 1991). Microaerophilic and ureolytic bacteria first colonize the rumen epithelium and rumen fluid while other species start to colonize the feed particles. A cellulolytic consortia forming on the feed particles develop during day three to four (Cheng *et al*., 1991). Monocentric and polycentric cellulolytic rumen fungi colonize between days 8 and 10 and join the microbial cellulolytic consortium. Protozoa are the

last microbial group to colonize between day 12 and 20 and are associated with methanogenic and cellulolytic consortia (Cheng *et al.*, 1991). The end result is a complex multispecie consortia developing on different locations and different substrates in the rumen. The consortia remain stable unless profound changes to the ruminant diet are introduced and the nutrient substrate of microbes changes as a result (Cheng *et al.*, 1991).

Bacteria represent the most numerous microbial group in the rumen ecosystem. More than 200 different species of rumen bacteria, mostly non spore forming anaerobes exist and number 10^9-10^{10}/ml of the rumen contents (McDonald *et al.*, 2002). Thirty of the 200 species are considered as predominant species; the most prominent fibriolytic bacteria being: *Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus,* (Miron *et al.*, 2001; Dehority, 2003). These species are able to digest both cellulose and hemicellulose. Though *Butyrivibrio fibrisolvens* is capable of cellulase production, Varga & Kolver (1997) suggests that it is mostly involved in hemicellulose hydrolysis together with *Prevotella ruminicola* (Dehority, 2003). Bacteria are classified according to function (Van Soest, 1994) or according to environmental existence (Cheng and Costerton, 1980; McAllistter *et al.*, 1994). Five different categories exist for the latter classification *i.e.*: I) free living bacteria associated with rumen liquid phase; II) bacteria loosely associated with feed particles; III) bacteria firmly attached to feed particles; IV) bacteria associated with rumen epithelium; V) bacteria attached to the surface of protozoa or fungal sporangia. Fibrolytic bacteria seems to focus on the more digestible structures like mesophyll cells, but are able to digest parenchyma bundle sheaths, epidermal cell walls and leaf sclerenchyma (Akin, 1989). During forage digestion cellulolytic bacteria and non-cellulolytic bacteria are responsible for synergistic actions. In a co-culture of *P. ruminocola* and *F. succinogenes,* Osborne and Dehority, (1989*)* reported a twofold increase in the utilization of orchardgrass hemicellulose and pectin over the utilization by *F. succinogenes* alone.

Protozoa number 10^6/ml of rumen contents and include more than 100 species. They are mostly ciliates (McDonald *et al.*, 2002) belonging to one of two families *i.e.* holotrichs or *Isotrichidae* and entodiniomorphs or *Ophryoscolecidae* (McDonald, *et al.*, 2002; Van Soest, 1994). The latter group represents the greater number of species (Van Soest, 1994). Protozoa represents a large proportion (20 - 40% of net microbial nitrogen) of the rumen biomass (Van Soest, 1994) and may equal bacteria in mass (McDonald *et al.*, 2002) despite lower numbers. Protozoa are larger in size. Output of protozoa may be minimal due to long generation time, slow turnover and high retention (Van Soest, 1994).

The third rumen micro-organism specie *i.e.* fungi, has only been discovered and studied since the 1970's (Theodorou *et al.,* 1992) due to the mistaken identification of fungi. Prior to the 1970's the zoospore was mistaken for flagellated protozoans. An insufficient method applied to prepare rumen contents also contributed to the late discovery of fungi. Because fungi are closely attached to

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ingested fibrous material fungi was unwittingly disposed of in the filtered fibrous materials of earlier rumen fluid preparations. Five genera exists *i.e. Neocallimastix, Caecomyces, Pyromyces and Orpinomyces, Anaeromyces* (Theodorou *et al*., 1992; Ho *et al*., 1990). Fifteen different species have been identified from the gut of herbivores of which seven are classed under the *Piromyces* genera.

2.4 Ruminal pH

Of the seven rumen conditions mentioned in the introduction ruminal pH has been studied most extensively and has proved to vary most of all (Dehority, 2003). Ruminal pH or hydrogen ion concentration is the result of a balance between production rate of short chain volatile fatty acids and hydrogen ion removal by absorption, neutralization, buffering and passage (Allen *et al*., 2006). Kolver and de Veth (2002) studied data from 121 pasture based studies and reported that the mean daily ruminal pH varied from 5.6 to 6.7 resulting in an average ruminal pH of 6.15. The lowest average ruminal pH ever reported for a pasture diet was 5.6 (Williams *et al*., 2001). Other than variance in mean daily ruminal pH, studies have also reported diurnal variation in ruminal pH. Fluctuations occur with time after feeding, nature of the feed and frequency of feeding (Dehority, 2003). Erasmus (2009) reported pH values of cows grazing westerwold ryegrass, supplemented with either 4 or 8 kg of concentrate (see Figure 2.2). The pH profile, measured over a 24 hour period, shows two rapid declines in pH that coincides with access to concentrate during milking (7:30 and 15:30) and access to fresh allocation of herbage following milking. The time of decline is similar than that reported by Wales *et al.* (2004). The lowest pH was measured 4 hours (19:30) after evening milking and was 5.91 and 5.69 for the treatments with 4 and 8 kg of concentrate supplement respectively. Carruthers *et al*. (1997), Kolver & de Veth (2002) and Wales & Doyle (2003) have reported diurnal pH variations from a minimum level of 5.5 to a maximum level of 6.8. Williams *et al*. (2005a) reported a lag effect in decline in ruminal pH following onset of grazing clover pasture. The same study reported a lag effect in ruminal pH increasing when ruminating time was longer than grazing time.

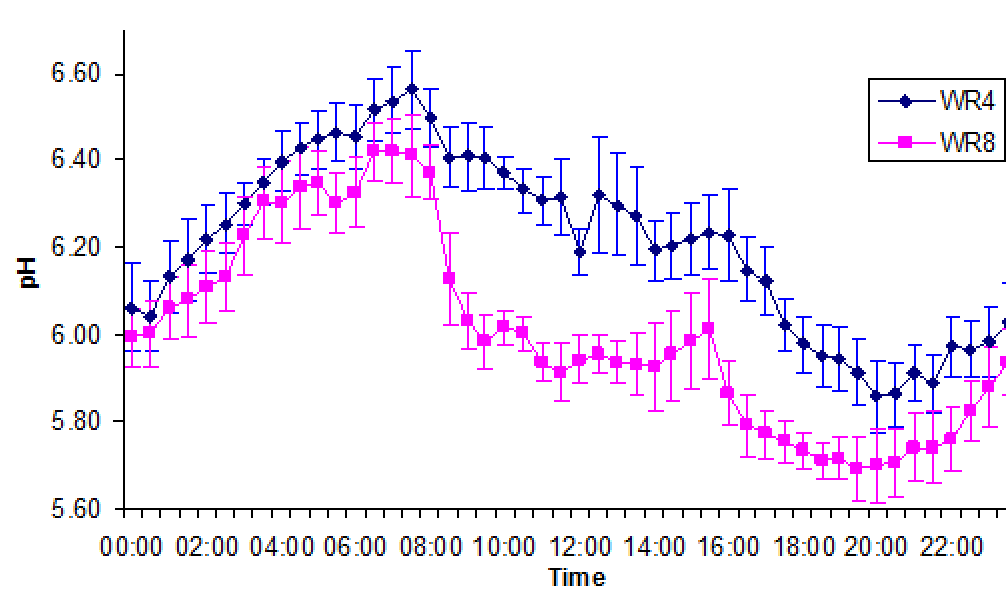

Figure 2.2 The mean rumen pH results of the cows that grazed westerwold ryegrass treatment and received either four or eight kilograms of concentrate per day, with standard error bars. WR4 = westerwold ryegrass, 4 kg of concentrate; WR8 = westerwold ryegrass, 8 kg concentrate (Source: Erasmus, 2009).

2.4.1 Factors influencing rumen pH

Different factors are responsible for shifts in ruminal pH. These factors are: I) plant factors, II) animal factors and III) management factors.

2.4.1.1 Plant factors

Plant breeders have recently turned attention to increasing water soluble carbohydrate (WSC) content of grass in an attempt to increase palatability and to synchronize energy with the highly degradable crude protein (CP) of pasture. However, higher levels of WSC content of pasture have been implicated in possible reductions in ruminal pH (Beerepoort *et al*., 1997). The study of Taweel *et al.* (2004) reported that feeding perennial ryegrass varieties with higher levels of WSC did not result in reduced ruminal pH. This differs from the *in vitro* study of Lee *et al.* (2003) that reported linear reductions ($P < 0.001$) in pH when sugar infusions of inulin and sucrose (80:20) was added to perennial ryegrass incubated in a RUSITEC system. Trevaskis *et al*., (2004) studied the effect of time of pasture allocation (morning vs. afternoon) on rumen pH based on studies like Fulkerson & Donaghy (2001) that showed that pasture contains more WSC in the afternoon than the same pasture in the morning. Although pasture had 52 g/kg DM more WSC than the same pasture in the morning,

Trevaskis *et al.* (2004) could not find a significant effect on mean ruminal pH. A significant difference ($P < 0.001$) on duration of ruminal pH at lowest values was, however reported. Minimum daily rumen pH (6.89 ± 0.06 (mean ± S.E.M.)) of cows that received fresh pasture in the morning was reached seven hours after commencement of grazing while this value (6.7 ± 0.06 (mean ± S.E.M.)) was reached three hours post commence of grazing for cows receiving fresh pasture in the afternoon. Van Vuuren *et al.* (1986) reported similar findings; ruminal pH of dairy cows grazing ryegrass pasture was lowest after evening milking. The effect of WSC content of pasture on ruminal pH needs to be investigated in more detail.

Forages have inherent buffering capacities. Three primary buffering systems exist to counter decreases in ruminal pH as a result of acid ingested or acid that is produced by microbial fermentation. The three systems are: I) buffers in saliva, II) buffering capacity (BC) of ingested feed and III) added dietary buffers (Erdman, 1988). System one and two will be discussed under more relevant sections *i.e.* section 2.4.1.2 and 2.4.1.3 respectively. The inherent buffering capacities of forages were studied by Playne & McDonald (1966) that showed that legumes had higher buffering capacities compared to grasses or whole corn plants when fresh forages were fed. Ensiling alfalfa and corn resulted in a two to three fold increase in buffering capacity in pH range 4 to 6. This was ascribed to the presence of organic acids from silage fermentation. Jasaitis *et al.* (1987) reported that cereal grains had low buffering capacities in pH range of 4 to 9 compared to hays and protein sources like soybean meal. The inherent buffering capacity seems to be important only when ruminal pH decrease to levels below 5.5. Most BC's of feed is at pH lower than normal ruminal pH which limits its usefulness. As pH decrease below 5.5 increasingly greater proportions of the total BC is used.

Plant species seem to influence ruminal pH differently. Williams *et al.* (2005a) reported that at equivalent DM intake Persian clover pasture resulted in lower ruminal pH and higher VFA concentrations compared to perennial ryegrass. Ruminal pH also remained below 6 for longer periods compared to when cows grazed ryegrass pasture. The lower ruminal pH observed when cows grazed clover pasture, is associated with a decrease in chewing as reported by Williams *et al.* (2001).

2.4.1.2 Animal factors

The volume of ruminal fluid determines dilution levels of hydrogen ions and affects the passage of hydrogen ions from the omasal orifice (Allen, 1997). Water flux into the rumen and from the rumen, therefore plays a role in ruminal pH. Water flux into the rumen occurs mainly via drinking, feed consumption and saliva (Allen, 1997). Saliva is primarily responsible for water flow into the rumen and may range from 180 (Van Soest, 1994) to 308 litres/day (Cassida & Stokes, 1986). Church (1988) reported that almost 70% of all water entering the rumen comes from saliva secretion. Water

intake of early lactation cows was shown to be dependent on feed consumption, environmental temperature, sodium intake and milk production in a study by Murphy *et al.* (1983). The influx of water from feed is estimated to be 2 - 14 L/d for cows consuming 20 kg DM/d with moisture contents of 10 to 70 % (Allen, 1997). Water flow from the rumen occurs when it passes through the omasal orifice or by flux across the rumen wall.

Volatile fatty acids are end products of the anaerobic microbial fermentation process and are closely related to ruminal pH. Volatile fatty acids provide the ruminant with a major source of metabolisable energy (Van Soest, 1994). The principal fatty acids in order of abundance are: acetic acid, propionic acid and butyric acid. Minor fatty acids include isobutyric, valeric and isovaleric acid (Van Soest, 1994). Ruminal acids are considered mild acids, have pKa's around pH 4.8 and are the primary acids involved in sub-acute ruminal acidosis (Allen *et al.*, 2006). Production rate and amount of VFA's vary diurnally as a result of eating patterns, organic matter (OM) intake and proportion of rumen degraded organic matter (RDOM) in the diet (Van Soest, 1994; Allen *et al.*, 2006). The quantity of fermentable organic matter (OM) in the rumen, and thus VFA production, in turn depends on dry matter intake (DMI), flow rate of OM from the rumen and rate of fermentation (Allen *et al.*, 2006).

Volatile fatty acids are removed from the rumen by means of: I) absorption across the rumen wall and II) passage through the omasal orifice. Removal of VFA's result in a net removal of hydrogen ions because little or no VFA's are absorbed in the ionized form (Ash & Dobson, 1963). Absorption rates vary at certain pH levels and depend on the type of VFA. At neutral pH the three primary VFA's are absorbed at similar rates (Allen, 1997) but with decreasing pH the absorption rates differ because of increases in molecular weight (Danielli *et al.*, 1945; Dijkstra *et al.,* 1993). Absorption rate of VFA is dependent on the VFA concentration gradient. Mixing of rumen content may result in increases in the VFA concentration at ruminal epithelium in turn resulting in increased VFA absorption (Allen, 1997). The effective surface area for absorption is another factor influencing absorption since it influences flux of VFA absorbed. Increase in ruminal fluid volume from 10 to 30 L showed a decrease in fractional rates of absorption (Dijkstra *et al.,* 1993). Ruminal papillae surface affects rates of VFA absorption and is influenced by diet (Allen, 1997). Passage of VFA's through the omasal orifice occurs mainly as part of the liquid fraction; increases in rate of liquid passage resulting in increased VFA passage. A considerable fraction of VFA is removed via the omasal orifice. Allen (1997) reported that for cows at maintenance intake and at four times maintenance intake 29% and 39% of VFA's were removed via the orifice, respectively.

Hydrogen ions are removed, not only by means of absorption of VFA's across the rumen wall, but also by means of a combination of alkalization and buffering. This process involves saliva that contains bicarbonate (126 meq/L) and hydrogen phosphate ions (26 meq/L) (Bailey & Balch, 1961). Hydrogen ions combine with bicarbonate and forms carbonic acid (H_2CO_3) that is converted to H_2O

and CO_2. The latter is constantly lost via eructation (Allen *et al*., 2006). The phosphate buffer system differs from the carbonate buffer system and is less of a buffer system than an alkalizer system because it is nearly completely complexed with hydrogen ions below pH 6 (Allen *et al*., 2006). At pH 6, 94% of the potential BC of hydrogen phosphate is utilized when it is complexed to dihydrogen phosphate and is removed by passage through the omasal orifice (Allen *et al*., 2006). Both systems depend on flow of saliva rather than composition of saliva because composition remains constant regardless of diet or feed intake (Erdman, 1988). Saliva flow varies with chewing activity and differs when cows eat or rest (Allen, 1997). Cassida & Stokes (1986) reported saliva flow values of multiparous Holstein cows. During times of rest saliva flow was 177 ml/min and during eating 151 ml/min. However, Allen (1997) argued that the former value might have been overestimated due to cardial stimulation during the collection. Saliva flow also differed as a result of the stage of lactation. Cassida & Stokes (1986) reported saliva flow increases from 130 to 173 ml/min between the fourth and the eighth week postpartum. The same study reported saliva flow, 1.8 times the measured value of the resting period for the ruminating period. Flow of saliva is stimulated by eating and ruminating so that total salivary flow is related to time spent eating and ruminating (Van Soest, 1994). Concentrate intake is more rapid than forage intake resulting in less time spent eating and thus less saliva per gram feed (Van Soest, 1994). This partially explains the lower rumen pH of cows fed concentrate.

Forage dry matter intake (DMI) and rate of forage intake seem to influence rumen pH of cows grazing under continuous stocking. Taweel *et al*. (2004) studied grazing behaviour during the three main grazing bouts *i.e*. dawn (6:00 to 12:00), afternoon (12:00 - 18:00) and dusk (18:00 - 24:00) and the effect on rumen fermentation. Total eating time (TET) ($P = 0.005$), biting rate (BR) ($P = 0.08$) and bite mass (BM) ($P = 0.026$) increased as the day progressed while chewing rate decreased ($P = 0.08$) resulting in increased DMI and rate of intake. The rumen pH decreased gradually ($P < 0.05$) as the day progressed from 6.5 to 6. This was explained by the increased concentrations of propionate and butyrate ($P < 0.05$) resulting from the increased DMI and rate of intake. Williams *et al*. (2005a) reported similar findings with regard to effect of DMI on rumen pH when they studied fermentation characteristics of cows grazing clover pastures. Williams *et al*. (2005b) reported that average daily rumen pH decreased linearly with increasing DMI of Persian clover (*Trefolium resupinatum L.*) from different PA. This is explained by the increase in VFA concentration observed at higher PA. Dry matter intake increased asymptotically as PA increased. More rapid declines in rumen pH were reported at high PA.

2.4.1.3 Management factors

The relationship between ruminal pH and quantity of concentrate supplemented to cows on pasture is complicated by inconsistent reports (Bargo *et al.,* 2003). Studies that fed different quantities of concentrate were reviewed by Bargo *et al*. (2003) to quantify the relationship. Concentrate

supplements caused reductions in ruminal pH in studies where cows grazed orchard grass and received more than 8 kg DM/day (Bargo *et al.*, 2002). Confined cows receiving ryegrass, supplemented with low (< 1.5 kg DM/d) levels of a non-structural carbohydrate (NSC) supplement (50:50 corn flour and dextrose monohydrate) had similar responses (Carruthers & Neil, 1997). Sayers *et al.* (2003) studied the effect of high (10 kg) vs. low (5 kg) concentrate level and high starch vs. high fibre type concentrate on rumen fermentation of cows on pasture. They reported decreased ruminal pH with increasing quantities of concentrate supplements. This is consistent with reports by Bargo *et al.* (2002). Contradictory to this, Garcia *et al.* (2000) found no effect on ruminal pH when winter oat pasture was partially supplemented (2.5 kg DM/d) with either ground corn or barley. Jones-Endsley *et al.*, 1997 also reported no decline in pH when concentrate level was increased from 5.6 to 8.4 kg DM/d. Bargo *et al.* (2003) speculated that inconsistent results between studies might have been the result of pasture quality differences or different timing of rumen sampling. When studies were divided into groups that contained high (< 50% NDF) and medium (> 50% NDF) quality pasture no pattern could be observed, eliminating pasture quality as cause.

The review of Bargo *et al.* (2003) studied the effect of different types of energy supplements on rumen pH by comparing starch vs. fibre based concentrates. Starch sources included: corn (Van Vuuren *et al.*, 1986), barley and wheat (Sayers, 1999) and oats (Khalili & Sairanen, 2000). Fibre sources included: beet pulp (Van Vuuren *et al.*, 1986) and citrus pulp (Sayers, 1999). The study of Sayers *et al.* (2003) reported lower rumen pH levels with high starch concentrate compared to high fibre concentrate (concentrates being equal in CP, ME and ERDP). This finding was confirmed by the study of Sayers (1999). In cases where moderate (5 kg DM/d) amounts were offered (Van Vuuren *et al.*, 1989; Khalili & Sairanen, 2000) similar findings were made. Lana *et al.* (1998) reported a linear decrease ($P < 0.001$) in rumen pH with increasing levels (0, 45, 90 % DMI) of concentrate. In studies where protein supplements were used for grazing cows, ruminal pH was not influenced (Sayers, 1999, Bargo *et al.*, 2001; McCormick *et al.*, 2001). The study of Delagarde *et al.* (1997) did, however report reduced pH when cows grazing ryegrass received 2 kg DM/d of soybean meal supplement.

The influence of forage supplement was evaluated by Elizalde *et al.* (1992) and Reis & Combs, (2000) that used corn silage and hay respectively. Elizalde *et al.* (1992) found that ruminal pH increased with corn silage supplements (5 kg DM/d) when cows grazed winter oats pasture. Supplementing cows, grazing grass legume pasture, with long alfalfa hay (3.2 kg DM/d) plus dry ground or steam-rolled corn had no effect on ruminal pH (Reis & Combs, 2000). Physical form of corn supplements does not seem to influence ruminal pH. Soriana *et al.* (2000) reported no differences in ruminal pH when cows on pasture received 6 kg DM/day of corn supplement in either coarsely ground or high moisture form.

Apart from concentrate level, form and type of concentrate, interactions between concentrate supplement (CS) and PA also influence ruminal pH. Bargo *et al.* (2002) reported a significant

interaction ($P < 0.05$) between CS and PA for ruminal pH. Decreases in ruminal pH were greater at low PA (6.57 vs. 6.25 pH) compared to high PA (6.40 vs. 6.23 pH) ($P < 0.05$). When no concentrate was given a high PA resulted in a lower ruminal pH (William *et al.*, 2005a). This is explained by the higher DMI observed at high PA leading to greater VFA production rates.

The effect of different levels of N fertilization of pasture on rumen fermentation and ruminal pH has been reported by Van Vuuren *et al.* (1993), Mackle *et al.* (1996) and Peyraud *et al.* (1997). Peyraud *et al.* (1997) reported no effect of pasture treatments with 0 and 80 kg N ha^{-1}. This was confirmed by the study of Van Vuuren *et al.* (1993) where fertilizer levels (275 kg and 500 kg N ha^{-1}/year) did not have an effect.

The form in which roughage is presented, *i.e.* pasture vs. stored forage (silage or hay), and forage particle size does have an effect on ruminal pH. Holden *et al.* (1994) studied the effect of the three different forms of the same grass specie. The mean ruminal pH of grazing cows was less than those of cows receiving stored forage. The most marked differences were observed during afternoon and evening hours. Forage particle size has an indirect effect on ruminal pH by means of influencing rumination. Coarse forage fibre affects ruminal pH by retaining digesta in the rumen. This provides the inherent buffering capacity of fibre to function and increase salivary buffer flow through stimulation of rumination (Allen *et al.*, 2006). A total mixed ration (TMR) study by Krause *et al.* (2002) reported that increasing the particle size of forages resulted in an increase in ruminating because of increases in number of ruminating periods per day and because a trend towards an increase ($P = 0.07$) in duration of each rumination period was observed. The study showed that forage particle size had a greater influence on mean rumen pH than level of RFC. Minimum daily ruminal pH decreased from 5.66 to 5.47 with increased RFC levels and decreased from 5.47 to 5.48 when forage particle size was decreased from 1.9 cm to below 1.9 cm. Time spent below the 5.8 pH level and area below pH 5.8 increased with increases in RFC level and decreases in particle size. These results however may not be applicable to pasture based diets and more research is required in that field.

Dairy operations make use of dietary buffers to prevent acidosis. Examples of dietary buffers include: magnesium oxide, potassium carbonate, potassium bicarbonate, sodium bicarbonate and sodium sesquicarbonate. Though MgO is often regarded as a buffer, it is in actual fact a neutralizing agent under rumen conditions. Dietary buffers and neutralizing agents range in acid consuming capacity due to different physical and chemical characteristics (Ruyet & Tucker, 1992). Ideally buffers and neutralizing agents should be released during the interval of most severe acid production or should provide a continuous release to prevent fermentation related increases in free proton concentration (Ruyet & Tucker, 1992). Ruyet & Tucker (1992) studied the effect of $NaHCO_3$, sodium sesquicarbonate, a multi-element buffer and MgO on rumen pH. $NaHCO_3$ and sodium sesquicarbonate had higher buffer value indexes compared to the multi-element buffer and MgO and

was active within a shorter time. The authors concluded that $NaHCO_3$ and sodium sesquicarbonate should be more beneficial in preventing short term postprandial increases in rumen fluid hydrogen ion concentration.

2.4.2 Effect of rumen pH on microbial growth and action

Ruminal pH influences microbial growth, action and competition to various extends depending on species involved. *Butyrivibrio fibriosolvens* for example is considered as the cellulolytic specie most resistant to low pH (Russell & Dombrowski, 1980). Russell *et al.* (1979) reported that the growth rate of *B. fibriosolvens* is not influenced by a decline in pH_e from 6.75 to 5.95. The three main cellulolytic bacteria (*R. albus, R. flavefaciens, F. succinogenes) (*Hungate, 1950) are however sensitive to low pH (Stewart, 1977; Russell & Dombrowski, 1980; Russell, 1987). Miyazaki *et al.* (1992) have shown that the growth rates of the three species were only half of the maximum rate at an extracellular pH (pH_e) of 6 whilst growth rate was extremely low at pH_e 5.6. Maximum growth rates for the different species were obtained at different pH_e. *Ruminococcus albus* showed maximum growth rates at pH_e6.4 whilst *F. succinogenes* reached peak growth rates at pH_e 6.8 (Miyazaki *et al.*, 1992). Differences in rate of reduced growth as a result of declining pH_e may also occur between species. *Selenomonas ruminatium* for example displayed a stepwise decline ($P < 0.05$) in growth rate with decline in pH_e in the ranges 6.7 - 6.5 and 5.9 – 5.4 (Russell *et al.*, 1979) while Russell and Dombrowski (1980) reported abrupt sensitivity of the three main cellulolytic bacteria to declining pH_e. In the latter case the authors reported that a decrease of 0.25 units often made the difference between maximum cell yield and washout. The sensitivity to declining pH_e was thought to be related to enzyme activity being inhibited by low pH_e or by VFA toxicity explained by a mechanistic approach based on "uncoupling". However, both theories had flaws. Russell & Wilson (1988) have reported that cellulases generally have low pH optima rendering the first approach inaccurate. Bacteria also differ in their sensitivity to VFA (Russell, 1992).

Despite the internal pH of the bacteria decreasing due to the decrease of pH outside the bacteria (as described above) the external pH is still substantially lower than the inside of the cells as the cells actively try to maintain their intracellular pH (pH_i). The chemiosmotic theory and the anion accumulation approach give an accurate explanation of the sensitivity. The theory was formulated by Peter Mitchell in the early 1960's and explains the concept that a proton concentration gradient serves as the energy reservoir for driving ATP formation (Horten *et al.*, 1996). The hypothesis is that most bacteria utilize membrane bound ATPases or electron transport chains to translocate protons from the intracellular space (Russell *et al.,* 1990). A chemical (ΔpH) and an electrical gradient ($\Delta \Psi$) develop

as a consequence. The driving force of the two gradients is predicted by the Nernst equation (Russell *et al.,* 1990):

$-2.3\ (RT/F) \times \log\ [in] / [out],$

With $-2.3\ (RT/F) = 60$ mV at 25°C, thus $\Delta\Psi$ has a force of:

$60mV \times \log\ [H^+]_{in} / [H^+]_{out}$

Because pH is already a log scale, the chemical gradient of protons ($Z\Delta pH$) becomes:

$-60mV \times \Delta pH$

The $Z\Delta pH$ is, therefore by convention a negative value. The sum of the two gradients forms the total driving force (Δp, the protonmotive force) (Russell *et al.*, 1990).

$\Delta p = \Delta\Psi - Z\Delta pH$

Protonmotive force of bacteria generally ranges from 140-160mV (Kashket, 1985). Since many neutrophilic bacteria maintain a pH gradient across the cell membrane (Padan *et al.*, 1981), lipid soluble VFA like acetate pass through the cell membrane and dissociate in the alkaline interior (Russel & Wilson, 1996) (see Figure 2.3). Anion accumulation occurs and is dictated by the Hendeson Haselbach equation, the logarithmic function:

$pH = pKa + \log\ ([anion] / [acid])$

(Russell & Wilson, 1996). The mechanism is, however, not successful in the case of cellulolytic bacteria like *R. albus* because growth tends to cease when pH_i is < 6.0 due to inhibition of cellular metabolism (Russell & Wilson, 1996). Miyazaki *et al.* (1992) concluded that cellulolytic bacteria use energy for growth in preference to regulating pH_i until pH_i is lowered to less than 6 and cellular metabolism is compromised resulting in inhibited growth.

The conclusion is based on data that showed that pH_i of *R. albus* decreased linearly with a decrease in pH_e but that the decrease was not linear when cellobiose was added suggesting that energy is utilized to maintain pH_i at 6 when pH_e is 5.6.

Studies suggest that ruminal pH might affect microbial action by influencing attachment to substrate. Microbial adhesion to feed particles (Cheng *et al.*, 1991) and close juxtaposition of consortia (Kudo *et al.,* 1990) is of pivotal importance to microbial colonization and digestion. Adhesion of cellulolytic bacteria occurs via a process where different mechanisms are used and is complicated by bacteria, substrate and rumen environmental related factors. The bacterial adhesion process is divided in four phases to distinct between specific and non-specific adhesion (Miron *et al.*, 2001). The phases are: I) transport of bacteria to fibrous substrates; II) nonspecific adhesion to proper sites on substrate. III) specific adhesion via extensive linkages and adhesions and IV) proliferation by cell division to the point of colonies on specific sites of the plant tissue. The phases follow in a successive order (Miron *et al.,* 2001).

Smith *et al.* (1973) reported an apparent interaction between pH and attachment. In this study the amount of cellulolytic enzyme adsorbed to a cellulose substrate was associated with pH. Miron *et al.* (2001) reported that the effect of pH on adhesion of cellulolytic bacteria to cellulose varied according to bacteria. An *in vitro* study with *F. succinogenes* showed that adhesion to cellulose differed at different pH levels (Roger *et al.,* 1990).

With increases in pH from 4.5 to 6 adhesion increased whereas stable levels of adhesion was observed between pH 6 and 7. Interestingly adhesion levels seem also to decline with increasing levels of pH. An abrupt decrease in adhesion was reported when pH reached levels above pH 7.5. Roger *et al.* (1990) studied adhesion of *R. flavefaciens* to cellulose and found that adhesion was stable between pH 3.3 to 7.5 but decreased at pH 8. The study of Shriver *et al.* (1986) showed a marked decrease of 43% in microbial attachment when pH was reduced from 6.2 to 5.8 with a corresponding decrease of 15% in total microbes. The *in vitro* study of De Veth and Kolver (2001a) refers to decreased adhesion as the reason for the largest reductions in digestibility and microbial population at suboptimal pH occurring at different times *i.e.* 4 h and 8 h. There are, however studies that contradict the ones mentioned. Gong & Forsberg (1989) reported that the adhesion of *F. succinogenes* were not affected over a pH range of 5.3 to 6.8 while Rasmussen *et al.* (1989) reported no difference in adhesion between pH 6 to 8. Mirion *et al.* (2001) ascribed the differences between studies to variations in the technique and bacterial strains.

No literature could be found that specify the mechanism by which lowered pH results in decreased microbial attachment. The question as to which of the four phases of attachment is influenced and why adhesion is negatively influenced at pH levels higher than 7.5 also remain unanswered.

Ruminal pH may also influence competition among rumen bacterial species. Russell *et al.,* 1979 reported that the maximum growth rate of *Selenomonas ruminantium* surpassed that of *Bacteroides. ruminicola* at pH_e 6.7. At pH_e 5.85 - 5.9 the opposite was true and at pH_e 5.6 - 5.4, *S. ruminantium* again had a higher maximum growth rate than *B. ruminicola*.

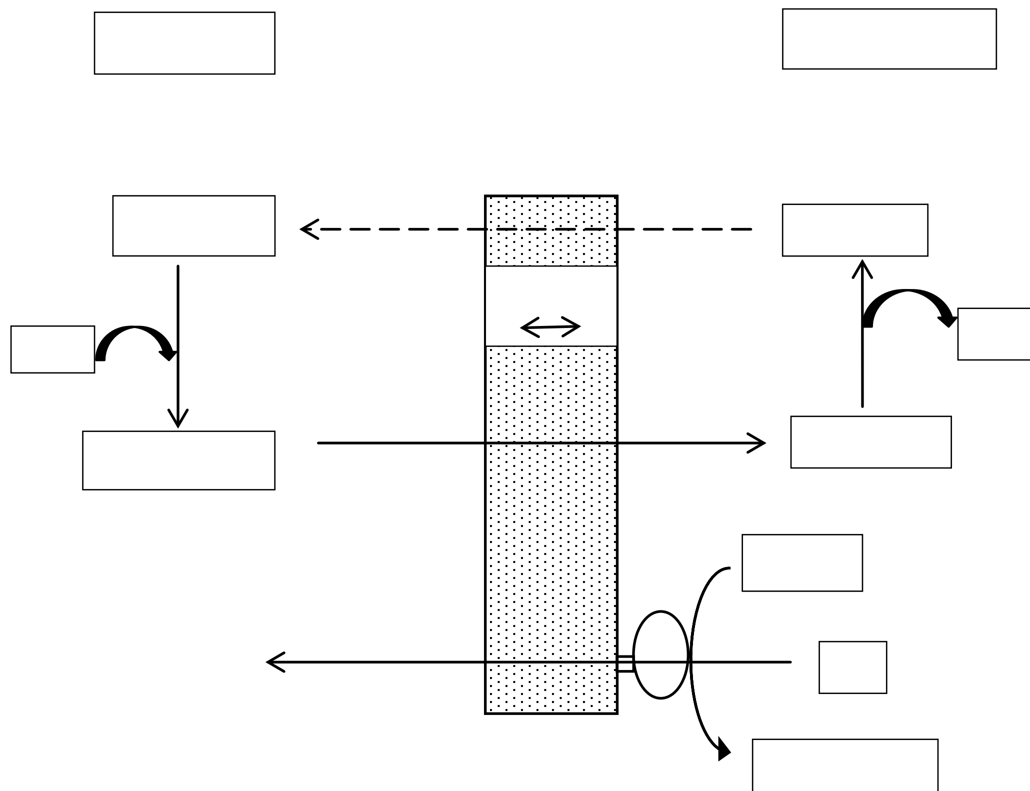

Figure 2.3 Schematic representation of acetate movement in bacteria in response to aZΔpH. The protonated species (XCOOH) should freely permeate the cell membrane and the concentration inside and outside should be the same. If the anion ($XCOO^-$) cannot penetrate the cell membrane, it will accumulate inside the cell in response to the ZΔpH. In fermentative species such as *S. bovis*, intracellular protons are expelled by membrane –bound ATPases. (Source: Russel, 1991).

2.4.3 Effect of pH on milk yield and composition

The effect of reduced pH on depressed digestibility of DM and subsequent influence on milk production was discussed by De Veth & Kolver (2001a). They evaluated the effect in terms of metabolic energy (ME) available for milk production. Using a regression equation to calculate DM

digestibility they showed that a decrease in pH from optimal (6.35) to threshold (5.8) represented a decrease of 4.8 % in DM digestibility. This represented a difference of 0.7 MJ of ME/kg DM of pasture. A further reduction in pH from 5.8 to 5.4 showed a 9.5% reduction in DM digestibility and a decrease in energy content of approximately 1.5 MJ ME/kg DM of pasture. De Veth & Kolver (2001a) calculated this to represent as much as 5 kg milk/d for a cow that consumes 16 kg of DM/d. Wales *et al.* (2004) calculated that the 10% unit decrease in OM, observed when culture pH was reduced from 6.1 to 5.6 would result in a decrease in energy supply of approximately 2.0 MJ of ME/kg of DM, equalling approximately 6.5 kg of milk for a grazing dairy cow consuming 18 kg of DM. The same workers calculated that additional energy equivalent to 2 kg of milk would be available should diurnal variation around a mean daily ruminal pH of 5.6 be removed. They concluded that feeding strategies that minimize diurnal variation in ruminal pH will give significant benefits when daily pH is low (5.6).

Literature on effect of ruminal pH on milk composition is not abundant though it might be possible to reason the indirect effect when one looks at VFA production as influenced by pH. For a more detailed discussion on the effect of ruminal pH on VFA production the reader is referred to section 2.4.5. At this stage it should suffice to say that ruminal pH has been shown to alter acetate production, and that intraruminal infusions of different VFA's have shown to alter milk composition. The study by Rook & Balch (1961) used continuous intraruminal infusions with energy values similar to concentrate supplements to determine the effect of acetate, propionate and butyrate on yield and composition of milk. Acetic infusions increased ($P < 0.05$) mean milk fat % by 0.40% and increased ($P < 0.05$) milk yield by 9-14%. This resulted in an increase in daily fat yield of 18 - 27%. Percentage solids-non-fat (SNF), lactose and protein were not influenced. Propionic infusions resulted in lower ($P < 0.05$) mean fat % than the control. Reductions ranged from 0.29 to 0.36% unit decreases and resulted in depressions in milk fat by up to 10%. Increases ($P < 0.001$) were reported in mean SNF % and milk protein % ranging from 0.21 to 0.30 and 6 to 17 percentage units respectively. No effect of propionic infusions on non-protein nitrogen (NPN) content of milk was reported. A different perspective on rumen pH and milk composition involves conjugated linoleic acid (CLA). Choi *et al.* (2005) determined the effects of ruminal pH on the production of *cis* - 9, *trans* - 11 and *trans* - 10, *cis* - 12 CLA isomers by mixed rumen bacteria from cows fed a high concentrate diet and a high forage diet. At pH values below 6 the level of *trans* - 10, *cis* - 12 CLA was higher than that of the *cis* - 9, *trans* - 11 CLA but at pH levels > 6 little *trans* - 10, *cis* - 12 CLA was detected. In contrast, the *cis* - 9, *trans* - 11 isomers increased in a steady fashion with increasing pH leading to levels representing up to 90% of the detected CLA at pH > 6.3. Choi *et al.* (2005) concluded that *cis* - 9, *trans* - 11 CLA are positively correlated to pH whereas *trans*-10, *cis*-12 CLA is inversely correlated to rumen pH. The work of Baumgard *et al.* (2000) compared the effect of *cis* - 9, *trans* - 11 and *trans* - 10, *cis* - 12 CLA isomers on milk fat synthesis by infusing the isomers into the abomasum. The study found that milk fat percentage and milk fat yield were substantially reduced when *trans* - 10, *cis* - 12 CLA isomers were infused while infusion of a similar quantity of *cis* - 9, *trans* - 11 CLA isomers had no effect on milk fat. None of the treatments had an effect on milk yield. The CLA isomer concentration found in the milk treatment groups corresponded with the CLA supplement applied. The *trans*-10, *cis* - 12 CLA content

of milk fat in the 10, 12 CLA treatment group increased from trace levels to 3.9 mg/g fat. Considering the studies of Choi *et al.* (2005) and Baumgard *et al.* (2000) it would be safe to conclude that pH has an indirect effect on milk fat synthesis by way of influencing the concentration of CLA isomer.

2.4.4 Effect of pH on fibre and NSC digestion

Mould *et al.* (1983/1984) described the effect of pH on digestion of fibre as biphasic. Decreases in pH from 6.8 to 6 results in moderate depressions in fibre digestion while decreases below pH 6 results in severe inhibition of fibre digestion. Reasons for the decrease in the first phase is unclear since activity of isolated fibrolytic enzymes are still high in this pH range (Morrison, 1976) and because numbers of cellulolytic micro-organisms have not been noticed to decrease with such small decreases in pH (Mackie *et al.,* 1978; Leedle *et al.,* 1982; van der Linden *et al.*, 1984). Reductions in digestibility can occur despite ruminal pH being well buffered (Mould *et al.*, 1983/1984). Added starch, maltose and manose can set about a so called 'carbohydrate' effect described by Hoover, (1986) leading to reduced fibre digestibility. The effect of pH on digestibility of high quality pasture may differ from TMR diets and from low quality pasture. De Veth & Kolver (2001a) reported that ruminal pH of cows grazing pasture only start to inhibit fibre digestibility and microbial protein synthesis once pH levels fall below 5.8. This is below the pH threshold of 6.2 suggested by the Cornell Net Carbohydrate and Protein System (CNCPS) model and below the pH 6 level reported by Hoover *et al.* (1984), Mould & Ørskov, (1984) and Shriver *et al.* (1986). The CNCPS model and other models assume steady state rumen conditions (De Veth & Kolver, 2001b) whereas, in fact, the ruminal pH and products of digestion varies within 24 - hour periods (Pitt & Pell, 1997; Gill *et al.*, 1999; Robinson, 1989). Work by De Veth & Kolver (2001a) established the optimal ruminal pH for digestion of pasture and synthesis of microbial protein and the threshold pH. The optimum pH for DM and OM digestion was established to be 6.35 and 6.38 respectively and compares well with the pH range of 6.0 to 6.3 recommended to optimize digestion of forage – concentrate diets (Hutjens *et al.*, 1996; Pitt *et al.,* 1996). The threshold pH was set at pH 5.8 since the greatest reduction in true digestibility of DM and OM occurred at this level. De Veth & Kolver (2001b) studied the relationship between rumen pH and fibre digestion *in vitro* with high quality pasture diets. Suboptimal and optimal pH was set at pH 5.4 and pH 6.3 respectively. Results showed that apparent digestibility of DM and OM decreased quadratically ($P < 0.05$) with longer intervals at suboptimal pH while true digestibility of DM and OM and apparent digestibility of neutral detergent fibre (NDF), acid detergent fibre (ADF) and NSC decreased linearly ($P < 0.05$) with increasing time at suboptimal pH. Exposure to suboptimal pH for 4 hours resulted in the largest reduction in digestibility of OM, DM and NDF. The results of De Veth & Kolver (2001b) also emphasize the importance of diurnal variance in pH. Decreases in digestion may occur as a result of short periods (4h) at suboptimal pH despite mean daily ruminal pH not reaching suboptimal levels. Wales *et al.* (2004) tested the hypothesis that diurnal variation in culture pH would reduce digestibility when average culture fluid pH is 5.6, but not when culture pH is 6.1. Four treatments were tested; I) a constant culture pH of 6.1 (C6.1), II) variation around culture pH 6.1 (V6.1), III) a

constant culture pH of 5.6 (C5.6) and IV) a variation around culture pH 5.6 (V5.6). Digestibility of NDF and ADF was highest ($P < 0.05$) and not different for the treatments C6.1 and V6.1. Intermediate digestibility was reported for the C5.6 and lowest digestibility for V5.6. Digestion coefficients of OM, NDF, and ADF were reduced by 15, 30 and 36% respectively when average daily culture pH was allowed to vary throughout the day in response to twice daily feeding at pH 5.6 vs. pH 6.1 (V5.6 vs. V6.1).

Mould *et al*. (1984) reported that high quality roughages (high digestibility) are influenced by low ruminal pH to a lesser extent than roughages with a low quality (lower digestibility). The reason for this can be speculated on. Quality or digestibility depends to a large extent on the composition of the forage. Forages contain compounds like lignin and tannins that influence the extent and rate of fermentation. The mechanism by which lignin inhibits microbial fermentation is considered to be by means of a physical barrier to rumen microbial enzymes; preventing reach of target polysaccharides (Buxton & Redfaern, 1997). Cellulolytic bacteria utilize an inside-out process to overcome this limitation (Cheng *et al.,* 1991). Plant cell walls, rich in lignin, are penetrated via stomas or parts mechanically disrupted by chewing or processing. Penetration allows access to the nutritionally rich inner parts of the plant cell supplying micro-organisms with the necessary energy to proliferate and function. Microbial digestion then proceeds back towards the lumen side of the plant cell wall. The lower the quality of the forage the longer it will take the micro-organisms to reach the inner parts, thus wasting energy and inhibiting the some cellulolytic bacteria to maintain cell metabolism at the low rumen pH as described by section 2.4.2.

Wales *et al*. (2004) observed that WSC and starch digestion were not affected by reductions in culture pH from 6.1 to 5.6. The study of De Veth & Kolver (2001a), confirms the finding since NSC was highly digestible over the range pH 4.5 to 7.5. The insensitivity to lowered pH was attributed to saccorolytic and amylolytic bacteria being able to survive and function under a wide range of ruminal pH.

2.4.5 Effect of pH on VFA absorption

Fractional rate of VFA absorption increases when ruminal pH decreases because of a greater fraction of VFA that is in the associated form (Allen, 1997). The associated form of VFA is more membrane permeable (Ash & Dobson, 1963). Volatile fatty acid absorption is a passive process of facilitated diffusion and is influenced by rumen movement that transfers VFA from the lumen to surface areas of rumen epithelium. Rumen movements are triggered by particulate matter in the rumen explaining in part why ruminal pH decreases when concentrate is fed (Lana *et al*., 1998). Absorption rates vary by

type of VFA, except at neutral pH when all VFA's are absorbed at equal rates. Dijkstra *et al*. (1993) reported that decreased ruminal pH from 7.2 to 4.5 had no influence on the fractional absorption rate of acetic acid (0.31/h) but did result in increased fractional absorption rate of buturyte (0.28 to 0.85/h) and propionate (0.35 to 0.68/h). The differences were explained by a greater concentration gradient between the rumen and the portal circulation for butyrate and propionate depending on the extent of metabolism in the rumen epithelium. At an initial pH of 4.5 fractional absorption rates of VFA were in the order ($P < 0.05$): butyric acid > propionic acid > acetic acid (Dijkstra *et al*., 1993). When pH was increased to 5.4 the order changed to butyric acid = propionic acid > acetic acid. At pH values between 6.3 and 7.2 no significant difference was detected.

2.4.6 Effect of rumen pH on VFA, methane and ammonia production

Esdale & Satter (1971) studied the effect of change in ruminal pH on VFA production *in vitro* and VFA concentrations *in vivo* with high forage diets and high concentrate diets. They concluded that within the pH range 6.8 to 6.2 *in vitro* VFA production and *in vivo* VFA concentration were unaffected. When pH reached levels of 5.6 acetate production was decreased resulting in A:P ratios changing from 2.8 at pH 6.2 to 1.3 at pH 5.6. Lana *et al.* (1998) found that ruminal pH was negatively correlated with VFA concentration and positively correlated with A:P ratio. The study of De Veth & Kolver, (2001b) showed that time exposed to suboptimal ruminal pH affected some of the individual VFA concentrations and the VFA ratios. A negative ($P < 0.05$) and positive ($P < 0.01$) quadratic relationship to time at suboptimal pH was reported for acetate and propionate concentration respectively. Acetate to propionate ration (A:P) displayed a negative ($P < 0.05$) quadratic relationship at suboptimal pH. Wales *et al*. (2004) reported a 25% decrease in total VFA concentration as culture pH was reduced *in vitro* from 6.1 to 5.6. De Veth and Kolver (2001a) reported a smaller (13 %) reduction over the same pH range.

Diurnal variation of culture pH reduced ($P < 0.01$) total VFA concentrations by 11% but did not affect the molar proportions of individual VFA's (Wales *et al*., 2004). Ammonia concentration and flow of ammonia N and non-ammonia N were not affected as time at suboptimal pH increased but quantity of microbial N flowing from the continuous culture system decreased linearly ($P < 0.01$) with increasing time at suboptimal pH. Diurnal variation in culture pH did not affect concentrations of NH_3-N but flow of total N was reduced ($P < 0.05$); the difference partially attributed to a decrease ($P < 0.01$) in the flow of microbial N. The *in vitro* study of Lana *et al.* (1998) showed rates of ammonia production decreased from 28 to 15 nmol.mg protein^{-1}.min^{-1} when pH was reduced from 6.5 to 5.7 but only when bacteria were obtained from cattle fed forage. The study also showed that decreasing *in vitro* pH from 6.5 to 5.7 resulted in a decrease ($P < 0.001$) in methane production from 48 to 7 nmol.mg

protein^{-1}.min^{-1} when a predominant concentrate diet was fed. When forage diets were used the reduction was less; 14 to 2 nmol.mg protein^{-1}.min^{-1}.

2.5 Rumen ammonia concentration

Optimum concentration of ammonia in rumen liquor varies between 85 to 300 mg/litre (McDonald *et al*, 2002). Low ammonia levels (50 mg/litre) or levels below optimum are generally observed when dietary protein resists degradation or when diets are deficient of protein. The result is slow proliferation of micro-organism and subsequent decrease in carbohydrate degradation (McDonald *et al.*, 2002). Cajarville *et al.* (2006) reported NH_3-N levels of 224 mg/litre for cows grazing pasture supplemented with grain, twice a day at a level of 33% of daily DM ingested. Berzaghi *et al.* (1996) found similar levels for cows grazing only pasture. Maximum NH_3-N concentrations were observed 8 hours after the morning supplementation. This is in accordance with findings of Van Vuuren *et al.* (1986) who reported peak rumen NH_3 concentrations seven hours after dairy cows commenced grazing ryegrass. Since efficiency of utilization of N in grazed herbage for milk protein is low (± 20 %; Van Vuuren, 1993) many workers advocate synchronizing the availability of available N in pasture and readily fermented carbohydrates from concentrates. In N fertilized pastures NSC: rumen degradable protein (RDP) ratio's can be ten times lower than the recommended 2:1 (Trevaskis & Fulkerson, 1999). Excess ammonia, resulting from degradation of RDP is absorbed across the rumen wall, rapidly converted to urea in the liver and excreted in the urine via the kidney and/or in the milk. The energy cost of detoxifying excess ammonia in the liver and excreting range between 0.035 to 0.052 MJ ME/g N excreted (Lobley & Malino, 1993).

2.6 Rumen temperature

Impema *et al.* (2008) studied rumen temperature as part of a pilot study using capsule based wireless technology. They reported that rumen temperature varied diurnally and that rumen temperatures during night time were higher compared to temperatures during the day. The mean, maximum and minimum temperatures were: 39.7°C; 41°C and 36.4°C respectively and occurred during different time periods. This represents a wider temperature range compared to the temperature range, 38-40°C, reported by Church, (1969, 1976) and Clarke (1977). The highest temperature was measured between 20:00 and 00:00 while the lowest temperatures were measured from 8:00 to 12:00. The study did not monitor rumen temperature of grazing animals. Both ambient temperature and water temperature can influence rumen temperature. Gengler *et al.* (1970) found that rumen temperature increased when ambient temperature increased from 18°C to 35°C. Rumen temperature tends to increase immediately after feeding when active fermentation occurs (Dehority, 2003). Gengler *et al.* (1970) also reported decreases in feed and water intake when rumen temperature was increased to

43°C using heating coils. The rumen, however, seldom reaches levels above 41°C. Cunningham *et al.* (1964) reported that decreases in rumen temperature are dependent on the temperature and quantity of water consumed. Depressions in rumen temperature of cows that consumed 20.8 ± 3.87 L of water with temperature of 1.1°C were: - 4.94 ± 12.38°C; - 12.04 ± 14.76°C and - 16.48 ± 16.87°C for the lower, middle and upper parts of the rumen respectively. Dehority (2003) suggested that ingestion of cold water or frozen forage may depress the rumen temperature from 5 to 10°C and that it may take 1 - 2 hours for the temperature to return to normal.

2.7 Osmotic pressure

Normal osmolality of roughage diets are below that of concentrate diets. Garza *et al.* (1989) have reported normal ruminal osmolality for roughage diets to be in the range of 240 - 265 mOsm/L and 240 - 300 mOsm/L for concentrate diets. The former is in line with the 250 mOsm/kg reported by Warner & Stacy (1965). Prior to feeding rumen fluid is hypotonic with respect to plasma. Immediately after feeding rumen fluid osmolality raises rapidly to values about 400 mOsm/kg and then declines gradually to hypotonic level compared to plasma in 8 - 10 hours (Warner & Stacy, 1965). The reason for the marked increase post feeding is related to the concentration of potassium ions (Dehority, 2003). Beauchemin & Buchanan-Smith (1990) reported that the increase in osmolality of dairy cows peaked 2 hours post feeding and then gradually decreased. The effect of increased osmolality is decreased feed intake (Carter & Grovum, 1990). Elevations beyond 350 mOsm/L have been associated with inhibited bacterial digestion of fibre and starch causing rumen contents to become stagnant. Bergen (1972) reported decreases in *in vitro* cellulose digestion when rumen osmotic pressure was > 400 mOsm/kg medium. Scott (1975) reported that removal of fluid and acid from the rumen is compromised when osmolality levels reach levels beyond 300mOms/L and the abomasum is distended. This is due to inhibition of outflow of rumen fluid and acids. Minerals, VFA, lactate and glucose are the primary solutes in rumen fluid contributing to osmolality (Owens *et al.*, 1998). When rumen osmotic pressures surpass normal blood osmotic pressure (285 – 310 mOsm/L) water in the blood shifts through the rumen wall to the rumen causing swelling of rumen papillae. This may cause damage to the rumen epithelium and subsequent parakeratosis or hyperkeratosis. The end effect is inhibited rates of VFA absorption (Owens *et al.*, 1998). High osmolality can be controlled by controlling contributors to osmotic pressure in feed and water (Owens *et al.*, 1998). These include: ammonia, soluble minerals like sodium, potassium and chloride. Increased input of saliva at ± 255 mOsm has also resulted in decrease osmotic pressure. High moisture diets or increased intake of water is unlikely to decrease osmolality because fermented diets often have high osmolality and water may partly flush past the rumen (Garza & Owens, 1989). Osmolality of rumen contents are maintained within narrow margins by means of the large volume of hypertonic saliva, rapid absorption of water from hypotonic solutions and iso-osmotic absorption of water along with sodium, chloride, VFA's and other substance (Van Soest, 1994).

2.8 Oxidation reduction potential and composition of gas

Oxidation reduction potential (ORP) can be regarded as a measure of microbial activity (Mishra *et al.*, 1970). Marden *et al.* (2005) reported the redox potential of dry Holstein cows receiving a TMR diet using a continuous monitoring technique. They found the Eh range to be -173.5 mV to -216.8 mV. The range differed from the range reported by using a conventional suction device (-111.3 to -139 mV) where no precaution was taken to avoid air contact with samples. Marden *et al.* (2005) also found that the redox potential increased 3 hours post feeding and then declined slowly. The reason for the increase was ascribed to the supply of oxygen directed towards the rumen during feed intake, mastication and water intake. Redox potential declined due to rapid uptake of oxygen by micro-organisms to maintain anaerobosis (Broberg, 1957b).

Major end products of microbial fermentation in the rumen include a variety of gases. Carbon dioxide and CH_4 represent 65 and 27% respectively of the total gas from fermentation. Minor (0.2%) quantities of H_2 are also present and are utilized by methanogenic bacteria to reduce CO_2 to CH_4 (Hungate, 1966; Clarke, 1977). Carbon dioxide formation also results from salivary bicarbonate, amino acids and organic acids (Dehority, 2003). Nitrogen represents 7% of the gas mixture with traces of CO and H_2S and low quantities of O_2 also occurring (Dehority, 2003).

2.9 Structure of digesta

Rumen contents are in the form of stratified layers with different physical qualities. Structure and composition of the contents are markedly influenced by diet; coarse hay, for instance, would produce a contents with large, dense floating layers, while concentrate would produce a more viscous content (McDonald *et al.*, 2002). The former is called the rumen matt. As fermentation proceeds, digestion and rumination decrease particle size of the rumen mat and particles become waterlogged and sink due to increased particle density (McDonald *et al.*, 2002). Proportion of mat (*i.e.* mat:non-mat ratio) and consistency of the rumen mat is important in regulating small particle retention in the rumen (Zalebi *et al.*, 2007). The rumen mat acts as a first-stage separator (Sutherland, 1988) retaining potential escapable fibre particles by means of mechanical entanglement and filtration. This ensures increased time for digestion (Welch, 1982; Weidner & Grant, 1994).

2.10 Conclusion

The rumen fermentation system is complex. It is not closed, but prone to influences from human interventions and factors related to rumen micro-organisms as well as factors related to the animal

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and feed. Within the fermentation system the rumen environment plays an important role. Optimal function and proliferation of rumen fibrolytic bacteria depend on rumen environmental parameters being maintained within limits. Ruminal pH is the rumen parameter that varies the most and is thus most likely to hinder optimal function and growth of microorganisms. With the aid of automated pH recording technology researchers have shown diurnal variation in ruminal pH of cows grazing pasture and supplemented with concentrate. This results in an uneven diurnal rumen pH profile. The current hypothesis is that the rumen pH profile can be stabilized within the pH range optimal for fibrolytic micro-organisms by following concentrate feeding strategies. A more stabilized rumen pH profile would result in higher milk yield, a change in milk composition and a change in fibre digestion.

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CHAPTER 3

General Materials and Methods

Note: Methods applied in this study are mentioned in relevant chapters. Some methods were modified to accommodate findings of new research. It was decided to discuss these methods in more detail in this chapter to familiarise the reader. Where no distinction is made between studies using kikuyu or ryegrass, methods or procedures are applicable in both cases.

3.1 Standard ethical norms; animal welfare

All actions were in accordance to the Stellenbosch University's policies regarding ethical research. A project proposal was submitted to the Ethics Committee of Subcommittee B of the University of Stellenbosch and ethical clearance (Reference: 2007B03007) was granted after evaluation of the proposal.

3.2 Pasture yield estimation, allocation and management

Available pasture (kg DM/ha) was estimated from linear regression equations [Y= aX +b, where Y = pasture mass in kg DM/ha and X = RPM reading]. At the initial stage of the trial, equations based on 2006 data were used. This was necessary since the RPM (Filip's folding plate meter, Jenquip, Rd 5, Fielding, New Zealand) used for measuring pasture height could only be calibrated during the course of the trial. Total grazing area (m^2) per allocation was calculated, taking into account, the total DM need per cow per day (10 kg DM) and DM availability per ha. A visual assessment was made of the pasture height after each day's grazing. This, in combination with the RPM reading taken after grazing, was used to adapt the new area of pasture allocated.

The RPM was calibrated by cutting three samples from three pasture height levels (low, medium and high) each. The different levels were measured with the RPM. Grass under the RPM plate was cut to a height of 3 cm above the ground using a sheep shear and a ring mounted on 3 cm legs. The ring had the same area (0.0985 m^2) as the RPM. Each sample was dried at 60°C for 72 hours in a drying oven and weighed to determine the moisture content. The data was used to estimate kg DM per hectare. In total, data from eight weeks were collected. A linear regression equation [Y= aX + b,

where Y = pasture mass in kg DM/ha and X = RPM reading] was fitted to the data with the LINEST function of Microsoft Excel. A final equation for ryegrass (Y = 76.84X - 742.29; R^2 = 78 %) and kikuyu (Y = 75.97X - 218.61; R^2 = 79 %) pasture was obtained and was used to calculate the real DM yield during the course of the trial.

Average DM intake per cow was calculated by taking one hundred pasture height readings before and after grazing of the daily grazing strip. Fitting daily data to the linear regression equation enabled the calculation of average daily DM intake per cow by subtracting DM available after grazing from that available before grazing. Grazing strips were closed after grazing to allow fertilizer to be applied. Fertilizer was applied at a 56 kg N (Limestone ammonium nitrate, 28 %) level per hectare. Pasture was irrigated with the aid of water tensiometers. Tensiometer reading at which irrigation commenced was set at – 25 kPA.

3.3 Data collection

3.3.1 Feed samples

Grass samples were taken on a weekly basis for eight weeks 12:00 . The samples were obtained by blindly casting a metal ring (35.4 cm in diameter; raised 5 cm from the surface) on the pasture. The grass inside the ring and adjacent was inspected to avoid faeces or foreign grass species contaminating the sample. Once it was firmly established that the grass inside the ring was pure ryegrass it was cut to a 5 cm level above ground using a sheep shear. Plant material was transported in an open transparent plastic bag. Each sample was divided in subsamples, weighed and put in paper bags. The bags were dried in an oven for 72 hours at 60°C. Subsamples were weighed and milled using a cutting mill (Retsch GmbH, RheinischeStraße 36, 42781, Haan, Germany) with 2 mm sieve. Once the subsamples were thoroughly mixed it was stored in airtight plastic jars and kept in a cold room at 4°C.

Concentrate samples were taken once a week for eight weeks. Samples were weighed and put in brown paper bags to dry in a drying oven for 72 hours at 60°C. On completion of drying, samples were weighed and stored in airtight plastic jars and kept in a cold room at 4°C. Samples were milled at the feed laboratory of the University of Stellenbosch using a cutter mill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa) fitted with a 2 mm sieve. Concentrate samples, of the ryegrass phase, were pooled to give a representative sample of concentrate from every second week.

3.3.2 Animal weight and body condition score

Body weight of cows in the production study was measured on two consecutive days during the start and the end of the trial. During these periods body condition scoring (BCS) was determined on the first day according to the five point score system of Wildman *et al.* (1982). This system consists of palpating areas on the cow's hind quarter, specifically the tuber sacrale (hip bone), tuber ischia (pin bone) and the anterior coccygeal vertebrae (tail head) areas. Scoring was done by an experienced evaluator. The same person did the scoring at each occasion.

3.3.3 Milk samples and milk yield

Milk samples of cows were taken every second week for eight weeks. The samples were a composite (ratio 9:15 ml) of milk from the afternoon milking session and that of the morning session. Samples were preserved with bronopol and couriered to Lactolab, ARC, Irene, to be analysed for butterfat, protein, somatic cells, lactose and milk urea. The butterfat, protein, lactose and milk urea was analysed using a Milkoscan FT 6000 (Foss, Denmark) and the SCC using a Fossomatic 5000 (Foss, Denmark). Milk yield was automatically recorded using Dairy Master weigh-all electronic milk meters (Dairy Master, Causeway Co. Kerry, Ireland).

3.3.4 Soil samples

Soil samples were taken at the end of the trial using a Beater soil sampling auger. The experimental plot (8.5 ha) was divided in three subplots. Two subplots consisted of 13 grazing strips with the remaining plot having 11 grazing strips. Subsamples were taken of each subplot at a depth of 10 cm in a zigzag pattern. Samples were transferred directly to transparent plastic bags for analysis at the soil laboratory of the Department of Agriculture, Western Cape at Elsenburg.

3.4 Analytical methods

3.4.1 Starch and glucose

Starch was determined with a two procedure process, *viz.* 1) starch gelatinization and hydrolysis and 2) glucose analysis. Procedure one was executed using a modification of the method described by Holm *et al*., (1986). Four subsamples (0.1 g, as is) were weighed in four 60 mL test tubes. Two tubes, containing starch, and one empty tube were included to serve as control and blank reagent

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respectively. Distilled water (10 mL) was added to each test tube using a pipette and mixed using a 220 V vortex (Heidolph Instruments GmbH & Co.KG. Walperdorfer Str. 12. 91128 Schwabach/Germany). Ten millilitres of distilled water were added to wash sample clinging to the side of test tubes. Heat stable alpha-amylase (Number A3306 in the dietary fibre kit; Sigma chemical Co., St. Louis, MO) (0.1 mL) was added and mixed thoroughly. Test tubes were sealed with rubber stops. Two, of the set of four test tubes, were placed in a water bath (Scientific Engineering (Pty) Ltd. Johannesburg, RSA) at 92 - 93°C for 1 hour. The remaining two were kept at room temperature. Tubes were removed from the water bath after 1 hour and placed on a lab bench to cool down for 15 min. Samples were filtered into 50 mL volumetric flasks using funnels filled with glass wool and filled to volume with distilled water. An aliquot (1 mL) of each sample was pipetted to 25 mL volumetric flasks and 8 mL of Sodium acetate buffer was added to each flask. Amyloglucosidase (50 µL/ test tube) was added, swirled gently and incubated in a water bath at 60°C for 30 minutes; swirling every 10 minutes. Samples were filled to volume and the second procedure was started.

Glucose was analysed according to a modification of the method of Karkalas (1985). Reagents that were used are shown in Table 3.1. Aliquots (0.5 mL) of samples and glucose standards were pipetted in duplicate test tubes. Glucose oxidase – peroxidase colour reagent (2.5 mL) was added to each test tube and was mixed. Test tubes were covered with single layer parafilm and placed in a water bath (Scientific Engineering (Pty) Ltd. Johannesburg, RSA) at 35 - 40°C for 45 minutes after which the tubes were allowed to cool at room temperature for 10 min. in the dark. Glucose was determined using a spectrophotometer; reading absorption at 505 nm.

3.4.2 Neutral detergent fibre

Neutral detergent fibre of milled grass and milled concentrate samples was determined using the method of Ankom Technology (Ankom, 2006b); a modification of the method of Van Soest *et al.* (1991). Samples were ground through a 1 mm sieve with a feed mill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa). Five grams of sample were weighed in Dacron bags (5 cm x 5 cm) and heat sealed. Fat was pre-extracted from concentrate samples by submerging Dacron bags containing sample in acetone. Concentrate samples were shaken 10 times and allowed to soak for 10 minutes. This was repeated in fresh acetone; acetone was removed and samples were allowed to air dry. Bags were transferred to the vessel of an Ankom 220 Fibre analyser (Ankom technologies, Fairport, NY, USA) and 2000 mL of ambient ND solution were added with 20 g of sodium sulphite where upon agitation and heat switches were turned on for 75 minutes. Sodium sulphite was added to reduce protein contamination of fibre. The hot solution was drained and samples were rinsed three times for 5 minutes with water (1900 mL; 70 - 90°C). Four millilitres of heat stable alpha-amylase (Number A3306 in the dietary fibre kit; Sigma chemical Co., St. Louis, MO) was added during the first two rinses.

Bags were gently pressed by hand to remove excess water and placed in 250 mL beakers. Acetone was added until bags were covered and soaked for 5 minutes. Bags were removed and placed on a wire screen to air dry and placed in a drying oven at 100°C for 4 hours for complete drying. Bags were placed in a desiccator for temperature to reach equilibrium, weighed and incinerated in a muffle furnace (Centrotec, 8 Tobago, Hout Bay) for 6 hours at 500°C.

Table 3.1 Reagents and quantities used to prepare 500 mL of glucose oxidase – peroxidase for glucose analysis

Reagent	Quantity (g)
Sodium phosphate, dibasic anhydrous	4.550
Potassium phosphate, monobasic	2.500
Phenol, solid loose, crystals, ACS	0.500
4-Aminoantipyrine [1]	0.075
Glucose oxidase [2]	0.146
Glucose oxidase [3]	0.021
Peroxidase [4]	0.036

[1]Sigma, A-4382), desiccated at room temperature; [2]Sigma, G 6125, 23 900 units/g solid, desiccated at - 20°C; [3]Sigma, G 2133, 165 000 units/g solid, desiccated at - 20°C, [4]Sigma, P-8125, 96 purpurogallin units/mg solid, desiccated at - 20°C. Source: Personal communication, F. Nherera (ARC, Private bag X2, Irene,0062, Pretoria. January 2009)

3.4.3 Acid detergent fibre

Acid detergent fibre of concentrate samples were determined with the procedure described by Ankom Technology (Ankom, 2006a). Dry samples were ground to pass through a 1 mm sieve with a feed mill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa). Samples were weighed in Dacron bags (5 cm x 5 cm), heat sealed and submerged in a container with acetone to pre-extract fat. The container with acetone was shaken 10 times. Bags were kept submerged for 10 minutes. The process was repeated with fresh acetone. Bags were retrieved from the acetone and placed on a wire screen to air - dry. After drying the bags were placed into the vessel of an Ankom 220 Fibre analyser (Ankom technologies, Fairport, NY, USA) and 2000 mL of ambient AD solution were added. Agitate and heat switches were turned on for 60 minutes after which the hot solution was drained. Bags were rinsed three times with water (70 - 90°C) for 5 minutes and removed from the vessel. Excess water was removed by gently pressing the bags and was placed in 250 mL beakers. The bags were

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submerged in acetone, soaked for 3 - 5 min. and placed on a wire screen to air - dry. Complete drying was done in drying oven at 104°C for 4 hours. The bags were weighed and incinerated in a muffle furnace (Centrotec, 8 Tobago, Hout Bay, South Africa) to report an ADF value exclusive of residual ash. The acid detergent fibre for grass samples was determined with the same procedure as above, except that the pre - extraction of fat with acetone was omitted.

3.4.4 Acid detergent lignin

Acid detergent lignin was determined with two processes. Firstly, acid detergent fibre was determined as described above, but without incinerating bags. Lignin was then determined with the method prescribed by Ankom Technology (Ankom, 2005a) by placing bags in Daisy^{II} digestion vessels (Daisy^{II}Ankom technology, Fairport, New York, USA). Bags were submerged in 500 mL of 72 % H_2SO_4 and vessels were placed in the Daisy^{II} incubator (Daisy^{II}Ankom technology, Fairport, New York, USA). The rotation switch was turned on for 3 hours and then the H_2SO_4 was poured off and bags were rinsed with tap water until the pH was neutral. Water was removed by rinsing bags with 250 mL of acetone for 3 minutes. Bags were then placed in a drying oven (105°C; 4 hours), placed in desiccator, weighed and incinerated in muffle furnace (Centrotec, 8 Tobago, Hout Bay) at 500°C for 6 hours) to report an ADL value exclusive of residual ash.

3.4.5 Non protein nitrogen and soluble crude protein

Non - protein nitrogen (NPN) was determined using a modification of the method described by Licitra *et al.* (1996) using tungstic acid. The modification consisted of determining residual N with a Dumas type N analyser (Leco FP-528, Leco Corporation, St Joseph, MI) instead of the prescribed Kjeldahl apparatus. The method of AOAC International (2005), Official Method 968.06 was applied. Residual N was adapted to exclude the N content of the filter paper (Whatman #54) by subtracting the N value of blank filter paper. The latter was obtained from analysis with the Leco P - 528 analyser. Soluble crude protein was determined with the method described by Licitra *et al.* (1996). The method was modified in the same manner as that for determining NPN. The method determines the insoluble protein fraction. Soluble protein was calculated by subtracting the insoluble protein value from the total crude protein.

3.4.6 Fibre associated nitrogen

Neutral detergent fibre nitrogen (NDF) was analysed using the NDF procedure of Ankom Technology (Ankom, 2006b). The AOAC International (2005), AOAC Official method number 968.06 was applied to determine N content. Ankom F57 bags were used for NDF analysis and filled with 0.7 g (as is) milled sample instead of the prescribed 0.5 g (as is). The increase in sample mass was to ensure an adequate sample mass for N analysis. Ankom F57 bags, filled and sealed with concentrate sample, were soaked in acetone for 12 hours. Neutral detergent fibre was determined using an Ankom 220 Fibre analyser (Ankom technologies, Fairport, NY, USA) and N with a Leco FP - 528 (Leco Corporation, St. Joseph, MI, USA) N analyser.

Acid detergent nitrogen was analysed using the procedure of Ankom Technology (Ankom, 2006a) and the AOAC International (2005), AOAC Official method number 968.06 for determining N. Ankom F 57 bags were used for analysis of the grass samples. Concentrate samples were analysed using Dacron bags (5 x 5 cm). Bags containing concentrate sample were soaked in acetone for 12 hours prior to ADF analysis. Sample mass was increased to 0.75 g (as is) to ensure adequate sample mass for N analysis.

3.4.7 In vitro organic matter digestibility

In vitro organic matter digestibility (IVOMD) was determined according to a modification of the method described by Ankom Technology (Ankom, 2005b). This method is based primarily on the two stage technique described by Tilley & Terry (1963). Dried samples were milled through a 2 mm sieve with a cutter mill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa). Samples were sieved for 5 minutes with a Retsch AS 200 (Retsch GmbH, RheinischeStraße 36, 42781, Haan, Germany) sieve shaker using a Kingtest sieve with 106 µm aperture. Samples (5 g) were weighed and placed in F57 Ankom bags. The bags were heat sealed. Medium was prepared using the method of Goering and Van Soest (1970). The medium consisted of tryptose, buffer, macro mineral solution, micro mineral solution, distilled water and resazurin and was heated to 39°C in a water bath (Model 416, Scientific Engineering (Pty) Ltd. Johannesburg, South Africa). Reducing solution used was modified according to Van Soest and Robertson (1985) and was made by preparing two separate solutions. Solution A consisted of potassium hydroxide pellets and cysteine HCL. Solution B consisted of sodium sulphide nonahydrate and distilled water. The two solutions were mixed one hour before incubation commenced. Digestion vessels were placed in the incubator (Daisy^{II} Ankom technology, Fairport, New York) and agitation and heat switches were turned on to pre-heat the vessels.

Rumen inoculum was collected at 9:00 am from two fistulated Holstein cows receiving a TMR diet. The diet consisted of oat and lucerne hay with wheat straw and concentrates and had a CP content of 11.2 % and a NDF content of 55.9 %. Cows were fed at 7:30 and 17:00. Rumen inoculum was collected by hand and filtered through two layers muslin cloth into two, 2 L thermos flasks. Both the muslin cloth and thermos flasks were pre-heated to 39°C. A fistful of the fibrous matt was included in each flask to ensure that the inoculum contained fibre associated rumen organisms. The pH of the rumen inoculum was measured before and after blending with a WTW pH 340i pH data logger with a WTW Sentix pH electrode. Blending (10 seconds at low speed) was done with a commercial blender (Waring commercial heavy duty blender, model CB 15T, Waring Laboratory 314, Ella T, Grasso Ave, Torrington, CT 06790). The blender bucket was flushed with CO_2 gas prior to blending and was kept at 39°C. The blended inoculum was then filtered twice through two layers of pre-heated muslin cloth in a thermos flask flushed with CO_2.

Medium (1076 ml) and reducing solution (54 ml) was transferred to each digestion vessel. Vessels were continuously flushed with CO_2 during the transfer and placed in the incubator. Filtered rumen inoculum (270 mL) was added to each digestion vessel once the medium was completely reduced. Sample bags (15) were added to each vessel and incubated for 48 hours. Samples were rinsed after incubation using cold water and then air dried. Following air drying, samples were analysed for NDF and incinerated in a muffle furnace (Centrotec, 8 Tobago, Hout Bay) at 500°C for 6 hours.

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CHAPTER 4

The effect of concentrate feeding strategies on rumen parameters, *in sacco* fibre degradation and milk production of Jersey cows grazing kikuyu (*Pennisetum clandestinum*) based ryegrass (*Lolium multiflorum)* pasture

Abstract

Ruminal pH is the rumen condition that varies most. Studying the cause and effect of diurnal variation offers means of improving ruminal conditions for rumen micro-organism with subsequent improvements in fibre degradation and milk yields. A research project was conducted at the Outeniqua research farm to study the effect of concentrate feeding strategies on the rumen environment of Jersey cows grazing annual ryegrass (*Lolium multiflorum*). The project consisted of two studies *i.e.* a rumen study and a production study. The former investigated the effect on rumen parameters (pH, VFA) and *in sacco* degradation of fibre. The production study quantified the effect of the concentrate strategy on milk production and milk composition. Treatments consisted of concentrate supplements being allocated in different weight ratios between morning and afternoon allocation. No differences were found in ruminal pH between treatments. The time (h) spent below the critical ruminal pH *i.e.* 5.8) was of short duration. The concentration of certain VFA's differed ($P < 0.05$) at times, but the differences were not portrayed in graphs and data of the ruminal pH. No differences were observed in *in sacco* fibre and DM degradation between treatments. The ruminal environment, especially the ruminal pH thus seemed not to have been altered to the extent that fibre degradability was influenced. Neither milk production nor milk composition was affected by the treatments. The fact that treatments did not differ in terms of milk yield and milk composition in the production study can be explained by the lack of differences between treatments in terms of ruminal pH in the rumen study.

4.1 Introduction

The efficiency of pasture based dairy systems is measured by milk output per unit of land (Clark & Kanneganti, 1998). High quality pastures (comprising 18 - 24% dry matter (DM), 18 - 25% crude protein (CP), 40 - 50% neutral detergent fibre (NDF) and 6.99 MJ/kg DM of NE_L) forms the base of such systems (Clark & Kanneganti, 1998) though pasture is seldom used as the only feed source. Low pasture DM intake being a major factor limiting milk production of grazing cows (Kolver & Muller, 1998) necessitate supplementing pasture to increase total DM and energy intake (Stockdale, 2000).

Secondary objectives of supplementary feeding include: increased stocking rate, increased length of lactation during shortage periods and maintenance of body condition score (BCS) important for reproduction. Attaining these objectives result in optimized profit per unit of land (Fales *et al*., 1995). Be it as it may, concentrate may not be fed without constraint since high levels (> 10 kg) may be detrimental to the metabolic health of the cow (Bargo *et al*., 2003). Apart from this the marginal milk response (MR) of concentrate has been described to have a curvilinear nature (Kellaway & Porta, 1993) with MR decreasing as concentrate DM intake increase (St - Pierre, 2001). Milk producers using pasture based systems therefore are limited by the extent with which milk yield can be improved using increased levels of concentrate. Logical alternatives are to improve the genetic potential of pasture species and/or to improve the utilization of current pasture species. The latter entails increased fibre digestion by creating a rumen environment that will ensure optimal proliferation and function of rumen micro-organisms. Since rumen pH is the rumen condition that varies the most (Dehority, 2003) rumen pH represents the area of research where much can be attained to create the ideal rumen environment. Fluctuations occur with time after feeding, nature of the feed and frequency of feeding (Dehority, 2003). The ruminal pH profile of cows on pasture generally has a bimodel peak form.

Erasmus (2009) reported on the pH values of cows grazing westerwold ryegrass, supplemented with either 4 or 8 kg of concentrate. The pH profile, measured over a 24 hour period, showed two rapid declines that coincided with access to concentrate during milking (7:30 and 15:30) and access to the fresh allocation of herbage following milking. The declines followed immediately after the peak periods. The most pronounced decline occurred during the morning period while minimum pH levels were reached during the second declining period in the afternoon. The profile behaviour can in part be explained by concentrate allocation and grazing behaviour. Abrahamse *et al.* (2009) showed that the main grazing bout is between 16:30 and 21:00. The second most aggressive bout occurs from 6:00 to 11:00. Since various authors (Van Vuuren *et al*., 1986; Orr *et al.,* 2001 and Delegarde *et al.,* 2000) have reported higher concentrations of water soluble carbohydrates (WSC) in pastures in the afternoon ruminal pH might decline in the afternoon partially as a result of the increased intake of WSC. Pronounced diurnal fluctuations in ruminal pH can be detrimental to rumen microbes since it may necessitate continuous metabolic readjustments (Mertens, 1979). The current study aimed to address the fluctuation in ruminal pH below the critical value, thus the fluctuation occurring during the afternoon period. The null hypothesis was that the fluctuation in ruminal pH during the afternoon (16:00) would be less pronounced by increasing the ratio of morning to afternoon concentrate allocation during milking. In order to test the hypothesis two studies *i.e.* a rumen study and a production study were conducted. The rumen study investigated changes in rumen pH, volatile fatty acid (VFA) concentration of rumen fluid and changes in neutral detergent fibre (NDF) degradation *in sacco*. The production study investigated mean milk yield and milk composition.

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4.2 Materials and Methods

4.2.1 General information

The study was conducted at the Outeniqua Research Farm (latitude: 33° 58' 41.98" S; longitude: 22°25'14.48''E; altitude: 190 m) in George in the Western Cape. The average monthly rainfall during the trial was 37.13 mm. Temperature (°C) during the trial was moderate ranging from a mean daily maximum of 19.73°C to a mean daily minimum of 8.93°C (Erasmus, 2009). See Addendum A (Table A1) for monthly temperature and rainfall data (Table A2). The experimental plot consisted of an irrigated pasture (8.5 ha) with an Estcourt soil type (see Addendum B, Table B1) (Soil Classification Working Group, 1991). The pasture species consisted of a kikuyu (*Pennisetum clandestinum*) grass base over sown with annual ryegrass (*Lolium multiflorum var. westerwoldicum*, cv Energa). The trial was conducted from late winter (1 August 2007) to early spring (14 October 2007). The first two weeks served as an adaptation period.

4.2.2 Experimental design and treatments

A randomized block design was used for both studies. Cows were blocked for parity, days in milk (DIM) and average milk production. Treatments consisted of the morning concentrate allocation being fed in different weight ratios to the afternoon allocation. The two treatments applied for the rumen study were:

1) Concentrate fed at 3 kg during morning and 3 kg during afternoon milking (RT3:3)
2) Concentrate fed at 5 kg during morning and 1 kg during afternoon milking (RT5:1)

The three treatments applied for the production study were:

1) Concentrate fed at 3 kg during morning and 3 kg during afternoon milking (RT3:3)
2) Concentrate fed at 4 kg during morning and 2 kg during afternoon milking (RT4:2)
3) Concentrate fed at 5 kg during morning and 1 kg during afternoon milking (RT5:1)

4.2.3 Animal management

The rumen study used six rumen cannulated Jersey cows per treatment. The cows were blocked according to DIM, parity and average milk production at 22 days prior to the trial and randomly allocated to two treatments. Six intact cows were used as filler cows. This enabled three groups of six animals each. The milk production study used 42 high producing Jersey cows (milk yield (kg per cow/day); 18.93 ± 0.417 S.E.M., days in milk (DIM): 95.71 ± 9.019 S.E.M., lactation number: 4.7 ±

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0.34 S.E.M.). The cows were blocked according to DIM, parity and average milk production at twenty two days prior to the trial and randomly allocated to one of three treatments (see Addendum C, Table C1 for blocking and allocation of cows to treatments). Cows in different treatment groups were identified with coloured ear tags, fastened to chains around the neck. Each cow had a transponder attached to the ear for identification required for electronic milk recording and feeding purposes. Treatment groups of both studies and of the same study grazed as a single unit to avoid variation in data due to pasture differences. A strip grazing system was employed with cows receiving a fresh break after each milking session. The cows were allowed access to pasture 24 hours a day, except during milking times. Fresh water was available *ad libitum*.

The average walking distance from pasture to milking parlour was 0.9 km (0.55 ± 1.18 km). Cows of both studies were milked at 06:00 AM and 16:00 PM with a 20 point swing over Dairy Master (Dairy Master, Causeway Co. Kerry, Ireland) milking machine. Concentrate feeding was done in the parlour with automated feeders, programmed according to treatments. Treatment groups received 6 kg (as is) concentrate per cow/day (see Addendum D, Table D1 and Table D2 for chemical and nutrient composition). Cows were kept in the parlour until all the fed concentrate had been consumed. A steel bailing system prevented cows from consuming concentrate in adjacent feed cribs. The feeders were tested on two occasions to ensure that the correct mass of concentrate was fed to each treatment group during each milking session.

4.2.4 Rumen pH data collection

Rumen pH was measured using WTW pH 340i pH data logger with a WTW Sentix pH electrode (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). The electrodes were calibrated with two buffer solutions with known pH values and protected by a metal capsule. The metal capsule was fitted to the cannula stop inside the rumen. The pH logger was fitted to a specially designed leather box on a leather harness. The harness was attached to the cannulated cows and the box was covered with a plastic shower cap to avoid rain damaging the equipment. The electrode was connected to the data logger via an electronic cable.

Data were captured during two runs. The first run stretched from 3 to 7 September and was divided into two parts. During the first part of the first run; 3 to 5 September, pH data were logged from three animals per treatment. During the next part data from remaining animals in each treatment were logged. The second run occurred from 10 to 14 September. The time between the two runs was sufficient to prevent cows from suffering from chafe from the leather harnesses. The procedure followed in run one was also followed in run two, but animals were allocated different loggers from the ones used in run one. The loggers were set to log the pH and rumen temperature at intervals of 10 minutes and were calibrated between runs.

4.2.5 Rumen sampling and manual pH data collection

Seventy-two rumen liquor samples were collected from 24 September to 26 September using a modified drain pump fitted with a plastic sample container. Sampling was done at six different times from six cannulated cows per treatment. Rumen liquor was obtained from animals of both treatments. Animals were kept on the pasture during sampling with minimal interference. Sampling started at 12:00 am (24 September) with eight hour intervals until 00:00 (26 September). Rumen pH was measured manually during sampling using a WTW pH 340i pH data logger with a WTW Sentix pH electrode (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Rumen liquor was filtered through a double layer of mutton cloth. Nine ml of each sample was preserved with 1 ml NaOH (10% m/v) and stored in an airtight plastic container. Samples were frozen for preservation purposes.

4.2.6 In sacco digestibility

The pasture substrate, used to determine *in sacco* dry matter (DM) and neutral detergent fibre (NDF) disappearance, was selected to ensure minimal contamination with foreign material like faeces. A 6 m x 5 m area was chosen and pasture height was measured with a rising plate meter (RPM). Pasture was cut with a cutter bar mower (Agria Compact 360B, Agria-werke, Gmbh export, Möckmühl, Germany) and plant material was raked and transferred to black plastic bags. Plant material was put in brown paper bags and allowed to dry for 72 hours at 60°C. The dried plant material was milled with a feed mill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa) fitted with a 5 mm sieve. A Retsch AS 200 (Retsch GmbH, Rheinische Straße 36, 42781, Haan, Germany) sieve shaker was used to sieve the milled plant material through a Kingtest sieve with 106 µm aperture. This was done to reduce the mass of DM that is lost instantaneously through the pores of the Dacron bag.

Large Dacron bags (10 x 20 cm; 53 ± 10 micron) (Bar Diamond Inc. Parma, Idaho, USA) were used and were marked with a laundry marker. The bags were weighed empty (as is), then filled with ± 5 g (as is) of plant substrate and weighed again. A cable tie (10 cm) was used to tie each bag closed. The mass of the bag with substrate and cable tie was recorded. Six empty bags were dried in a drying oven for 72 hours at 60°C to determine the dry matter content of the bags and six samples of substrate were dried to determine dry matter content. Four bags containing substrate were used as time zero bags to determine the lag phase. The method of Cruywagen (2006) was used to retrieve the bags from the rumen. This system consisted of two components *viz*. an accommodation vessel and a receptacle. The accommodation vessel was an opaque nylon stocking (44 decitex yarn and 17 decitex lycra) (Burhose, Brackenfell Industria, Cape Town, South Africa), capable of carrying up to six

Dacron bags. The accommodation vessel was filled with Dacron bags placed in tandem position, starting from the bottom of the accommodation vessel. A tight knot was tied between adjacent Dacron bags. A glass marble (33 mm diameter) was placed at the bottom of the stocking and isolated with a knot from the Dacron bags. The weight (50 g) of the marble ensured that the accommodation vessel was kept in position. The receptacle consisted of a shorter stocking, named a catcher. The catcher had a loop on the end where the accommodation vessel was attached. The other end of the catcher was tied to the cannula plug. Dacron bags were inserted in the rumens at the same time and removed after specified incubation times. The bags were removed from the free end of the accommodation vessel. The remaining bags were not exposed to the environment and thus did not influence fibre degradation.

Incubation times (hours) were 2, 4, 6, 13, 15, 29, 48 and 72. Two bags were used for each time except for times 29, 48 and 72 where three bags were used to ensure adequate residue for further analysis. Bags were rinsed with cold water until water appeared clear and then frozen in a box freezer at -16°C. The bags were allowed to thaw and were dried in a drying oven for 72 hours at 60°C prior to analysis.

4.2.7 Volatile fatty acid analytical methods

Rumen samples were analysed at Nutrilab, University of Pretoria for volatile fatty acids (VFA's) (acetic, propionic, butyric, iso-butyric and valeric acids) using a Varian 3300 FID Detector Gas Chromatograph (Varian Associates, Inc. 1985, USA). The gas chromatographic method described by Webb (1994) was used for the analysis.

4.2.8 In sacco residue analytical methods

Dacron bags were weighed following the drying process mentioned in section 4.2.6. Residue was carefully retrieved and placed in air tight plastic containers. Neutral detergent fibre of *in sacco* plant substrate and residue was determined with an Ankom 220 Fibre analyser (Ankom technologies, Fairport, NY, USA), applying the method of Ankom Technology (Ankom, 2006b); a modification of the method of Van Soest *et al.* (1991). Neutral detergent fiber disappearance data was fitted to the non-linear model of Khorasani *et al.* (1994):

$$Y = a + b(1-e^{-c(t-L)})$$

where Y is the NDF disappearance (%) at time t

a is the soluble and rapidly degradable NDF fraction (%)

b insoluble fermentable NDF fraction (%)
c is the rate at which b is degraded (%/h)
L represents the lag phase (h)

The same model was used to fit DM disappearance data. Effective degradability (ED) of NDF was calculated using the formula of Ørskov & McDonald (1979):

$$ED = a + ((b \times c)/(k + c))$$

where k is the rumen outflow rate, assumed to be 5 %/h.

4.2.9 Analytical methods

Feed samples were analysed as follows: calcium (Ca) and phosphorus (P) (ALASA, 1998, macro – and trade elements method no. 6.1.1, using an iCAP 6000 ICP spectrometer, Thermo Electron Corp, USA), ash (AOAC Official method number 942.0, AOAC International, 2002), gross energy (GE) (MC-1000 modular calorimeter, operator's manual), total nitrogen (N) (AOAC Official method number 968.06, AOAC International, 2005, using a Leco FP-528 N analyser, Leco Corporation, St. Joseph, MI, USA), crude protein (CP) (AOAC Official method number 920.39, AOAC International 1995), neutral detergent fibre assayed with heat stable amylase, exclusive of residual ash (a NDF OM) and acid detergent fibre (ADF) (methods described by Ankom technology, Ankom, 2006b; Ankom, 2006a).

Soluble crude protein and non protein nitrogen (NPN) (modification of the method described by Licitra *et al.* (1996), *in vitro* organic matter digestibility (IVOMD) (modification of Ankom technology method, Ankom, 2005b), acid detergent lignin (ADL) (Ankom technology method, Ankom, 2005a), neutral detergent fibre nitrogen (NDF-N) and acid detergent fibre nitrogen (ADF-N) (NDF and ADF methods plus AOAC Official method number 968.06, AOAC International, 2005), starch (modification of Holm *et al.*, 1986), glucose (Karkalas, 1985), fat (Ether extract) (AOAC Official method number 920.39, AOAC International, 2002) using a Soxtec System HT 1043 Extraction Unit, Tecator AB, Höganäs, Sweden), dry matter (DM) (AOAC Official method number 934.01, AOAC International, 2002). Metabolisable energy (ME) was determined by means of the formula: ME = (0.84 x GE x IVOMD) of Robinson *et al.* (2004).

4.2.10 Statistical analyses

Data were subjected to a one - way analysis of variance (ANOVA) with the aid of the GLM procedure of SAS, version 9.2 (SAS, 2008). The structure of the underlying linear model was:
$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, (Clewer and Scarisbrick, 2006)

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where y_{ij} = j^{th} observation from i^{th} treatment

μ = overall mean

α_i = effect of the ith treatment where i = 1,2,….a, and

ε_{ij} = difference of jth observation from the ith mean, *i.e.* residual (i,j).

The assumption for the model is $\varepsilon_{ij} \sim N(0,\sigma_e^2)$.

The null hypothesis was Ho: $\mu_1 = \mu_2 = \mu_3 = \ldots\ldots \mu_a$. The F-ratio and the P value were used as indication to reject or accept H_0. The null hypothesis was rejected where $P < 0.05$. Students *t*-tests (Ott, 1993) were used to confirm the results of the ANOVA. Shapiro Wilk tests (Shapiro and Wilk, 1965) were used to test for non normality.

4.3 Results

4.3.1 Rumen study

4.3.1.1 Ruminal pH

The results of the diurnal ruminal pH, measured by means of automated data logger, are presented in Table 4.1. No differences were observed between treatment means of maximum pH, minimum pH and mean daily ruminal pH. The times that daily ruminal pH in the different treatments spent below ruminal pH 5.8 are displayed in Table 4.1. No differences were observed between treatment means. The diurnal ruminal pH profiles of the two treatment groups (n = 6) are illustrated in Figure 4.1. A two peak profile was observed for both treatment profiles. Both treatment profiles followed similar trends with declines following close to the time when concentrate was allocated during milking.

Table 4.1 The ruminal pH of lactating Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture supplemented with 6 kg (as is) of concentrate per cow / day

Item	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
Max. pH	6.47	6.49	0.076	0.813
Min. pH	5.66	5.73	0.046	0.357
Mean daily pH	6.03	6.04	0.034	0.787
Time (h) < 5.8	3.42	2.92	1.207	0.776

[1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

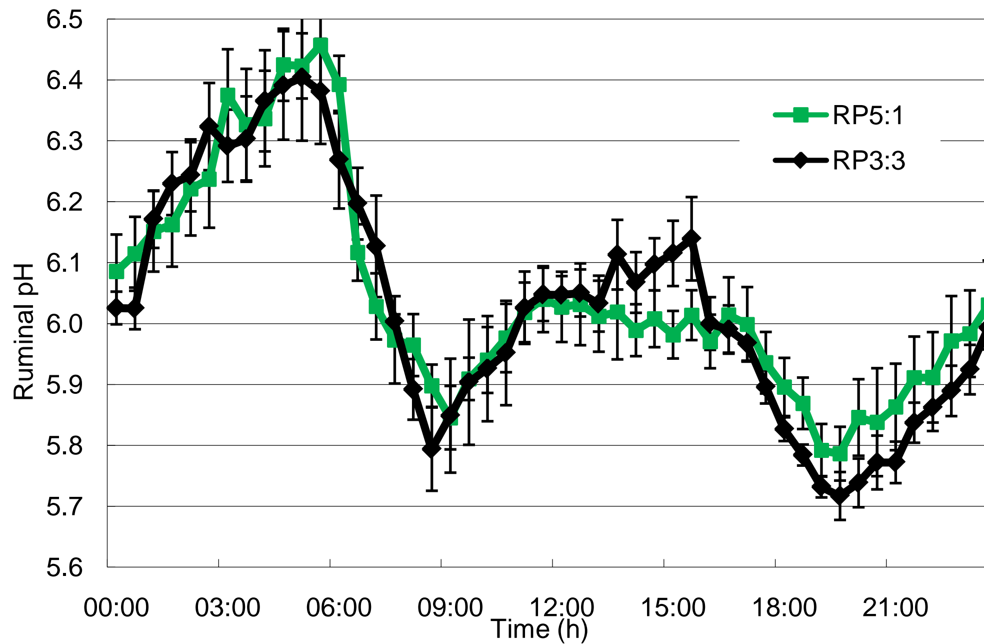

Figure 4.1 Ruminal pH profile with S.E.M. bars based on 24 hour diurnal basis of Jersey cows (n = 6) receiving 6 kg (as is) concentrate per cow per day and grazing ryegrass (*Lolium multiflorum*) pasture ([1]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon).

The minimum diurnal pH for both treatments occurred during the evening around 19:30. The maximum pH of RP3:3 occurred a half hour earlier (5:30 am) compared to the maximum pH of RP5:1. Two periods of declining pH were visible for both treatments. The onset and end points in time/24 hour cycle of period 1(P1) compared similar between treatments (onset P1RP3:3 = 5:00 am; P1RP5:1 = 5:30 am; end P1RP3:3 = 8:30 am; P1RP5:1 = 9:00 am). The same was true for period 2 (P2) (onset P2RP3:3 = 15:30 pm; P2RP5:1 = 16:30 pm; end P2RP3:3 = 19:30 pm; P2RP5:1 = 19:30 am).

Table 4.2 and Table 4.3 display the ruminal pH data for the two treatments from midnight to 12:00 noon and from 12:00 to 23:30 pm respectively. Each data point represents an average of three measurements recorded every 10 minutes to give a representative figure of ruminal pH during each half hour. No differences were observed between treatments during any time of the 24 hour recording period.

Table 4.2 Effect of concentrate feeding strategies on ruminal pH profile from 00:00 to 12:00 of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	6.02	6.09	0.047	0.382
00:30	6.03	6.11	0.057	0.329
01:00	6.17	6.15	0.062	0.815
01:30	6.23	6.16	0.059	0.470
02:00	6.24	6.22	0.073	0.855
02:30	6.32	6.24	0.086	0.523
03:00	6.29	6.38	0.072	0.442
03:30	6.31	6.33	0.111	0.895
04:00	6.37	6.34	0.104	0.839
04:30	6.39	6.43	0.094	0.811
05:00	6.41	6.42	0.092	0.903
05:30	6.38	6.46	0.094	0.595
06:00	6.27	6.39	0.050	0.144
06:30	6.20	6.12	0.029	0.104
07:00	6.14	6.03	0.067	0.266
07:30	6.01	5.97	0.060	0.743
08:00	5.89	5.97	0.056	0.400
08:30	5.80	5.90	0.061	0.283
09:00	5.85	5.85	0.062	0.971
09:30	5.91	5.91	0.0648	0.972
10:00	5.93	5.94	0.042	0.832
10:30	5.95	5.98	0.041	0.726
11:00	6.03	6.02	0.043	0.875
11:30	6.05	6.04	0.049	0.908
12:00	6.05	6.03	0.049	0.765

[1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; $P < 0.05$ was considered significant.

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Table 4.3 Effect of concentrate feeding strategies on ruminal pH profile from 12:00 to 23:30 of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
12:00	6.05	6.03	0.049	0.765
12:30	6.05	6.03	0.055	0.823
13:00	6.04	6.01	0.049	0.765
13:30	6.12	6.02	0.077	0.417
14:00	6.07	5.99	0.051	0.325
14:30	6.10	6.01	0.059	0.348
15:00	6.12	5.98	0.060	0.177
15:30	6.14	6.02	0.063	0.219
16:00	6.00	5.97	0.052	0.718
16:30	5.99	6.01	0.067	0.815
17:00	5.97	6.00	0.059	0.747
17:30	5.90	5.94	0.047	0.589
18:00	5.83	5.90	0.044	0.330
18:30	5.78	5.87	0.036	0.156
19:00	5.73	5.79	0.033	0.247
19:30	5.72	5.79	0.046	0.351
20:00	5.74	5.85	0.059	0.258
20:30	5.77	5.84	0.069	0.506
21:00	5.77	5.86	0.052	0.280
21:30	5.84	5.91	0.038	0.244
22:00	5.86	5.91	0.049	0.515
22:30	5.89	5.97	0.051	0.320
23:00	5.93	5.98	0.063	0.539
23:30	5.99	6.03	0.066	0.724

[1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; $P < 0.05$ was considered significant.

Results of the manual recorded ruminal pH are presented in Table 4.4. No differences were observed during five of the six times of pH recording while a strong tendency (P = 0.058) was observed during 16:00. The minimum pH (5.66) of RP3:3 was reached at 20:00 pm while that of RP5:1 (5.81) was reached at 12:00 am. The maximum pH of both treatments was reached at 04:00 am.

Table 4.4 Mean manual recorded ruminal pH of rumen fluid (n = 6) during different times from different treatment groups

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	5.92	5.96	0.095	0.784
04:00	6.37[b]	6.65[a]	0.053	0.015
08:00	5.97	5.96	0.074	0.919
12:00	5.85	5.81	0.069	0.723
16:00	5.93	6.17	0.071	0.058
20:00	5.66	5.82	0.098	0.308

[a,b] Means in the same row with different superscripts differ (P < 0.05); [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; P < 0.05 was considered significant.

4.3.1.2 Volatile fatty acid concentration of rumen fluid

Results of acetic acid concentration (mmol/L), measured during six different times, are presented in Table 4.5. Mean ± S.E.M. acetic acid concentration (mmol/L) per 24 hour period were 58.0 ± 1.51 (60.1 ± 2.68 mol/100 mol) and 47.5 ± 1.33 (61.9 ± 0.52 mol/100 mol) for RP3:3 and RP5:1 respectively. Figure 4.2 represents a graphic illustration of the acetic acid concentration at the sampling times. Differences (P < 0.05) between the two treatment means were observed at four of the six sampling times. The maximum concentration (mmol/L) was measured at different times for the two treatments. This was also the case for the minimum concentration level.

Table 4.6 presents the propionic acid concentration (mmol/L) and Table 4.7 the butyric acid concentration (mmol/L). Graphic illustrations of the propionic acid and butyric acid concentration results are provided by Figure 4.3 and 4.4 respectively. Differences (P < 0.05) in treatment means were observed for propionic acid concentration. Mean ± S.E.M. propionate concentration (mmol/L)

Table 4.5 Effect of concentrate feeding strategies on acetic acid concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	55.2[a]	46.7[b]	1.63	0.014
04:00	61.0	54.7	2.46	0.126
08:00	58.1[a]	40.8[b]	1.71	0.001
12:00	50.2	50.6	3.65	0.946
16:00	69.1[a]	47.6[b]	3.08	0.004
20:00	54.4[a]	44.8[b]	1.38	0.004

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

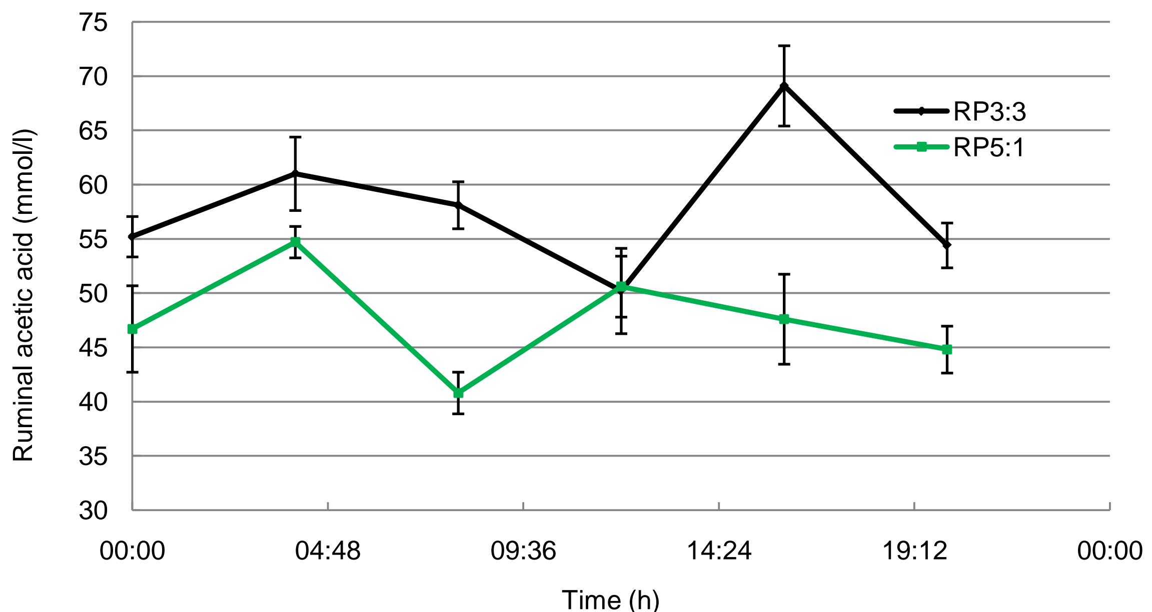

Figure 4.2 Mean ruminal acetic acid concentration (mmol/L) with S.E.M. bars of treatment groups during six times during a 24 hour period ([1]RP5:1 = 5 kg (as is) concentrate fed in morning;1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning;3 kg (as is) concentrate fed in afternoon).

per 24 hour period for RP3:3 and RP5:1 were 21.5 ± 1.05 (22.1 ± 1.76 mol/100 mol) and 15.5 ± 0.71 (20.03 ± 0.86) respectively. During 00:00, 08:00, 12:00 and 16:00 the propionic acid concentration

was higher ($P < 0.05$) in rumen fluid samples of RP3:3. Butyric acid concentration (mmol/L) was higher ($P < 0.05$) during 08:00, 12:00 and 16:00 in rumen fluid samples of cows receiving RP3:3.

Table 4.6 Effect of concentrate feeding strategies on propionic acid concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	21.1[a]	14.4[b]	1.13	0.009
04:00	19.4	20.2	1.13	0.643
08:00	17.4[a]	11.5[b]	1.04	0.010
12:00	32.6[a]	18.9[b]	2.19	0.007
16:00	20.3[a]	14.1[b]	1.68	0.048
20:00	18.2	14.1	1.33	0.080

[a,b] Means in the same row with different superscripts differ ($P < 0.05$), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; $P < 0.05$ was considered significant.

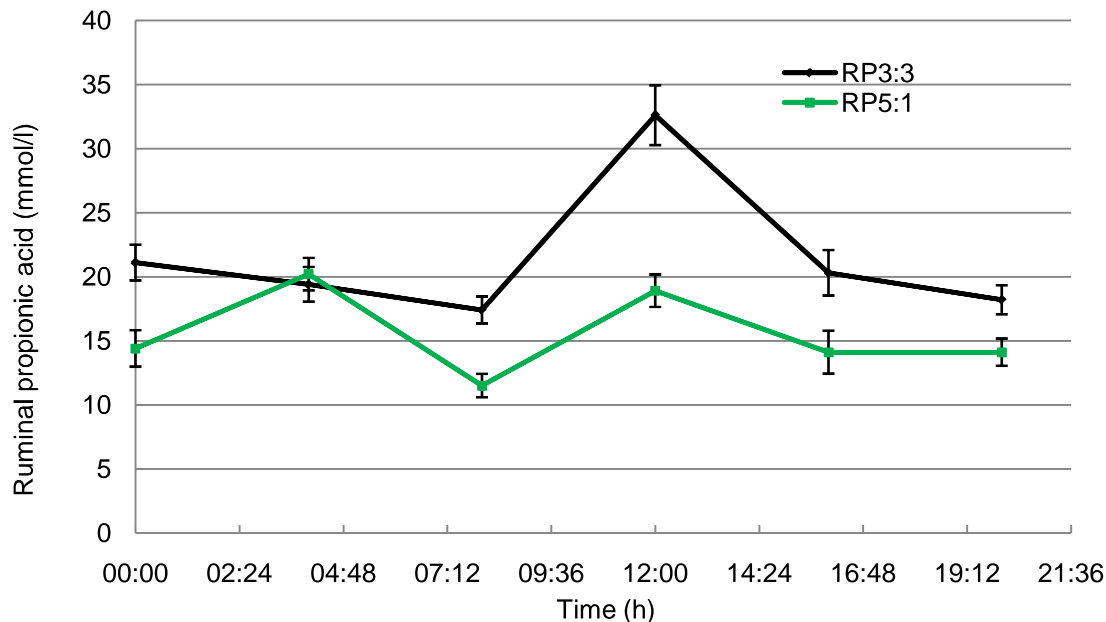

Figure 4.3 Mean ruminal propionic acid concentration (mmol/L) with S.E.M. bars of treatment groups during six times during a 24 hour period ([1]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon).

Table 4.7 Effect of concentrate feeding strategies on butyric acid concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	12.6	11.7	0.60	0.334
04:00	13.6	13.0	0.94	0.626
08:00	13.9[a]	10.5[b]	0.72	0.019
12:00	18.5[a]	11.4[b]	1.39	0.015
16:00	15.1[a]	11.1[b]	0.65	0.008
20:00	12.8	11.2	0.92	0.261

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; P < 0.05 was considered significant.

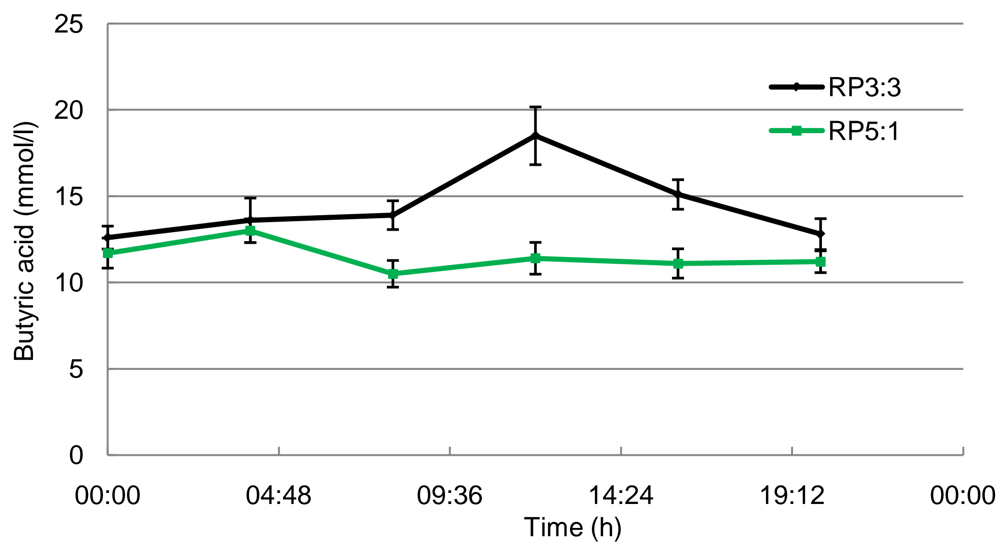

Figure 4.4 Mean ruminal butyric acid concentration (mmol/L) with S.E.M. bars of treatment groups during six times during a 24 hour period ([1]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon).

Results of iso-butyric acid concentration (mmol/L) and valeric acid concentration (mmol/L) are presented in Tables 4.8 and 4.9. Differences (P < 0.05) in iso-butyric acid concentration (mmol/L)

Table 4.8 Effect of concentrate feeding strategies on iso - butyric acid concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	0.99[a]	0.74[b]	0.062	0.0340
04:00	0.99	1.09	0.061	0.2825
08:00	0.88[a]	0.53[b]	0.064	0.0120
12:00	0.80	0.80	0.050	0.5663
16:00	0.99	0.79	0.078	0.1278
20:00	0.76	0.71	0.066	0.6217

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; P < 0.05 was considered significant.

Table 4.9 Effect of concentrate feeding strategies on valeric acid concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	1.92[a]	1.21[b]	0.038	< 0.0001
04:00	1.45	1.66	0.074	0.1095
08:00	1.19	0.987	0.074	0.1105
12:00	5.41[a]	1.38[b]	0.417	0.0210
16:00	1.29	1.03	0.120	0.1907
20:00	1.26	0.96	0.084	0.0519

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

were observed between treatment means of samples taken at 00:00 and 08:00. Differences (P < 0.05) in valeric acid concentration (mmol/L) were observed in samples taken at 00:00 and 12:00.

The mean acetic – propionic acid (A:P) ratio of samples from the two treatments, taken at the six different times are presented in Table 4.10. Differences (P < 0.05) in the ratio were observed for samples taken at 00:00, 04:00 and 12:00. During 00:00 and 12:00 the ratio was higher (P < 0.05) in samples from cows receiving RP5:1. Results of the acetic - total acid concentration (mmol/L) ratio

Table 4.10 Effect of concentrate feeding strategies on acetic:propionic acid (A:P) ratio (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	2.65[a]	3.28[b]	0.148	0.0311
04:00	3.16[a]	2.74[b]	0.097	0.0279
08:00	3.36	3.61	0.169	0.3545
12:00	1.54[a]	2.72[b]	0.127	0.0012
16:00	3.46	3.43	0.149	0.8787
20:00	3.03	3.23	0.199	0.5080

[a,b] Means in the same row with different superscripts differ ($P < 0.05$), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, $P < 0.05$ was considered significant.

and propionic-total acid concentration (mmol/L) are presented by Tables 4.11 and 4.12 respectively. Differences ($P < 0.05$) in the acetic - total acid concentration (mmol/L) ratio were observed at 04:00 and 12:00 while differences ($P < 0.05$) in propionic - total acid concentration (mmol/L) were observed during 00:00, 04:00, and 12:00. During 00:00 and 12:00 the propionic – total acid concentration (mmol/L) ratio were higher ($P < 0.05$) in samples from cows receiving RP3:3. Table 4.13 presents the results of the total volatile fatty acid (VFA) concentration (mmol/L). Differences occurred at 00:00, 08:00, 16:00 and 20:00. Treatment means were higher ($P < 0.05$) for samples taken from cows receiving RP3:3 during all of the mentioned times.

Table 4.11 Effect of concentrate feeding strategies on acetic:total VFA ratio (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	0.60	0.62	0.008	0.1159
04:00	0.63[a]	0.60[b]	0.006	0.0189
08:00	0.64	0.62	0.014	0.3672
12:00	0.47[a]	0.61[b]	0.009	0.0001
16:00	0.64	0.64	0.006	0.3304
20:00	0.62	0.63	0.012	0.8951

[a,b] Means in the same row with different superscripts differ ($P < 0.05$), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, $P < 0.05$ was considered significant.

Table 4.12 Effect of concentrate feeding strategies on propionic:total VFA ratio (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	0.23[a]	0.19[b]	0.009	0.0299
04:00	0.20[a]	0.22[b]	0.005	0.0348
08:00	0.19	0.17	0.0089	0.2039
12:00	0.30[a]	0.23[b]	0.006	0.0003
16:00	0.19	0.19	0.007	0.9195
20:00	0.21	0.20	0.009	0.3768

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

Table 4.13 Effect of concentrate feeding strategies on total VFA concentration (mmol/L) in rumen fluid of Jersey cows (n = 6) grazing ryegrass (*Lolium multiflorum*) pasture

Time	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
00:00	91.8[a]	74.8[b]	2.68	0.006
04:00	96.6	90.6	4.36	0.377
08:00	91.5[a]	66.6[b]	2.08	< 0.001
12:00	107	83.0	7.10	0.058
16:00	107[a]	74.7[b]	5.50	0.009
20:00	87.4[a]	78.8[b]	3.44	0.024

[a,b] Means in the same row with different superscripts differ (P < 0.05), [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

4.3.1.3 In sacco fibre degradation

Results of the DM disappearance parameters *i.e.* a, b, c and L are presented in Table 4.14. No differences were observed between treatments for any of the parameters. The percentage *in sacco* DM disappearance per time (h) is illustrated in Figure 4.5. The same non - linear pattern was observed for both treatments.

Table 4.14 Mean DM disappearance parameters of *in sacco* trial using Jersey cows (n = 6) on ryegrass (*Lolium multiflorum*) pasture supplemented with concentrate

Parameter	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
a	47.3	45.5	0.79	0.136
b	44.3	45.8	0.88	0.257
c	0.069	0.066	0.0450	0.646
L	1.35	0.72	0.348	0.231

a = Soluble degradable DM fraction; b = Insoluble fermentable DM fraction; c = Rate at which b is degraded; L = Lag phase (h) (Khorasani *et al.*, 1994); [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

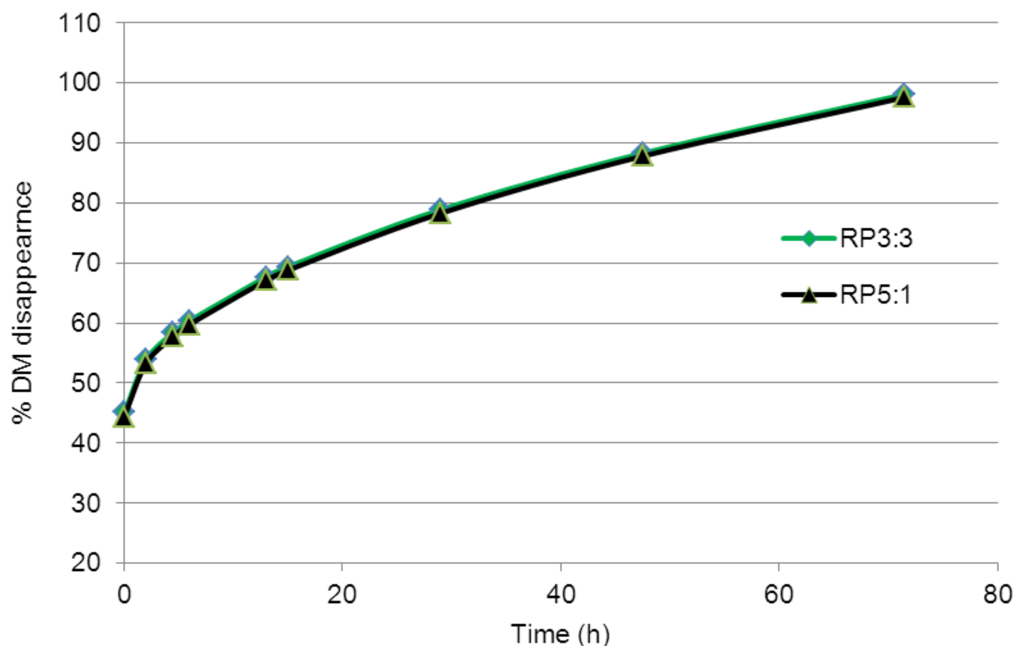

Figure 4.5 Percentage DM disappearance, *in sacco,* per time (h) of different treatment groups ([1]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon).

Table 4.15 presents the NDF disappearance parameters. No S.E.M. or P-value was available for the first parameter (a) because this value was set at 5.3 (0 h washing value) and therefore no variance occurred. No differences were observed between parameters of the two treatments. Figure 4.6

illustrates percentage NDF disappearance per time (h). Curves of both treatments followed the same non - linear pattern.

Table 4.15 Effect of concentrate feeding strategies on mean NDF disappearance parameters of *in sacco* trial using Jersey cows (n = 6) on ryegrass (*Lolium multiflorum*) pasture

Parameter	RP3:3[1]	RP5:1[2]	S.E.M.[3]	P - value
a	5.3	5.3	N/A	N/A
b	79.8	80.7	0.75	0.406
c	0.068	0.066	0.0030	0.4954
L	0.025	0.003	0.0166	0.3537

a = Soluble degradable DM fraction; b = Insoluble fermentable DM fraction; c = Rate at which b is degraded (%/h); L = Lag phase (h) (Khorasani *et al.*, 1994); [1]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means, $P < 0.05$ was considered significant.

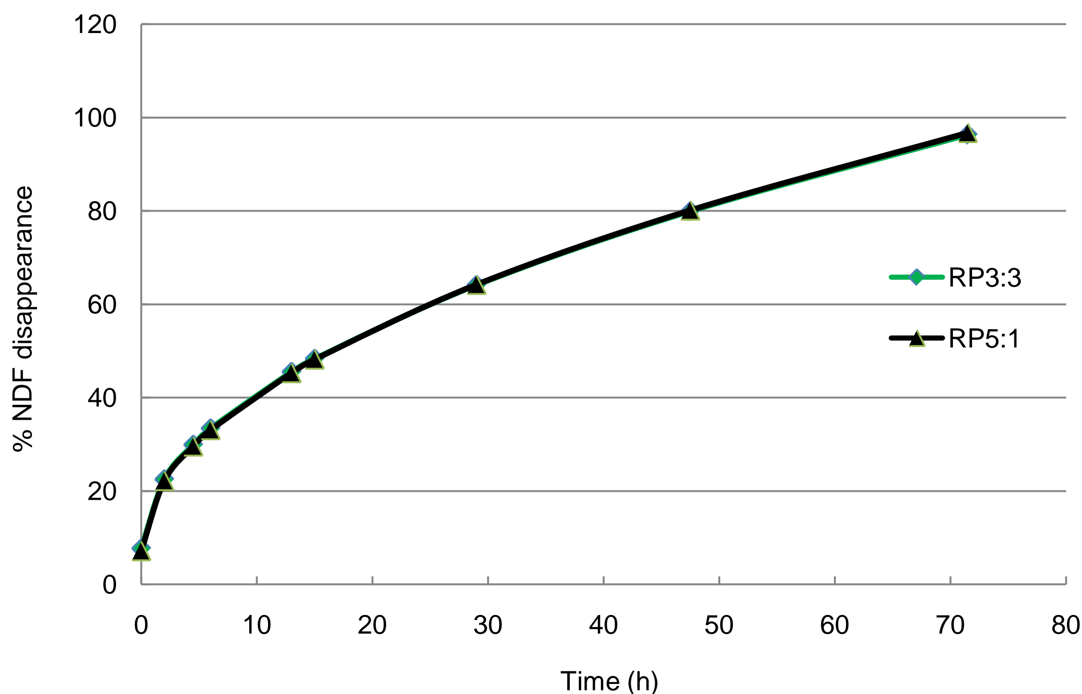

Figure 4.6 Percentage NDF disappearance, *in sacco*, per time (h) of different treatments ([1]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon).

Table 4.16 presents the results of the Effective Degradability (ED) of Neutral Detergent Fibre (NDF). No differences were observed between treatment means.

Table 4.16 Effect of concentrate feeding strategies on mean Effective Degradability (ED) of Neutral Detergent Fibre (NDF), *in sacco,* using Jersey cows (n = 6) on ryegrass (*Lolium multiflorum*) pasture

Parameter	RP3:3[2]	RP5:1[3]	S.E.M.[4]	P - value
ED[1]	51.3	51.0	0.84	0.812

[1]ED = Effective Degradability (a + ((b x c)/(k + c)) where a = Soluble degradable NDF fraction; b = Insoluble fermentable NDF fraction ; c = Rate at which b is degraded (Khorasani *et al.*, 1994); k = rumen outflow rate, assumed 0.05 (Ørskov and McDonald, 1979); [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [3]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [4]S.E.M. = Standard Error of the Means, P < 0.05 was considered significant.

4.3.2 Production study

Mean ± S.E.M. pasture intake (kg DM per cow /day) for August to mid October is presented in Table 4.1. Intake was calculated using the regression equation given in Chapter 3, section 3.2 with data of pasture height measured before grazing and after grazing.

Table 4.17 Mean ± S.E.M.[1] (kg DM per cow/day) intake of ryegrass (*Lolium multiflorum*) pasture from August 2007 to October 2007

Date	Intake ± SEM[1] (kg DM per cow/day)
August	5.64 ± 0.29
September	7.13 ± 0.30)
October	6.76 ± 0.56

[1]S.E.M = Standard Error of the Means.

Results of pasture management are presented in Table 4.18 and Table 4.19. Rising plate meter readings are reported in Table 4.18 for the pre - graze and post - graze periods. The mean yield (tDM/ha) are reported in Table 4.19 for the months August until October.

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Table 4.18 Mean RPM[1] reading, pre - graze and post – graze for ryegrass (*Lolium multiflorum*) pasture from August 2007 to October 2007

Month	n	Mean RPM[1] reading			
		Pre -graze	S.E.M[2]	Post - graze	S.E.M.
August	31	26.9	0.89	9.3	0.34
September	28	31.5	0.90	8.9	0.23
October	17	30.4	1.70	11.2	0.49

[1]RPM = Rising Plate Meter; [2]S.E.M. = Standard Error of the Means.

Table 4.19 Mean pasture yield (t DM/ha, calibrated to 3 cm pasture height), pre - graze and post - graze of ryegrass (*Lolium multiflorum*) pasture allocated from August 2007 to October 2007

Month	n	Mean yield (t DM/ha)			
		Pre - graze	S.E.M[1]	Post - graze	S.E.M.
August	31	1.33	0.068	-0.03	0.026
September	28	1.68	0.069	-0.06	0.017
October	17	1.59	0.131	0.12	0.038

[1]S.E.M. = Standard Error of the Means.

The results of the mean ± S.E.M. nutrient composition of ryegrass (*Lolium multiflorum*) pasture and concentrate pellets are provided in Table 4.20. All values, except for dry matter (DM), are reported on DM basis. Mean milk yield (kg per cow/day), mean 4 % fat corrected milk (4 % FCM) yield (kg per cow/day) and mean milk composition of the three treatments are reported in Table 4.21. No differences were observed between treatment means of either mean milk yield or 4 % FCM.

No differences were observed for fat % or total fat (kg) nor was any difference observed for protein %, total protein (kg), lactose, milk urea nitrogen (MUN) or somatic cell count (SCC).

Table 4.20 Mean ± S.E.M.[1] nutrient composition of ryegrass *(Lolium multiflorum)* pasture (n = 8) and concentrate pellets (n = 4)

Nutrient	Pasture	Concentrate
	Mean ± S.E.M.	Mean ± S.E.M.
DM (g/kg AS IS)	127 ± 4.9	912 ± 5.9
CP (g/kg DM)	298 ± 1.8	148 ± 6.8
Sol. CP (g/kg CP)	304 ± 32.9	824 ± 33.5
ME (MJ/kg DM)	11.8 ± 0.12	12.98 *
a NDF OM (g/kg DM)	470 ± 11.8	94.0 ± 3.56
NDF-N (g/kg NDF)	3.56 ± 26.5	8.62 ± 0.423
ADF (g/kg DM)	345 ± 14.8	45.2 ± 3.73
ADF-N (g/kg ADF)	3.87 ± 24.7	N/A
ADL OM (g/kg DM)	78.6 ± 6.45	153 ± 0.6
NPN (g/kg Sol. CP)	438 ± 32.9	168 ± 46.4
NPN (g/kg DM)	39.68 ± 2.21	N/A
NFC (g/kg DM)	189 ± 20.0	648 ±133.6
IVOMD (%)	83.7 ± 1.02	99.7 ± 0.02
Starch (g/kg DM)	72.1 ± 11.90	487 ± 40.6
EE (g/kg DM)	41.4 ± 1.51	30.2 ± 0.41
Ash (g/kg DM)	128 ± 4.5	80.3 ± 2.59
Ca (g/kg DM)	4.13 ± 0.190	16.7 ± 0.85
P (g/kg DM)	4.46 ± 0.152	4.18 ± 0.151
Ca:P	0.93 ± 0.065	3.87 ± 0.261

[1]S.E.M. = Standard Error of Means; DM = Dry Matter, CP = Crude Protein, Sol. CP = Soluble Crude Protein, ME = Metabolisable Energy (0.84 x GE x IVOMD) (Robinson et al., 2004), a NDF OM = Neutral Detergent Fibre assayed with heat stable amylase, exclusive of residual ash, NDF-N = Neutral Detergent Fibre Nitrogen, ADF = Acid Detergent Fibre, ADF-N = Acid Detergent Fibre Nitrogen, ADL OM = Acid Detergent Lignin, exclusive of residual ash, NPN = Non Protein Nitrogen, NFC = 100-(CP + a NDF OM + EE + Ash); IVOMD = *In Vitro* Organic Matter Digestibility, EE = Ether Extract, Ca = Calcium, P = Phosphor. *ME value as supplied by Nova (see Addendum D; Table D2).

Results of changes in mean ± S.E.M. body weight (BW) (kg) and body condition score (BCS) are presented in Table 4.22 and Table 4.23. The body condition score is in increments of one, starting from a minimum of 1 to a maximum of 5. No differences were observed between treatment means for either the BW or BCS.

Table 4.21 Effect of concentrate feeding strategies on mean ± S.E.M.[1] daily milk yield (kg/cow), daily 4 % fat corrected milk yield (kg/cow) and milk composition of Jersey cows (n = 14) allocated ryegrass *(Lolium multiflorum)* grass pasture

Item	Treatment			S.E.M.	P - value
	RP3:3	RP4:2	RP5:1		
Daily Milk yield (kg/cow)	19.9	20.1	20.3	0.51	0.813
Daily 4% FCM yield (kg/cow)[2]	20.8	20.8	20.6	0.40	0.912
Fat (%)	4.34	4.26	4.11	0.135	0.478
Fat (kg)	0.856	0.851	0.830	0.0204	0.655
Protein (%)	3.53	3.46	3.39	0.060	0.304
Protein (kg)	0.696	0.694	0.686	0.0154	0.892
Lactose (%)	4.60	4.57	4.66	0.033	0.180
MUN (mg/dl)[3]	16.8	17.9	16.9	0.50	0.241
SCC (x 1000/ml)[4]	389	339	153	119.2	0.352

[a,b] Means in the same row with different superscripts differ (P < 0.05); [1] S.E.M. = Standard Error of Means; [2] 4% FCM = 4 % Fat Corrected Milk (0.4 x kg Milk + 15 x kg Milk fat) (NRC, 2001); [3]MUN = Milk Urea Nitrogen; [4] SCC = Somatic Cell Count; [5]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [6]RP4:1 = 4 kg (as is) concentrate fed in morning; 2 kg (as is) concentrate fed in afternoon [7]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon.

Table 4.22 Effect of concentrate feeding strategies on mean ± S.E.M.[1] BW of Jersey cows (n = 14) allocated ryegrass (*Lolium multiflorum*) pasture

Item	RP3:3[2]	RP4:2[3]	RP5:1[4]	S.E.M.	P - value
Start BW[5] (kg)	380	363	364	8.7	0.333
End BW (kg)	390	378	383	10.0	0.708
Change BW (kg)	9.95	14.8	19.2	4.16	0.306

[a,b] Means in the same row with different superscripts differ (P < 0.05); [1]S.E.M. = Standard error of the means; [2]RP3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [3]RP4:1 = 4 kg (as is) concentrate fed in morning; 2 kg (as is) concentrate fed in afternoon [4]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [5]BW = Body Weight.

Table 4.23 Effect of concentrate feeding strategies on mean ± S.E.M.[1] BCS[2] (1 - 5) of Jersey cows (n = 14) allocated ryegrass (*Lolium multiflorum*) pasture

Item	RP3:3[3]	RP4:2[4]	RP5:1[5]	S.E.M.	P - value
Start	2.41	2.54	2.36	0.100	0.443
End	2.39	2.59	2.43	0.104	0.378
Change	-0.0179	0.0536	0.0714	0.0789	0.703

[a,b] Means in the same row with different superscripts differ (P < 0.05); [1]S.E.M. = Standard error of the means; [2]BCS = Body Condition Score; [3]RP3:3 = 3 kg (as is) concentrate fed in morning; 3kg (as is) concentrate fed in afternoon; [4]RP4:1 = 4 kg (as is) concentrate fed in morning; 2 kg (as is) concentrate fed in afternoon [5]RP5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon.

4.4 Discussion

4.4.1 Rumen study

4.4.1.1 Ruminal pH

The maximum daily pH of RP3:3 (6.47) and RP5:1 (6.49) were similar to the maximum daily pH (6.56) reported by Erasmus (2009) for cows grazing annual ryegrass (*Lolium multiflorum*) supplemented with 4 kg (as is) per cow/day of a maize based concentrate. The maximum values were reached ½ hour to an hour prior to concentrate feeding for RP5:1 and RP3:3 respectively. Studies by Cajarville *et al* (2006), Malleson (2008) and Erasmus (2009) reported maximum ruminal pH occurring at similar times relative to concentrate feeding times. The mean daily ruminal pH of both treatments corresponded with the mean pH (6.00) of cows on ryegrass pasture supplemented with 5 kg (as is) corn based supplement (Sayers, 1999). Reis & Combs (2000) reported a higher mean daily ruminal pH (6.72) at a concentrate level of 5 kg (as is) per cow/day. However, the pasture was a mixture of ryegrass, red clover and lucerne and 1 kg less concentrate was allocated than in the current study. Bargo *et al.* (2003) studied 10 different pasture based studies and found that ruminal pH of supplemented (1.1 - 10 kg DM per cow/day) ranged between 5.7 - 6.29. The minimum daily ruminal pH of both treatments were below the critical level of 5.8 (De Veth and Kolver, 2001) and below the minimum (5.86) reported by Cajarville *et al.*, (2006) for cows receiving grain supplement at a level of 6 kg per cow/day. The minimum pH of both treatments occurred 3 ½ hours after the afternoon (16:00) concentrate and pasture allocation. Studies by Williams *et al.* (2005), Cajarville *et al.* (2006) and Erasmus (2009) reported a similar lag between minimum daily pH and time of supplementation. The time (h) during which ruminal pH was below 5.8 was higher than the 80 minutes below pH 5.8 reported by the study

of Erasmus (2009). Since more concentrate was allocated per cow/day than in the study by Erasmus (2009) this might be due to the effect of concentrate level on pH described by Bargo *et al.* (2002b). The diurnal ruminal pH profile (see Figure 4.1) of both treatments followed the same pattern with no treatment differences visible at neither of the two periods of pronounced declining ruminal pH. The two periods of decline (P1 and P2) coincided with the two important grazing bouts indicated by Abrahams *et al.* (2009). The first bout occurred from 6:30 - 11:00 while the longest grazing bout occurred between 16:30 - 21:00. This is in accordance with findings of Rook *et al.* (1994) that reported the main grazing activity to take place before sunset. The longest ruminating action occurred at 21:00 – 01:30 and from 01:30 – 06:00 (Abrahams *et al.* (2009). Chemical composition of pasture during the main grazing bout have been reported to differ from composition during other bouts (Wilkinson *et al.*, 1994; Taweel *et al.*, 2006) as a result of loss of moisture and increased exposure to sunlight resulting in increased levels of products of photosynthesis. Taweel *et al.* (2006) has compared chemical content of pasture in the afternoon (P14) (14:00 pm) with the same pasture in the morning (P7) (07:00 am) under strip grazing conditions. The findings were: a) higher ($P < 0.05$) WSC concentrations (g/kg DM) (P14 = 112 vs. P7 = 89) and b) higher ($P < 0.05$) DM content (g/kg) in the afternoon (P14 = 247 vs. P7 = 185). Neutral detergent fibre content (g/kg DM) was lower ($P < 0.05$) in the afternoon (P14 = 456 vs. P7 = 521. Some of the change in chemical composition may explain the drop in pH observed in the afternoon. Increased WSC concentrations of perennial ryegrass (*Lolium multiflorum*) pasture does not influence ruminal pH (Taweel, 2004) as was argued by Beerepoort *et al.* (1997) nor does it increase DMI (Taweel, 2004). Taweel, (2004) argues that because of a high rate of intake of grass rich in WSC the release of WSC from plant cells may limit rapid fermentation of the sugar. Since the lower concentrate level in RP5:1 (1 kg vs. 3 kg) at 16:00 did not influence ruminal pH, starch levels at the 487 ± 40.6 g/kg DM level (see Table 4.20) does not seem to contribute to the decreasing pH either. The explanation should, therefore lie in reduced levels of saliva and buffer levels from decreased peNDF content. Mertens (1997) recommends a peNDF level of 22% of DMI to maintain a ruminal pH of 6.0.

However, the higher expected intake in pasture NDF between treatments as a result of less substitution of pasture would lead one to expect that the ruminal pH of RP5:1 should be higher in the afternoon, despite decreased levels of NDF content in pasture. This was not the case in the current study, thus eliminating chemical content of pasture as the cause of decreasing pH in the afternoon. Considering all factors *i.e.* diurnal variation in quality of pasture, increased absorbance of some VFA's at low ruminal pH (Dijkstra *et al.,* 1993) and increased grazing activity close to dusk (Abrahams *et al.* (2009) might lead one to believe that the decreased ruminal pH is an evolutionary adaptation to fully exploit increased supply of nutrients.

The results of mean ruminal pH values obtained from manual recordings (Table 4.4) differed from results of the logger recordings (Table 4.2, Table 4.3 and Figure 4.1). Figure 4.1 shows the minimum daily ruminal pH of both treatments being reached at 19:30. The manually recorded pH for RP3:3

show that the minimum value is reached more or less at the same time *i.e*. 20:00. However, for RP5:1 the minimum ruminal pH, according to the manual recording, was reached at 12:00. The value (pH = 5.81) at 12:00 in Table 4.4 does not correspond with the value reported in Table 4.3 (pH = 6.03). Differences (P < 0.05) reported in Table 4.4 do not correspond with results of Table 4.2 and Table 4.3. In the latter two tables no differences were reported. Since the manually recorded pH results are a crude representation of automated recording, and since the aim of the manual recording was to compare pH data with VFA data, results of the manual recording should only be applied as intended.

The differences in Table 4.4 should reflect in the results of the volatile fatty acids since the relationship between ruminal pH and VFA concentration is inversely related (Lana *et al.,* 1998).

4.4.1.2 Volatile fatty acids

Mean (n = 36) acetic acid concentration per 24 hour period of both treatments were in the order reported by Berzaghi *et al*. (1996) (62.4 ± 0.50 mol/100 mol) and Jones – Endsley *et al*. (1997) (63.54 mol/100 mol). In these studies the concentrate had a maize base and the level of allocation was 6.4 kg per cow/day. The values expressed in mmol/L do not compare well with the value reported by Malleson (2008) (81.5 mmol/L) where annual ryegrass (*Lolium multiflorum*) was supplemented with 6 kg (as is) concentrate per cow/day. Erasmus (2009) reported concentrations (mmol/L) of 74.4 and 77.0 for treatment groups receiving 4 kg (as is) concentrate with either annual (*Lolium multiflorum*) or perennial (*Lolium perenne*) ryegrass respectively. The study of Reis and Combs (2000), using a pasture mix (alfalfa, red clover and ryegrass) and 5 kg corn base concentrate supplement reported a similar acetic acid concentration (63.1 mmol/L). The higher (P < 0.05) acetic acid concentration reported for RP3:3 in the current study (see Table 4.5) at 00:00, 8:00, 16:00 and 20:00 correspond with differences (P < 0.05) in total acid concentration (mmol/L) (see Table 4.13). During these times the total VFA concentration (mmol/L) was higher (P < 0.05) for RP3:3. Therefore, it seems that the differences in acetic acid concentration were the result of increases in total volatile acid concentration rather than a shift in the proportions of individual fatty acid concentrations. This is confirmed by the results of acetic:total acid ratios in Table 4.11. No difference was reported in the ratio during 00:00, 8:00, 16:00 and 20:00.

The values for mean propionic acid concentration (mmol/L) reported by Berzaghi *et al*. (1996) (19.1 ± 0.10), Malleson (2008) (22.8) and Erasmus (2009) (20.5) compare well with the mean ± S.E.M. of propionate concentration (mmol/L) for RP3:3 *i.e.* 21.5 ± 1.05. Berzaghi *et al.* (1996) and Jones - Endsley *et al.* (1997) reported concentrations (mol/100 mol) similar to the current study *i.e.* 19.1 ± 0.10 and 21.17. The higher (P < 0.05) propionic acid concentrations at times 00:00, 08:00, 12:00 and 16:00 for RP3:3 (see Table 4.6) can in part be explained in the same manner in which the higher

acetic acid concentration was explained. Since total VFA concentration (mmol/L) was higher ($P < 0.05$) in RP3:3 at time 08:00 and 16:00, one can explain the higher propionate concentration as an effect of the increase in total VFA concentration rather than a shift in the proportion of individual VFA toward propionic acid. This is confirmed by the ratio of propionic acid:total acid. No difference in the ratio was reported at 08:00 and 16:00. At 00:00 the total VFA concentration was higher ($P < 0.05$) for RP3:3 and the propionic acid:total acid ratio was bigger ($P < 0.05$). This indicates that the higher propionate concentration at this time for RP3:3 can be explained both by an increase in total VFA concentration and a proportional shift in individual VFA toward propionate.

Mean ± S.E.M. butyric acid concentration (mmol/L) per 24 hour period for RP3:3 *i.e.* 14.4 ± 0.53 (14.2 ± 0.73 mol/100 mol) were in the same order as reported by Berzaghi *et al.* (1996) (13.5 ± 0.1) and Erasmus (2009) (14.0 mmol/L). The higher ($P < 0.05$) mean concentration reported for RP3:3 at times 8:00 (see Table 4.7) can be explained by a proportional shift of VFA's toward butyric acid. Differences ($P < 0.05$) reported at 12:00 and 16:00 were possibly due to an increase in total VFA concentration since at these times the concentration of total VFA was higher ($P < 0.05$) for RP3:3. The same applies for the higher ($P < 0.05$) concentrations (mmol/L) of iso - butyric (See Table 4.8) and valeric acid (see Table 4.9) reported for RP3:3 at 00:00.

The higher concentration of total VFA for RP3:3 reported in Table 4.13 could have explained differences in ruminal pH in Table 4.4 since ruminal pH and total VFA concentration are inversely related (Lana *et al.*, 1998). However, the only pH difference that coincides with an increased total acid concentration was reported during 16:00. The implication of the higher ($P < 0.05$) acetate, propionate and butyrate acid concentrations reported for RP3:3 should reflect in the milk yield and the milk composition. Higher concentrations of acetic acid are associated with higher fat content in milk (Thomas & Martin, 1988; Ishler *et al.*, 1996 and Taweel, 2004). Higher concentrations of propionate are in turn associated with increases in protein content of milk (Thomas & Martin, 1988; Taweel, 2004). This is explained by the increase in glucose concentration, resulting from metabolism of propionate, meaning that less plasma amino acids are oxidised and more is directed to protein production in the mammary gland. Ishler *et al.* (1996) reported increases in lactose concentration in milk with increases in propionate concentration in ruminal fluid.

4.4.1.3 In sacco fibre degradation

The soluble degradable DM fraction (a-fraction) of pasture represents readily available energy for micro-organisms (McNabb *et al.,* 1996). The data presented in Table 4.14 shows a high a-fraction for both treatments *i.e.* RP3:3 = 47.3% and RP5:1 = 45.5%. These values are higher than values reported for *in sacco* studies with perennial ryegrass (*Lolium perenne*) (Chaves, 2003 (42%) and annual ryegrass (*Lolium multiflorum* - cv Energa) (Lehmann *et al.,* 2007) (43.1%) (Lingnau, 2011)

(32%) and can be attributed to different methodologies used in sample preparation. Similar values have been reported by Hoffman *et al*. (1993) (46.7%) for perennial ryegrass (*Lolium perenne*).

Van der Merwe *et al*. (2005) reported differences ($P < 0.05$) in A_{DM} fractions between treatments of different substrate preparation methodologies. This is confirmed by different values reported for bromegrass (*Bromus inermis*) and orchardgrass (*Dactylis glomerata*) that was cut in 1 cm pieces (Bargo, 2000b) (a = 8.7%) instead of dried and milled (Hoffman *et al.*1993) (a = 36.3%). The insoluble fermentable DM fractions (B_{DM} -fraction) (see Table 4.14) were lower compared to values reported by Lehmann *et al*. (2007) (51.4%) and Lingnau (2011) (56.3%) but compare well with values reported for perennial ryegrass (*Lolium perenne*) (Hoffman *et al*., 1993) (45.1%). The rate at which the b-fraction is degraded, represented by the c-value (see Table 4.14) is close to the 0.05 value reported by Lehmann *et al*., 2007. Lingnau (2011) reported a c-value of 0.080. The differences reported between studies might be explained by differences in plant maturity since the c-value decreases with advanced plant maturity (Chaves, 2003). The high a-fraction means that a high proportion total DM consisted of WSC and/or soluble degradable NDF. Interpreting kinetic parameters of DM disappearance for the purpose of evaluating fibre degradation is of limited value since the a-fraction does not distinguish between WSC and soluble degradable NDF. This necessitates the interpretation of DM degradation data along with NDF degradation data. The percentage DM disappearance for the two different treatment groups per 72 hour period is illustrated in Figure 4.5. The lag period (L) for both treatments (see Table 4.14) is small to the extent that it does not appear in Figure 4.5. In the study of Lingnau (2011) no lag period appears in graphical illustrations either. The non - linear curve in the current study did not reach a plateau at the 60 hour mark like the typical *in sacco* degradation curve for forage DM illustrated in the study by Chaves (2003) and the graph illustrated by Lingnau, 2011.

The a-fraction of NDF (see Table 4.15) was small compared to the value (15%) reported by Hoffman *et al*. (1993) for perennial ryegrass (*Lolium perenne*) at boot stage. Chaves (2003) reported an a - fraction of 22% in perennial ryegrass (*Lolium perenne*) at 49 days regrowth level while Lehmann *et al*. (2007) reported a value of 17.2% for annual ryegrass (*Lolium multiflorum*). The value does, however compare favourably with the value (7.41%) reported by Lingnau (2011). The a - fraction would be expected to approach 0, because NDF is not water soluble. The b - fraction value was similar to values reported by Lehmann *et al*. (2007) (77.3%) and Lingnau (2011) (76.0%) while the c - value was similar to the value (0.050) reported by Lehmann *et al*. (2007). Lingnau, (2011) reported a slightly higher c-value *viz*. 0.080. The percentage NDF disappearance per 24 hour period is illustrated in Figure 4.6. Similar to the DM disappearance graph (Figure 4.5), no lag period was visible.

The effective degradability of NDF (see Table 4.16) was similar to the value (51.55%) calculated from kinetic parameters reported by Lehmann *et al.* (2007) for annual ryegrass (*Lolium multiflorum*) supplemented with 7.2 kg concentrate with a barley base. Calculating the ED of NDF using kinetic parameters of Lingnau (2011) for annual ryegrass (*Lolium multiflorum*) and a rumen outflow rate of 5 %/h resulted in a slightly higher ED of 54.18. The fact that no differences were observed between treatments for any of the kinetic parameters for DM nor NDF and for ED of NDF can be explained by the fact that ruminal pH did not drop below the critical point *i.e.* 5.8 (De Veth & Kolver, 2001) long enough to affect the fibre digestion.

4.4.2 Production study

4.4.2.1 Feed intake and feed composition

The pasture dry matter intake (PDMI) for August and October (see Table 4.17) was low compared to the mean PDMI (7.6 kg DM per cow/day) for annual ryegrass (*Lolium multiflorum*) reported by Malleson (2008). However, the regression equation that was applied in the latter study had a low R^2 value (0.4). Lingnau (2011) reported a mean PDMI (kg per cow/day) of 6.5 kg that is similar to the intake value for October in the current study. Table 4.18 shows the mean RPM readings for the trial period. The post - graze values for the three months were below the desired 10 - 12 RPM reading proposed by Fulkerson *et al.* (1998). However, high levels of utilization of pasture and maintaining ideal post graze RPM readings are challenging and not always achievable. The negative values of post - graze mean yield (t DM/ha) in Table 4.19 could either mean that the pasture allowance was not adequate or it may be that the regression equation was not accurate in estimating post - graze yield. Reeves *et al.* (1996), for instance, proposed using different regression equations for estimating post and pre - graze yield. The results of the weight change and changes in BCS (see Table 4.22 and Table 4.23) indicate that the regression equation probably underpredicted post - graze yield.

The ME value of concentrate, determined by the formula (ME = 0.84 x GE x IVOMD) of Robinson *et al.* (2004), was considered unreliable. The ME value supplied by the feed manufacturer *i.e.* 12.98 MJ/kg, was used instead. The total ME required and total ME supplied by the feed for the different months were calculated according to AFRC (1993) guidelines (see Addendum E). The difference between ME intake and ME requirement *i.e.* the ME balance is shown in Table E4. During the three month period a positive ME balance was maintained during September and October. Rumen degradable protein (RDP) and rumen undegradable protein (RUP) values of the pasture were determined according to formulae supplied by NRC (2001) (see Addendum F). Table F1 displays the RDP and RUP balance for the months August to October. A negative RDP balance occurred in August. However, the RDP and RUP requirement is based on a cow with BW (kg) 454 kg and DMI of 16.5 kg. In the current study the mean mass of cows was 376.33 kg and total DMI was < 16.5 kg.

The nutrient composition of the pasture (see Table 4.20), was in agreement with other studies involving annual ryegrass (*Lolium multiflorum*) and showed both similarities and differences. Dry matter content (g/kg as is) was similar to the value of Erasmus (2009) (120 g/kg as is) but slightly lower than values reported by Meeske *et al*. (2006) (140 g/kg as is), Malleson (2008) (137.3 g/kg as is) and Lingnau (2011) (147 g/kg as is). The CP content (g/kg DM) was higher than values reported by Meeske *et al*. (2006) (251 g/kg DM), Malleson (2008) (265 g/kg DM) and Lingnau (2011) (259 g/kg DM). This may be because pasture was probably at an earlier stage of maturity. As grass mature, CP content decreases (Van Vuuren *et al.,* 1991) The ME (MJ/kg DM) was similar to that of Lingnau (2011) (11.37 MJ/kg DM) and Meeske *et al.* (2006) (10.8 MJ/kg DM). The aNDFom content was below 500 g/kg DM, indicating that the ryegrass was of high quality (Clark & Kanneganti, 1998). Malleson (2008) reported a NDF value of 452 g/kg DM. The ADF value (g/kg DM) was much higher than values reported by Meeske *et al*. (2006) (241 g/kg DM). This means that hemicellulose contributed to a lesser extent to the NDF content in the current study. The IVOMD (g/kg DM) was high; confirming that the pasture was of a high quality. Meeske *et al*. (2006) reported a similar IVOMD (g/kg DM) value (802 g/kg DM). Calcium (g/kg DM) and P (g/kg DM) levels were similar to levels reported by Erasmus (2009) *i.e.* 4.1 g/kg DM and 4.2 g/kg DM, respectively. The Ca:P ratio was smaller than the 2:1 ratio recommended by McDonald *et al*.(2002). Since the Ca:P ratio in the concentrate was 3.87:1, the ratio of total Ca:P would be adequate.

4.4.2.2 Milk yield and composition

Volatile fatty acids contribute approximately 70% of the energy needs of ruminants (Bergman, 1990). The increased VFA concentration observed for RP3:3 may, therefore manifest in increased milk production and/or BCS, depending on VFA absorption. However, no increases in milk production were observed in the current study. Since treatments did not affect the ruminal pH profile and did not influence *in sacco* degradation of fibre a response in milk yield is unlikely to have occurred.

Milk fat percentage and protein percentage was similar than values reported by Meeske *et al*. (2006) for grazing cows supplemented with 6 kg concentrate/day. The values were higher than the range reported for milk fat (3.29 % - 3.82%) and protein (2.93% - 3.11%) by Bargo *et al*. (2002a) using Holstein cows. Differences between the two breeds might have resulted in different milk fat and protein percentages. An increase in milk fat was expected for RP3:3 since a higher ruminal acetic acid concentration was found for this treatment. However, since a proportional increase in ruminal propionic acid was also observed, the effect of higher acetic acid concentration might have been obscured. The somatic cell count of RP3:3 and RP4:2 was high and was above the 300 000/ml considered abnormal and indicative of subclinical mastitis (De Villiers *et al.,* 2000). Lactose concentrations (see Table 4.21) were similar to the range of values reported by Lingnau (2011) *viz.*

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4.59 - 4.71. The MUN values were higher that the 10 mg/dl reported for diets deficient of protein but below the range (18 - 20 mg/dl) reported for diets having excessive protein concentrations (Jonker *et al*., 1999). The fact that treatments did not differ in terms of milk yield and milk composition in the production study confirms the general lack in response in the rumen study.

4.4.2.3 Body weight and body condition score

The body weight of all the treatment animals increased, confirming the finding that the energy balance was positive (see Table 4.22). Body condition score (scale 1 - 5) (see Table 4.23) was slightly less for RP3:3 at the end of the trial, but since it is a subjective assessment, using BCS as sole assessment is risky.

4.5 Conclusion

Findings from this study lead to the conclusion that the null hypothesis should be rejected. In theory ruminal parameters, especially ruminal pH can be manipulated by concentrate feeding strategies since decreases in ruminal pH are often associated with increased starch intake. Feeding less starch close to the point of minimum daily ruminal pH while still maintaining the same total level of concentrate intake was anticipated to increase the ruminal pH and decrease diurnal fluctuation in ruminal pH. A more stable ruminal environment in terms of ruminal pH should have been reflected in increased levels of fibre degradation which in turn should have influenced milk yield and milk composition as well as BCS and BW. In practice the feeding strategies did not result in any of the expected results. The ruminal pH did not reach levels below pH 5.8 for sufficient periods of time to influence fibre digestion. The reason as to the zero influence treatments had on ruminal pH can only be speculated upon until further research might explain the highly complicated nature of the rumen.

4.6 References

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CHAPTER 5

The effect of concentrate feeding strategies on ruminal pH, *in sacco* fibre degradation and milk production of Jersey cows grazing kikuyu (*Pennisetum clandestinum*) pasture

Abstract

Dairy producers of the Southern Cape region in South Africa that produce from kikuyu (*Pennisetum clandestinum*) based pastures use supplements) to compensate for the low nutritive value of kikuyu and low pasture dry matter intake (PDMI) limiting milk production . A research project was conducted to study the effect of concentrate feeding strategies on the ruminal pH and concomitant effects on fibre degradation, milk yield and milk composition of Jersey cows grazing kikuyu pasture. The project consisted of two studies *i.e.* 1) a rumen study; to determine the effect of treatments on ruminal pH and *in sacco* degradation of NDF and DM and 2) a production study; quantifying the effect of treatments on milk yield and milk composition. Treatments were:

1) Concentrate fed: 5 kg during morning; 1 kg during afternoon (PKT5:1)
2) Concentrate fed: 4 kg during morning; 2 kg during afternoon (PKT4:2)
3) Concentrate fed: 3 kg during morning; 3 kg during afternoon (PKT3:3)

No differences were found in either daily maximum, minimum or mean ruminal pH between treatment means. Time (h) spent below the critical ruminal pH *i.e.* 5.8 (de Veth and Kolver, 2001a) was of a long duration (7.1 hours) but did not differ between treatments. No differences were observed between treatment means for *in sacco* NDF and DM degradation. Mean milk yield (kg per cow/day) was not affected by the treatments. Except for milk fat % that was higher ($P < 0.05$) for PKT3:3 compared to PKT5:1 treatment means did not differ for other milk composition parameters. The lack of response in the production study can be explained by the lack of response observed in the ruminal pH. The latter can, however not be readily explained and emphasize the complex nature of the rumen environment.

5.1 Introduction

Kikuyu (*Pennisetum clandestinum*) grass is well adapted to the main milk producing areas of the Southern Cape (Botha *et al.,* 2008) and the Natal Midlands of South Africa where it is used as summer grazing (Marais, 1990). However, quality seems to be the primary limitation to animal production from pastures based on kikuyu grass (Fulkerson *et al.*, 1998 despite high annual DM yield potential (28.2 t per ha/year) (Gherbin *et al.*,2007). Kikuyu has low nutritive qualities (Reeves, 1997); it contains nitrogenous compounds in excess of animal requirements, has low levels of readily digestible non - structural carbohydrates, contains anti-nutritional compounds such as oxalic acids, is deficient in sodium and is prone to mineral imbalances (Marais, 2001). Other than nutrient deficiencies pasture dry matter intake (PDMI) is generally low, resulting in lower milk yield (Kolver & Muller, 1998). For these reasons cows receiving a predominantly kikuyu grass diet need to be supplemented (Botha *et al.*, 2008). Be it as it may, concentrate may not be fed without constraint to achieve better total dry matter intake and milk yield since high levels (> 10 kg) of concentrate may be detrimental to the metabolic health of the cow (Bargo *et al.*, 2003). Apart from this, the marginal milk response (MR) of concentrate has been described to have a curvilinear nature (Kellaway & Porta, 1993) with MR decreasing as concentrate DM intake increases (St - Pierre, 2001). Milk producers using pasture based systems are thus limited by the extent with which milk yield can be improved using increased levels of concentrate. Alternatives are to improve the genetic potential of pasture species and/or to improve the utilization of current pasture species. The latter entails creating a rumen environment that would ensure optimal proliferation and function of rumen micro - organisms thereby increasing fibre digestion. Since rumen pH is the rumen condition that varies the most (Dehority, 2003) rumen pH represents the area of research where much can be attained to create the ideal rumen environment. Time after feeding, nature of the feed and frequency of feeding may result in fluctuations in ruminal pH (Dehority, 2003). The ruminal pH profile of cows on pasture generally has ruminal pH profiles containing two peaks.

Malleson (2008) reported pH values of cows grazing kikuyu, supplemented with 5.5 kg (DM basis) of concentrate. The pH profile, measured over a 24 hour period, showed two rapid declines that coincided with access to concentrate during milking (6:00 and 14:30) and access to fresh herbage following milking. The declines followed immediately after the peak periods. The most pronounced decline occurred during the morning period. Minimum pH levels were reached during the second declining period in the afternoon. The profile behaviour of this study can in part be explained by concentrate allocation and grazing behaviour. Abrahamse *et al.* (2009) showed that the main grazing bout is between 16:30 and 21:00. The second most aggressive bout occurred from 6:00 to 11:00. Since various authors (Van Vuuren *et al.*, 1986; Orr *et al.,* 2001 and Delegarde *et al.,* 2000) have reported higher concentrations of water soluble carbohydrates (WSC) in pastures in the afternoon, ruminal pH might decline in the afternoon partially as a result of the increased intake of WSC.

Pronounced diurnal fluctuations in ruminal pH can be detrimental to rumen microbes since it may necessitate continuous metabolic readjustments (Mertens, 1979).

The current study aimed to address the fluctuation in ruminal pH below the critical value (pH 5.8) (De Veth & Kolver, 2001a) occurring during the afternoon period. The hypothesis was that the fluctuation in ruminal pH during the afternoon (± 4:00 pm) would be less by increasing the ratio of morning to afternoon concentrate allocation during milking. In order to test the hypothesis two studies *i.e.* a rumen study and a production study were executed. The rumen study evaluated changes in rumen pH and changes in neutral detergent fibre (NDF) degradation *in sacco*. The production study determined mean milk yield and milk composition.

5.2 Materials and Methods

5.2.1 General information

The research trial was conducted at the Outeniqua Research Farm (latitude: 33° 58' 41.98" S longitude: 22°25'14.48'' E, altitude: 190 m) in George in the Western Cape province of South Africa from late summer (February) to early autumn (April) of 2008. The experimental plot (8.5 ha) consisted of an Estcourt soil type (see Addendum B, Table B2) (Soil Classification Working Group, 1991) and an established kikuyu (*Pennisetum clandestinum*) grass pasture irrigated by means of overhead sprinklers. The average monthly rainfall during the trial was 55 mm and temperature (°C) was moderate ranging from a mean daily minimum of 13.6°C to a mean daily maximum of 23.5°C (see Addendum A for monthly temperature and rainfall data) (Erasmus, 2009).

5.2.2 Experimental design and treatments

A randomized block design was used for both studies. Cows were blocked according to days in milk (DIM), parity and average milk production measured twenty-two days prior to the trial and randomly allocated to treatments. Treatments consisted of the morning concentrate allocation being fed in different weight ratios (as is basis) to the afternoon allocation. The two treatments applied in the rumen study were:

1) Concentrate fed: 5 kg during morning; 1 kg during afternoon (RKT5:1)

2) Concentrate fed: 3 kg during morning; 3 kg during afternoon (RKT3:3)

The three treatments applied in the production study were:

1) Concentrate fed: 5 kg during morning; 1 kg during afternoon (PKT5:1)

2) Concentrate fed: 4 kg during morning; 2 kg during afternoon (PKT4:2)

3) Concentrate fed: 3 kg during morning; 3 kg during afternoon (PKT3:3)

Concentrate was fed during milking for both studies.

5.2.3 Animal management

Six rumen cannulated cows per treatment were used in the rumen study. The cows were blocked according to days in milk (DIM), parity and average milk production measured twenty-two days prior to the trial and randomly allocated to treatments. For practical purposes, six intact cows were used as filler cows to enable three groups of six animals each. Cows in each of the six strata were randomly allocated to treatments.

Forty - two high producing Jersey cows (milk yield (kg per cow/day); 19.36 ± 0.30 S.E.M., days in milk (DIM): 83.57 ± 5.52 S.E.M., lactation number: 3.3 ± 0.20 S.E.M.) were used in the milk production study. Cows were blocked and randomly allocated to treatments (see Addendum C, Table C2 for the blocking and allocation of cows to treatments). Management of cows were similar to that explained in section 4.2.3. The average walking distance from pasture to milking parlour was 0.9 km (0.55 ± 1.18 km). Milking times and concentrate level (kg per cow/day) were similar to that in section 4.2.3 (see Addendum D, Table D3 and Table D4 for chemical and nutrient composition of the concentrate). Cows were kept in the parlour until all the concentrate offered had been consumed. A steel bailing system prevented cows from consuming concentrate in adjacent feed cribs. Feeders were tested on two occasions to ensure that the correct mass of concentrate was fed.

5.2.4 Automated rumen pH data collection

Rumen pH was recorded by means of an automated recording apparatus. The apparatus consisted of a M-12 pT100 pH probe (Figure 5.1) (Endress + Hauser Conducta Inc. Anheim, California, USA) and a pH - HR logger (Intech Instruments Ltd. Riccarton, Christchurch, New Zealand). The logger was protected in a stainless steel casing. The apparatus was put in a tight fitting transparent rubber hose (20 mm diameter). The pH sensitive membrane and pT100 temperature sensor of the probe protruded from the one open end of the hose (Plate 5.1). The other open end protruded through the

cannula plug and was kept in position with two plastic clamps. The probe was calibrated in accordance with the instructions manual of the manufacturer. Data logging was done in two runs (13 to 15 and 20 to 22 February) using six loggers per treatment per run. Different loggers and probes were paired with different cows in the two runs. Temperature and rumen pH data were logged every 10 minutes to obtain an average rumen pH for every half hour. Temperature data was used to verify the reliability of the probe and to dispose of suspect values. Data was downloaded after each run using Omnilog software version 1.6 (Intech Instruments Ltd. Riccarton, Christchurch, New Zealand). Probes were re - calibrated after each run.

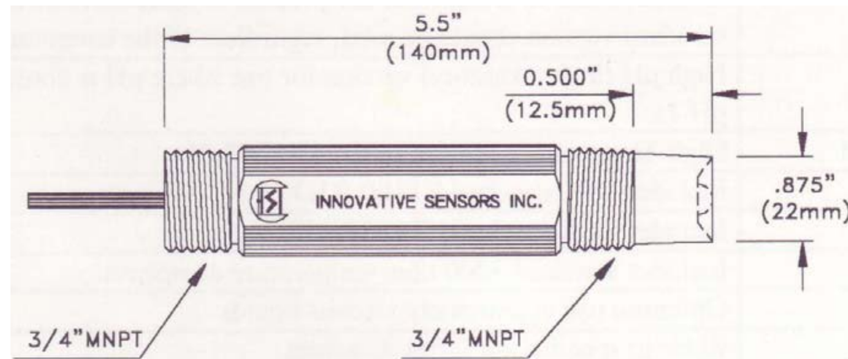

Figure 5.1 Diagram of M-12 pT100 pH probe (Intech Instruments Ltd. Riccarton, Christchurch, New Zealand).

Plate 5.1 Data logger, pH probe and other components of the ruminal pH measuring apparatus.

5.2.5 In sacco digestibility

The grass sample used in the *in sacco* trial was selected at the experimental plot from a 30 m^2 area during the experimental period and was carefully screened by eye to ensure minimum contamination with faecal and foreign matter. Grass height was measured with a rising plate meter (RPM) before manual cutting of the sample at a height of 5 cm above ground. The sample was put in paper bags and dried (72 hours at 60°C) in a conventional drying oven. The dried plant material was cut to 5 cm pieces using a guillotine type paper cutter. Five grams (as is) of the sample was placed in a large Dacron bag (10 x 20 cm; 53 ± 10micron) (Bar Diamond Inc. Parma, Idaho, USA) and marked with laundry marker. A cable tie (10 cm) was used 4 cm from the open end to close the bag. Six empty bags and substrate samples were dried (72 hours at 60°C) to account for moisture accumulation during processing.

The retrieval method of Cruywagen (2006) was used to retrieve bags from the rumen. Two components were used *viz*. an accommodation vessel and a receptacle. The accommodation vessel was an opaque nylon stocking (44 decitex yarn and 17 decitex lycra) (Burhose, Brackenfell Industria, Cape Town, South Africa) capable of carrying up to six Dacron bags with a glass marble (33 mm diameter) tied at the bottom. The accommodation vessel was filled with Dacron bags placed in tandem position, starting from the bottom of the accommodation vessel. A tight knot was tied between adjacent Dacron bags.

The receptacle consisted of a shorter stocking, called a catcher. The catcher had a loop on the end where the accommodation vessel was attached. The other end of the catcher was tied to the cannula plug. Dacron bags were inserted in the rumens at different times and removed simultaneously. Incubation times were 18 and 30 hours. Four bags per cow were used for each time to ensure adequate residue for analysis and six bags containing grass sample was used as time "zero" bags to determine the lag phase. All bags were machine washed, 5 times at 1 min/rinse (NRC, 2001) and frozen at -16 °C in a box freezer. Prior to analysis the bags were thawed, dried (72 hours at 60 °C) and weighed to determine DM disappearance. The residue of each bag was milled using a feedmill (Scientific Engineering (Pty) Ltd. Johannesburg, South Africa) with 2 mm sieve to ensure a homogenised sample. Residues were sieved using a Retsch AS 200 (Retsch GmbH, Rheinische Straße 36, 42781, Haan, Germany) sieve shaker with a Kingtest sieve with 106µm aperture and placed in air tight plastic containers.

5.2.6 Analytical methods applied to in sacco residue

Dacron bags were weighed following the drying process mentioned in section 5.2.5. Residues were carefully retrieved and placed in air tight plastic containers. Neutral detergent fibre (NDF) of *in sacco* plant sample and residue was determined with an Ankom 220 Fibre analyser (Ankom technologies, Fairport, NY, USA), applying the method of Ankom Technology (Ankom, 2006b); a modification of the method of Van Soest *et al*. (1991).

5.2.7 Analytical methods applied to feed samples

Analytical methods followed to analyse feed samples were the same as the methods applied in section 4.2.9.

5.2.8 Statistical analysis

The statistical analysis and model were the same as in section 4.2.10.

5.3 Results

5.3.1 Rumen study

5.3.1.1 Ruminal pH

Results of the rumen study are presented in Table 5.1 and Figure 5.2. No differences were reported between treatment means for minimum, maximum and mean daily pH as well as for time that ruminal pH spent below pH 5.8. The ruminal pH profiles of the two treatment groups (n = 6) are illustrated in Figure 5.1. A two peak profile, following similar trends with pronounced declines close to the milking times, was observed for both treatments and compared well with the pH profile of the control used by Malleson (2008). The first period of pronounced decline (P1) for the RKT5:1 treatment occurred from 5:00 am to 11:00 am. Ruminal pH decreased from a high of 6.33 to a low of 5.80. Similar to RKT5:1, ruminal pH of RKT3:3 started dropping at 5:00 am but the lower level of the period was reached at 8:30 am. The ruminal pH of RTK3:3 decreased from a high of 6.21 to a low of 5.81. The second period (P2) started at 4:00 pm from pH 5.97 for both treatments. The minimum daily pH of both treatments was reached at different times *i.e.* 7:00 pm (RKT3:3) and 8:00 pm (RKT5:1).

5.3.1.2 In sacco fibre degradation

Table 5.2 displays the results of the *in sacco* trial for dry matter (DM) and NDF disappearance. No differences were observed between the treatments for DM degradation at 18 and 30 hours. Similarly, no differences were observed for NDF degradation at 18 and 30 hours.

Table 5.1 The ruminal pH of lactating Jersey cows (n = 6) grazing kikuyu (*Pennisetum clandestinum)* pasture supplemented with 5.5 kg (DM basis) of maize based concentrate per cow per day

Item	RKT3:3[1]	RKT5:1[2]	S.E.M.[3]	P - value
Min. pH	5.60	5.72	0.09	0.373
Max. pH	6.29	6.35	0.076	0.596
Mean pH	5.97	5.92	0.078	0.627
Time (h) < 5.8	6.33	7.92	2.688	0.686

[1]RKT3:3 = 3 kg (as is) concentrate fed at 06:00; 3 kg (as is) concentrate fed at 16:00; [2]RKT5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; P < 0.05 was considered significant.

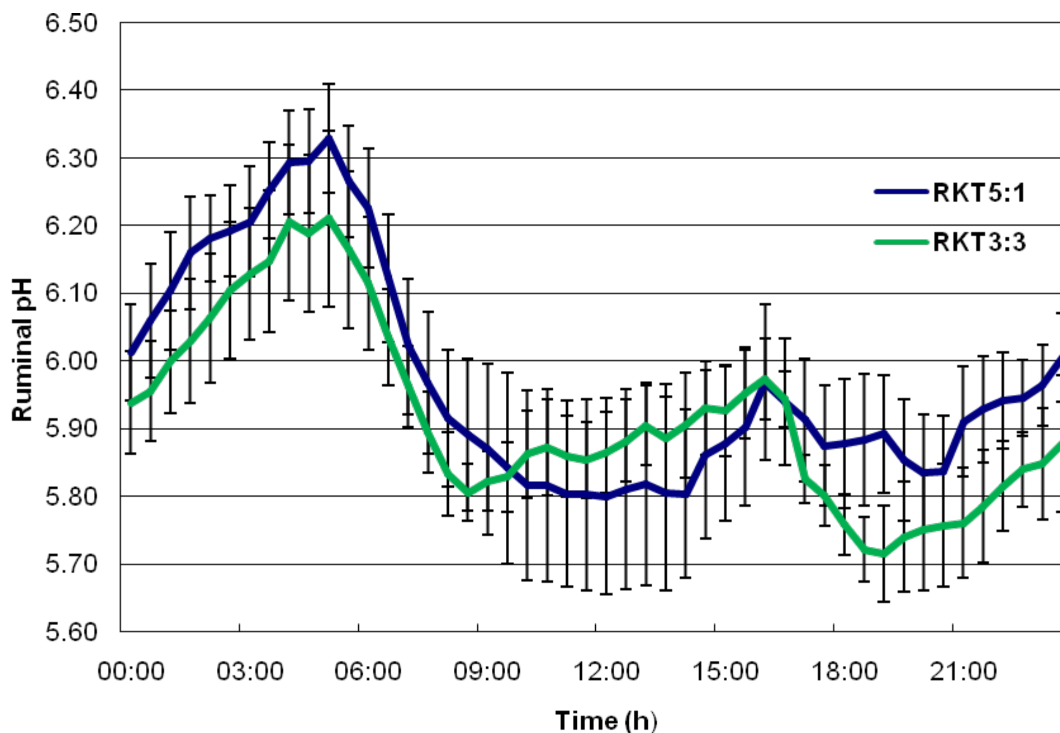

Figure 5.2 Ruminal pH profile (diurnal basis; continuous data logging) with S.E.M. bars of Jersey cows (n = 6) grazing kikuyu (*Pennisetum clandestinum*) pasture and receiving 5.5 kg (DM basis) of maize based concentrate per cow per day ([1]RKT3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RKT5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon).

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Table 5.2 Mean percentage DM and NDF degradation *in sacco* of kikuyu (*Pennisetum clandestinum*) grass using Jersey cows (n = 6) grazing kikuyu supplemented with 5.5 kg (DM basis) of maize based concentrate per cow / day

Treatment	RKT3:3[1]	RKT5:1[2]	S.E.M.[3]	P - value
DM				
18 hours	34.90	37.30	0.923	0.126
30 hours	38.58	40.76	1.357	0.308
NDF				
18 hours	26.62	27.92	1.438	0.552
30 hours	34.90	37.30	0.923	0.126

[1]RKT3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [2]RKT5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [3]S.E.M. = Standard Error of the Means; P < 0.05 was considered significant.

5.3.2 Production study

Mean pasture allocation and intake (kg DM per cow/day) for January until April is presented in Table 5.3. Intake and allocation was calculated using the regression equation given in section 3.3.

Table 5.3 Mean ± S.E.M.[1] (kg DM per cow/day) estimated allocation and intake of kikuyu (*Pennisetum clandestinum*) pasture from January 2008 to March 2008

Date	Mean ± S.E.M.[1] Allocation	Mean ± S.E.M. Intake
Jan.	9.85 ± 0.523	7.07 ± 0.457
Feb.	8.66 ±0.300	5.59 ± 0.283
March	9.06 ± 0.241	6.04 ± 0.213

[1]S.E.M. = Standard Error of the Means.

Information on pasture management is presented in Table 5.4 and Table 5.5. Rising plate meter readings are reported in Table 5.4 for the pre - graze and post - graze periods. The mean yield (t DM/ha) are reported in Table 5.5 for the months January until March. Figure 5.3 provides a graphic illustration of estimated pasture allocation and intake.

Table 5.4 Mean pre and post - graze RPM[1] readings of kikuyu (*Pennisetum clandestinum*) grass pasture from January 2008 to March 2008

Month	n	Mean RPM[1] reading			
		Pre - Graze	S.E.M.[2]	Post - Graze	S.E.M.
Jan.	12	37.47	1.835	12.90	0.579
Feb.	33	33.27	1.054	13.54	0.397
March	35	34.69	0.844	13.52	0.280

[1]RPM = Rising Plate Meter; [2]SEM = Standard Error of the Means.

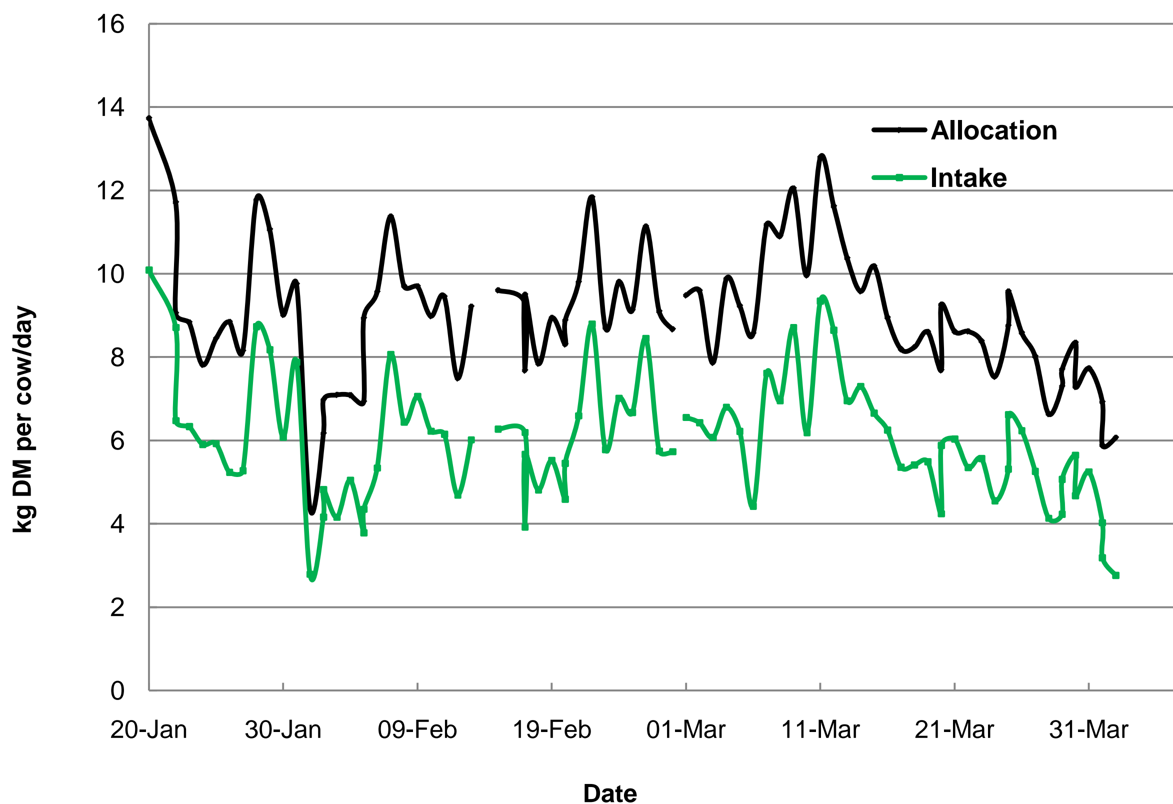

Figure 5.3 Pasture allocation (kg DM per cow/day) and intake (kg DM per cow/day) of Jersey cows grazing kikuyu (*Pennisetum clandestinum*) pasture supplemented with 5.5 kg (DM basis) per cow/day of maize based concentrate pellets.

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Table 5.5 Mean pre - graze and post - graze pasture yield (t DM/ha) of kikuyu (*Pennisetum clandestinum*) pasture allocated from January 2008 to March 2008

Month	n	Mean yield (t DM/ha)			
		Pre-Graze	S.E.M.[1]	Post-Graze	S.E.M.
Jan.	12	2.63	0.14	0.76	0.044
Feb.	33	2.35	0.07	0.81	0.031
March	25	2.42	0.41	0.81	0.021

[1]S.E.M. = Standard Error of the Means.

Table 5.6 Mean ± S.E.M.[1] nutrient composition of kikuyu *(Pennisetum clandestinum)* pastures (n = 8) and maize based concentrate pellets. (n = 8)

Nutrient	Pasture	Concentrate
	Mean ± S.E.M.	Mean ± S.E.M.
DM (g/kg AS IS)	161.22 ± 3.392	924.65 ± 2.692
CP (g/kg DM)	204.51 ± 4.18	127.35 ± 5.264
Sol. CP (g/kg CP)	686.94 ± 22.73	725.12 ± 45.30
ME (MJ/kg DM)	9.03 ± 0.184	12.6*
a NDF OM (g/kg DM)	697.28 ± 9.887	118.09 ± 5.485
NDF-N (g/kg NDF)	5.57 ± 0.279	9.92 ± 0.793
ADF (g/kg DM)	307.17 ± 4.573	62.76 ± 3.673
ADF-N (g/kg ADF)	5.48 ± 0.272	N/A
ADL OM (g/kg DM)	45.98 ± 3.601	19.97 ± 1.222
NPN (g/kg Sol. CP)	360.56 ± 45.32	140.14 ± 18.00
IVOMD (%)	64.54 ± 1.134	93.54 ± 0.159
Starch (g/kg DM)	60.80 ± 5.583	369.68 ± 9.882
EE (g/kg DM)	21.55 ± 0.915	38.15 ± 2.016
Ash (g/kg DM)	105.22 ± 3.892	102.36 ± 6.276
Ca (g/kg DM)	3.86 ± 0.126	26.44 ± 2.217
P (g/kg DM)	4.45 ± 0.155	3.62 ± 0.215
Ca:P	0.88 ± 0.05	7.63 ± 1.010

[1]S.E.M. = Standard Error of Means DM = Dry Matter, CP = Crude Protein, Sol. CP = Soluble Crude Protein, ME = Metabolisable Energy (0.84 x GE x IVOMD) (Robinson *et al.*, 2004), a NDF OM = Neutral Detergent Fibre assayed with heat stable amylase, exclusive of residual ash, NDF-N = Neutral Detergent Fibre Nitrogen, ADF = Acid Detergent Fibre, ADF-N = Acid Detergent Fibre Nitrogen, ADL OM = Acid Detergent Lignin, exclusive of residual ash, NPN = Non Protein Nitrogen; IVOMD = *In Vitro* Organic Matter Digestibility, EE = Ether Extract, Ca = Calcium, P = Phosphor, *ME value supplied by Nova (see Addendum D, Table D4).

The nutrient composition of kikuyu pasture and concentrate pellets are provided in Table 5.6. All values, except for dry matter (DM), are reported on DM basis.

Mean milk yield (kg per cow/day) and mean 4 % fat corrected milk (4 % FCM) yield (kg per cow/day) are reported in Table 5.7. No differences were observed between treatment means of either mean milk yield or 4 % FCM. Results of the effect of treatments on mean milk composition are presented in Table 5.8. Milk of PKT 3:3 had a higher ($P < 0.05$) percentage milk fat and milk fat yield (kg) compared to PKT5:1. No differences were observed for protein %, total protein (kg), lactose, milk urea nitrogen (MUN) or somatic cell count (SCC).

Table 5.7 Effect of concentrate feeding strategies on mean ± S.E.M.[1] daily milk yield and composition of Jersey cows (n = 14) allocated kikuyu *(Pennisetum clandestinum)* grass pasture receiving 5.5 kg (DM basis) of maize based concentrate

Parameter	PKT5:1[1]	PKT4:2[2]	PKT3:3[3]	S.E.M.[4]	P - value
Daily Milk yield (kg/cow)	18.45	17.97	18.42	0.334	0.530
Daily FCM[5] yield (kg/cow)	18.30	18.25	19.45	0.542	0.224
Fat (%)	3.96[a]	4.17[ab]	4.45[b]	0.164	0.0136
Fat (kg)	0.728[a]	0.744[ab]	0.808[b]	0.0265	0.0095
Protein (%)	3.22	3.18	3.32	0.095	0.568
Protein (kg)	0.591	0.568	0.605	0.0173	0.310
Lactose (%)	4.62	4.55	4.60	0.034	0.416
MUN (mg/dl)[6]	12.19	12.76	12.45	0.363	0.545
SCC (x 1000/ml)[7]	222.25	176.89	386.88	77.32	0.150

[a,b] Means in the same row with different superscripts differ ($P < 0.05$); [1]PKT5:1 = 5 kg (as is) concentrate fed in morning; 1 kg (as is) concentrate fed in afternoon; [2] PKT4:2 = 4 kg (as is) concentrate fed in morning; 2 kg (as is) concentrate fed in afternoon [3]PKT3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon; [4]S.E.M. = Standard Error of the Means; $P < 0.05$ was considered significant; [5]FCM = 4% Fat Corrected Milk(0.4 x kg Milk + 15 x kg Milk fat) (NRC, 2001); [6]MUN = Milk Urea Nitrogen; [7]SCC = Somatic Cell Count.

The mean body weight (BW) (kg) and body condition score (BCS) are presented in Table 5.9. The body condition score was determined in increments of one, starting from a minimum of 1 to a maximum of 5. No differences were observed between treatment means for either the BW or BCS.

Table 5.8 Effect of concentrate feeding strategies on mean ± S.E.M. BW and BCS of Jersey cows (n = 14) allocated kikuyu (*Pennisetum clandestinum*) pasture

Parameter	PKT5:1[1]	PKT4:2[2]	PKT3:3[3]	S.E.M.[4]	P - value
BW[5]					
Start BW (kg)	386.42[a]	356.55[b]	343.68[b]	7.606	0.002
End BW (kg)	386.04	372.31	365.20	7.685	0.170
Change BW (kg)	-0.37	15.76	21.52	2.179	< 0.0001
BCS[6]					
Start	2.23	2.23	2.21	0.0648	0.927
End	2.20	2.18	2.18	0.0465	0.952
Change	-0.036	-0.054	-0.054	0.0465	0.952

[a,b] Means in the same row with different superscripts differ (P < 0.05); [1]PKT5:1 = 5 kg (as is) concentrate fed in morning; 1kg (as is) concentrate fed in afternoon; [2] PKT4:2 = 4 kg (as is) concentrate fed in morning; 2 kg (as is) concentrate fed in afternoon [3]PKT3:3 = 3 kg (as is) concentrate fed in morning; 3 kg (as is) concentrate fed in afternoon [4]S.E.M = Standard error of the Means; [5]BW = Body Weight; [6]BCS = Body Condition Score**.**

5.4 Discussion

5.4.1 Rumen pH study

Literature on ruminal pH relevant to the kikuyu pastures and level and type of supplement are limited. Minimum, maximum and mean pH for both treatments were numerically lower than the values reported for the corresponding treatments in the ryegrass phase. The minimum daily ruminal pH of both treatments was below the critical level of 5.8 where fibre digestion is impaired (de Veth and Kolver, 2001b) and below the minimum (5.86) reported by Cajarville *et al.* (2006) for cows receiving grain supplement at a level of 6 kg per cow/day. The time (h) that pH was below 5.8 was numerically longer in the current study compared to that in the ryegrass phase in Chapter 4. In the study of Malleson, 2008 ruminal pH of a control group grazing kikuyu and receiving 5.5 kg (DM basis) of concentrate never reached levels below 5.8. The opposite was anticipated relative to what was observed in the ryegrass phase for the minimum daily pH and time spent below pH 5.8 since NDF increases when pastures switch from kikuyu - dominant to kikuyu only (Botha *et al.*, 2008) and since ruminal pH and NDF content is positively related (Kolver and de Veth, 2002).

Following the declining period P1 (Figure 5.2) the ruminal pH of RKT5:1 exhibited a more or less constant pH from 11:00 am to 2:00 pm whilst the pH of RTK3:3 started to ascend immediately from 8:30 am. During the period from 11:00 am to 4:00 pm the line of the curve of RTK3:3 was located above that of RTK5:1. The opposite occurred during P2. During P1 cows receiving RKT5:1 probably consumed less pasture due to substitution. This might have resulted in less intake of NDF and the

onset of the "cascade of events" leading to a decrease in animal performance *i.e.* decreased chewing activity, less salivary buffer secretion and lower ruminal pH (Mertens, 1997). During P2 NDF intake of RKT5:1 should have been higher compared to NDF intake by cows receiving RTK3:3 which would explain the higher ruminal pH in the RTK 5:1 treatment group.

Reasons why treatments did not elicit a significant ($P < 0.05$) response in ruminal pH parameters (mean, min, max etc.) nor a significant ($P < 0.05$) improvement in ruminal pH profile over an extended time period cannot be readily explained and is possibly due to the same factors that prevented a response in the ryegrass (*Lolium multiflorum*) phase in Chapter 4. The effect of total NDF intake on ruminal pH presented as a possible cause of decreases in ruminal pH is also not without flaw since Kolver and de Veth, (2002) concluded that "no single dietary variable or group of variables could be used to reliably predict ruminal pH". Even when the interaction between pasture dry matter intake (PDMI), pasture quality, amount and type of concentrate supplemented were reviewed to quantify the effect on ruminal pH no consistent pattern could be found (Bargo *et al.*, 2003) Factors such as: diurnal variation in quality of pasture, increased absorbance of some volatile fatty acids (VFA's) at low ruminal pH (Dijkstra *et al.,* 1993) and increased grazing activity close to dusk (Abrahams *et al.*,2009) might lead one to believe that the decreased ruminal pH observed during P2 is an evolutionary adaptation to fully exploit increased supply of nutrients.

5.4.2 In sacco fibre degradation

Ruminal pH affects fibre digestion especially at levels below pH 6 where severe inhibition has been reported (Mould *et al.*, 1983/84). The critical pH level for cows on pasture receiving concentrate supplement was determined to be 5.8 (de Veth and Kolver, 2001a). Fibre digestibility and microbial protein synthesis are both inhibited below this level (de Veth and Kolver, 2001a). Decreases in fibre digestion may also occur due to exposure to short periods (4h) of ruminal pH < 5.8 despite a mean ruminal pH being at optimal levels (de Veth and Kolver, 2001b). In the current study the time (h) that ruminal pH was below the 5.8 levels was longer than 4 hours irrespective of treatment. Considering this and the fact that lower quality (low digestibility) roughages like kikuyu are influenced more by a low ruminal pH should signal that there are opportunities to improve digestibility of kikuyu pasture by improving the ruminal pH level. The reason why treatments did not elicit a significant ($P < 0.05$) effect on DM or NDF degradation during the two incubation times could be attributed to the zero response that was found for the ruminal pH.

5.4.3 Production study

5.4.3.1 Pasture management, intake and nutrient composition

Pasture DM allocation was lower than the recommended level of twice the expected PDMI (Bargo *et al*., 2002a) (Table 5.3). This recommendation is, however, made when pasture yield is measured from ground level. In the present study pasture yield was measured above 3 cm. Optimal pasture utilization was reached with mean RPM readings (post – grazing) being close to the proposed reading of 10 – 12 on the RPM as proposed by Fulkerson *et al*. (1998) (Table 5.4). Values of post - graze yield (t DM/ha) (Table 5.5) indicate that cows consumed sufficient amounts of DM to satisfy the feeding drive and rumen fill. Dry matter intake (DMI) can be calculated using the assumption of Bargo *et al*. (2002b) that cows can consume NDF equivalent to 1.3% of BW. Using this assumption and the mean body weight (BW) of 386.36 kg with concentrate dry matter intake (CDMI) values means cows should have been able to consume 6.27 kg pasture DM and 5.02 kg total NDF. Using the NDF values of the feed (Table 5.6) and DMI intake values (Table 5.3) the estimated NDF intake for January, February and March was 5.58 kg, 4.55 kg and 4.86 kg respectively.

The nutrient composition of the pasture compares well to the values cited in literature. The DM content was close to the value (159 ± 44 g/kg as is) reported by Meeske *et al*. (2006) while crude protein (CP) (g/kg DM) was close to values reported by Meeske *et al*. (2006) (196 g/kg DM ± 57.1 S.E.M.) and Botha *et al*. (2008) (237 g/kg DM). The NDF value was slightly high even though it is within the range reported by Marais (2001) *i.e.* 581 -741 kg. When applying the formula NSC (g/kg DM) = 100 – (CP + (NDF – NDFprotein) + EE + Ash) (McDonald *et al*., 2002) and using values in Table 5.6 the result is a negative value indicating an overestimation of one of the five nutrients used in the formula. Miles *et al*. (2000) reported a mean NSC concentration of 38.7 g/kg DM (range 10.4 – 90.8 g/kg DM) for kikuyu in the Eastern Cape. Replacing NDF values with values of Botha *et al*. (2008) gives a NSC concentration of 45.4 g/kg DM and it, therefore, seems likely that NDF was overestimated. The ME value of the concentrate, calculated by the formula of Robinson *et al*. (2004), was considered unreliable and was replaced by the ME value (12.6 MJ /kg) supplied by the feed manufacturer.

5.4.3.2 Milk yield and composition

Mean milk yield of the treatments were 18.28 kg per cow/day and was the same value reported by Malleson (2008) for cows under the same feeding conditions. The higher fat % and fat yield of PKT 3:3 compared to PKT5:1 could not be readily explained since no supporting data on volatile fatty acids concentration were available to verify the data on milk fat %. Increases in milk fat % could be

explained by shifts in the proportion in volatile fatty acids. A shift from acetate and butyrate might have resulted in decreased milk fat since milk fat synthesis could not be supported without adequate quantities of these fatty acids (Bauman and Griinari, 2003).

5.4.3.3 Body condition score and BW

The BW and BCS were included in the study to determine if cows were utilizing body reserves to maintain or increase milk production. No differences were observed between treatment means in change of body weight. Values were positive, indicating the positive energy balance of animals.

5.5 Conclusion

Main findings from this study were similar to the findings of the ryegrass phase. Based on the findings the null hypothesis should be rejected. Feeding less starch close to the point of minimum daily ruminal pH while still maintaining the same total level of concentrate intake per cow per day does not increase the mean daily ruminal pH and does not decrease diurnal fluctuation in ruminal pH during P2. A more stable ruminal environment in terms of ruminal pH would have been expected to reflect in increased levels of fibre degradation which in turn should have influenced milk yield and milk composition as well as BCS and BW. Neither of these anticipated effects occurred. The reason as to the zero influence treatments had on ruminal pH can only be speculated upon at this stage. Further research is needed to explain the highly complicated nature of the rumen and micro-organism ecosystem.

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CHAPTER 6

General conclusion

Findings from both studies in both pasture phases showed unequivocally that feeding a larger proportion of a total daily 6 kg (as is) allocation of a maize based concentrate per cow/day in the morning relative to in the afternoon did not result in:

- Less variation in ruminal pH during the afternoon (± 4:00 pm)
- Increased minimum ruminal pH on a daily basis
- Less time (h) that ruminal pH spend below the critical pH (5.8)
- Increased degradation of neutral detergent fibre (NDF) or dry matter (DM)
- Increased milk yield/cow/day nor changes in milk composition
- Changes in body weight (BW) nor body condition score (BCS).

The zero response in fibre degradation, milk yield, composition and body weight was not surprising since it was anticipated that such a response would have to be preceded by a response in ruminal pH. The reason why no response was observed for ruminal pH cannot be readily explained but it is speculated that the total level of concentrate feeding per cow/day might have been too low to have elicited a response.

Literature showed increased absorbance of some volatile fatty acids at low ruminal pH; higher levels of non – structural carbohydrates in pasture during the afternoon and one of the main grazing bouts occurring during the afternoon. Based on these findings one might further speculate that the lower ruminal pH observed during the afternoon may be an evolutionary adaptation to ensure optimal use and absorbance of increased levels of non-structural carbohydrates in grass in the afternoon.

Based on the findings of both phases it can be concluded that the null hypothesis should be rejected.

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CHAPTER 7

Critical review of materials and methods applied in studies utilising concentrate feeding strategies and kikuyu *(Pennisetum clandestinum)* or ryegrass *(Lolium multiflorum)* pasture

7.1 Introduction

Hindsight should serve to identify opportunities, shortcomings, and challenges. This chapter attempts to attain this objective for the purpose of future research within the field of dairy production from planted pastures. In many ways the time frame of the field and laboratory work was as such that it did not allow more tasks. Time was limited due to the growth seasons of the different pasture species. The suggested methods mentioned in this chapter should, therefore be carefully evaluated. Criteria such as: 1) ease of implementation, 2) functionality, 3) reliability, 4) and repeatability, should be used against the backdrop of the research setup with the main aim of attaining maximum information with minimum effort and time expenditure. Should the opportunity have presented itself and with sufficient funding, a research assistant assigned to the project might have allowed implementation of the suggestions mentioned in this chapter.

7.2 Suggestions for future research

7.2.1 Estimating individual cow dry matter intake

Estimating DMI in studies concerned with rumen fermentation and fibre degradation is important since forage DMI and rate of intake may influence ruminal pH (Taweel *et al*., 2004). Grazing studies rely on indirect methods to estimate DMI. The RPM method is less exact than the direct measuring of feed fed minus feed orts applied to estimate DMI in a TMR system. It seems that the exact mass (kg) of pasture consumed per cow per day is, and remains, a relative unknown variable despite methods such as the use of internal and external markers. In the studies described in preceding chapters, pasture DMI was estimated using a RPM (see chapter 3, section 3.2). This was mainly used as an aid in estimating the stocking rate and for allocating sufficient areas of pasture per grazing break. Still, only an average pasture DMI for the herd could be calculated and it was thus not possible to determine the average pasture DMI of treatment groups.

Individual pasture DMI can be calculated with a certain level of accuracy using internal markers and data of total faecal output. Faeces are collected with the aid of a bag attached to the animal weighed and then dried (Van der Merwe & Smith, 1991). Faecal and pasture samples are then quantitatively analysed for the specific internal marker. Dry matter intake is calculated applying the equation of Van der Merwe & Smith (1991):

$$PDMI = \frac{FO \times IMF}{PMP}$$

where:

PDMI = Pasture Dry Matter Intake
FO = Faecal Output (DM basis)
IMF = Internal Marker in Faeces (DM basis)
PMP = Percentage Marker in Pasture (DM basis)

Since the use of faecal collection bags might be too laborious and might influence movement of animals, total faecal output can alternatively be estimated by external markers as described by Van Soest, (1994). For the purpose of the studies described in the preceding chapters individual PDMI of a representative sample size of cows from each treatment group could be determined to restrict variation in intake between cows.

7.2.2 Microbial count

The effect of concentrate feeding strategies on microbial numbers should be investigated if one wants to evaluate treatment effects on overall rumen fermentation (Dehority, 2003). In the studies in preceding chapters the effect of treatments on microbial numbers were not determined. Analysing total rumen samples, *viz.* rumen fluid including digesta, for changes in rumen microbial numbers at different times should be a valuable parameter to measure the effect of treatments on rumen fermentation.

Two approaches to microbial count are available *viz.* direct counts and culture counts (Dehority, 2003). The direct count approach seems to be more simplistic and more relevant to the purpose of this study. Both approaches are preceded by accurate and representative sampling of total ruminal contents. Site differences have been reported in the rumen of cattle with regard to bacterial numbers (Munch-Peterson & Boundy, 1963). Sampling in cattle via the cannula should thus involve taking samples at different locations (Dehority, 2003). A beaker can be used for this purpose. With the

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direct count procedure a dilution of the sample is made as described by Hungate (1966). A known quantity is then transferred and spread over a measured area of a slide. The slide is dried, fixed and stained. Two strips, perpendicular to the smear are counted. Using the diameter of the microscope field, area of the smear and the volume of diluted sample spread over the area, it is possible to determine the number of rumen bacteria (Dehority, 2003). For the purpose of future studies, involving ruminal pH and micro - organisms, samples should be taken during times where high and low daily ruminal pH values are anticipated or during times in the day where much diurnal variation in ruminal pH may occur. Alternatively the sampling can be done simultaneously with the *in sacco* study to determine if changes in bacterial numbers are responsible for changes in fibre degradation.

7.2.3 In sacco study

The *in sacco* study described in Chapter 5 used two incubation times *viz*. 18 and 30 hours. In order to calculate the rate of NDF disappearance using the rate calculator of Van Amburgh *et al.* (2003), an additional incubation time *i.e.* a 6 hour time, should be included. Extracting bags at different times involves removing bags from the end of the string. The string becomes shorter after subsequent extractions and the position of the string relative to the ventral rumen position change. Using one string per incubation time may prevent bags positioned in different rumen strata during different times as a result of extraction. The disadvantage of inserting at different times is that the bags are not subject to the same rumen conditions from the start (Nozière & Michalet-Doreau, 2000).

Plant samples should be cut in 50 mm pieces as described in Chapter 5 to prevent losses occurring from washout and to represent a physical form closer to masticated material. Bags should be pre-soaked to simulate ingested feed more accurately and to facilitate attachment of rumen micro-organisms to feed (Nozière & Michalet-Doreau, 2000).

7.3 Retrospect on project management

Project management is an important aspect of research and involves time, personnel, resource and logistic management. In all the studies, time frames were drafted for time management purposes. In the studies described in preceding chapters, relative inexperience and the lack of a sufficient benchmark concerning time expenditure needed to complete tasks, made time frames less effective in earlier studies. Check lists were set up to confirm completion of tasks and to avoid communication errors.

In the kikuyu study a new automated pH recording apparatus was introduced. Minor adjustments had to be made to the equipment during the recording period. A dummy run to test functionality of the

apparatus prior to the recording period would have prevented time and energy being diverted during the recording period.

Positive aspect of the project was that students with similar projects were encouraged to assist with one another's projects. This proved valuable. Many challenges were addressed from perspectives of different students and of these the best ideas were used. Assistance was always at hand and students had access to all the necessary resources that were required. Students were also encouraged to assist in working with the trial animals, doing routine work like allocating pasture breaks, milking *etc*. This stimulated learning and debating different aspects of dairy farming and identifying future research questions. Students had the opportunity to interact with leading dairy producers and had access to information from a multi disciplinary research team of the Department of Agriculture Western Cape. In order to fully exploit knowledge of the scientific community a platform should be established for formal debate about the research protocol of each project. This will allow students to draft contingency plans to address problems that might occur. The platform might be in the form of a blog to allow national and international debate from specialists.

7.4 Conclusion

Suggestions for future research include: more funding to allow more thorough investigation of research problems. Ideally research questions should be handled by a multidisciplinary research team that investigate different fields of the same project. Students from fields such as animal nutrition, pasture science and rumen microbiology should form part of the research team if possible. A platform for research debate among students and experts is highly recommended. Much emphasis should also be placed on ways to maintain excellent project management. A pre-trial briefing about project management could help students achieve this objective.

Positive aspects of the project were: valuable opportunities that were granted to students and excellent access to assistance, experience and equipment. All things considered the project proved a most valuable academic and practical learning experience.

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ADDENDUM A

Mean monthly minimum, maximum and average daily temperatures (°C) and mean monthly rainfall (mm) for the George area from June 2007 to April 2008

Table A1 Mean monthly minimum, maximum and average daily temperatures (T) for the George area from June 2007 to April 2008

Month	Minimum T[1] (°C)	Maximum T (°C)	Average daily T
June	8.2	20.6	14.7
July	7.2	18.7	12.5
August	7.6	18.8	12.9
September	8.8	19.9	14.1
October	10.4	20.5	15.3
November	11.9	21.3	16.4
December	14.5	23.4	18.8
January	15.5	23.3	19.3
February	15.7	24.6	19.8
March	14.1	23.9	18.5
April	11.0	22.0	16.0

[1]T = Temperature

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Table A2 Mean monthly rainfall (mm) for the George area from June 2007 to April 2008

Month	Mean monthly rainfall (mm)
June	35.8
July	43.9
August	33.5
September	34.3
October	43.6
November	491.4
December	156.5
January	90.6
February	69.3
March	61.9
April	36.3

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ADDENDUM B

Chemical composition and quality of soil samples collected during the ryegrass (*Lolium multiflorum*) and kikuyu (*Pennisetum clandestinum*) studies

Table B1 Chemical composition and quality of soil from ryegrass (*Lolium multiflorum*) pasture

Analyses	L 1-13	L14-27	L28-39	Unit
pH	5	4.9	4.7	--
Resistance	150	180	180	Ohms
Texture	Sand	Sand	Sand	--
Acidity	1.38	1.53	1.65	cmol(+)/kg
Calcium	3.94	4.20	4.22	cmol(+)/kg
Magnesium	1.90	1.95	1.75	cmol(+)/kg
Potassium	200	169	172	mg/kg
Sodium	170	145	122	mg/kg
P (citric acid)	118	154	137	mg/kg
Total cations	8.47	8.74	8.59	cmol(+)/kg

L1-13 = Lane 1-13; L14-27 = Lane 14-27; L28-39 = Lane 28-39

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Table B2 Chemical composition and quality of soil from kikuyu (*Pennisetum clandestinum*) pasture

Analyses	**L1-13**	**L14-27**	**L28-39**	**Unit**
pH	5.8	5.3	5.3	--
Resistance	260	210	210	Ohms
Texture	Sand	Sand	Sand	--
Calcium	4.73	1.11	4.83	cmol(+)/kg
Magnesium	2.54	2.78	2.48	cmol(+)/kg
Potassium	261	233	173	mg/kg
Sodium	153	106	141	mg/kg
P (citric acid)	158	189	147	mg/kg
Total cations	8.61	10.18	9.37	cmol(+)/kg
Copper	2.29	2.01	1.78	mg/kg
Zink	26.13	29.15	22.16	mg/kg
Manganese	38.10	44.99	34.66	mg/kg
Boron	0.45	0.46	0.39	mg/kg
Carbon	3.67	4.37	3.59	%
Sulphur	7.05	8.14	7.22	mg/kg

L1-13 = Lane 1-13; L14-27 = Lane 14-27; L28-39 = Lane 28-39

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ADDENDUM C

Blocking and allocating cows used in the ryegrass (*Lolium multiflorum*) and kikuyu (*Pennisetum clandestinum*) studies to treatments

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Table C1 Blocking and allocating cows to experimental treatments for the ryegrass *(Lolium multiflorum)* study

Name	Av. milk production*	DIM**	Lactation number	Block	Treatment
Amsa 3	23.36	89	6	1	1
Alta 24	22.36	51	7	1	2
Dora 103	22.7	39	4	1	3
Japn 58	21.42	91	5	2	1
Bert 6	22.90	79	5	2	2
Magd 76	22.20	41	4	2	3
Sall 1	21.81	114	5	3	1
Elize 72	22.49	92	5	3	2
Magd 72	22.02	111	6	3	3
Max	18.88	46	7	4	1
Lin	19.65	50	6	4	2
Amsa 13	20.38	95	3	4	3
Bert 18	17.51	21	4	5	1
Lin 5	16.50	23	5	5	2
Bell 118	12.45	18	5	5	3
Santa 1	N/A	13	6	6	1
Susa 18	16.84	26	4	6	2
Bert 36	20.00	23	2	6	3
Wand	19.41	140	4	7	1
Tes 2	19.57	160	3	7	2
Bert 13	19.04	100	4	7	3
Etna 2	17.99	146	2	8	1
Wand 5	17.73	142	2	8	2
Susa 23	18.60	159	2	8	3
Elize 62	20.00	66	9	9	1
Lua	19.46	99	6	9	2
Alet 71	20.42	55	9	9	3
Blon 37	17.59	184	7	10	1
Tes 3	17.52	215	2	10	2
Hes 2	17.02	160	3	10	3
Paul 2	16.57	197	4	11	1
Amsa 8	16.50	176	4	11	2
Max 1	16.91	192	6	11	3
Lua 10	16.00	101	3	12	1
Liz 8	21.99	99	4	12	2
Bert 29	19.21	45	2	12	3
Wanda 7	9.88	15	2	13	1
Susa 27	18.00	32	2	13	2
Lin 8	18.87	87	3	13	3
Marl 31	18.90	203	9	14	1
Santa	17.86	136	5	14	2
Mart 89	17.68	89	11	14	3

* **Average calculated from data of 22 days; **DIM: Days In Milk**

Table C2 Blocking and allocating cows to treatments for the kikuyu *(Pennisetum clandestinum)* study

Name	Av. milk Production*	DIM**	Lactation number	Block	Treatment
Amsa 27	16.70	115	2	1	3
Etna 3	16.13	139	2	1	2
Hes 4	16.58	96	2	1	1
Amsa 29	16.13	89	2	2	3
Max 18	16.94	91	2	2	2
Berta 39	16.21	92	2	2	1
Berta 4	20.03	97	6	3	3
Dora 89	20.95	92	7	3	2
Mona 1	20.14	88	3	3	1
Lin 6	21.50	70	3	4	3
Lin 7	21.30	46	4	4	2
Amsa 20	21.28	65	3	4	1
Susa 31	22.40	26	2	5	3
Santa 2	21.56	25	4	5	2
Liz 14	22.72	47	2	5	1
Susa 14	22.98	36	4	6	3
Max 7	22.96	38	5	6	2
Susa 15	22.45	18	3	6	1
Berta 20	17.61	117	4	7	3
Susa 29	17.80	124	2	7	2
Wanda 8	17.14	146	2	7	1
Lin 4	18.97	106	5	8	3
Liz 6	18.26	107	4	8	2
Amsa 18	18.99	117	3	8	1
Susa 24	18.77	135	2	9	3
Amsa 31	18.05	135	2	9	2
Lua 12	18.18	130	2	9	1
Santa 3	18.89	117	3	10	3
Max 14	18.94	129	3	10	2
Arna 7	18.44	96	2	10	1
Lass 4	18.63	30	4	11	3
Lin 10	18.29	55	3	11	2
Susa 17	18.03	25	4	11	1
Amsa 5	19.93	90	5	12	3
Amsa 11	19.10	80	5	12	2
Amsa 15	19.43	94	4	12	1
Mart 127	19.27	67	5	13	3
Berta 21	19.19	67	4	13	2
Berta 40	19.72	74	2	13	1
Paul 5	20.79	102	3	14	3
Max 15	20.59	40	3	14	2
Arna 2	20.98	57	5	14	1

*** Average calculated from data of 21 days; **DIM: Days In Milk**

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ADDENDUM D

Ingredient and nutrient composition of concentrate pellets allocated in the ryegrass (*Lolium multiflorum*) and kikuyu (*Pennisetum clandestinum*) studies

Note: Information of the exact composition of some of the ingredients *i.e*. Premix, Megalac, Mepron Amino Acids and Acid buf (See Table D1 and D3) were omitted due to a confidentiality clause by the manufacturer.

Table D1 Ingredient composition (g/kg DM) of concentrate pellets allocated in the ryegrass *(Lolium multiflorum)* study

Ingredient	**Content (g/kg DM)**
Maize meal	759.5
Soybean oilcake	107.0
Wheat bran	23.0
Molasses	60.0
Feed lime	21.5
Salt	12.0
MgO	3.1
Lactating cow premix	9.3
Rumi buf	4.6

Table D2 Nutrient composition of concentrate pellets as formulated for the ryegrass *(Lolium multiflorum)* study

Nutrient	Content
DM (g/kg)	870.0
CP (g/kg DM)	129.9
RUP (g/kg CP)	410.0
ME (MJ/kg DM)	12.9
NSC (g/kg DM)	658.6
NDF (g/kg DM)	87.4
ADF (g/kg DM)	26.4
EE (g/kg DM)	35.6
Ash (g/kg DM)	69.0
Ca (g/kg DM)	13.2
P (g/kg DM)	3.4

Table D3 Ingredient composition (g/kg DM) of concentrate pellets allocated in the kikuyu (*Pennisetum clandestinum*) grass study

Ingredient	Content (g/kg DM)
Barley	248.0
Maize meal	488.0
Megalac	56.3
Soybean oilcake	47.5
Fishmeal	46.9
Molasses	6.0
Feedlime	21.1
Mepron Amino acids	1.1
Acidbuf	8.4
MgO	3.5
Salt	11.0
Urea	5.7
Premix	2.8

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Table D4 Nutrient composition (g/kg DM) of concentrate pellets as formulated for the kikuyu *(Pennisetum clandestinum)* study

Nutrient	Content (g/kg DM)
CP	145.3
RUP	516.0
ME (MJ/kg DM)	12.6
NDF	71.6
EE	68.0
Ca	16.0
P	3.0
Mg	4.0

ADDENDUM E

Calculating ME requirement and ME intake of Jersey cows receiving ryegrass (*Lolium multiflorum*) pasture and concentrate supplement

Assumptions:

Mean cow mass (kg): 376.33
Mean milk yield (kg): 20.1
Mean milk fat content (g/kg): 42
Activities:

Table E1 Energy cost and energy (MJ per kg/day) expended of physical activity of trial animals

Activity	Energy cost	Energy expended (MJ per kg/day)
Standing(14 hours)	5.83 kJ per kg/d	0.0058
Positional changes (9)	0.26 kJ/kg	0.0023
Walking (2300m)	2.0 J per kg/m	0.0046
Total Source: AFRC (1993)		0.0127

Source : AFRC (1993)

Table E2 Dry matter intake of concentrate and pasture for the period Aug. to Oct.

Month	CDMI (kg) per cow/day	PDMI (kg) per cow/day	CDMI:Total DMI ratio	PDMI:Total DMI ratio
Aug	5.46	5.64	0.49	0.51
Sept	5.46	7.13	0.44	0.57
Oct.	5.46	6.76	0.45	0.55

CDMI = Concentrate Dry Matter Intake PDMI = Pasture Dry Matter Intake

Metabolizability (q_m) of feed = [ME]/[GE] (AFRC, 1993)

q_{mAUG} = PDMI:total DMI x metabolizability of pasture + CDMI:total DMI x metabolizability of concentrate

= (0.51)(0.6856)+(0.49)(0.8393)

= 0.7609

$q_{mSEPT.}$ = (0.57)(0.6856) + (0.44)(0.8393)

= 0.7516

$q_{mOCT.}$ = (0.55)(0.6856) + (0.45)(0.8393)

= 0.7522

ME need for maintenance (M_m):

$M_m = E_m/k_m$ (AFRC, 1993)

= 0.53 x $(\text{mass}/1.08)^{0.67}$ + (Energy expended by activity)(mass)]/(0.35)(q_m) + 0.503

ME need for lactation (M_p):

$M_p = E_l/k_l$ (AFRC, 1993)

= [(mean milk yield)(1.509)+(0.406)(milk fat content)]/(0.35)(q_m) + 0.402

ME need for maintenance and production (M_{mp}) adapted for level of feeding with a correction factor (C_l):

$C_l = 1 + 0.018(M_p/M_l)$ (AFRC, 1993)

$M_{mp} = (M_p+M_m)(C_l)$ (AFRC, 1993)

$M_{mpAUG} = (M_{pAUG} + M_{lAUG})(C_l)$

$= 143.49$ MJ ME per cow/day

$M_{mpSEPT.} = (M_{pSEPT.} + M_{lSEPT.})(C_l)$

$= 144.18$ MJ ME per cow/day

$M_{mpOCT.} = (M_{pOCT.} + M_{lOCT})(C_l)$

$= 144.13$ MJ ME per cow/day

Table E3 Total MEI (MJ per cow/day) during the period August 2007 to October 2007

Month	CDMI	MEc	MEI	PDMI	MEp	MEI	Total MEI
Aug	5.46	12.9	70.43	5.64	11.8	66.55	136.98
Sept	5.46	12.9	70.43	7.13	11.8	84.13	154.56
Oct.	5.46	12.9	70.43	6.76	11.8	79.77	150.20

CDMI = Concentrate Dry Matter Intake (kg); MEc = Metabolisable Energy Content of Concentrate (MJ/kg DM); MEI = Metabolisable Intake (MJ per cow/day); PDMI = Pasture Dry Matter Intake (kg); MEp = Metabolisable Energy Content of Pasture (MJ/kg DM).

Table E4 Total MEI and total ME need for the months August to October

Month	ME need	MEI	Balance
Aug	143.49	136.98	-6.51
Sept	144.18	154.56	10.38
Oct.	144.13	150.20	6.07

ME = Metabolisable Energy; MEI = Metabolisable Intake (MJ per cow/day); Balance (MJ ME per cow/day) = MEI – ME need.

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Reference

AFRC, 1993. Energy and Protein Requirements of Ruminants and advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB INTERNATIONAL, Wallingford, UK.

ADDENDUM F

Calculating RDP and RUP requirement and intake of Jersey cows receiving ryegrass (*Lolium multiflorum*) pasture and concentrate supplement

Assumptions:

Protein fractions according to CNCPS (Sniffen *et al.,* 1992)

A = NPN x (0.01) x (Sol. CP)
B1 = Soluble CP - A
B2 = 100 – A – B1 – B3 - C
B3 = (NDF-CP) – (ADF–CP)
C = ADF-CP
kp = 7.1 %/h; kd = 15 %/h (Berzaghi *et al*., 1996)

F 1 Protein fractions of ryegrass (*Lolium multiflorum*) pasture

NDF-N (g/kg NDF) = 3.56 (see Table 4.20)
NDF (g/kg DM) = 470
NDF-N (g/kg DM) = 1.67
CP (g/kg DM) = 298 (see Table 4.20)
NDF-N (% CP) = 0.56
NDF- CP (% CP) = 3.51

ADF-N (g/kg ADF) = 3.87 (see Table 4.20)
ADF (g/kg DM) = 345
ADF-N (g/kg DM) = 1.335
CP = (g/kg DM) = 298
ADF-N (% CP) = 0.448
ADF-CP = 2.80

A (% CP) = (43.8) x (0.01) x (30.4)
= 13.31

B1 (% CP) = 30.4-13.31

= 17.09

B2 (% CP) = 100 – 13.31 - 17.09 – 0.71 – 2.80

= 66.09

B3 (% CP) = 3.51 – 2.80

= 0.71

C (% CP) = 2.80

F2 Pasture RDP and RUP intake

RDP intake$_{AUG}$ = ((Pasture kd/(Pasture kd + kp)) x (((Pasture B fraction/100) x (Pasture CP/100)) x Pasture DM fed))) + (((Pasture A-fraction/100) x (Pasture CP/100)) x Pasture DM fed)

= ((15/15+7.1) x (((83.89/100) x (29.8/100)) x (5.64))) + (((13.31/100) x (29.8/100)) x (5.64)

= (0.679) x (1.409) + (0.224)

= 1.18 kg DM

RUP intake $_{AUG}$ = CP intake – RDP intake

= ((29.8/100) x (5.64)) – 1.18

= 0.50 kg DM

RDP intake $_{SEP}$ = ((Pasture kd/(Pasture kd + kp)) x (((Pasture B fraction/100) x (Pasture CP/100)) x Pasture DM fed))) + (((Pasture A-fraction/100) x (Pasture CP/100)) x Pasture DM fed)

= ((15/15+7.1) x (((83.89/100) x (29.8/100)) x (7.13))) + (((13.31/100) x (29.8/100)) x (7.13)

= (0.679) x (1.782) + (0.283)

= 1.49 kg DM

RUP intake $_{SEP}$ = CP intake – RDP intake

= ((29.8/100) x (7.13)) – 1.49

= 0.63 kg DM

RDP intake $_{OCT}$ = ((Pasture kd/(Pasture kd + kp)) x (((Pasture B fraction/100) x (Pasture CP/100)) x Pasture DM fed))) + (((Pasture A-fraction/100) x (Pasture CP/100)) x Pasture DM fed)

= ((15/15+7.1) x (((83.89/100) x (29.8/100)) x (6.76))) + (((13.31/100) x (29.8/100)) x (6.76)

= (0.679) x (1.689) + (0.268)

= 1.41 kg DM

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RUP intake $_{Oct}$ = CP intake – RDP intake

= ((29.8/100) x (6.76)) – 1.49

= 0.52 kg DM

F3 RDP and RUP intake of concentrate

RUP (% CP) = 41 % (see Addendum D, Table D2)

RDP (% CP) = 100 % - 41 %

= 59 %

RUP intake = RUP x CP conc. x CDMI

= (41/100) x (14.8/100) x 5.46

= 0.33 kg DM

RDP intake = RDP x CP conc. x CDMI

= 0.59 x 0.148 x 5.46

= 0.477 kg DM

F4 Total RDP and RUP intake and requirement

Table F1 Total RDP and RUP intake, requirement and balance (kg DM) from August to October

Month	RDPI	RDPR[1]	RDP balance	RUPI	RUPR[2]	RUP balance
Aug.	1.657	1.730	- 0.073	0.830	0.720	0.110
Sept.	1.967	1.730	0.237	0.960	0.720	0.240
Oct.	1.887	1.730	0.157	0.850	0.720	0.130

[1,2]NRC, 2001; RDPI = Rumen Degradable Protein Intake; RDPR = Rumen Degradable Protein Requirement; RDP balance = RDPI – RDPR; RUPI = Rumen Undegradable Protein Intake; RUPR = Rumen Undegradable Protein Requirement; RUP balance = RUPI – RUPR.

F5 References

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