Lucerne hay supplementation to Jersey cows grazing kikuyu/ryegrass pastures

By

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Submitted in partial fulfillment for the degree

MSc (Agric) Animal Science: Nutrition Science

Department of Animal and Wildlife Sciences
Faculty of Natural and Agricultural Sciences
University of Pretoria
2012

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DECLARATION

I declare that this dissertation for the degree of MSc (Agric): Animal Nutrition at the University of Pretoria, has not beem submitted by me for a degree at any other University.

L. Müller

Pretoria

May 2011

ACKNOWLEDGEMENTS

I would like to thank the following people for their contribution towards making this project a success:

Firstly I want to thank my parents and brothers for their support and encouragement throughout the project.

My fiancé, Fanie van Vuuren, for all his love and support and encouraging me to write the thesis as quickly as possible, while he waited patiently for me to come back home.

A special word of thanks to the Agricultural Research Trust and Dr. Ilse Trautman from the Western Cape Department of Agriculture for allowing me to do my project at the Outeniqua Research Farm and for funding of the project. This was such an enriching experience and I am very grateful for the opportunity.

Prof. Robin Meeske, one of my supervisors, for the advice, motivation and support that he gave throughout the project and Dr. Philip Botha for his motivation and support while working at the Outeniqua Research Farm.

Pieter Swanepoel, Carmen Coetzee, August Lignau and Janke van der Colf for always being willing to give advice and help during the trial, even if it meant getting up at all hours of the night to do rumen sampling and early in the mornings to help with milk sampling.

Mr. Gerrit van der Merwe for everything that I learned from him. His many years of experience were invaluable and he helped me so much with the practical side of the trial.

The milking team at the Outeniqua Research Farm was always willing to help and they really made my life so much easier. I want to thank them all for their positive attitude towards the trial and being patient with me while I had to learn to do the things they do every day. Titus, Justin, Isaac, Themba, Abraham, David, Marius, Daniel, Terence, Siena, Emerentia and Dina it was such a pleasure to work with you and I will dearly miss you all.

To all the other people who work at the Outeniqua Research Farm, thank you for the support and for making my MSc project such an enjoyable experience. I will always think of you all with fond memories.

Thank you to Prof. Lourens Erasmus, my other supervisor, for his advice and contributions towards the project. And thank you to Mr. Roelf Coertze who helped with the statistical analysis of the data. Thank you to all the staff in the Department of Animal and Wildlife Science at the University of Pretoria, for always being friendly and willing to help.

Elise Ferreira and the staff at Nutrilab made the laboratory analysis easy and enjoyable and because of their dedication and help I was able to complete the analyses quickly and effectively.

I apologise if I forgot to mention anyone else that contributed towards the success of this project.

ABSTRACT

Lucerne hay supplementation to Jersey cows grazing kikuyu/ryegrass pastures

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During spring kikuyu-ryegrass pasture has a low dry matter (DM) content (10-12%), is highly digestible and has high levels of soluble carbohydrates. Low rumen pH values have been recorded for cows grazing these pastures even when supplemented with low levels of concentrate. The rumen environment and extent of rumination may therefore be sub-optimal. Supplementation of the pasture with dry roughage may improve rumination, the rumen environment and therefore also milk production performance.

The aim of the study was to determine if strategic supplementation of lucerne hay will improve milk production, milk composition and the rumen environment of cows grazing high quality kikuyu/ryegrass during spring and receiving low levels of concentrate.

Forty eight high producing Jersey cows were blocked and randomly allocated to one of the following treatments: control (no supplemental roughage), supplementation of 1.0 kg lucerne hay and supplementation of 2.0 kg lucerne hay after morning milking. Cows received 5 kg of dairy concentrate per day during milking. Cows grazed as one group and pasture was allocated to ensure a post grazing height of 10-12 on the rising plate meter (5-6 cm). The average post grazing pasture height for the experimental period was 10.83±1.68 (n=73) on the RPM (5.42 cm).

There were no differences (P >0.10) between the treatments for 4% fat corrected milk production, which were 22.2 kg/d for the control, and 22.5 kg/d and 22.9 kg/d for the 1 kg and 2 kg lucerne treatments respectively. Milk fat and protein percentage was not affected (P >0.10) by supplementation of lucerne hay. The milk lactose content of cows receiving the control and 1 kg

lucerne hay treatments were higher (P <0.05) than those of cows receiving the 2 kg lucerne hay treatment.

Eight rumen cannulated Jersey cows were randomly allocated to either the control or the 2 kg lucerne hay treatment in a cross-over design. These cows grazed together with the cows of the production study and received the same dairy concentrate. Rumen pH was measured for 48 hours with 10 minute intervals using an automated pH logging system. Rumen samples were taken at 08:00, 14:00, 20:00 and 02:00 and were analysed for ammonia-nitrogen (NH_3-N), volatile fatty acids (VFA) and pH. An *in sacco* study was conducted to determine DM and neutral detergent fibre (NDF) disappearance of ryegrass.

There were no differences (P >0.10) between treatments in overall mean pH, measured with either the logging systems or with the portable pH meters averaging 6.18 and 6.11 for cows receiving the control and the 2 kg lucerne treatment respectively. The mean rumen NH_3-N did not differ (P >0.10) between treatments. The mean concentration of acetic acid and the total VFA concentration was higher (P <0.05) for cows receiving the 2 kg lucerne treatment compared to the control. There were no differences (P >0.10) between treatments in the DM or NDF disappearance of ryegrass after 24 hours.

Supplementation of lucerne hay to cows grazing well managed kikuyu-ryegrass pasture during spring did not improve milk production, milk composition, rumen pH or *in situ* NDF disappearance of ryegrass

Results suggest that cows grazing kikuyu/ryegrass pastures supplemented with low levels of concentrate consume sufficient eNDF to maintain a favourable rumen environment and normal milk composition.

LIST OF ABBREVIATIONS

ADF	acid detergent fiber
BCS	body condition score
BF	butter fat
BUN	blood urea nitrogen
BW	body weight
Ca	calcium
CP	crude protein
Cr_2O_3	chromium oxide
DIM	days in milk
DM	dry matter
EE	ether extract
FCM	fat corrected milk
GE	gross energy
ha	hectare
H_2SO_4	sulphuric acid
H_3PO_4	phosphoric acid
IVOMD	*in vitro* organic matter digestibility
K	potassium
Lys	lysine
ME	metabolisable energy
Met	methionine
MCP	mono calcium phosphate
Mg	magnesium
MgO	magnesium oxide
MUN	milk urea nitrogen
N	nitrogen
NDF	neutral detergent fiber
NE	nett energy
NFC	non-forage carbohydrate
NH_3-N	ammonia nitrogen
NPN	non-protein nitrogen

NSC	non-structural carbohydrate
OM	organic matter
P	phosphorous
PA	pasture allowance
peNDF	physically effective neutral detergent fiber
PSPS	Penn State particle size separator
RDP	rumen degradable protein
RPM	rising plate meter
RUP	rumen undegradable protein
SCC	somatic cell count
SEM	standard error of means
SR	substitution rate
TMR	total mixed ration
VFA	volatile fatty acid
$W^{0.75}$	metabolic live weight
WOL	week of lactation

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CHAPTER 1

INTRODUCTION AND MOTIVATION

The climate in the Southern Cape region of South Africa is ideal for pasture based dairy production (Meeske *et al.*, 2006). Even though milk production on pasture based systems is generally lower than on total mixed ration (TMR) systems, the profitability of pasture systems may be more than that of TMR in these regions, because grazed forage is the cheapest source of nutrients for the dairy cow (Clark and Kanneganti, 1998; Peyraud and Delabey, 2001).

The most popular pastures for milk production are temperate species, which are described as high quality or young and leafy pastures (Clark and Kanneganti, 1998). Although the use of pasture is a profitable low cost feeding system (Bargo *et al.*, 2003), there are some nutrient limitations to milk production and supplements must be considered to correct for nutritional imbalances and deficiencies in pasture.

Botha (2003) reported differences in growth and constituents of ryegrass pastures in the Southern Cape over seasons. During spring, which is the active growing season, pasture will have lower dry matter (DM) content, lower neutral detergent fiber (NDF) values and elevated levels of non-structural carbohydrates (NSC) (Reis and Combs, 2000). Non-structural carbohydrates in the diet are readily fermentable and will influence the fermentation parameters in the rumen, because fibrolytic micro-organisms must compete with the carbohydrate fermenters for available substrates (Dixon and Stockdale, 1999). Rumen pH is reduced by rapid fermentation of NSC which produces volatile fatty acids (VFA). This decrease tends to be linearly related to the level of NSC ingested (Kennedy and Bunting, 1992).

Neutral detergent fiber in the ration is important in stimulating chewing activity, maintain rumen pH, optimize the rumen environment for digestion, increase acetate:propionate ratio, prevent milk fat depression and avoid metabolic disorders (Allen, 1997; Mertens, 1997). Generally there exists an inverse relationship between rumen pH and dietary NDF concentration. The NDF fraction has a slower fermentation rate than the NFC, and because most of the NDF in the diet originates from forages with a physical structure, chewing and saliva production is promoted which has buffering capabilities (NRC, 2001). Sufficient NDF therefore ensures overall cow and rumen health

Feeding young and highly digestible forage may therefore not provide sufficient physical structure and effective fiber to prevent low rumen pH and sub-clinical acidosis (Carruthers *et al.*,

1997). Previous grazing studies conducted on highly digestible ryegrass pasture in the Southern Cape region have shown that low rumen pH might be expected, with values as low as 5.7 observed when 4-5.5 kg of concentrate was fed (Malleson, 2008; Erasmus, 2009).

The purpose of this trial which was conducted at the Outeniqua Research Farm, near George in the Southern Cape, was to determine if supplemental roughage, such as lucerne hay, will increase intake of effective NDF and therefore potentially improve the production performance and rumen environment of grazing dairy cows.

A production study was performed to investigate the effect of supplemental lucerne hay on daily milk production, fat corrected milk (FCM) production, and milk composition as well as the effect on body weight and body condition score (BCS) changes. The hypotheses tested were:

H_0 = Supplementation of lucerne hay to Jersey cows grazing kikuyu-ryegrass pasture in spring will increase milk production and/or improve composition.
H_1 = Supplementation of lucerne hay to Jersey cows grazing kikuyu-ryegrass pasture in spring will not increase milk production and/or improve composition.

A rumen study was performed to investigate the effect of supplemental lucerne hay on rumen pH, rumen ammonia-nitrogen (NH_3-N), volatile fatty acid (VFA) profile and fiber digestion. The hypotheses tested were:

H_0 = Supplementation of lucerne hay to Jersey cows grazing kikuyu-ryegrass pasture in spring will improve rumen fermentation and fiber digestion.
H_1 = Supplementation of lucerne hay to Jersey cows grazing kikuyu-ryegrass pasture in spring will not improve rumen fermentation and fiber digestion.

In the following chapter a literature review on the effect of supplementing concentrates and dry roughages to grazing cows is presented. This is followed by a chapter on a production performance study and then a chapter on a rumen fermentation study using cannulated cows.

CHAPTER 2

LITERATURE REVIEW:

SUPPLEMENTARY FEEDING OF COWS ON PASTURE WITH PARTICULAR REFERENCE TO SUPPLEMENTATION OF DRY ROUGHAGE

2.1 INTRODUCTION

The main characteristic of pasture based dairy production systems is high milk output per unit of land whereas in confinement systems the focus lies in milk output per cow (Clark and Kanneganti, 1998). The most popular pastures for dairy production systems are temperate species, which are described as high quality or young and leafy pastures (Clark and Kanneganti, 1998). Although the use of pasture is a profitable low cost feeding system (Bargo *et al.*, 2003), there are some nutrient limitations to milk production and therefore supplements must be considered to correct for nutritional imbalances and deficiencies in pasture.

In order to incorporate effective supplementation strategies on pasture systems, knowledge of the effect that the supplement might have on dry matter intake (DMI), animal performance, and digestion is required (Bargo *et al*., 2003). The goal of supplementation is to provide nutrients in the supplement that complement the pasture and meet requirements of the dairy cow (Bargo *et al*., 2003). Achieving this goal is challenging, because of the continual change in quantity and quality of pasture and the difficulty of quantifying intake (Jones-Endsley *et al.*, 1997; Bargo *et al.*, 2003).

The energy content of pasture and metabolisable energy intake is believed to be the most important factors that limit milk production from pasture diets (Kolver and Muller 1998; Kolver, 2003). Energy supplementation has therefore received the most attention in supplementation studies. A few studies have reported results on roughage supplementation, and these will be discussed in detail in this literature review.

The profitability of supplementation is largely determined by the response in milk production (Bargo *et al*., 2003). Milk response is expressed as milk output (in kg) per kg of supplement intake (Kellaway and Porta, 1993). The response in milk solids is also economically important and must be evaluated to determine the success of supplementation. Many supplementation studies have

investigated the response in milk production, milk composition, digestion and rumen parameters, and these will be discussed in the literature review that follows.

2.2 NUTRIENT REQUIREMENTS OF THE DAIRY COW IN EARLY LACTATION

Nutrient requirements of the cow vary with stage of lactation and gestation (Erasmus *et al.*, 2001). During the different stages in the cow's production cycle, requirements for milk production and composition, body weight and condition, maintenance and pregnancy change constantly and determine the nutritional requirements of the cow.

Feeding standards have been developed that give guidelines for feeding the cow throughout her production cycle to meet requirements. The problem with these standards is that they apply to the average cow and to optimal conditions and considerable variation may therefore be expected across different scenarios (Stewart *et al.*, 1995).

After calving, milk production increases rapidly and the cow struggles to increase feed intake at a sufficient level and rate to meet nutrient requirements (Erasmus *et al.*, 2001). Table 2.1 gives the nutrient requirement guidelines from Erasmus *et al.* (2001) for cows in early lactation.

Energy is a major limiting factor during these early stages of lactation and body tissue will be mobilised to meet requirements for milk production. The ME content of the diet during this stage of lactation should be 11.3 - 11.5 MJ/kg DM (Mahanna, 1993; Erasmus *et al.*, 2001). Grazing further increases the energy requirements of cows. Energy required for maintenance of grazing cows is 10-30% higher than that of non-grazing cows (Muller and Fales, 1998; Muller, 2003a). This is largely due to the extra requirement for walking much more during grazing than non-grazing cows (Bargo *et al.*, 2002b).

Protein supply is very important during this stage to ensure sufficient use of mobilised body tissue for milk production (Erasmus *et al.*, 2001). Crude protein (CP) levels should be between 16-19% on DM basis and 35-40 % of CP should be rumen-undegradable protein (RUP) (Mahanna, 1993; Erasmus *et al.*, 2001; Hutjens, 2008). Milk urea nitrogen (MUN) levels over 20 mg/dL may indicate improper balancing of degradable and undegradable protein (Mahanna, 1993). The ratio of protein to carbohydrate plays an important role in ensuring optimal microbial protein synthesis and N-flow to the small intestine (Jones-Endsley *et al.*, 1997).

Fiber is important in maintaining a healthy rumen environment. A minimum of 18-20% acid detergent fiber (ADF) and 28-30% neutral detergent fiber (NDF) is needed for this purpose (Erasmus *et al.*, 2001; Hutjens, 2008). The physical form of fiber is important and a minimum of

20% of NDF should be physically effective i.e. should be provided from roughage that is 5cm or longer in length (Erasmus *et al*, 2001).

As cows get closer to mid-lactation and reach peak DMI, they should not have any difficulty in meeting requirements from their diet. In this time cows should either maintain or gain body weight and condition (Erasmus *et al.*, 2001).

Table 2.1 Guidelines for nutrient requirements (DM) in the diet of cows in early lactation (Erasmus *et al.*, 2001)

Nutrient (% DM)	Recommended level
CP	16-19
RUP	30-35
ME (MJ/kg)	11.5
ADF (min)	18
NDF (min)	28-32
Effective NDF	20-24
NSC	35-40
Fat	5.0-7.0
Ca	0.8-1.0
P	0.38-0.42

DM = dry matter; CP = crude protein; RUP = rumen-undegradable protein; ME = metabolisable energy; ADF = acid detergent fiber; NDF = neutral detergent fiber; NSC = non-structural carbohydrates; Ca = Calcium; P = Phosporous

2.3 PASTURE COMPOSITION AND DRY MATTER PRODUCTION

Pasture quality and quantity determine the nutritional value to the animal. Quality of pasture depends on many factors, such as geographic location, season and environmental conditions, species differences, plant maturity and grazing management (Muller, 2003b).

Kikuyu (*Pennisetum clandestinum*) is the predominant summer and autumn pasture species used for milk production in the Southern Cape region of South Africa (Botha *et al.*, 2007). Fulkerson *et al.*, (1998) illustrated that the DM production of kikuyu and annual ryegrass (*Lolium multiflorum*) both show a seasonal pattern and furthermore that these species reach their peak production at different times during the growing season. Kikuyu tends to have higher growth rates

during the summer months and low production potential in the winter and spring (Fulkerson *et al.*, 1998; Botha, 2003) when ryegrass performs comparatively well. The temperatures in sub-tropical and tropical areas during the colder months are ideal for temperate species (cool season, C_3 grasses), which make the winter-spring period the best time for growth of annual ryegrass (Fulkerson *et al.*, 1998). A possible solution to the problem of low winter production of kikuyu based pasture systems is to over-sow temperate species into kikuyu pastures (Botha, 2003).

Results reported by Botha (2003) revealed that kikuyu-ryegrass pasture had a higher mean annual ME content than pure kikuyu, with values of 9.28 MJ/kg DM for kikuyu-ryegrass and 8.55 MJ/kg DM for kikuyu. The NDF fraction in kikuyu-ryegrass pastures were lower (60.9%) than in pure kikuyu pasture (63.7%), while CP concentrations did not differ between kikuyu and kikuyu-ryegrass pastures.

During the cooler months, a larger proportion of kikuyu over-sown with ryegrass pasture would be predominantly ryegrass because of low production of kikuyu and ryegrass being in peak growth (Fulkerson *et al.*, 1993). When the grass is at peak growth stage the NDF declines while CP and non-fiber carbohydrate (NFC) increases and nutritional value of the grass therefore increases (Muller, 2003b). Nutrient quality of intensively managed pasture is usually higher. Well-managed spring pasture can have CP concentrations of as high as 25% and NDF concentrations of less than 40% (Muller, 2003a).

2.4 FACTORS LIMITING MILK PRODUCTION FROM PASTURE

The dairy cow needs pasture nitrogen (N) for milk production. Crude protein (as determined by N content multiplied by 6.25) in plants are mostly available in the form of non-protein nitrogen (NPN). Peptides, free amino acids and nitrates largely contribute to the NPN concentration in grass forage and are highly degradable which release ammonia during proteolysis (NRC, 2001). The ammonia cannot effectively be captured and utilized by the microbial population if the dietary carbohydrates supply is inadequate, and over 70-80% of the forage N is lost from the rumen (Miller *et al.*, 2001; Muller, 2003c). An imbalance in energy and protein supply in grass leads to high rumen ammonia concentrations, elevated blood urea nitrogen (BUN) and MUN levels and consequently a high excretion rate of N from the urine (Peyraud *et al.*, 1997; Kolver *et al.*, 1998; Gehman *et al.*, 2006).

Absorption of ammonia and detoxifying ammonia to urea is an energy costly process, the energy used for this purpose (referred to as “urea cost”) is not available for milk production and

causes animal performance to be less than optimal with less than 20% of dietary N appearing in milk (Muller, 2003c). Other consequences of excess protein and inadequate energy concentration in pasture are fast passage rate through the rumens of cows, loose manure, reduced milk fat percentages and loss of body condition (Muller, 2003c).

Some minerals, including Ca and magnesium (Mg), are usually not available in adequate levels in pasture. Potassium (K) on the other hand may be in oversupply of requirements (Muller, 2003a).

Fiber content of high quality pastures may be too low. Spring pasture can have NDF concentrations of less than 40% and furthermore, a large proportion of the NDF in pasture is fermentable fiber and not effective NDF. The effective NDF is that portion of fiber that stimulates rumination, which increases chewing activity and salivation and aids in buffering the rumen pH (Muller, 2003a; 2003c). Sub clinical rumen acidosis may be a problem in cows grazing lush pasture without sufficient effective fiber (Carruthers *et al.*, 1997). Consequences of sub clinical acidosis include decreased fiber digestion, inconsistent feed intake, decreased milk fat and diarrhea (Nocek, 1997; Owens *et al.*, 1998).

2.5 DETERMINATION OF PASTURE YIELD USING THE RISING PLATE METER

Pasture measurement is imperative in order to manage pasture well and properly allocate grazing to cows. Frequent assessment of forage mass and growth of pasture is therefore important (Sanderson *et al.*, 2001).

Direct methods for measuring forage mass, such as the standard clip and weigh method, are laborious and expensive (Sanderson *et al.*, 2001), indirect measurements, although less accurate, are therefore preferred by most farmers, as they are less time consuming and easier (’t Mannetjie and Jones, 2000).

Forage disc meters estimate forage mass by the height of the pasture canopy and the resistance to compression (Harmoney *et al.*, 1997). Earle and McGowan (1979) developed an automated rising plate meter (RPM), which gives a cumulative measure of height recorded on a counter and can be used to determine average height of the area measured. The RPM is calibrated by directly measuring the height and dry matter yield of a number of quadrats (± 0.2 m^2) of pasture and correlating the height with a certain yield. The relationship of pasture yield (Y = $kg.ha^{-1}$) to height (H = cm) is linear and can be described by the following model: Y = a + bH (Earle and McGowan, 1979).

The accuracy of pasture measurements with a RPM is greatly influenced by pasture characteristics, such as density, growth stage and variation in botanical composition, and by operator variability.

2.6 DRY MATTER INTAKE AND PASTURE ALLOWANCE

Although energy is the major limiting nutrient in pasture systems, it appears that the limitation is rather low DMI (therefore low energy intake) of pasture and not energy content of the pasture as such (Reis and Combs, 2000; Kolver, 2003). Hodgson and Brooks (1999) described the three factors affecting DMI on pasture as 1) nutrient requirement 2) physical satiety or capacity constraints and 3) behavioural constraints associated with pasture and animal factors that affect grazing behaviour. Grazing behaviour can further be described by three factors namely bite mass, grazing time and biting rate (Mc Gilloway and Mayne, 1996). Bite mass is mostly influenced by pasture characteristics such as pasture height (Mc Gilloway *et al*., 1999), and pasture density (Rook, 2000). Grazing time and biting rate are controlled by animal factors such as genetic merit and milk production (Rook, 2000). These two parameters are mainly for compensatory regulation to avoid drop in DMI when bite mass decreases (Rook, 2000).

With forage intake a lot of the available energy (10-35%) is lost because of the inability of the animal to digest a great proportion (20-70%) of the cellulose (Varga and Kolver, 1997). The metabolisable energy intake therefore seems to limit milk production from forage diets (Kolver and Muller 1998; Kolver, 2003). Fiber from forage diets resides in the rumen and may limit the capacity for DMI (Allen, 1996). Kolver (2003), however, suggested that low DMI from pasture can be attributed more to grazing time and bite mass constraints than to rumen fill, because of the high *in vivo* digestibility of pasture NDF. Moisture content of pasture may also have a limiting effect on DMI (Hodgson and Brookes, 1999).

Pasture allowance (PA) has a major influence on intake (Dalley *et al.*, 1999; Stockdale, 2000). In order to achieve high DMI and therefore high milk production large amounts of pasture must be offered, the relationship between PA and DMI, however is asymptotic (Dalley *et al.*, 1999). Dry matter intake will increase as PA increases, but with a declining rate until a plateau in DMI is reached (Peyraud *et al.*, 1996; Bargo *et al*., 2002a). Bargo *et al.* (2003) combined data from seven studies to describe the relationship between pasture DMI and PA. They found the optimum PA to be around 110 kg DM/cow per day, and the DMI increased 0.26 kg for every kg increase in PA up to 110kg DM per cow per day.

Providing high PA to increase DMI also implies low pasture utilisation (Mc Giloway and Mayne, 1996) and high postgrazing residual pasture heights, which can result in deterioration of pasture quality because of accumulation of stems and dead material (Peyraud and Delaby, 2001; Lee *et al.*, 2007) and may also lead to lower pasture productivity (Lee *et al.*, 2008; Macdonald *et al.*, 2008) and lower consumption of pasture (Dalley *et al.*, 1999; Macdonald *et al.*, 2008). It is therefore important to offer an optimum amount of pasture to ensure sufficient DMI but also prevent pasture deterioration and wastage. Bargo *et al.* (2002a) suggested a PA of twice the expected pasture DMI or 25 kg DM per cow per day when cows are also fed supplements.

The type and quality of grass species on offer will also have an effect on voluntary intake of pasture (Romney and Gill, 2000). As the plant matures, intake will decline, because of inpalatability and lower digestibility (Hodgson and Brookes, 1999; Minson, 1990). There may also be differences in intake between tropical and temperate species, which can be related to the difference in digestibility between species (Minson, 1990).

Pasture intake is therefore a dynamically controlled process driven by the requirements of the cow, but influenced by pasture management and pasture characteristics.

2.7 PREDICTING DRY MATTER INTAKE

Estimating DMI of grazing animals are much more challenging than for animals in confinement. Reeves *et al.* (1996) conducted a study to compare three prediction techniques for grazing animals. They compared an animal-based technique, a pasture-based technique, and a technique that makes use of equations.

2.7.1 Animal-based techniques

Animal-based techniques make use of the relationship between faecal output and the digestibility of the diet (Stockdale and King, 1983; Bargo *et al.*, 2002a). The faecal production is estimated by dosing indigestible markers such as chromium oxide (Cr_2O_3) (Stockdale and King, 1983; Peyraud, 1998; Schor and Gagliostro, 2001; Gehman *et al.*, 2006) or using naturally-occuring markers like alkanes present in the cuticular wax of plants (Reeves *et al.*, 1996; Fulkerson *et al.*, 2005). Faecal grab samples are then analysed for the concentration of the marker and faecal output (kg DM/cow/day) estimated by relating this to the dosage rate. The *in vitro* digestibility and faecal output can then be used in the equation DMI = faecal output/ (1-*in vitro* DM digestibility) to determine intake.

As with any estimation, some inaccuracy may be expected. Some authors found that DMI was overestimated when using Cr_2O_3 as a faecal marker (Bargo *et al.*, 2002a; Gehman *et al.*, 2006). The alkane technique seems to be more reliable and Stakelum and Dillon (1990) found very similar values for actual intake and predicted intake when using the alkanes for housed animals. Reeves *et al.* (1996) also found the use of alkanes to be more accurate than using the RPM (see section 2.5).

In grazing systems the animal-based techniques for estimating DMI are labour intensive and have various sources of error (Bargo *et al.*, 2003). Criticism against this technique is that *in vitro* digestibility is used. Reeves *et al.* (1996) listed between animal variation, diet composition, level of intake and physiological status of the animal as sources of error not taken into account when using these *in vitro* digestibility values.

2.7.2 Pasture-based techniques

Stockdale and King (1983) suggested that sampling pasture pre- and post-grazing and using the difference between the two measurements to determine pasture DMI was likely to give a more reliable estimate for grazing cows than the animal-based method, provided that grazing periods are short, stocking densities are high and sampling is adequate.

Direct sampling can be done by cutting 10 to 12 quadrats (Hodgson *et al*., 1999) at random sites on the area that is allocated for grazing. Earle and McGowan (1979) suggested a quadrat size of 0.2 m^2 to be most satisfactory. The height at which the grass is cut vary through studies, from ground level (Schor and Gagliostro, 2001; Bargo *et al.*, 2002a; 2002b; Meeske *et al.*, 2006) to 5 cm above ground (Fulkerson and Slack, 1993; Reeves *et al.*, 1996; Delaby *et al.*, 2001) and is based on the assumption of the residual height below which cows will not remove any more material.

The DM content of the pasture can then be calculated by drying samples in an oven and weighing these samples before and after drying. The suggested temperature for drying the pasture samples is 55°C (Kolver and Muller, 1998; Bargo *et al.*, 2002a; 2002b). To ensure that all moisture is removed from the samples, a drying time of 72 hours is suggested (Meeske *et al.*, 2006). Some authors dried samples at 100°C for 24 hours (Earle and McGowan, 1979; Dalley *et al.*, 1999). DM weight is then used to determine kg pasture yield per hectare (Earle and McGowan, 1979).

Direct methods for estimating DMI on pasture are physically limiting as many samples must be cut over many different sites to overcome within-pasture variability (Earle and McGowan, 1979). Indirect measurements are easier to use and are less time consuming.

Pasture available before and after grazing can be measured indirectly with the use of a rising plate meter (RPM) which records pasture height in 5mm increments with a counter (Sanderson *et*

al., 2001). The pasture mass available can then be determined with the use of a regression equation, which is also important for calibration of the RPM (see section 2.5). Correct calibration is especially important when dealing with stoloniferous species such as kikuyu to compensate for the dense pasture mat (Fulkerson and Slack, 1993).

The disadvantage of indirect pasture-based techniques is that DMI is estimated for a group and not individually. It is, however, a useful way in which many measurements can be taken in a short time to overcome errors in variability within paddocks (Earle and McGowan, 1979; Reeves *et al.*, 1996). Malleson (2008), however, found that the difference in DMI between cows on different treatments could not accurately be determined by RPM measurements of pre- and post-grazing pasture.

2.7.3 Prediction equations

Equations to predict DMI of grazing cows have been developed by Caird and Holmes (1986), Vazquez and Smith (2000) and NRC (2001). The equations by Caird and Holmes (1986) and Vazquez and Smith (2000) differ from the NRC (2001) equation in that the latter is based on animal variables only while the other two equations include animal, pasture and supplement variables. The Caird and Holmes (1986) and Vazquez and Smith (2000) equations were developed by using data from many other experiments.

The NRC (2001) predicts DMI from an equation using only 4% fat corrected milk (FCM, kg/d), body weight (BW) and week of lactation (WOL):

$$DMI = [(0.372*FCM) + (0.0968*BW^{0.75})] * [1-e^{(-0.192*(WOL+3.67))}]$$

Bargo *et al.* (2003) compared intake measured by Cr_2O_3 as faecal marker with the intake estimated by the above three equations, and found that the Caird and Holmes (1986) and NRC (2001) equations were accurate, but that the equation by Vazquez and Smith (2000) predicted higher intake.

2.7.4 Other methods for predicting intake

Neutral detergent fiber concentration in the diet is commonly used as chemical predictor of voluntary intake (Kolver and Muller, 1998), and could be used to define the upper and lower bounds of dry matter intake (Mertens, 1994). At high NDF concentrations rumen fill will limit DMI. Constraints on capacity will cause a decline in DMI when NDF concentration in the diet increases beyond 25 % of total intake (NRC, 2001).

Kolver and Muller (1998) showed that grazing cows consumed more NDF (as % BW) than cows fed a total mixed ration (TMR). Pasture has a higher concentration of NDF and therefore grazing cows had an NDF intake of 1.5% BW as to 1.2% for TMR fed cows. The TMR value agrees with the NDF intake of 1.3% of BW found by Bargo *et al.*, (2002b) for diets consisting of 60 % pasture and 40% concentrate. The 1.5% of BW (Kolver and Muller, 1998) is similar to results from Fulkerson *et al.* (1998) who reported an NDF intake of 1.4% of BW when cows grazed lower quality pasture such as kikuyu. Results from Vazquez and Smith (2000) also suggest that a higher NDF intake can be expected when cows consume a diet consisting only of pasture, 1.51 % of BW, than when concentrate is supplemented, 1.38% of BW.

Rayburn and Fox (1993) found that the inclusion of NDF in the prediction models for DMI, would make the model more accurate and unbiased and the resulting model would also be superior in predicting DMI than by using constant NDF intake as 1.2% BW. The reason for improved accuracy of such a model is that NDF intake increases with increased NDF in the ration, increased FCM production and increased DIM, and would therefore be described better.

Another method of estimating intake is back calculation. Published standards for the requirements of cows under various different conditions and circumstances are available; an example of such a publication is the NRC (2001). By using the accepted energy requirements for maintenance, production, liveweight change and physiological status, it is possible to predict herbage intake in reverse (Reeves *et al.*, 1996).

The principle relies on the calculation of ME requirements of the cows and the knowledge of ME intake from the supplements given (concentrate, protein or roughage). The amount of energy not supplied by the supplements must then come from the pasture and the pasture intake can therefore be estimated.

The studies by Reeves *et al.* (1996) suggested an increase in the error of this method with higher levels of concentrate being fed. A significant reduction in estimated pasture intake was observed for each 3 kg increase in concentrate. Lower predictions (16-19%) were observed with concentrate levels of 0-3 kg/cow/d, when compared to the RPM estimations. Predictions were 35 % higher than RPM predictions for cows receiving 3-6 kg/d, the predicted substitution (see section 2.8.2) were therefore much more pronounced with higher levels. Reeves *et al.* (1996) further stated that before this method can be adopted, accurate data on animal production parameters and feed quality must be obtained. The animal parameters must also be evaluated over time, in order to compensate for any fluctuations and to obtain representative data. Between animal variation for efficiency of utilisation or changes in the efficiency with which animals can use food when different

levels of concentrate are being fed, will cause further inaccuracy in this method (Reeves *et al.*, 1996).

2.8 SUPPLEMENTATION ON PASTURES

2.8.1 Type of supplement

2.8.1.1 Energy

As discussed earlier (see section 2.4) there exist imbalances in protein and energy supply from pasture-only diets and energy is thought to be the first limiting nutrient in pasture-based systems (Peyraud *et al.*, 1997; Kolver *et al.*, 1998; Gehman *et al.*, 2006). It is therefore common practice to supplement grazing cows with a high energy concentrate to match degradation of pasture N and carbohydrate supply and degradation (Gehman *et al.*, 2006).

Different types of energy supplementation have been researched. The most common source of energy supplementation is starch-based supplements composed of grains (Muller, 2003a).

Concentrates containing non-forage fiber sources as an additional supply of fermentable fiber may be beneficial. These non-forage fiber sources can be obtained from soy hulls, beet pulp, distiller's grain, citrus pulp, cottonseed hulls and many other by-products (Muller, 2003a). Fiber-based concentrate was found to increase pasture and total DMI when it replaced starch-based concentrates (Meijs, 1986; Sayers, 1999; Gehman *et al*., 2006). A concentrate blend containing non-forage fiber will provide rapidly and slowly fermentable carbohydrates and therefore could improve the milk response (Muller, 2003a).

Increased levels of concentrate feeding may result in a smaller margin over feed cost (Meeske *et al.*, 2006). Very high levels of concentrate feeding would therefore not make economical sense and often more conservative levels are more profitable depending on the milk response per kilogram of concentrate fed.

2.8.1.2 Protein

Protein supply from pasture is already high and grazing cows are thought to require little supplementary protein (Muller, 2003a; 2003c). With high quality pasture, a concentrate supplying 12-14% CP should be adequate in ensuring the total diet contains a level of 16-18% CP/kg DM (Muller, 2003a; 2003c). With 70-80% of pasture N being degradable in the rumen, there will be no

benefit in supplying extra RDP for cows grazing pasture containing 14% CP or more (Schor and Gagliostro, 2001).

Most supplementation strategies incorporating protein supplementation is based on providing more RUP to improve the amino acid profile of the diet, particularly methionine (Met) and lysine (Lys). Supplying RUP in pasture-systems should therefore be considered, especially for high producers (NRC, 2001; Muller 2003a). Examples of feedstuffs high in RUP are roasted soybeans, corn distillers, dried brewers grain and fishmeal (Muller, 2003a).

2.8.1.3 Forage

As previously mentioned (see section 2.4), spring pasture is often low in total fiber and effective fiber, and lush pasture can consist of 80-85% moisture, which can lead to fast passage rates through the digestive tract (Muller, 2003a). In addition to this, lush pasture also has high NSC and fermentable fiber concentrations that may have a negative effect on rumen pH (Dixon and Stockdale, 1999).

Long hay can add some effective fiber and slow down the passage rate to help maintain feed intake and milk fat percentage (NRC, 2001). Increasing the fiber content of the diet with dry roughage supplementation, such as hay or straw, will increase chewing activity and rumination time (Allen, 1997; Beauchemin *et al*., 2003). The increased chewing time will in turn increase saliva flow to the rumen, which contains bicarbonate and phosphate and have buffering effects on low rumen pH (Allen, 1997).

The most popular source of supplemental roughage is maize silage, because of its high energy and fiber concentration; it complements pasture and helps with utilization of high protein in spring pasture (Muller, 2003a). Maize silage will also allow lower levels of grain in concentrate to be fed (Muller, 2003a).

2.8.2 Supplementation effect on pasture intake and grazing behaviour

When pasture diets are supplemented, substitution of the pasture intake by the supplement is expected. The substitution rate (SR) is the decline in pasture DMI when grazing cows are fed a supplement (Kellaway and Porta, 1993). Substitution rate can be calculated as follows:

SR (kg/kg) = (pasture DMI in unsupplemented treatment – pasture DMI in supplemented treatment) / supplement DMI. When the SR is equal to 1kg/kg then total DMI stays constant. SR >

1kg/kg indicates a reduction in total DMI and SR < 1kg/kg shows that DMI in the supplemented treatment is higher than in the unsupplemented treatment (Bargo *et al*., 2003).

There are two theories with regards to what causes substitution with supplementation. It can either be caused by negative associative effects in the rumen (Dixon and Stockdale, 1999), or by a reduction in grazing time (Mc Gilloway and Mayne, 1996). Interaction in the rumen between the digestion of the supplement and forage can be implicated in causing substitution. This is mainly due to pH disturbances and effects on the rumen environment, and therefore fermentation in the rumen.

Pasture allowance also plays a significant role in detemining the SR when supplements are fed. The SR will generally increase with higher PA (Hodgson and Brookes, 1999; Peyraud and Delaby, 2001; Bargo *et al.*, 2002a; 2003). The positive effect of supplementation on total DMI will therefore be more pronounced at low PA (Hodgson and Brookes, 1999; Bargo *et al.*, 2002a, Wales and Doyle, 2003).

The type of supplement given is important factor that determines the SR. It is generally accepted that forage supplementation, such as supplementation of hay or corn silage, results in a greater depression of pasture DMI than compared to concentrate supplementation (Mayne and Wright, 1988; Stockdale, 2000; Reis and Combs, 2000).

Supplementing fermentable carbohydrates, such as barley, will reduce ruminal pH and decrease the activity of cellulolytic bacteria. This will result in a decreased rate of NDF digestion and pasture DMI will therefore decrease accordingly (Hodgson and Brookes, 1999; Bargo *et al.*, 2003). Bargo *et al.* (2003) summarized the effects of different types of supplements and stated that those with slower fermentation rates would have a smaller effect on the substitution and decline in voluntary pasture intake.

The amount of concentrate fed has an effect on pasture intake and in most studies the SR increased with the level of supplementation (Kellaway and Porta, 1993; Sayers, 1999; Bargo *et al.*, 2002a). Jones-Endsley *et al.* (1997) and Peyraud and Delaby (2001), however, found no consistent effect of supplementation on the SR. This may suggest that high producing cows seldom reach maximum voluntary intake under grazing conditions (Peyraud and Delaby, 2001). The total DMI increases with supplementation of concentrate (Sayers, 1999; Bargo *et al.*, 2002a).

Limited research has been done on supplementing dry roughage to high producing dairy cows grazing high quality pastures. The effect of hay supplementation on total DMI and SR differs among studies. Reis and Combs (2000) reported that total DMI did not change with lucerne hay supplementation, even though pasture DMI declined. The substitution rate in this study ranged from 0.81 to 0.97 kg pasture/kg hay. In contrast Stockdale (1999) reported a SR of 0.33 kg/kg when hay

was supplemented to cows on pasture and total DMI was therefore increased. Bargo *et al.* (2003) stated that the increase in total DMI when fiber-based supplement are fed may be attributed to a higher ruminal pH which will favour fiber digestion. Rearte *et al*. (1986b) reported that hay supplementation had no effect on pasture or total DMI. The situation may be changed with lower quality roughage and Ferris *et al*. (2000) investigated the effect of straw supplementation in grass or grass-silage based diets. Dry matter intake followed a quadratic curve, with total intake declining as level of straw inclusion increased.

Pasture and supplement characteristics are not the only factors controlling SR. Some animal factors, such as physiological state and energy balance of the cow, will also influence the SR. When energy intake from pasture is low in relation to the cow's requirements a low SR can be expected. Therefore, with high producing cows, who have a genetic merit for intake and milk production, as well as cows in early lactation, who often experience negative energy balance, SR would generally be low (Peyraud and Delaby, 2001; Bargo *et al.*, 2002a). Energy availability is also influenced by grazing intensity, stocking rate and pasture digestibility, and therefore these factors will play a role in the SR observed (Peyraud and Delaby, 2001).

2.9 NEUTRAL DETERGENT FIBER AND PHYSICALLY EFFECTIVE FIBER

Neutral detergent fiber in the ration is important in stimulating chewing activity, maintain rumen pH, optimize the rumen environment for digestion, increase acetate:propionate ratio, increase milk fat concentration, and avoid metabolic disorders (Allen, 1997; Mertens, 1997). There exists an inverse relationship between rumen pH and dietary NDF concentration, because of the slower fermentation rate of NDF than that of NFC and because most of the NDF in the diet is from forages with a physical structure that promotes chewing and saliva production which has buffering capabilities (NRC, 2001). Sufficient NDF therefore ensures overall cow and rumen health

Recommendations by the NRC (2001) suggest that dairy cows being fed a TMR should receive a diet containing a minimum of 25% NDF and 19% NDF from forages, providing that forage has an adequate particle size and that the predominant starch source is dry maize. In the case of grazing systems where cows receive concentrate twice daily and separately from forage, the NDF requirements would probably be higher, but exact requirements are unknown (NRC, 2001).

Neutral detergent fiber as a chemical component of the diet, however, measures chemical characteristics and not physical characteristics of fiber. These physical characteristics, such as particle size and density, have an influence on nutrient utilization, ruminal fermentation, and animal

production that are not dependent on the amount or composition of the NDF in the diet (Mertens, 1997). Mertens (1997) therefore suggested the introduction of physically effective NDF (peNDF) as a fiber portion of the diet.

Graf *et al.* (2005) speculated that systems for evaluating feed on "structural fiber" or "effective fiber" may have limitations because it reflects interactions with other feed components only to a limited degree. Some variables suggested as indicators for effective fiber content in diets have given unsatisfactory results. These variables include milk fat percentage, which was not a repeatable response variable across different diets (Clark and Armentano, 1997) or differents stages of lactation (Allen, 1997). Allen (1997) suggested ruminal pH to be the most valuable response variable for predicting effectiveness of dietary fiber. Graf *et al.* (2005), however, argued that in most cases, punctual data from occasionally collected rumen samples are used and that it does not take diurnal variation into consideration and would be insufficient as the only indicator of sufficiency of fiber effectiveness. Chewing activity has also been used as indicator of effective fiber content of the diet (Graf *et al.*, 2005; Yang and Beauchemin, 2007a), but results from these studies are conflicting.

There are uncertainties regarding the most effective means of determining the peNDF content of a diet (Yang and Beauchemin, 2006). Particles should be retained in the rumen in order to be effective and the critical size theory states that particle size determines passage rate through the rumen. According to Poppi *et al.* (1980) particles longer than 1.18 mm have the greatest resistance to passage and are largely responsible for stimulating chewing and rumination.

Mertens (1997) proposed a laboratory method for determining peNDF of a diet that is based on particle size. It uses the proportion of DM retained on a 1.18 mm sieve multiplied by the dietary NDF content.

A practical method that is easy to use in routine on-farm evaluations was developed by Lammers *et al.* (1996). A device known as the Penn State Particle Separator (PSPS) is used to determine peNDF by multiplying the proportion of DM retained on a 19- and 8 mm sieve with the dietary NDF content. Kononoff *et al.* (2003a) further refined the PSPS by adding a 1.18 mm sieve that would be consistent with the system used by Mertens (1997). The peNDF is then determined as a proportion of DM retained on 19-, 8- and 1.18 mm sieves multiplied by the dietary NDF content.

Comparison between the original PSPS with 2 sieves and the new 3-sieve PSPS show that the original PSPS better describes the variation in physical effectiveness of the diet and the potential to promote chewing activity and control rumen pH (Yang and Beauchemin, 2006). The difference between the two methods can be attributed to a much smaller range in physically effective fractions

of the three sieves of the new PSPS, therefore no significant increase in peNDF would be observed with increased forage particle length as is the case with the 2-sieve PSPS (Kononoff and Heinrichs, 2003a; Yang and Beauchemin, 2006; 2007b).

Yang and Beauchemin (2006) also investigated the use of fractional NDF content of each sieve rather than the dietary NDF for determining peNDF, and found the estimated peNDF values to be higher with use of fractional NDF. The classification of diets was, however, not changed and a major disadvantage of using the fractional NDF is that it requires more laboratory analysis which restricts its application.

The current methods of using particle size as estimate for peNDF can easily be applied to dry diets such as total mixed rations, silage based diets and hays, but its application does not extend to the diets of grazing animals consuming fresh pasture (Kolver and de Veth, 2002). A reverse calculation approach was introduced by Kolver and de Veth (2002) that uses the relationship between ruminal pH and peNDF described in the Cornell Net Carbohydrate and Protein System (CNCPS) equation. They reported that between pH 5.8 and 6.0 the effective fiber intake was estimated at 29% and at 78% for pH between 6.6 and 6.8. These authors could however not find any further confirmation of the reliability of this method in the literature.

It therefore seems that determining the effectiveness of fiber in diets of grazing animals is still a challenge, and that pH values and milk fat alone may not be reliable indicators of the peNDF intake.

2.9.1 Effect of peNDF on milk production and composition

Many studies were conducted to determine the effect of increased dietary peNDF on milk production (Krause *et al.*, 2002a; Kononoff *et al.*, 2003a; Teimouri Yansari *et al.*, 2004; Yang and Beuchemin, 2005; 2007b). Results were somewhat inconclusive due to differences in measuring the peNDF (Yang and Beauchemin, 2006).

Milk yield does not seem to be affected by forage particle length in silage-based diets (Kononoff and Heinrichs, 2003a; 2003b; Yang and Beachemin 2005; 2007b) and any response in milk production to altered forage length would more than likely reflect changes in DMI or energy intake (Krause *et al.*, 2002a).

Increasing peNDF of diets based on maize silage, had no effect on milk fat, protein or lactose percentages or yield (Yang and Beauchemin, 2005). Diets containing adequate NDF to meet fiber requirements would likely not show any response in milk fat when additional peNDF is supplemented (Krause *et al.*, 2002a; Kononoff and Heinrichs, 2003b; Yang and Beauchemin,

2007b). From the studies by Allen (1997) is appears that peNDF is a poor predictor of milk fat because many factors have an influence on the fat content of milk.

2.9.2 Effect of peNDF on fiber digestion

An increase in the peNDF component of a diet low in effective fiber will likely have an influence on nutrient digestion (Yang and Beauchemin, 2005). By increasing the forage particle length of a ration, and thereby also increasing peNDF intake, the total tract and ruminal digestion of NDF and ADF may be improved (Yang *et al.*, 2002; Teimouri Yansari *et al.*, 2004; Yang and Beauchemin, 2007b). Some studies, however, did not show a significant increase in total tract fiber digestion with increased peNDF intake (Krause *et al.*, 2002a; Kononoff and Heinrichs, 2003b).

2.9.3 Effect of peNDF on chewing activity and rumen pH

The peNDF stimulates chewing activity and is responsible for ruminal mat formation. Increased peNDF in the diet can cause an increased ruminal pH, in this way the risk of ruminal acidosis is minimised (Krause *et al.*, 2002b; Temouri Yansari *et al.*, 2004). Some studies, however, had the opposite result, where increased peNDF in the diet did increase chewing activity, but had no effect on the rumen pH, this was particularly prevelant in diets containing highly fermentable carbohydrates (Kononoff *et al.*, 2003b; Kononoff and Heinrichs, 2003a; Beauchemin and Yang, 2005).

Yang and Beachemin (2007a) showed that increasing the peNDF intake by increasing forage particle length caused an increase in both the mean and ruminal pH. Altering the ratio of forage to concentrate (F:C) in order to increase peNDF intake showed a much greater response in pH variables, with elevated mean, maximum and minimum ruminal pH, and duration that pH <5.8 or pH < 5.5 with increased F:C. This showed that not only the fiber length of the forage but also the forage proportion of the diet is very important in maintaining ruminal pH.

2.9.4 Effect of peNDF on rumen fermentation

Yang and Beauchemin (2007a) found no effect on rumen ammonia nitrogen (NH_3-N) when forage particle length was increased, an increase in F:C significantly raised the concentration.

Yang and Beauchemin (2007a) also reported no significant response to increased peNDF intake from increased forage particle length in terms of total volatile fatty acid (VFA) concentration, acetate, propionate, acetate:propionate or butyrate concentrations. Increased forage component of the diet will cause decreased fermentability and therefore total VFA will decrease.

Acetate concentration is likely to increase with increased forage and propionate concentration will decrease (Yang and Beauchemin, 2007a).

2.10 SUPPLEMENTATION WITH DRY ROUGHAGE

2.10.1 Response to roughage supplementation

2.10.1.1 Milk response

The profitability of supplementation is largely determined by the response in milk production (Bargo *et al*., 2003). Milk response is expressed as kg milk / kg supplement intake (Kellaway and Porta, 1993). There is a negative relationship between SR and milk response, because a large SR will cause no increase or a small increase in total DMI and therefore a low response in milk production. Kellaway and Porta (1993) also stated that the increase in milk produced per kg of supplement, decreases as the amount of supplement given increases.

Most studies exploring forage supplementation to grazing cows, was done with maize silage as source of roughage. The effect on production when maize silage is supplemented is positive when the amount of pasture on offer is low. At high pasture allowance the SR is in the order of 1kg/kg, total DMI remain constant and no positive effect on milk production would be observed (Phillips, 1988; Stockdale, 1994; Graf *et al.*, 2005).

Milk production response to hay supplementation differed between studies. Some authors found no response when hay was supplemented to early lactation cows grazing highly digestible pasture (Rearte *et al*., 1986b; Reis and Combs, 2000; Wales *et al*., 2001; Graf *et al.*, 2005), while in other studies higher milk production have been recorded (Beauchemin and Buchanan-Smith, 1990; Rearte *et al*., 1986a; Stockdale, 1999).

High SR can be expected with hay supplementation (see section 2.8.2). Graf *et al.* (2005) found that substitution rate of pasture was 1 kg per kg of hay fed and because the nutritional quality of lucerne was similar to that of the pasture no significant change in nutrient intake was observed. This ensured that milk production was not affected by hay supplementation, even though the supplementation rate was relatively high (almost 40 % of total DMI) at 5.5kg/cow/day.

Particle size played a significant role in results reported by Rearte *et al*. (1986a), who found higher milk production when long lucerne hay was supplemented, but when hay was chopped and fed with the concentrate, production was similar between supplemented and unsupplemented treatments.

Method of supplementation also seems to influence milk response and Beauchemin and Buchanan-Smith (1990) observed that cows on a silage-based diet achieved higher productions when hay was fed before the concentrate was given.

Straw usually has a low nutrient density and a decrease in ME intake can be expected with supplementation of straw to cows grazing highly digestible pasture. The decrease in ME intake will cause a linear decline in milk yield (Ferris *et al*. 2000). In contrast, Wales and Doyle (2003) found no difference in ME intake with increased levels of straw supplementation. The milk response obtained from grain supplementation to cows grazing a highly digestible pasture was however decreased with supplementation of straw. This suggested that factors other than NDF concentration determine the marginal-milk response (Wales and Doyle, 2003).

2.10.1.2 Milk composition

Milk fat and protein can be altered by nutrition, with milk fat being more sensitive to dietary changes than protein (Varga and Ishler, 2007). It is generally assumed that milk fat is regulated by the fiber content of the diet (Allen, 1997; NRC, 2001; Varga and Ishler, 2007). In most studies roughage supplementation had no effect on milk fat (Reis and Combs, 2000; Wales and Doyle, 2003; Graf *et al.*, 2005).

Only Rearte *et al*. (1986a) found a response in milk fat and reported that milk fat was decreased with long hay supplementation. The decrease in milk fat may partly be attributed to increased milk production in this study.

No change in milk protein percentage was found with hay supplementation (Rearte *et al*., 1986a, 1986b; Reis and Combs, 2000; Stockdale, 1999). Straw supplementation however caused variable responses, with either decreasing protein concentration or having no effect (Ferris *et al*., 2000; Wales and Doyle, 2003).

The response in lactose and minerals that make up the other constituents of solids in milk is not as easy to predict with dietary changes as milk fat and protein (Varga and Ishler, 2007).

Milk urea nitrogen reflects the protein levels in the diet and the efficiency of fermentable carbohydrates for microbial protein synthesis. In the study of Graf *et al.* (2005) pasture had higher CP levels than lucerne hay or maize silage supplements and therefore MUN values were higher in the cows that only received pasture than in the cows supplemented with hay or silage.

2.10.1.3 Supplementation effect on digestion and fermentation in the rumen

2.10.1.3.1 Rumen pH

There is evidence pointing to a higher tolerance of low rumen pH on low starch pasture diets with highly fermentable fiber (Kolver and de Veth, 2002). Studies have shown that rumen pH of cows grazing highly digestible pasture full-time did not have the expected negative impact on production and digestion (Graf *et al.*, 2005), and that supplementary roughage feeding therefore may have a limited or no effect on the mean ruminal pH (Reis and Combs, 2000; Wales and Doyle, 2003; Graf *et al.*, 2005). Graf *et al.* (2005), however, did mention that the situation might be changed with concentrate feeding and that lower pH values may be expected. This was confirmed by findings in the study of Wales and Doyle (2003) where cows grazing highly digestible clover pasture with low NDF content were given 5 kg of cereal grain and different levels of straw. The ruminal pH of grain supplemented cows was significantly lower than that of cows not receiving grains, and differing amounts of straw did not change the rumen pH. The argument therefore remains that the low pH observed in situations where cows graze highly digestible and lush pasture is doubtfully due to the effect of the grass on the rumen, but rather the effect of concentrate high in grains.

Significant diurnal variation is observed in rumen pH of grazing cows (Carruthers *et al.*, 1997; Kolver and de Veth, 2002; Wales and Doyle, 2003), which can partly be explained by the change in diurnal grazing patterns and the subsequent change in DMI and rumination (Wales and Doyle, 2003; Graf *et al.*, 2005). Wales *et al.* (2004) investigated the effect of diurnal variation on digestibility of high quality pasture that causes low average daily rumen pH. They found that at low rumen pH (5.6), diurnal variation had a significant effect and OM, NDF and ADF digestion was lower than at a constant pH of 5.6. At higher pH (6.1), digestion was not affected by diurnal variation with similar values between a constant pH and fluctuating pH.

Apart from the diurnal variation observed, there seems to be differences in the time spent below a critical pH. Graf *et al.* (2005) reported lower mean pH during the day when cows were fed supplemental lucerne hay, the pH also remained under 5.8 for a longer period during the day. The authors suggested that this observation in low daytime pH for hay supplemented cows can be attributed to more aggressive grazing during the morning because cows were restricted in feed intake during the night, whereas full-time grazing cows had a better distribution of intake throughout the day and night. This would then likely cause more intensive ruminal fermentation during the day for supplemented cows when they are on pasture.

Wales and Doyle (2003) found that straw supplementation had no effect on the diurnal pattern of ruminal pH and that the rate in pH decline for all treatments were similar following the two major periods of grazing during the day. These findings confirmed that of Graf *et al.* (2005), and it seems that a more even distribution of pasture intake is needed and that supplemental roughage does not seem to have a positive effect on pH in the rumen.

2.10.1.3.2 Rumen ammonia-nitrogen

Rumen NH_3-N is largely dependent on the protein content of the diet and the rate of protein degradation in the rumen. Graf *et al.* (2005) found that the effect that hay supplementation had on rumen NH_3-N differed between times of day and that this could be linked to changes of nutrient intake throughout the day. In this study, cows receiving hay during the night was restricted in feed intake and compensated for it during the day by grazing more aggressively, the higher protein intake from pasture during the day caused the higher rumen NH_3-N observed.

If the diets of supplemented cows and unsupplemented cows contain more or less the same amount of protein, such differences in rumen NH_3-N would not be expected (Reis and Combs, 2000; Wales and Doyle, 2003).

2.10.1.3.3 Volatile fatty acids

Volatile fatty acid concentration fluctuates during the day and is linked to rumen pH fluctuations, as the pH is largely dependent on the concentration of acids in the rumen (Owens *et al.*, 1998). In the study of Graf *et al.* (2005), daytime differences were observed in all the traits describing VFA concentration and profile between full-time grazing cows and hay supplemented cows. Total VFA concentration for roughage supplemented cows were lower in the morning which could be expected because cows received supplements during the night and grazed during the day which caused ruminal fermentation to increase during daytime.

Treatment effects on total VFA concentration and profile are insignificant regardless of daytime differences observed (Wales and Doyle 2003; Graf *et al.*, 2005).

2.10.1.3.4 Fiber digestion

The rate of digestion is primarily determined by the NDF content of feed, with a negative relationship existing between these variables (Mc Donald *et al.*, 2002). There are four factors that regulate fiber digestion in the ruminant: 1) plant structure and composition, as this influences the bacterial access to nutrients within the plant cells; 2) the predominant type and population density

of fiber digesting micro-organisms in the rumen; 3) microbial factors that control adhesion and hydrolysis; and 4) animal factors such as mastication, salivation and digestion kinetics that largely influence the exposure of nutrients to micro-organisms (Cheng *et al.*, 1991).

Rumen kinetics and digestion in the grazing cow supplemented with roughage has been investigated. Reis and Combs (2000) found that starch and free glucose digestibility increased with hay supplementation. Their results suggested that hay supplementation reduced the liquid rate of passage and therefore also the particle passage rate, lowering the starch and free glucose found in the faeces. The authors also recorded higher degradability values for pasture forage organic matter and ADF for cows fed supplemental hay. Since no changes were seen in the rumen environment or in rumen fermentation, the authors speculated that the positive effect of hay on digestion was largely due to changes in rumen digestion kinetics.

Beauchemin and Buchanan-Smith (1990) did *in sacco* studies and recorded improved fiber digestion when hay was supplemented, this was in agreement with findings by Reis and Combs (2000).

Wales and Doyle (2003) found no improvement in DM or NDF degradability with straw supplementation of cows grazing highly digestible pasture low in NDF.

2.10.1.4 Chewing activity and the influence on pH

By supplementing additional roughage the chewing activity, saliva production and therefore ruminal fermentation can be altered (see section 2.8.1.3).

Wales and Doyle (2003) observed that cows grazing highly digestible pasture with low NDF content and receiving 5kg of high grain concentrate ruminated more when supplemented with straw and that rumination activity increased with increasing levels of straw supplementation. The increased rumination time, however, did not seem to have any effect on the ruminal pH. This is in agreement with studies on the effect of peNDF on rumen pH of cow fed TMR diets (see section 2.9.3; Kononoff *et al.*, 2003b; Kononoff and Heinrichs, 2003a; Beauchemin and Yang, 2005).

The grazing pattern and behaviour is changed with supplementation (Graf *et al.*, 2005). The chewing activity and rumination patterns are therefore also changed. Graf *et al.* (2005) reported that full-time grazing cows spent more time eating than cows supplemented with hay over night and being restricted in DMI. The increased eating time in full-time grazing cows resulted in a more even distribution of daily feed portion and this could explain the lack of a detrimental effect of highly digestible pasture on rumen pH. The primary reason for the lack of an effect on rumen pH in full-time grazing cows may therefore not be mainly due to increased saliva flow to the rumen, because

prolonged eating time is not necessarily associated with higher average pH (Wales and Doyle, 2003) and saliva excretion is lower during eating than rumination (Baily and Balch, 1961).

Therefore cows grazing highly digestible and young pasture may be less prone to sub-clinical acidosis than assumed, and effective forage supplementation must ensure a more even intake pattern over night (Graf *et al.*, 2005). Wales *et al.*, (2001) showed that cows were able to consume sufficient NDF (370 g NDF/kg DM) from irrigated perennial ryegrass pasture, supplemented with grain, to maintain a good milk production response. Rumen pH of cows grazing highly digestible pastures that are high in fermentable fiber may not be as low as expected (Graf e*t al.*, 2005).

CHAPTER 3

PRODUCTION PERFORMANCE OF JERSEY COWS GRAZING KIKUYU/RYEGRASS PASTURE SUPPLEMENTED WITH LUCERNE HAY

3.1 MATERIALS AND METHODS

3.1.1 Location, climate and soil

The study was conducted at the Outeniqua Research Farm near George in the Western Cape province of South Africa. The farm is situated at an altitude of 201m at 33° 58' 38'' S and 22° 25' 16'' E. The long term average rainfall in this area is 728 mm per annum. The total rainfall for the duration of the trial period was 106.7 mm (daily measurements on farm) and the average daily maximum and minimum temperatures were 20.4°C and 8.8°C respectively (ARC, 2010). The soil where the pasture was established was classified as a Westleigh soil form (Fey, 2010).

3.1.2 Duration of the experimental period

The experimental period was from 3 August 2010 to 15 October 2010, allowing the cows to adapt during the first 14 days of this period. Measurements and samples were taken from 17 August 2010 to 15 October 2010.

The selection and blocking of cows used in the production study was done during July 2010.

3.1.3 Cows and experimental treatments

3.1.3.1 Cows

Forty eight high producing multiparous, Jersey cows were blocked according to the average 4% fat corrected milk production (FCM = [0.4 * milk production] + [15 * milk production * BF%/100) during the three weeks prior to the start of the study, lactation number and days in milk (DIM).

3.1.3.2 Experimental treatments

Cows within blocks were randomly allocated to one of the three different treatments (16 cows per treatment).

Treatment 1: Kikuyu-ryegrass pasture + 5 kg Dairy concentrate (Control)

Treatment 2: Kikuyu-ryegrass pasture + 5 kg Dairy concentrate + 1.0 kg Lucerne hay (Low lucerne, LL)

Treatment 3: Kikuyu-ryegrass pasture + 5 kg Dairy concentrate + 2.0 kg Lucerne hay (High lucerne, HL)

3.1.3.3 Body weight and body condition scoring

The body weight and body condition score of each cow was measured on two consecutive days at both the beginning and end of the trial period. This was done before afternoon milking and values were used to determine the average weight gain and condition change of each treatment group.

The body weight was measured with a Tru-Test EziWeigh 1 scale (Tru-Test Limited, Auckland, NZ), and body condition scores were awarded according to a scale of 1 to 5, with 1 representing emaciation and 5 representing obesity (Wildman *et al.*, 1982). The scores were awarded by the same person on each occasion to minimise variation.

3.1.3.4 Management

The cows in the trial grazed together as one group, therefore they had to be separated into their three different treatment groups before milking. This was done by fitting each cow with a light neck chain carrying a different colour tag for each treatment. The block to which each cow belonged was also written on the tag around its neck.

Each cow received 5 kg of a dairy concentrate per day (2.5 kg per milking on as is basis). Table 3.1 shows the ingredients composition of the dairy concentrate as provided by NOVA feeds (NOVA Feeds George, Saagmeule Str, George Industria, George, 6529).

Table 3.1 Ingredients composition (DM basis) of the dairy concentrate fed to control, LL and HL cows[1]

Raw material	% Inclusion
Maize (ground)	80
Soya Bean Oil cake	9.31
Wheat Bran	3
Molasses	4
Feed lime	1.58
Salt	0.74
Mineral Premix	0.4
MCP	0.69
MgO	0.28

[1]LL = low lucerne treatment; HL = high luceren treatment
MCP = mono calcium phosphate
MgO = magnesium oxide

Concentrate was accurately weighed out (HICO PC.15 scale, capacity 15 kg, divisions 0.005 kg) and put into plastic bags. Concentrate was then fed in individual troughs inside the milking parlour for each cow while being milked.

After morning milking the cows that received supplemental lucerne were put into individual stalls. Plastic bags containing 1 kg or 2 kg lucerne were accurately weighed out (HICO PC.15 scale, capacity 15 kg, deviation 0.005 kg), the 1 kg and 2 kg bags were kept separate to avoid confusion. Lucerne was placed in each individual stall's feeding trough and cows were allowed sufficient time to consume all of what was allocated to them.

The refusals of each cow were removed from the feeding troughs every day and three days' refusals were weighed during the week to estimate daily intake. In order to facilitate refusal collection each stall was marked with the cows name and cows were put in the same stall every day.

The cows from the control group were kept in a holding area while the other cows were consuming the hay. These cows waited 30 minutes per day on average and afterwards all the cows were allowed to return to the pasture as a group.

The cows grazed 24 hours per day, except during milking times, and had access to clean water *ad libitum* while they were grazing. The time spent away from the pasture was kept to a minimum and cows were returned to pasture as soon as possible after milking.

3.1.3.5 Lucerne hay preparation

Lucerne hay was prepared in a Seko Samurai5 500/33 mixer wagon before feeding it to cows. An average mixing time of 10 minutes for 400 kg lucerne was allowed and the mixer wagon kept on mixing for a further 5 minutes while the hay was removed from the mixer.

After mixing representative lucerne hay samples of about 500 g were taken for each week and pooled for every two weeks. The particle length of the five pooled samples was determined with a Penn State Particle Size Separator (Nasco, Fort Atkinson, Wisconsin, USA).

Each sample was weighed and placed in the box with the largest perforations. The three trays were then stacked on top of each other. The boxes were then shaked horisontally, on a flat and smooth surface. The horisontal movement in one direction was repeated five times, whereafter the boxes were turned 90° and the five times shaking procedure was repeated. The shaking procedure was repeated a total of eight times, with the boxes rotating 360° twice (Lammers *et al.*, 1996).

After shaking the sample, the content of each tray was weighed and expressed as a percentage of the original total mass of the sample.

3.1.3.6 Pasture

Cows grazed Italian ryegrass (cultivar Jeanne) over-sown into kikuyu pasture. A Nobili Model BNU160 mulcher was used to create a seedbed for the ryegrass. The ryegrass was then over-sown at a density of 15 kg/ha into the kikuyu base by using an Aitchison Seedmatic 3116C seeder (Aitchison Industries LTD, Mosston Road, Wanganui, NZ), after which the pasture was rolled.

The cows strip grazed pasture and a new strip was allocated after each milking. A rising plate meter (Filips Folding Plate Pasture Meter, Jenquip, Reidline East, NZ) was used to estimate pasture yield before grazing and pasture was allocated at 9.80 kg DM/cow measured above 50mm stubble height, for each grazing. Pasture height of each grazing strip was determined by taking 100 RPM readings per grazing strip both before and after grazing. The aim was to keep the RPM reading above 10 (50mm pasture height) after grazing.

Irrigation was scheduled by means of a tensiometer and fertilizer was applied at a rate of 42 kg N/ha to each strip after it was grazed. Cows were not allowed to graze a strip again for a minimum of 28 days after fertilizer was applied.

3.1.3.7 Dry matter production and pasture yield

Dry matter production was estimated using the difference between pre- and post-grazing mass estimated with a rising plate meter (RPM) (Stockdale and King, 1983; Fulkerson and Slack, 1993).

The RPM was calibrated by developing a linear regression between the disc meter reading and the herbage DM mass. To predict pasture mass the equation y = mx + b was used, with y = yield, m = factor, x = pasture height and b = constant. During the trial period 36 circles with an area of 0.098m^2 were cut at a stubble height of 50mm. Three cuttings each of pasture estimated to be low, medium and high were cut during four random days throughout the trial. Pasture yield was estimated by using pasture height and the cumulative regression to determine the kilograms of DM present before grazing and left after grazing.

3.1.4 Milk production and composition

Cows were milked twice daily at 06:00 and 14:00 using a 20 point Dairymaster swing over milking parlour with weigh-all electronic milk meters (Dairymaster Milking Systems NZ limited, Stratford East 4332, NZ). Daily milk production was recorded electronically and data was downloaded once a month.

Individual milk samples were taken monthly to be analysed for composition. Composite samples were taken, comprising of 8 ml from afternoon milking and 16 ml from morning milking, resulting in a 24 ml representative sample. Samples were preserved with sodium dichromate and were send via an overnight courier service to Lactolab (ARC, Irene) for analyses of butter fat % (BF), protein %, lactose % and milk urea nitrogen (MUN) by means of infrared technology using a Milkoscan 6000 (Foss Integrated Milk Testing FT 6000, Foss Electric, Hillerod, Denmark). Somatic cell count (SCC) was also determined for each sample by means of flow cytometry using a Fossomatic 5000 (Foss Electric, Hillerod, Denmark).

3.1.5 Sampling pasture, hay and concentrate

To determine nutritive value of the pasture, a representative sample of wet material was taken from three cuttings of a 0.098m^2 circles at a stubble height of 50mm. Samples were taken weekly and were then pooled for every two weeks, resulting in five pasture samples to be analysed. The samples were weighed (Precisa 3100C scale, capacity 3100.00 g) and dried at 60°C for 72h (Botha *et al.*, 2007), then weighed again to determine DM content. Each sample was then milled through a hammer mill with a 1mm sieve (Scientific manufacturing cc, Killarney Gardens, RSA) and kept in airtight containers until analyses.

Grab samples of the concentrate and lucerne hay were taken on Mondays, Wednesdays and Fridays to get a representative sample for each week of the trial period. The concentrate samples were taken directly from the 50 kg bags as they arrived from the feeding mill. The lucerne hay grab samples were taken after preparation in the Seko Samurai5 500/33 mixer wagon. After mixing, the lucerne hay was stored in large bags. The grab samples were taken from different bags at the bottom, middle and top of the bags to ensure that the leafy material that might have accumulated at the bottom was also included in the sampling. Concentrate and hay samples were pooled for every two weeks resulting in five samples each for the concentrate and the lucerne hay. Each sample was milled through a 1mm sieve and stored in an airtight container until analyses.

3.1.6 Laboratory analyses

The following analyses were performed on all feed samples at the UP Nutrilab (University of Pretoria, Hatfield Campus, Agricultural Building, Floor 10, Pretoria, 0002): dry matter (DM; AOAC 2000, procedure 934.01), ash (AOAC 2000, procedure 942.05), ether extract (EE; crude fat, AOAC 2000, procedure 920.39), gross energy (GE; MC – 1000 Modular Calorimeter, Operators manual), crude protein (CP) calculated from N*6.25 (Leco N analyser, model FP-428, Leco Corporation, St Joseph, MI, USA), neutral detergent fibre (NDF; ANKOM 2000 Automated fiber analyser, ANKOM technologies, Macedon, NY, USA), acid detergent fibre (ADF; ANKOM 2000 Automated fiber analyser, ANKOM technologies, Macedon, NY, USA), *in vivo* organic matter digestibility (IVOMD; Tilley and Terry, 1963), using rumen fluid from a rumen cannulated sheep fed lucerne), calcium (Ca; AOAC 2000, procedure 965.09) and phosphorous (P; AOAC 2000, procedure 965.17). Metabolisable energy (ME; MJ/kg DM) was calculated using the following equation: ME = 0.84(GE * OMD) for concentrates and for forages ME = 0.81(GE * OMD) (ARC, 1984; MAFF, 1984).

3.1.7 Experimental design and statistical analysis

The experiment was conducted as a randomized complete block design with three treatments randomly allocated within each of the 16 blocks. The data were analysed according to the described experimental design. An analysis of variance was performed with the GLM model to determine differences between experimental treatments (SAS, 2008).

3.2 RESULTS and DISCUSSION

3.2.1 Pasture regression

Pasture DM production was determined by cutting samples of grass and drying the samples. Weight before and after drying was used to determine the DM content of the pasture and the average over the trial period was 12.3% ± 1.35 (n=10).

Pasture yield was determined with a regression equation of y = 70.47x -212.4 (R^2=0.61). Figure 3.2 illustrates this regression equation that relates pasture height as measured by the RPM with pasture yield.

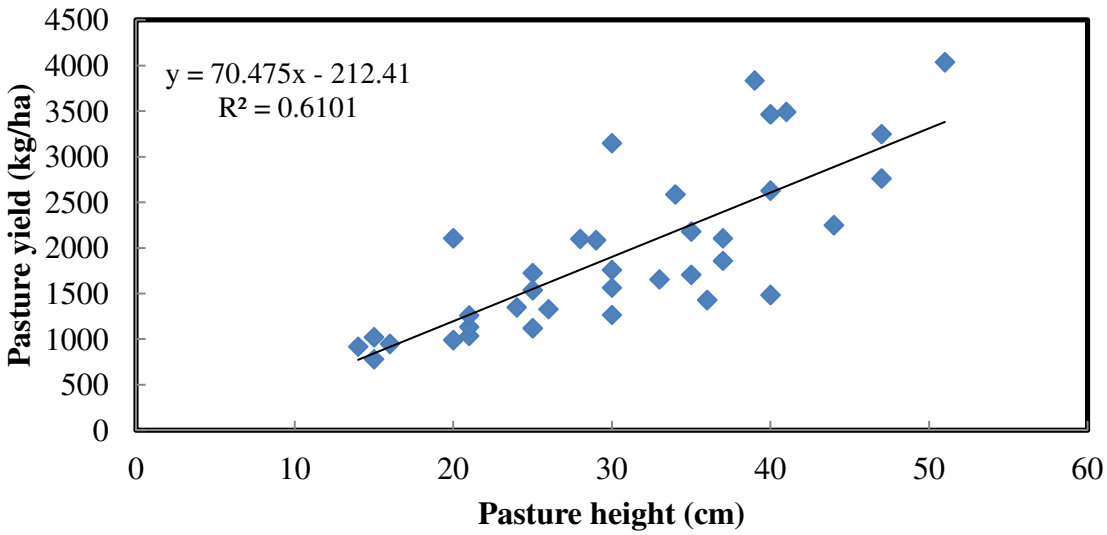


Figure 3.1 Illustration of regression used to relate pasture height with pasture yield

3.2.2 Dietary ingredients and chemical composition

Analyses of nutrient composition of pasture, lucerne and concentrate were done on the grab samples taken weekly and mean values are show in Table 3.2.

Table 3.2 Mean chemical composition (± SD) of pasture, lucerne hay and dairy concentrate[1]

Nutrient (%DM)	Pasture	Lucerne hay	Concentrate
DM	12.4 ± 1.35	89.2 ± 0.3	88.5 ± 0.17
CP	23.3 ± 2.50	21.6 ± 0.78	12.0 ± 0.13
ME (MJ/kg)	11.4 ± 0.35	10.2 ± 0.51	13.3 ± 0.12
NDF	47.2 ± 1.12	43.0 ± 3.36	9.23 ± 0.26
ADF	28.5 ± 3.53	36.5 ± 2.36	3.57 ± 0.12
IVOMD (%)	82.0 ± 1.82	72.4 ± 3.41	93.1 ± 1.29
Ash	11.4 ± 1.53	9.71 ± 1.28	5.02 ± 0.14
EE	3.90 ± 0.38	1.84 ± 0.14	2.71 ± 0.16
Ca	0.37 ± 0.02	1.26 ± 0.06	1.01 ± 0.04
P	0.37 ± 0.08	0.25 ± 0.01	0.44 ± 0.03

[1]Mean value of 5 samples except pasture DM (n=10);DM = dry matter; CP = crude protein; ME = metabolisable energy; NDF = neutral detergent fibre; ADF = acid detergent fibre; IVOMD = *in vitro* organic matter digestibility; EE = ether extract; Ca = calcium; P = phosphorous

3.2.2.1 Pasture composition

The pasture DM content was quite low at 12.3%, and similar to spring and autumn values for ryegrass pasture reported by authors performing studies in the same area. Botha (2003) reported an average DM content of 12.3% for ryegrass pasture in the spring over 3 years, and Malleson (2008) reported 13.7% DM for ryegrass pasture.

CP content reported in this study differed from that in other studies. Botha (2003) found values of 21.3% CP, whereas Malleson (2008) reported much higher values of 26.2%. The CP content of the ryegrass pasture evaluated in this study was intermediate, at 23.2%. These values all fell into the range of 18-25% CP as reported by Clark and Kanneganti (1998) for high quality temperate pasture species.

Metabolisable energy content of the pasture was in the same range as reported by other authors for ryegrass. An average values of 11.3 MJ ME/kg DM was reported by Botha (2003) and the ME reported by Malleson (2008) corresponded with this at 11.3 MJ ME/kg DM.

The NDF content of pasture grazed in this study was 47.2% which was within the range of 40-50% reported by Clark and Kanneganti (1998) for temperate grasses. NDF content of ryegrass in spring months reported in the literature was 46.3% (Malleson, 2008) to 48.1% (Botha, 2003).

Calcium content in the pasture was much lower than reported values from other studies. Ryegrass grazed in the studies of Botha (2003) and Malleson (2008) had a Ca content of 0.53% and 0.52% respectively, whereas the average Ca content in the pasture from this study was 0.37%. The P content of pasture in this study was also lower than the reported values in the studies of Botha (2003) and Malleson (2008). The ratio of Ca:P were no different in this study (1:1) than reported in Botha (2003), a slightly higher ratio of 1.28:1 was found in the ryegrass pasture grazed in the study of Malleson (2008).

3.2.2.2 Lucerne hay composition

The nutrient composition and therefore the quality of lucerne hay is largely dependent on factors such as time of harvesting, cultivar, climate, soil conditions, water supply and fertilization, leaf losses during mechanical processing, storage and feeding, disease and insects, weeds and moisture content during storage (Scholtz *et al.*, 2009). Lucerne hay quality can therefore be very variable and analysis of key components such as energy, protein and fibre content is critically important before formulating diets.

The nutrient concentrations of the lucerne hay supplemented to cows is shown in Table 3.2. The mean DM content was 89.2% which is similar to the mean value (92.7%) for South African lucerne hay as reported in the study of Scholtz *et al.* (2009). Graf *et al.* (2005) reported a DM content of 91% for grass hay and in the study of Reis and Combs (2000) lucerne hay with DM of 86.5% was used. The DM content of hay was naturally much higher than that of fresh high quality pasture.

CP was slightly lower at 21.6% than the CP concentration in the pasture, but fell within the same range. The protein content was high and comparable to the content of the lucerne hay used in the study of Reis and Combs (2000) who reported 21% CP. The mean CP content of South African lucerne hay is 20.7% (Scholtz *et al*., 2009).

The ME content in the lucerne hay was lower than that of the pasture. With high SR a lower ME intake would be expected by the supplemented cows compared to unsupplemented cows.

Neutral detergent fiber content of the hay was lower than that of pasture. The NDF concentration as chemically analysed, gives no indication of the physical effectiveness of fiber

however. The NDF concentration is in agreement with the mean value of 44.1% for South African lucerne hay as reported by Scholtz *et al*. (2009).

The Ca:P ratio of hay is 5.04:1 which is much higher than the ratio in the pasture and the hay would therefore most probably cause an improvement in the Ca:P ratio of the total diet.

The mixing time of the lucerne hay in the mixer wagon was regulated to ensure that the lucerne was not chopped too finely and would supply sufficient eNDF. The largest proportion of particles from the lucerne hay was therefore long. Using the Penn State Particle Size Separator the percentage of particles left on each sieve was determined for each of the five samples. Figure 3.2 a-c show the particle length of hay retained on the upper and middle sieve and bottom pan of the PSPS and the results are shown in Table 3.3.

Figure 3.2a Lucerne hay particles retained on upper sieve of PSPS

Figure 3.2b Lucerne hay particles retained on middle sieve of PSPS

Figure 3.2c Lucerne hay particles retained on bottom pan of PSPS

Table 3.3 Average percentage of lucerne hay particles on each of the upper - and middle sieve or bottom pan of the Penn State Particle Size Separator

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Average**	**Target for TMR**
% Upper sieve	37.0	38.0	35.0	37.0	32.0	35.8	> 6-10
% Middle sieve	21.0	18.0	17.0	19.0	23.0	19.6	30 - 50
% Bottom Pan	42.0	44.0	48.0	44.0	45.0	44.6	< 60

By using the PSPS we could ensure that the lucerne hay fed throughout the trial period had a constant particle length. A comparison between the proportions of lucerne hay retained on the each sieve of the PSPS with the targets suggested for TMR diets were made to give an indication of the effectiveness of the lucerne to promote rumination, chewing activity and overall rumen health. A lower proportion of particles were retained on the middle sieve than the suggested percentage for TMR diets, but the lower percentage on the bottom pan and the higher percentage on the upper sieve suggest that a big proportion of the particles were long and therefore we could assume that the lucerne hay supplied a reasonable amount of effective fiber.

3.2.2.3 Concentrate composition

Nutrient composition of the concentrate is shown in Table 3.2. The ME content of the concentrate was high at 13.3 MJ ME/kg which could be attributed to the high inclusion rate of ground maize at 80% (see Table 3.1). The concentrate had a low NDF content because no byproducts as sources of non-forage fiber were included and wheat bran inclusion was minimal.

Soybean oil cake as a protein source in the concentrate provided RUP. The CP content of the concentrate was 12.0%. From the studies of Schor and Gagliostro (2001) the CP content in this concentrate seemed to be adequate. With pasture already containing high levels of degradable N it would have been unneccesary to provide higher levels of protein.

3.2.2.4 Digestibility of diet components

In Table 3.2 te IVOMD of the different dietary ingredients is shown. The IVOM of pasture grazed in this study is similar to reported values for ryegrass found by Malleson (2008). Concentrate had a very high IVOMD which can be expected because of its high grain content. The lucerne hay, as expected, was not as digestible as pasture, but still had a reasonable IVOMD value at 72.4% and is comparable to values found by others (Stockdale, 1999; Teimouri Yansari *et al.*, 2004). The lower digestibility of the hay is also reflected in a lower ME of the hay (10.2 MJ/kg DM) compared to the pasture (11.3 MJ/kg DM).

3.2.3 Intake of dietary ingredients

3.2.3.1 Lucerne hay intake

Supplemental lucerne intake for individual cows was measured three times a week for the duration of the trial and the average intake for cows in each treatment was determined.

Cows receiving 1 kg of supplemental lucerne had an average daily lucerne intake of 0.96 kg ± 0.12 (n=352) and the cows receiving 2 kg had an average intake of 1.59 kg ± 0.39 (n=352) per day.

3.2.3.2 Concentrate intake

The concentrate supplementation rate was relatively conservative at 5 kg (as is) per cow per day. The cows therefore managed to consume the 2.5 kg of concentrate pellets allocated to each cow per milking time. The average concentrate intake per cow was 5 kg/cow/day.

3.2.3.3 Pasture intake measured with rising plate meter

The calculation of pasture intake as estimated with the RPM readings is given in Table 3.4. The mean pasture height before and after grazing over the trial period was determined from daily pasture measurements with the RPM. The average pasture yield over the entire trial period was then calculated by the regression equation y = 70.47x-212.4 (see section 3.2.1). Pasture yield was estimated at 2000 kg/ha and the average pasture removed was estimated at 1536 kg/ha.

From the estimated figures for pasture yield and pasture removed, the average DM available per grazing strip could be estimated before and after grazing and therefore the DM intake of the group of cows. The assumption made using this method of pasture intake calculation was that all cows had equal opportunity for pasture intake and that they did consume the same amount of pasture each day. The average individual intake could therefore be estimated by dividing the daily pasture removed by the amount of cows on the pasture.

The average daily intake from pasture was calculated to be 7.42 kg/cow/day. This seems unrealistically low and cows would not have been able to maintain body weight and condition had this been the actual DMI. Previous research done with the RPM as an estimator of pasture intake has shown that it is an unreliable method and usually under predicts the pasture intake (Malleson, 2008). The assumption of equal pasture intake between groups is also unlikely and substitution of pasture is expected with supplementation of lucerne hay. The pasture intake of the cows in the control group, therefore is expected to be higher than the intake of the cows fed lucerne supplement.

Table 3.4 Pasture intake calculations using the rising plate meter for cows of all three treatments

Pasture height before grazing	31.73 ± 4.53 (n=73)
Pasture height after grazing	10.83 ± 1.68 (n=73)
Yield (kg/ha)	2000.48 ± 332.63 (n=73)
Left after grazing (kg/ha)	464.44 ± 123.66 (n=73)
Total pasture removed (kg/ha)	1536.04 ± 258.33 (n=73)
Average daily pasture intake (kg/cow)	7.42

3.2.3.4 Pasture intake measured using the back calculation technique

The NRC (2001) formulation software was used to calculate the energy requirements of an average cow within each treatment group. The inputs for each group are given in Table 3.5.

The energy requirements estimated by the NRC (2001) software are shown in Table 3.6. The daily concentrate and lucerne intake is known and therefore the energy supplied by concentrate and lucerne could be calculated. The energy supplied by pasture is calculated by subtracting the energy supplied by the concentrate and lucerne from the total energy requirement. Pasture intake, total intake and intake as percentage of body weight could therefore be estimated and is given in Table 3.6.

Pasture intake was estimated to be higher in the control group than in the other two treatments. The estimated intake values were 9.06 kg, 8.60 kg and 9.59 kg for the LL, HL and control groups respectively. These values were more realistic than the intake values estimated with the RPM and agreed with finding of Reis and Combs (2000) who reported pasture intake of 10.8 kg/d for grazing cows receiving concentrate and 8.2 kg/d for grazing cows receiving concentrate and supplemental hay.

Supplemental hay seemed to increase total intake which agrees with findings of Stockdale (1999) but is different from what is reported in other studies (e.g. Mayne and Wright, 1988; Stockdale, 2000; Reis and Combs, 2000). Roughage supplementation is assumed to cause a high SR (Mayne and Wright, 1988; Stockdale, 2000; Reis and Combs, 2000) and therefore the substitution in this study was also expected to be high. The substitution rate in the LL and HL groups was estimated to be 0.55 kg/kg and 0.62 kg/kg respectively, which is much lower than SR reported by Reis and Combs (2000) and higher than the 0.33 kg/kg reported by Stockdale (1999).

The intake as percentage of body weight was within an acceptable range for all treatment groups with estimated values of 3.66 %, 3.81 % and 3.84 % for the control, LL and HL treatment respectively.

Table 3.5 Inputs used for the calculation of energy requirements using the NRC (2001) formulation software

	Treatments[1]		
Animal Input	**Control**	**LL**	**HL**
Lactation	4	4	4
Current age (months)	66	66	66
Age at first calving (months)	24	24	24
Calving interval (months)	12	12	12
Current weight (kg)	399	395	395
Mature weight (kg)	399	395	395
BCS	2.4	2.3	2.4
DIM	119	124	121
Milk production (kg/d)	21.6	21.8	21.8
Milk fat %	4.2	4.31	4.39
Milk protein %	3.6	3.6	3.5
Lactose %	4.68	4.73	4.49
Environmental Input			
Temperature (°C)	20.4	20.4	20.4
Coat condition	Clean/Dry	Clean/Dry	Clean/Dry
Heat stress	None	None	None
Distance walked from pasture to milking parlour (m)	1000	1000	1000
Topography	Flat	Flat	Flat

[1]Control = no lucerne hay; LL = 1 kg lucerne hay; HL = 2 kg lucerne hay
BCS = body condition score; DIM = days in milk

Table 3.6 Calculation of pasture intake and total intake from energy requirements estimated using the NRC (2001) software

	Treatments[1]		
Requirements	**Control**	**LL**	**HL**
ME for maintenance (MJ/d)	15.09	14.98	14.98
ME for lactation (MJ/d)	109.2	111.8	110.5
ME for BCS change (MJ/d)	3.51	5.14	7.57
Total ME requirement (MJ/d)	175.8	179.6	180.7
Provided in diet			
ME from Concentrate (MJ/d)	(66.50)	(66.50)	(66.50)
ME from Lucerne (MJ/d)	(0.00)	(9.80)	(16.2)
ME from Pasture (MJ/d)	109.3	103.3	98.00
Pasture intake (kg/d) (ME*11.4MJ/kg)	9.59	9.06	8.60
Total intake (kg/d)	14.59	15.02	15.19
Intake as % BW	3.66	3.81	3.84

[1]Control = no lucerne hay; LL = 1 kg lucerne hay; HL = 2 kg lucerne hay
ME = metabolisable energy

3.2.3.5 Total Nutrient intake

Table 3.7 show the daily nutrient intake for all three treatment groups as was calculated from the nutrient composition of pasture, lucerne hay and concentrate (Table 3.3) and the NRC (2001) estimations for pasture and total intake (Table 3.7). Crude protein, ME, NDF, ADF and P intake of all three treatments fell within the acceptable ranges as proposed by Erasmus *et al.* (2001).

A higher pasture intake for the LL than for the HL group would explain the higher intake of most of the nutrients for the LL group. Substitution rates of 0.55 kg/kg and 0.62 kg/kg for the LL and HL groups respectively caused a higher total intake for these groups and therefore the intake of most nutrients, with ME and fat being the exceptions, were higher in these groups than in the control.

Lucerne supplementation seemed to increased the fiber intake in supplemented groups, but a higher NDF intake for the LL treatment than the HL treatment suggests that the higher fiber intake for supplemented groups may largely be attributed to higher intake of pasture, because pasture NDF % is 47.2% with lucerne hay NDF concentration being only 43.0%. ADF intake in the HL treatment was higher than in the LL treatment, which could be expected because lucerne hay has a higher ADF concentration (36.5%) than the pasture (28.45%).

The fat content of the diets for all treatments may have been a bit low compared to the recommended fat percentages of 5.0-7.0 % suggested by Erasmus *et al.* (2001). This recommendation however is aimed more towards a maximum inclusion level as opposed to a minimum requirement. The fat content of pasture (3.9% EE) was higher than that of lucerne hay (1.84% EE) and the higher fat intake in control cows which had the highest pasture intake may therefore be expected.

Although the P content of diets in all three treatments was adequate, the Ca was lower than the 0.8-1.0 % suggested by Erasmus *et al.* (2001). The latter recommendation, however, is for high producing cows in early lactation consuming a TMR. The mean Ca level of 0.65% is in agreement with the NRC recommendations (NRC, 2001) This Ca level, however, caused a low Ca:P ratio in all three treatments with ratios of 1.5:1, 1.6:1, and 1.8:1 calculated for the control, LL and HL groups respectively. The Ca:P ratio can however be easily rectified by changing the mineral profile of the concentrate. Lucerne hay supplementation seemed to increase the Ca content of the diet, with the highest Ca intake observed for the HL treatment group.

Table 3.7 Mean nutrient profile of the total diet (pasture, concentrate and lucerne hay) fed to cows consuming the control, LL and HL diets

	Treatment[1]		
Nutrient (%DM)	**Control**	**LL**	**HL**
CP	19.4	19.4	19.4
ME (MJ/kg)	12.1	12.0	11.9
NDF	34.2	34.3	34.3
ADF	20.0	20.7	21.1
EE	3.49	3.37	3.29
Ca	0.59	0.64	0.67
P	0.39	0.39	0.38

[1]Control = no lucerne hay; LL = 1 kg lucerne hay; HL = 2 kg lucerne hay
CP = crude protein; ME = metabolisable energy; NDF = neutral detergent fibre; ADF = acid detergent fibre; EE = ether extract; Ca = calcium; P = phosphorous

3.2.4 Body weight and condition score change

The change in body weight and body condition score is shown in Table 3.8. There were no significant differences ($P > 0.10$) in the starting weights of cows receiving the control, LL and HL diets, with weights being 391kg, 388 kg and 383 kg respectively.

The end weights of cows for the control, LL and HL treatments were 407 kg, 401 kg and 408 kg respectively and there were no differences between these weights ($P > 0.10$).

The cows in the HL treatment group gained 25.5 kg over the trial period which was higher ($P < 0.05$) than the weight gain of 13.0 kg for the LL supplemented cows. There was also a trend ($P = 0.055$) towards higher weight gain in the HL treatment compared to the control group with gain in the control group being 15.25 kg.

Energy intake between groups (Table 3.7) could not satisfactorily explain the difference in weight gain. The HL treatment cows gained the most weight over the trial period, although the energy contents of the dietary treatments were very similar. It is possible that the HL treatment group had a better energy balance, and therefore gained more weight. One should, however, keep in mind that the cows were only weighed four times (twice at the start and twice at the end of the trial) and that many factors, such as fetal growth and water intake, may influence the weights and cause some error. As a % of BW these changes were biologically insignificant.

Table 3.8 The effect of lucerne hay supplementation on body weight (kg) and BCS change of cows grazing kikuyu-ryegrass pasture and receiving 5 kg of concentrate per day

	Treatments[1]				
Item	**Control**	**LL**	**HL**	**SEM**	**P-value**
Body weight (kg)					
Start	391	388	383	9.43	0.803
End	407	401	408	8.50	0.831
Change	$+15.3_{c}$	$+13.0^{a}$	$+25.5^{b}{}_{d}$	3.64	0.047
Body condition score					
Start	2.30	2.23	2.23	0.100	0.878
End	2.42	2.42	2.59	0.115	0.486
Change	+0.13	+0.19	+0.36	0.113	0.331

[1]Control = no lucerne hay; LL = 1 kg lucerne hay; HL = 2 kg lucerne hay

[ab] Means in the same row with different superscripts differ at ($P < 0.05$)

[cd] Means in the same row with different subscripts differ at ($0.05 < P < 0.10$)

SEM = standard error of means

There were no differences ($P > 0.10$) in body condition score (BCS) between treatments at the beginning or end of the experiment. The BCS change between the control (+0.13), LL group (+0.19) and HL group (+0.36) also were no different ($P > 0.10$).

3.2.5 Milk production and composition

3.2.5.1 Milk production

The average daily milk production during the trial (60 days) is illustrated as five different 12 day periods in Figure 3.3. There were no differences in milk productions between treatments during any of these periods ($P > 0.10$) and the lactation curve followed the same trend.

The average days in milk at the start of the trial were 89, 94 and 91 days for the control, LL and HL treatments respectively. A peak in milk production was observed between 29 August and 9 September for all the treatment groups, which corresponded with the expected 100 day peak in production. A steady decline in milk production after the initial peak was observed, which was in

agreement with the decline in production after peak lactation as predicted by the general lactation curve for dairy cows (Erasmus *et al.*, 2001).

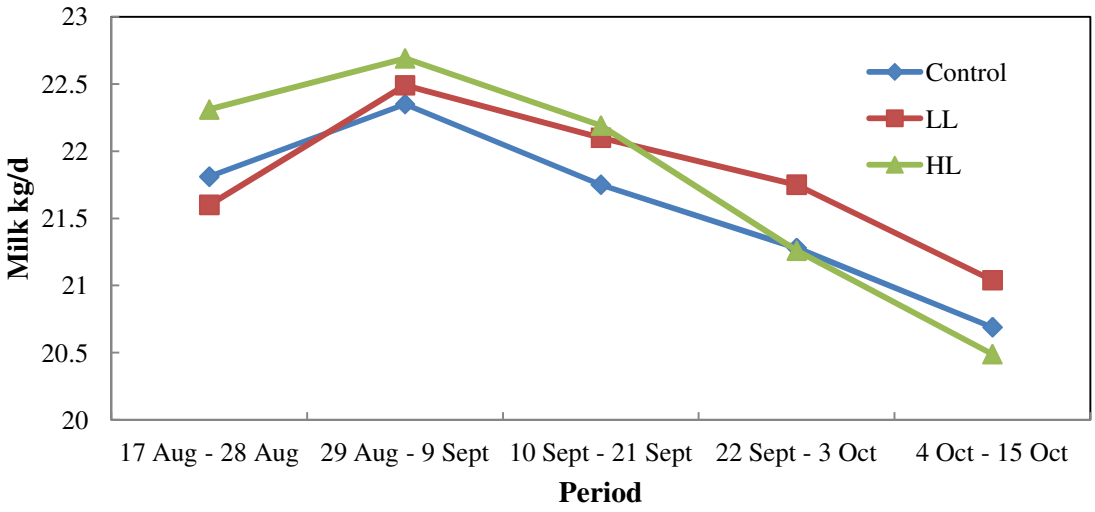


Figure 3.3 Mean daily milk production for cows receiving no supplemental lucerne, cows receiving 1kg of supplemental lucerne and 2 kg lucerne

The production parameters for the cows receiving the control, LL and HL treatments are shown in Table 3.9. There were no differences ($P > 0.10$) between treatments for average daily milk production or 4 % FCM over the trial period.

The lack of a response in milk production when hay is supplemented is in agreement with results reported in other studies (Rearte *et al.*, 1986b; Reis and Combs, 2000; Graf *et al.*, 2005). All these studies differed from the present study in that there were less grazing pressure and therefore higher post-grazing heights. In the study of Graf *et al.* (2005) an average post grazing height of 12 cm (reading of 24 on RPM) was recorded and pasture allocations in the studies of Rearte *et al.* (1986b) and Reis and Combs (2000) were much higher than in the present study. Stockdale (1994) reported that supplementation of maize silage to grazing cows only had a positive effect on milk production with low PA, but there was no response in milk production at higher PA. The substitution rates in all these roughage supplementation studies were high, but did not significantly influence total DMI. Therefore it seems that roughage supplementation does not improve milk production when excess pasture is available and cows are able to meet energy requirements.

There are exceptions to these findings in the literature. Rearte *et al.* (1986a) reported an increased in milk yield with long hay supplementation, but no difference in FCM production was reported. Stockdale (1999) found that milk production improved with lucerne hay supplementation in spring for cows grazing pasture composed mainly of white clover and ryegrass. The pasture in

the study of Stockdale (1999) had a lower ME value (10.3MJ/kg), NDF concentration (41.5%) and IVOMD (72.4%) than the ryegrass pasture grazed in the present study. Stockdale (1999) also reported that the cows receiving lucerne hay was highly selective and that the ME intake from hay was probably much higher than the 8.9 MJ ME/kg DM expected.

High SR with high quality roughage supplementation resulted in no milk response in the study of Graf *et al.* (2005). These authors reported no significant change in nutrient intake and therefore no response in milk production. It may therefore be surprising that in the present study no response in milk production was observed, because a low SR and therefore higher total intake for supplemented cows were expected to cause an increased production.

Table 3.9 Response in milk production and milk composition to supplementation of lucerne hay to cows grazing kikuyu-ryegrass pasture and receiving 5 kg of concentrate per day

	Treatments[1]				
Item	**Control**	**LL**	**HL**	**SEM**	**P-value**
Milk yield (kg/d)	21.6	21.8	21.8	0.46	0.932
4% FCM (kg/d)	22.2	22.5	22.9	0.53	0.666
Protein %	3.60	3.59	3.48	0.053	0.255
Kg protein	0.78	0.77	0.75	0.017	0.492
Butterfat %	4.20	4.31	4.39	0.150	0.677
Kg butterfat	0.90	0.92	0.95	0.030	0.588
Lactose %	4.68[a]	4.73[a]	4.49[b]	0.033	< 0.0001
Kg milk solids	1.68	1.69	1.70	0.043	0.951
MUN (mg/dL)	15.8	16.7	16.0	0.457	0.404
SCC (thousand cells/ml)	234	162	270	57.6	0.417

[1]Control = no lucerne hay; LL = 1 kg lucerne hay; HL = 2 kg lucerne hay

[ab] Means in the same row with different superscripts differ at $P < 0.05$

4% FCM = four percent fat corrected milk productionSEM = standard error of means;

MUN = milk urea nitrogen; SCC = somatic cell count

Milk yield in the present study averaged 21.7 kg/d over treatments, which was higher than values reported by Graf *et al.* (2005) and lower than values reported by Reis and Combs (2000). The reason for the differences is that the cows in the study of Graf *et al.* (2005) did not receive any concentrate and concentrate is known to increase energy intake of cows on pasture and would

therefore increase milk production (Meeske *et al.*, 2006). In the study of Reis and Combs (2000) Holstein-Fresian cattle was used which has the genetic ability to produce more milk than Jersey cows. The milk production reported in this study is acceptable for the stage of lactation and similar to that reported by Malleson (2008) who conducted a study under the same conditions using Jersey cows grazing annual ryegrass.

3.2.5.2 Milk composition

Table 3.9 shows milk composition parameters for cows receiving control, LL and HL diets. Milk protein percentage and yield as well as butterfat percentage and yield showed no difference between treatments ($P > 0.10$).

The lack of response in milk fat is surprising as it is generally assumed that the fat content is susceptible to any changes in the structural fiber content of the diet (Mertens, 1997). That, however, would only be true if milk fat was depressed and in this study there is no indication of milk fat depression. Other authors, however, also reported no milk fat response with roughage supplementation to cows grazing highly digestible pasture (Stockdale, 1999; Ferris *et al*., 2000; Reis and Combs, 2000; Wales *et al*., 2001; Wales and Doyle, 2003; Graf *et al.*, 2005).

The only authors who reported a different milk fat response was Rearte *et al*. (1986a), they found a decrease in milk fat percentage which was most likely due to higher milk yield with long hay supplementation.

Milk protein depression occurs after a severe deficiency in protein supply or if a significant amino acid imbalance is rectified by high supplementation levels of undegradable protein, increased microbial protein synthesis or supplementation of rumen protected amino acids. None of these condition existed in this study and therefore no change was seen in milk protein levels when lucerne hay was supplemented to cows. Milk protein responses to roughage supplementation in the literature also agree with results in the present study. Hay supplementation did not have any effect on milk protein content in the studies of Rearte *et al*. (1986a), (1986b), Reis and Combs (2000), Stockdale (1999) or Wales *et al*. (2001).

The percentage lactose in the milk from HL cows (4.49 %) was lower ($P < 0.05$) than that of LL cows (4.73 %) and of control cows (4.68 %). Lactose content of milk is generally assumed to remain unchanged with changes in the diet (Varga and Ishler, 2007). No reports in the literature could be found on changed lactose percentage in milk with roughage supplementation to grazing cows. There is also no reason to suspect a much lower supply of propionic acid between any of the treatments, since the level of concentrate supplementation was the same.

There were no differences (P > 0.10) in MUN or SCC between treatments. The MUN of all three treatments fell within the acceptable range of 12 – 18 mg/dl. The pasture and lucerne hay had comparable CP and ME content as seen in Table 3.2. The MUN values reflect that the relationship between protein and energy intake of different treatment groups was similar and that efficiency of utilization of nitrogen for microbial protein synthesis was acceptable.

The lack of any difference in production performance suggest that supplementation with 2.5 kg concentrate twice per day did not cause an unfavourable rumen environment which could lead to milk fat depression, reduced microbial synthesis or subclinical acidosis. The extra buffering and peNDF supplied by the lucerne hay , therefore, was not necessary under the conditions of this study. Futhermore, the substitution effect of lucerne hay was not sufficient to cause a difference in energy intake and therefore milk production. Additional lucerne hay might be beneficial at higher levels of concentrate intake.

CHAPTER 4

EFFECT OF LUCERNE HAY SUPPLEMENTATION ON THE RUMEN ENVIRONMENT OF COWS GRAZING KIKUYU/RYEGRASS PASTURES

4.1 MATERIALS AND METHODS

4.1.1 Location, climate and soil

The location, environmental conditions and soil type was the same as discussed in section 3.1.1

4.1.2 Duration of the experimental period

The rumen study was conducted in parallel with the production study. The experimental period was from 3 August 2010 to 15 October 2010. The cows were allowed to adapt during the first 14 days, followed by a 5 day measurement period, then they were switched over and the process was repeated. The selection and blocking of cows included in the rumen study was done during July 2010.

4.1.3 Cows, feeding and management

Eight lactating rumen fistulated Jersey cows from the Outeniqua Research Farm herd were randomly allocated to either the control or the 2 kg lucerne hay (HL) supplement treatment. The groups were balanced according to days in milk (DIM), milk production and lactation number. The cows from the rumen study grazed together with the cows from the production study. The management and feeding of these cows were exactly the same as the cows from the production study. See sections 3.1.4.3 – 3.1.4.5 for further detail.

4.1.4 Data collection and ruminal pH logging system

Data was collected during a 5 day sampling period. After the cows were adapted to the dietary treatments for 14 days, an automated pH logging system was used to collect information on rumen pH changes. The pH was measured in 10 minute intervals for 96 hours by inserting TruTrack Data Loggers (Model pH-HR mark 4, Intech instruments LTD, New Zealand) in the rumen. Systems were calibrated before each measurement period, using Omnilog Version 1.64 software (Intech instruments LTD). Data was then downloaded from the loggers with the same software program.

4.1.5 Rumen samples

Once the data loggers were removed from the cows' rumens, rumen fluid sampling started. Samples were taken by using a modified drain pump (see Figure 4.1) with a collection bottle attached at the end (see Figure 4.2). Sampling was done at 08:00, 14:00, 20:00, and 02:00.

The rumen fluid samples were taken for analyses of rumen ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) concentrations as well as rumen pH. The pH was measured with a portable pH meter (WTW pH 340i pH data meter/data logger connected to WTW SenTix41 pH electrode) directly after the rumen fluid was collected. Portable pH meters were calibrated before each measurement period by using buffers, pH 4 and pH 9.

The rumen samples were strained through two layers of cheese cloth to remove solid feed particles from the rumen liquor. Samples were then preserved for the two different analyses by adding 15 ml of rumen fluid to 2.5ml of a 50% H_2SO_4 solution for NH_3-N analyses and 18ml of rumen fluid to 2ml of a 25% H_3PO_4 solution for VFA analyses.

All the samples were kept in 20ml plastic bottles. The bottles were clearly marked with the time, date, cow name and type of sample (NH_3-N or VFA) and were frozen immediately. There were 8 samples per cow for each of the four sampling times, resulting in 128 samples in total for both sampling periods. Samples were analysed at the UP Nutrilab (University of Pretoria, Hatfield Campus, Agricultural Building, Floor 10, Pretoria, 0002).

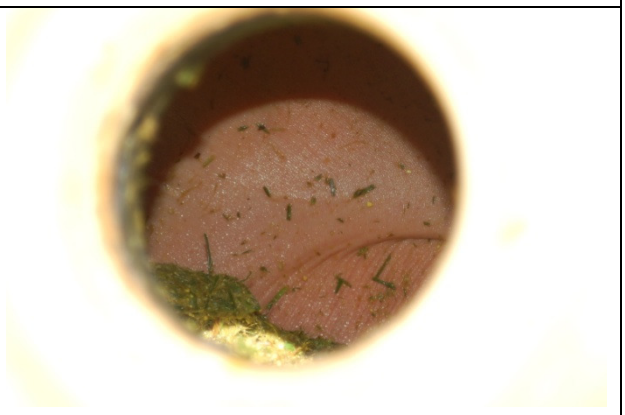
Figure 4.1 Janke van der Colf and August Lingnau collecting rumen fluid with a modified drain pump

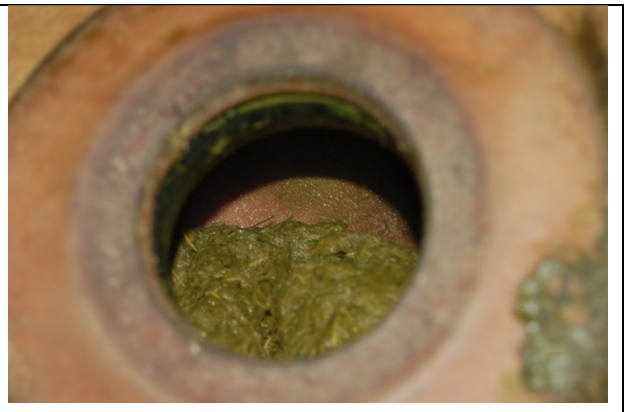
Figure 4.2 Plastic collection bottle attached to the end of the drain pump used for rumen fluid collection

4.1.6 Rumen fill investigations

A visual scoring system was used during the rumen sampling. At each of the sampling times, 08:00, 14:00, 20:00, and 02:00 a score was awarded to each cow according to how full her rumen was. A score of 1, 1.5, 2, 2.5 or 3 could be awarded and Table 4.1 describes the criteria of each of the scores.

Table 4.1 Criteria and photograph describing each level of the rumen score used to evaluate rumen fill

Rumen score	Criteria	Photo
1	Most of rumen wall exposed	
1.5	Significant part of rumen wall exposed	

2	Some part of rumen wall exposed	
2.5	Very little of rumen wall exposed	
3	Filled to capacity	

From the information gained while doing the rumen visual scoring, 14:00 was identified as a representative time to determine average rumen fill. Rumen fill was measured by manual evacuation of rumen content (Huhtanen *et al.*, 2007). The rumen content of all eight cows was removed and the total weight and volume was recorded for each cow. Two representative samples were taken from the rumen content of each cow, weighed (Satorius L420P scale, capacity 420g, error 0.001g) and dried in an oven at 60°C for 72 hours after which it was weighed again to determine the DM. The dried samples were then milled through a 1mm sieve and the two samples for each cow were pooled. The ash content of each sample was analysed in duplicate (AOAC 2000,

procedure 942.05) and the percentage organic matter (OM) in the rumen content could be determined and expressed on the basis of kg OM per kg metabolic live weight ($W^{0.75}$).

4.1.7 *In sacco* DM and NDF disappearance of ryegrass

An in sacco study was conducted to determine dry matter (DM) and neutral detergent fibre (NDF) disappearance of ryegrass. Three kilograms of ryegrass was cut at a height of 30 mm with 1.2 ton DM/ha of grass available above 30mm. The grass was then dried at 60˚C for 72 hours (Botha *et al*., 2007) and cut with scissors in 5 - 10 mm pieces (Taweel *et al*., 2004).

Fifty one dacron bags measuring 10cm by 20cm in size and with a pore size of 53 micron were marked with a cow name and bag number (six bags per cow and three zero time bags). The bags were placed in an oven at 60˚C for 72 hours and weighed. Five grams of the cut grass was accurately weighed out and put into each dacron bag. Each bag plus the grass was then weighed again before closing the ends with a cable tie. The bags plus cable tie were then weighed again so that the exact weight of the incubated bag was known.

Three samples of the cut grass were weighed and placed in an oven at 60˚C for 72 hours and then weighed again to calculate a correction factor for the moisture uptake from the environment with which the weight of grass in the Dacron bags could be corrected.

Six bags were then placed into a 44 decitex stocking (Cruywagen, 2006), with three bags per leg. A big glass marble, weighing an average of 52 g, was placed in the bottom of each stocking leg to weigh the bags down and prevent them from floating on top of the rumen content. A knot was made between each bag to separate them. Each stocking was then thightly tied to a numbered cannula plug to be inserted into the rumen of the cow (see Figure 4.3).

The bags were inserted after morning milking. Twelve hours after the bags were inserted into the rumen, three bags were removed from each cow by cutting one of the stocking legs and placing the plug with the remaining leg back into the rumen. The bags were rinsed after removing them from the stocking and the bag number, cow name and time of removal of each bag were recorded. The bags were then placed in a freezer to prevent any further microbial activity. After a further 12 hours the remaining bags were removed, 24 hours after incubation. The bags were rinsed and the bag number, cow name and time of removal were also recorded.

Both the 12 hour bags and the 24 hour bags were then washed with cold water in a washing machine (DEFY Twinmaid, DTT 131, DEFY Appliances (PTY) LTD, 135 Teakwood Road, Jacobs, 4052, Durban, South Africa). Bags were washed for an average of 15 minutes until the

water that drained was clear. The bags were spun to remove excess water and were then dried at 60˚C for 72 hours. After drying the bags were removed from the oven, six at a time, and weighed immediately. The three zero time bags were washed, dried and weighed in exactly the same manner as the other bags, and were used to determine DM loss at zero hours.

After the DM disappearance of each bag was calculated, the residues were pooled to give one sample for 12 hours and one for 24 hours per cow. These samples were milled through a 1 mm sieve and placed in airtight plastic bottles, clearly marked and kept in the freezer until it could be analysed for NDF content at the UP Nutrilab (University of Pretoria, Hatfield Campus, Agricultural Building, Floor 10, Pretoria, 0002). A representative sample of the cut grass before incubation was also kept to analyse the initial NDF of the samples and determine disappearance of NDF for each of the times.

The procedure was exactly replicated for the second incubation period once the cows were switched over and adapted again.

Figure 4.3 Stocking containing six nylon bags and tied to cannula plug is inserted into the rumen of a cow

4.1.8 Experimental design and statistical analysis

The experimental design was a switch over design, with two treatments. A repeated measures analysis of variance with the GLM model was used to determine treatment effects at different collection times (SAS, 2008).

4.2 RESULTS AND DISCUSSION

4.2.1 Ruminal pH

4.2.1.1 Results from pH data loggers

The variation in rumen pH over 24 hours for the control and HL treatment are shown in Figure 4.4. This is the average pH of both experimental periods taken at 10 minute intervals over eight days (four days per period) and pooled for every 30 minutes.

The pH results show distinct diurnal variation for both treatment groups and is influenced by grazing behaviour and feeding patterns throughout the day. The pattern of the graph follow the same trend as in the study of Coetzee (2011), which was conducted under the same conditions as the present study. The lowest rumen pH values for both groups were recorded during the day which is in agreement with the findings of Wales and Doyle (2003) and Graf *et al.* (2005). The low daytime pH coincides with the two major periods of grazing which occur after milking times each day. The rumen pH in both groups show steady increase from 21:00 to 05:00, this is the period when cows are assumed to have spent most of the time ruminating. The flow from saliva when cows ruminate has a buffering effect on the rumen pH (Allen, 1997).

Rumen pH showed a decline after 05:00 in both treatments, which continued up to 11:00. This decline can be attributed to the effect of the highly fermentable energy concentrate being fed during milking at 06:00 and aggressive grazing behaviour as cows returned to the pasture after milking. Both these factors contributed to an increased rate of ruminal fermentation in both groups.

A trend towards higher pH values for the control group between 07:00 ($P = 0.059$) and 08:00 ($P = 0.074$) was found, but only the 07:30 pH value of the control were significantly higher ($P = 0.039$) compared to HL treatment group. The difference in pH between the two treatment groups at these times is difficult to explain, as both groups received the same amount of concentrate at milking and the effect of the supplemental lucerne on rumen pH would not be expected to decrease rumen pH. A possible explanation may be that the cows receiving lucerne hay decreased the time

spent grazing when returning to pasture after morning milking and that the control cows therefore spent more time eating and the subsequent flow of saliva to the rumen buffered the negative effect of the concentrate fermentation on rumen pH. The pH of the control cows at 09:30 (P = 0.064) and 10:30 (P = 0.053) also tended to be higher than the HL group, which indicate that the probable influence that supplementation had on changes in grazing behaviour had a prolonged effect.

Figure 4.4 show that the difference in rumen pH between groups disappeared after 12:00 when buffering due to rumination likely started to play a role in both groups. There were no differences (P >0.10) in rumen pH between the treatment groups for the remainder of the measurement period. Taking into account the full 24 hour period, the relative small difference in rumen pH between 07:00 and 12:00 is of no real biological significance.

Rumen pH started to decline again in both groups between 14:00 and 15:00 which coincides with the afternoon milking time. Concentrate feeding and increased grazing activity can explain an increase in rumen fermentation at these times. The pH only started to increase again after 20:00, when cows probably spent more time ruminating than grazing.

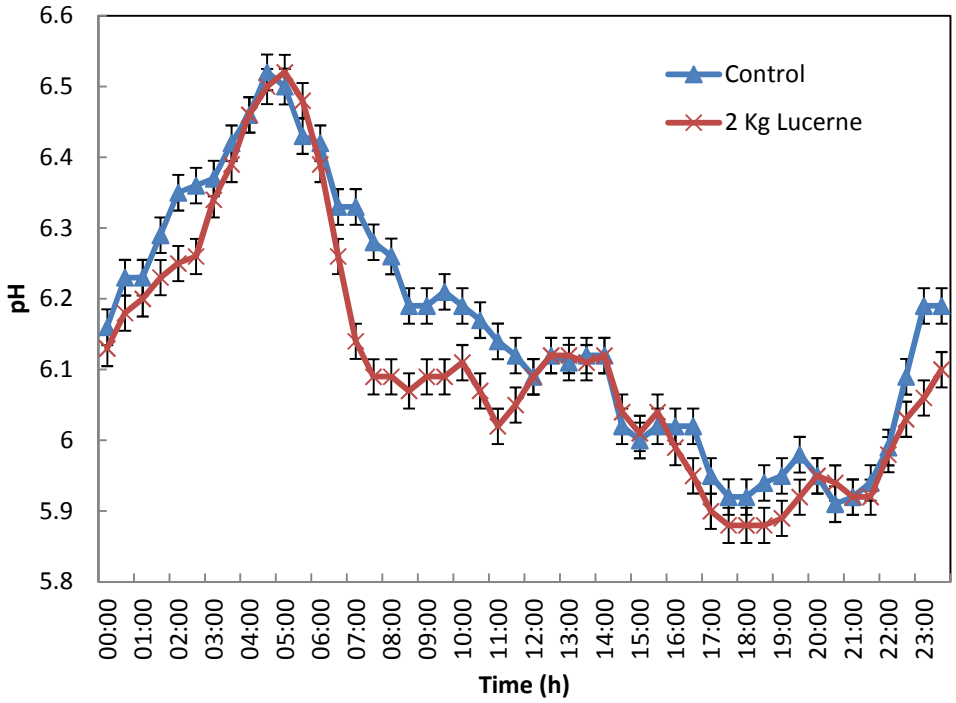


Figure 4.4 Ruminal pH results measured over 24 hours for cows that received 2 kg of supplemental lucerne and cows that received no supplemental lucerne

The average time (in minutes) that the ruminal pH was below 6.00 and 5.80 is shown in Table 4.2 for both treatments. The critical pH for ruminants The overall mean pH for the 24 hours is also given in Table 4.2. There were no differences in time below pH 6.00 or pH 5.80 between the control and the HL treatment (P <0.10). The overall mean pH over 24 hours did also not differ between the treatments (P <0.10).

The rumen fluid pH that defines sub-acute rumen acidosis is a controversial subject, with various threshold values suggested, ranging from 5.5 to 6.0. The time for which the pH must remain below this threshold to be regarded as causing sub-acute rumen acidosis has not been properly defined (Gozho *et al.*, 2005). Although there were no treatment differences observed in the overall mean pH over 24 hours, a significant amount of time was spent below pH 6 for both treatments when comparing to results from Graf *et al.* (2005). The difference is most likely due to concentrate fed in the present study, which contributed to lower pH values. This effect was predicted by Graf *et al.* (2005), who speculated that lower pH values may be expected with concentrate feeding compared to full time grazing. The lack of an effect of supplemental roughage on low pH, however, agrees with the findings of Graf *et al.* (2005) and Wales and Doyle (2003) who also did not manage to find any improvement in pH with roughage supplementation. Graf *et al.* (2005) reported that full-time grazing did not cause severely low pH for extended periods. Graf *et al.* (2005) and Wales and Doyle (2003) both concluded that supplementing dry roughage to increase the intake of effective fiber may not provide the expected benefit of increasing rumination and buffering in the rumen.

Table 4.2 Average time (in minutes) during a 24 hour period that the ruminal pH was below 6.00 and 5.80, as well as the overall mean ruminal pH during this period, for cows receiving 2 kg supplemental lucerne and cows receiving no supplemental lucerne

	Treatments[1]			
	Control	**HL**	**SEM**	**P-value**
Time below pH 6 (min/d)	410	506	102	0.534
Time below pH 5.8 (min/d)	190	126	99.5	0.672
Overall mean pH	6.18	6.11	0.058	0.430

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

SEM = standard error of means

4.2.1.2 Results from portable pH meter

The ruminal pH measured by the portable pH meter directly after rumen fluid collection is shown in Table 4.3. There was no difference (P <0.10) between the values for the control and the HL group for any of the collection times. These results may support the theory of Graf *et al.* (2005) who suggested that punctual pH data collected with rumen samples may be insufficient to indicate fiber effectiveness, because these point measurements do not give any indication of diurnal variation.

Table 4.3 Ruminal pH measured with a portable pH meter at four collection times for cows receiving 2 kg supplemental lucerne and cows receiving no supplemental lucerne

	Treatments[1]			
Ruminal pH measured at :	**Control**	**HL**	**SEM**	**P-value**
08:00	6.50	6.31	0.074	0.127
14:00	6.31	6.26	0.026	0.278
20:00	5.94	5.88	0.063	0.531
02:00	6.45	6.46	0.048	0.847
Overall means	6.30	6.23	0.037	0.217

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

SEM = standard error of means

4.2.2 Rumen ammonia nitrogen concentrations

The mean rumen NH_3-N concentrations (mg/dL) for each treatment group at four collection times are shown in Table 4.4 and Figure 4.5. The only difference (P <0.05) in NH_3-N concentration between the control and the HL group occured at 08:00. The average NH_3-N concentration in HL group at this time was 10.46 mg/dL and 6.02 mg/dL in the control group. There were no differences (P >0.10) in NH_3-N concentrations between the two groups for any of the other collection times or for the overall mean.

Results from the present study indicate the same trend as in the study reported by Graf *et al.* (2005). The fluctuation in NH_3-N concentration is dependent on dietary protein concentration, rate of ruminal protein degradation, availability of energy substrate and changes in grazing behaviour and resulting changes in protein intake can partly explain differences in NH_3-N concentrations throughout the day.

Higher concentrations of NH_3-N observed at 08:00 for cows receiving lucerne hay supplementation can be explained by the high CP (21.6%) content of the lucerne hay which was given just after morning milking at 06:00. Furthermore the rumen degradability of proteins in lucerne hay is high as reported by Cronje (1983) (79.7%) and Cruywagen *et al.* (2011) (73-77%). The high degradability of proteins in the rumen can contribute to high NH_3-N levels. Peltekova and Broderick (1996) reported a ruminal protein degradability of 44.3% for lucerne hay, with a fractional degradation rate of 0.103/h. Lower NH_3-N concentrations may be expected in control cows, because for practical reasons they did not return to pasture after morning milking before rumen fluid samples were collected. The supplemented cows that received lucerne hay after milking therefore had the opportunity to consume more protein which may have influenced the NH_3-N concentration at this time. Furthermore one can deduce from the rumen fill investigation (see section 4.2.4) that pasture intake was low during the night because cows' rumens were relatively empty in the mornings. Lower NH_3-N concentration in the rumens of control cows may therefore be expected in the morning.

For the rest of the day we can assume that protein intake between supplemented cows and unsupplemented cows were similar and therefore no differences were observed in NH_3-N concentration for any of the other collection times or for the average total concentration. This is in agreement with the findings of Reis and Combs (2000) and Wales and Doyle (2003). The assumption that cows from the control group compensated for intake with more aggressive grazing at returning to pasture and a relatively high SR (see section 3.2.3.5) may explain the lack of a response in NH_3-N concentration for any of the other collection times.

The highest daily NH_3-N concentration was observed at 20:00 for both treatments, about 6 hours after afternoon milking. This was also the time at which cows were the fullest (see section 4.2.4) and aggressive pasture intake can be assumed to have occurred before this time and would explain high NH_3-N concentrations. The NH_3-N concentration is seen to decrease gradually after 20:00 and the lowest concentration was observed at 02:00 for both treatments. Nocturnal grazing patterns may explain this observation and from the rumen fill investigation (see section 4.2.4) we can conclude that cows grazed less aggressively at night and likely spent more time ruminating.

High energy concentrate fed during milking supplies highly fermentable carbohydrates which are important for protein degradation by the rumen microbes, the gradual increase in NH_3-N concentration after each milking time, at 06:00 and 14:00, can therefore be expected as the rate of protein degradation is assumed to increase after these times.

Table 4.4 Ruminal NH_3-N (mg/dl) for cows receiving 2 kg supplemental lucerne and cows receiving no supplemental lucerne measured at four collection times

	Treatments[1]			
Ruminal NH_3-N (mg/dL) measured at :	**Control**	**HL**	**SEM**	**P-value**
08:00	6.02[a]	10.46[b]	1.002	0.020
14:00	9.87	9.35	1.240	0.774
20:00	11.5	11.4	1.005	0.954
02:00	6.57	6.60	0.352	0.954
Overall means	8.50	9.45	0.744	0.380

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

[ab] Means in the same row with different supercripts differ at $P < 0.05$

SEM = standard error of means

NH_3-N = ammonia nitrogen

4.2.3 Ruminal volatile fatty acid concentrations

The different VFA concentrations in the rumen (mmol/L) for four different collection times are shown in Figure 4.5. Daytime differences in VFA concentrations were expected as predicted by the study of Graf *et al.* (2005). The diurnal variation is influenced, like in the case of rumen pH, by grazing behaviour and pasture intake. The most aggressive grazing is expected after milking times and during the day and therefore higher VFA concentrations would be observed at day time which gradually decreases during the night. Concentrate feeding during milking also contributes to increased fermentation and therefore increased VFA concentrations at 08:00 and 14:00.

The only time at which differences ($P < 0.05$) between the control cows and HL cows were observed was at 08:00. Higher concentrations for all the VFAs, except for butyric acid, were recorded for the HL treatment. The total VFA concentration at 08:00 was also higher ($P < 0.05$) in the HL treatment than the control. It is difficult to explain these differences from data obtained in other published studies, but it is assumed that supplementary lucerne fed after morning milking may have altered the fermentation in the rumens of these cows. The control cows did not get the opportunity to take in any food after morning milking on collection days. The cannulated cows were

kept behind for sample collection before returning to pasture. The samples for control cows were therefore collected on relatively empty rumens in comparison to the lucerne treatment group.

There were no differences (P <0.10) between the two treatments in any of the VFA concentrations or total concentration for any of the other collection times.

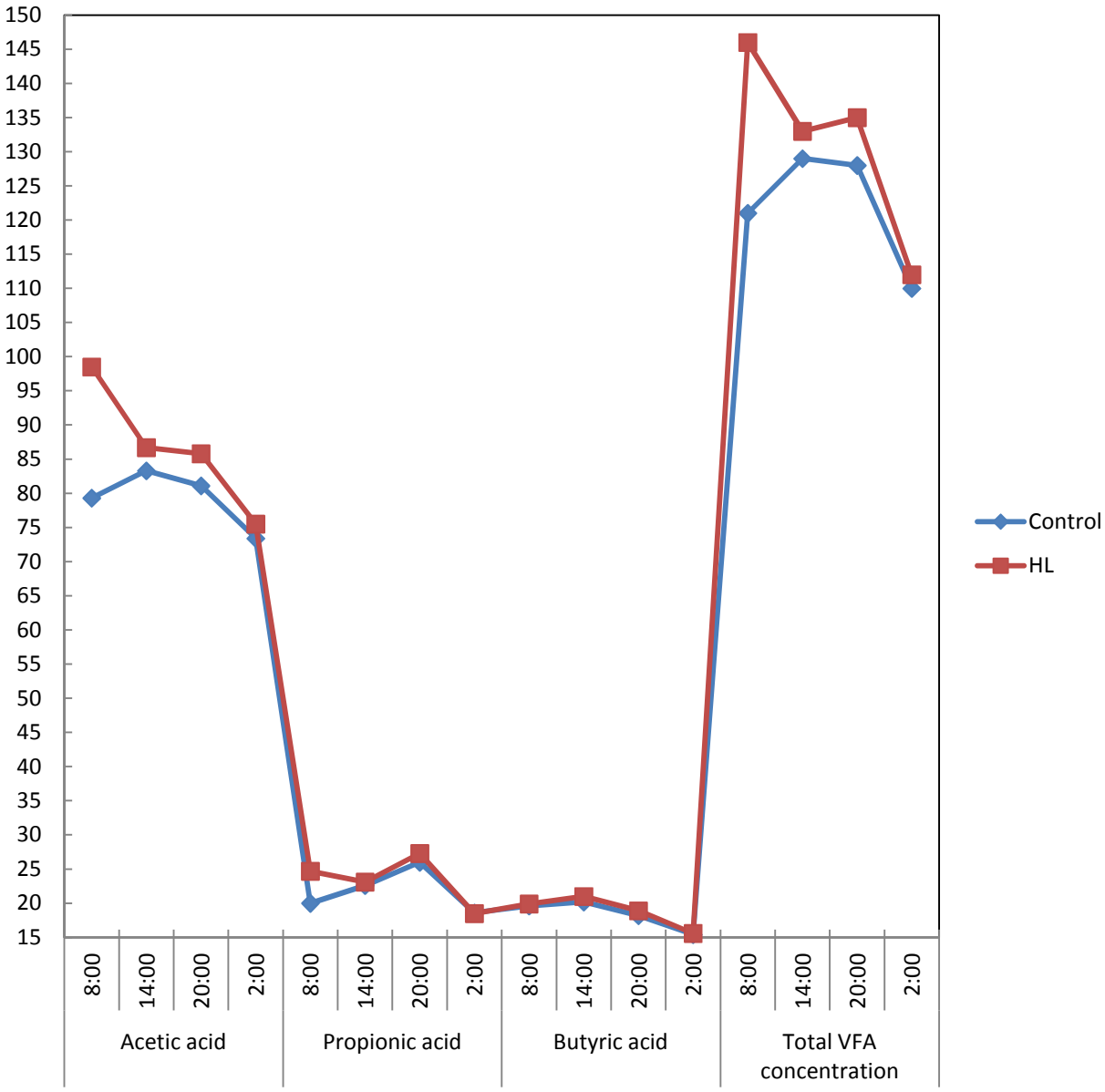


Figure 4.5 Volatile fatty acid concentrations (mmol/L) at four collection times for cows receiving no supplemental lucerne and cows receiving 2 kg supplemental lucerne

The mean concentrations of acetic acid, propionic acid, butyric acid, propionate:acetate and total VFA concentration over all the collection times are given in Table 4.5. Results for VFA concentrations were comparable to those reported in other grazing studies (e.g. Carruthers *et al.*, 1997; Reis and Combs, 2000; Wales and Doyle, 2003). Results reported in the study of Graf *et al.* (2005) were much lower than in the present study and can be attributed to the fact that no concentrate was fed to cows in that study.

Treatment differences were observed for acetic acid concentration and the total VFA concentation. This could be expected since the fermentation end-product of structural carbohydrates is acetic acid, whereas the end-product of NSC fermentation is propionic acid. The acetic acid concentration of the HL treatment (86.7 mmol/L) was higher ($P < 0.05$) than that of the control (79.3 mmol/L). The total VFA concentration of the HL treatment was also higher ($P < 0.05$) at 131 mmol/L than the control (122 mmol/L).

There were no differences in concentrations of propionic acid, butyric acid or acetate:propionate between the two treatments ($P < 0.10$).

These results differed from those found in literature. Studies performed by Wales and Doyle (2003) and Graf *et al.* (2005) suggest that treatment effects on total VFA concentration and VFA profile are insignificant regardless of observations of daytime differences. In the present study these daytime fluctuations (especially for acetic acid) had an effect on the average acetic acid concentration and the total VFA concentration, with higher concentrations reported for the HL cows than for control cows.

Higher total VFA concentrations would generally be expected to cause decreased rumen pH as indicated by Owens *et al.* (1998), the rumen pH in the present study were, however, no different ($P > 0.01$) between the two treatment for any of the collection times (see Table 4.3). The pH at 08:00 as measured with the portable pH meter was numerically higher for control cows, which may have been caused by the lower ($P < 0.05$) total VFA concentration in control cows at 08:00. The difference in rumen pH was not significant, however. One should also bear in mind that there are differences in the effect of different VFAs on rumen pH. Lactic acid is a much stronger acid compared to acetic acid and in this study, high levels of lactic acid accumulation in the rumen was unlikely because of the relatively low concentrate supplementation applied. The increase in acetic acid concentration and the consequent increase in total VFA concentration was not sufficient to affect rumen pH.

The acetate:propionate ratio in this study was 3.65:1 in the control treatment and 3.74:1 in the HL treatment, this is in agreement with other studies where cows were grazing kikuyu/ryegrass

pasture and received a similar level of concentrate supplementation (Malleson, 2008; Erasmus, 2009). The general rule of thumb is that an acetate:propionate ratio below 2.2:1 is indicative of milk fat depression (Emery, 1976). The rumen VFA data supports the production data in the sense that the acetate:propionate ratio was above 2.2:1 and no milk fat depression occurred.

Table 4.5 Mean concentrations of acetic acid, propionic acid, butyric acid, acetate:propionate and total VFAs for cows receiving 2 kg supplemental lucerne and no supplemental lucerne

	Treatments[1]			
VFA concentration (mmol/L)	**Control**	**HL**	**SEM**	**P-value**
Acetic acid	79.3[a]	86.7[b]	1.78	0.012
Propionic acid	21.8	23.4	0.78	0.180
Butyric acid	18.4	18.8	0.43	0.462
Acetate: Propionate	3.65:1	3.74:1	0.008	0.620
Total concentration	122[a]	131[b]	2.74	0.028

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

[ab] Means in the same row with different superscripts differ at $P < 0.05$

SEM = standard error of means

VFA = volatile fatty acid

4.2.4 Rumen fill investigation

A scoring system (see Table 4.1) was developed to describe rumen fill and the average scores allocated to each of the treatment groups are given in Table 4.6. Scores were awarded to each of the cannulated cows at each of the rumen sample collection times, 08:00, 14:00, 20:00 and 02:00. There were no differences ($P > 0.10$) between the rumen scores observed in the control group and the HL group at any of the collection times.

The highest score was observed at 20:00 for both treatment groups and one can therefore assume that the most aggressive grazing behaviour would have occurred before 20:00 in both groups. This is supported by the rumen pH data where rumen pH declined throughout the day and only started to increase after 20:00 (Figure 4.4), total VFA production also only started to decline after 20:00 (Figure 4.5).

The score decreased between 20:00 and 02:00 and a further decline was observed after 02:00 until the lowest score was recorded for both treatment groups at 08:00. From this observed decline

in rumen fill after 20:00 it is likely that cows spent most of the night ruminating and that pasture intake was minimized at night.

Table 4.6 Rumen scores and total rumen content of cows receiving 2 kg supplemental lucerne and cows receiving no supplemental lucerne

	Treatments[1]			
Rumen Score at:	**Control**	**HL**	**SEM**	**P-value**
08:00	2.06	2.25	0.085	0.168
14:00	2.50	2.50	0.125	1.000
20:00	2.81	2.88	0.122	0.730
02:00	2.31	2.38	0.085	0.620
Rumen content (kg OM / kg $W^{0.75}$)	0.49	0.51	0.031	0.684

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

SEM = standard error of means

OM = organic matter; $W^{0.75}$ = metabolic live weight

The total rumen OM content of the 2 kg lucerne treatment group and the control is shown in Table 4.7. There were no differences in rumen OM content between the two treatments ($P > 0.10$), and we can therefore assume that all the cows had equal opportunity to consume sufficient food to fill the rumens and that the pasture allocation was sufficient.

4.2.5 *In sacco* DM and NDF disappearance of ryegrass

The DM and NDF disappearance after 12 hours and 24 hours for both the control and HL treatments are given in Table 4.7. A trend could be observed in DM disappearance and NDF disappearance after 12 hours, with disappearance from the rumens of cows receiving the control diet being higher ($0.05 < P < 0.10$) than that of the HL treatment for both fractions. The disappearance of DM and NDF was no different ($P < 0.10$) between treatments after 24 hours.

These results differed from published results on fiber digestion with supplemental hay. Beauchemin and Buchanan-Smith (1990) and Reis and Combs (2000) recorded improved fiber digestion when hay was supplemented. In both these studies the base diet differed from the present study and contained lower levels of fiber. In the study of Beauchemin and Buchanan-Smith (1990) the base diet was lucerne silage supplemented with concentrate and had a NDF content of 25%

which is much lower than the 47.2% of the pasture grazed in our study. Similarly the NDF content (35.8%) of the pasture grazed in the study of Reis and Combs (2000), which was a 50:50 mix of grass and legume, was lower than the NDF content of our study. There probably was a shortage of effective fiber in these two studies, which was not the case in our study, as confirmed by the rumen pH data.

In the present study, there were no or minor changes seen in the rumen environment and rumen fermentation with lucerne hay supplementation. The lack of a positive effect of supplemental lucerne on fiber digestion could therefore be expected.

Table 4.7 The % DM disappearance and % NDF disappearance from an *in sacco* study, after 12 hours and 24 hours for cows receiving 2 kg supplemental lucerne and cows receiving no supplemental lucerne

	Treatments			
Item	**Control**	**HL**	**SEM**	**P-value**
DM disappearance (%)				
12h	68.4_{c}	65.3_{d}	1.042	0.085
24h	80.0	79.4	0.956	0.711
NDF disappearance (%)				
12h	54.1_{c}	50.3_{d}	1.360	0.096
24h	68.9	68.4	1.408	0.798

[1]Control = no lucerne hay; HL = 2 kg lucerne hay

[cd] Means in the same row with different subscripts differ at $0.05 < P < 0.10$

SEM = standard error of means

DM = dry matter

NDF = neutral detergent fiber

Results of the fermentation study confirm the lack of response observed in the production study. The rumen environment of the control cows was not unfavourable to the extent that it depressed ruminal pH or decreased fiber digestion and therefore production performance. The positive contribution of more effective fiber was relatively small and not detectable under the conditions of this study.

CHAPTER 5

CONCLUSION

5.1 Production study

Supplementation of 1 kg or 2 kg lucerne hay to high producing, multiparous Jersey cows in early to mid lactation, grazing highly digestible, well managed kikuyu-ryegrass pasture and receiving 5 kg (as is) of a maize-based dairy concentrate did not affect milk production, fat corrected milk production, milk fat or milk protein concentration.

The lack of any difference in production performance suggest that supplementation with 2.5 kg concentrate twice per day did not cause an unfavourable rumen environment which could lead to milk fat depression, reduced microbial synthesis or subclinical acidosis. Pasture intake appears to be sufficient to sustain a high production when 5kg of concentrate is being fed and post-grazing heights are between 10 and 12 on the rising plate meter (5-6 cm).

The extra buffering and peNDF supplied by the lucerne hay, therefore, was not necessary under the conditions of this study. Futhermore, the substitution effect of lucerne hay was not sufficient to cause a difference in energy intake and therefore milk production. Additional lucerne hay might be beneficial at higher levels of concentrate intake.

5.2 Rumen study

Diurnal variation in pH is observed for high producing, multiparous Jersey cows in early to mid lactation, grazing highly digestible kikuyu/ryegrass pasture and receiving 5 kg (as is) of a maize-based dairy concentrate. The diurnal pattern correspond with milking times and concentrate feeding and treatment differences observed may be attributed to changes in grazing behaviour. A more severe decline in pH after milking times was caused by lucerne hay supplementation, but the time spent below critical pH of 5.8 did not differ between treatments. The overall effect of lucerne hay supplementation on pH seems to be insignificant.

Although rumen NH_3-N concentration, some VFA concentrations and *in situ* DM and NDF disappearance was affected by treatment, it was relatively small changes and only for short time intervals. Considering a full 24 hour fermentation period, the differences and trends that did occur, were biologically insignificant.

Results of the fermentation study confirm the lack of response observed in the production study. The rumen environment of the control cows was not unfavourable to the extent that it depressed ruminal pH or decreased fiber digestion and therefore production performance. The positive contribution of more effective fiber was relatively small and not detectable under the conditions of this study.

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CHAPTER 6

CRITICAL EVALUATION

7.1 Production study

The results of this trial are specifically applicable to farming situations where good pasture management practices are employed in the Southern Cape during the spring months.

A major limitation of this trial was that individual pasture intake for each of the treatments was not known and therefore the SR and any differences in nutrient intake could only be roughly estimated. The RPM is a useful tool to manage pasture, but it may not be accurate enough to estimate pasture intake. The intake calculation was based on a regression equation with R^2 of 0.61 which introduces some error when calculating the DM available on the pasture.

The cows from different treatments grazed as one group and therefore intake differences between groups could not be estimated with the RPM. The reason the cows grazed together was that the current system on the farm would have made it difficult to separate the cows and pasture allocation would have been labour intensive.

Alkanes are another option that may be considered for determining individual intake. This method is labour intensive and stressfull to the cows, especially the daily faecal sample collection. Results from other grazing studies done with alkanes were unsatisfactory and the method has some limitations that need to be considered.

A back calculation was used to give some indication as to individual pasture intake. This method has many limitations, because many assumptions were made in order to estimate the energy requirement of an average cow within each treatment group. These assumptions mere made to keep the calculation less complicated, but we may have oversimplified the situation. In the end the results must be regarded as only an indication and an attempt to explain the situation that may have been expected with regards to pasture intake and SR.

Body weight inputs used for the back calculation may have significant error and variation as it was based on only four measurements and factors like fetal growth and water intake can have a large influence on the accuracy.

Because of the great variability in body weight only the change in BCS was used for the purpose of estimating energy required for body reserves. The equation used was derived from values found in NRC (2001), but significant variation and error may be possible. Body weight may

have given a completely different picture, but some replication may occur when using both body weight and BCS.

To simplify the inputs, cows were assumed to not be pregnant, because it would have been difficult to get a value for the average days pregnant for each treatment group.

Energy required for lactation was given in NE by the NRC (2001) software and the factor used to convert it to ME may need some revision. The NE value was multiplied by 4.184 to convert Mcal to MJ and then devided by 0.64 which is the factor suggested by the NRC (2001) for efficiency of energy used for milk production.

The feed library in the NRC (2001) feed formulation program does not include standards for local raw materials and pastures and therefore many of the ration inputs were only more or less the same as what was actually included in the trial diets. This probably also caused some error because the digestibility of the diet and diet type has an influence on ME requirements.

The pasture and total intake and the SR as estimated by means of back calculation may therefore be somewhat inaccurate.

Pasture was assumed to be purely kikuyu-ryegrass pasture, but in practice there are always some other plant species present in the pasture. A botanical composition determination of the pasture samples, which were collected weekly, may have given a clearer picture of the variation in pasture quality throughout the trial.

Lucerne hay intake showed considerable variation throughout the trial. The variation may be attributed to quality changes in pasture and lucerne hay and also to changes in pasture availability. Better management in terms of pasture allocation would have minimised the variation in hay intake. Care was taken to keep variation in hay quality as low as possible. The best bales from a batch were used for the trial and the time allowed for grinding the lucerne hay in the mixer feeder was standardised for each batch, to minimise variation in particle size between batches.

Environmental change is a factor that can not be controlled but may have a considerable influence on intake. Cows tend to decrease intake on warmer days compared to cold days.

There was also considerable individual variation and it seemed that some cows are more willing to eat the lucerne hay than others. Some individual cows ate very little of the hay each day, while others ate all the hay allocated to them every day. The situation on a farm may therefore be difficult to manage because cows will not be fed hay individually and some cows will therefore consume far more than the amount allocated per cow and others will probably not even touch the hay.

Average hay intake between the 1 kg and 2 kg lucerne hay treatments only differed by 630 g per day and it seems unlikely that most cows will consume 2 kg of dry roughage when sufficient pasture is available. The average intake of the 2 kg lucerne treatment was 1.59 kg and therefore 1.5 kg per day seems like a more achievable intake.

The inclusion of poorer quality roughage and other sources of roughage in the trial may be beneficial as this could provide a completely different scenario. Initially we wanted to compare responses of wheat straw with that of lucerne hay, but cows refused to eat the straw and the trial was adapted. Palatability of poor quality roughage therefore may limit intake and cause minimal responses. Another factor that must be considered with inclusion of poor quality roughage is that it would probably cause higher SR and therefore may cause lower milk response.

The control group was kept in a separate holding area after morning milking until the cows receiving hay had finished eating so that the whole group could return to pasture at the same time. Although this waiting time was no more than 30 minutes per day, one can argue that these cows did not have the same opportunity to consume food and that this cause the differences observed in intake. We assumed that the control cows compensated for this waiting period by aggressive grazing on returning to pasture, but this could only have been confirmed if a behavioural study was included. The milk production of the control group was not lower than that of the lucerne supplemented groups indicating that pasture intake was not limiting.

Care was taken to keep the milk sampling technique the same throughout the trial and for each sample taken. Some mechanical errors caused variation because the sample collection bottles were not filled with exactly the same volume of milk each time. This problem was managed by cleaning the pore where milk is let through from the collection bottle to the sample bottle for each milking station before every sample collection. We accept that the machine used was calibrated and accurate and that human error was negligible.

7.2 Rumen study

The number of cannulated cows on the farm is limited and as a result the cows that were available for the rumen study had large variation in age, production and days in milk. It would have been favourable if the cannulated cows could have resembled the cows from the production study more closely and a clearer picture may have been obtained.

For the rumen fill investigation it may have been an advantage to do the rumen evacuation at different times of the day and on more than one occasion per switch over. The purpose of the evacuation was, however, only to give an indication of average rumen fill at a representative time to

evaluate pasture allocation and differences in intake between treatments. Furthermore, the rumen fill investigation was not part of the main objectives of the study and the evacuation procedure is labour intensive and time consuming and the rumen environment is disturbed.

One can expect some error with regards to the sample collection of rumen content in the rumen fill investigation. To collect a representative sample of rumen content is very difficult because of difficulty in mixing the liquid portion and solid portion of the rumen content.

The *in sacco* method used needs revising. Three nylon bags were put in each leg of a stocking, but due to limited availability of large sizes there were variation in the size of stocking used for each cow. This can cause significant variation and increase the error of the procedure as some bags were tied very closely to the cannula plug and it is therefore unlikely that these bags were in contact with the rumen content at all times. On the contrary, when considering the rumen fill investigation it seems that for some part of the day these bags were completely above the rumen content and this may caused considerable variation in disappearance of NDF and DM between the bags. If this method is to be used one must take care to only use the largest sizes of stockings available in order to ensure that the bags go in deep enough and one must not put too many bags into a stocking leg so that the top bags are tied too close to the cannula plug. An alternative method that can be considered and may minimize error is the disc used in the past. Bags are tied to a disc and therefore are all incubated at the same depth in the rumen. This method was replaced in many studies by the stocking method because it is believed to be more difficult and laborious, but it may still be more accurate.

The rate of disappearance may have given a more accurate picture of fiber digestion in the rumen if a 36 hour and 48 hour removal time was included in the *in sacco* study. The procedure is laborious and the analysis expensive and seeing that no significant differences were seen at 24 hour removal further incubation probably would have been unnecessary.

Fermentation rate and protein degradation in the rumen is influenced by pasture intake, and therefore grazing behaviour may have had a significant influence in the rumen sample concentrations of VFA and NH_3-N and the pH. A behavioural study to investigate grazing patterns and rumination frequency would have provided a clearer picture and may have provided explanations for some of the results that differed from what was expected.

Differences in rumen fermentation and degradation observed at the 08:00 collection time may have been due to other factors than treatment effects. The cannulated cows were kept behind after morning milking. For practical reasons these cows were only allowed to return to pasture after the

collection was done. The control cows were therefore left without food for much longer than the lucerne supplemented cows and this may have caused variation in the results.

From the above discussion it is clear that the trial could be refined if it had to be repeated and that many sources of error and variation may have contributed to inaccuracy of results. The bottom line, however, is still that the control diet did not create an unfavourable rumen environment resulting in low pH, potential sub-acute rumen acidosis or milk fat depression. The diet apparently did not lack effective fiber and responses might only be observed if high levels of concentrate are fed. This aspect deserves further investigation. Future studies may focus on individual pasture intake, hay intake and quality, different sources of roughage and grazing behaviour.

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APPENDIX

SELECTION OF TRIAL COWS

All the lactating cows in the herd at the Outeniqua Research Farm were reviewed for selection for use in the trial. All first lactation cows, cows that were not functionally sound, cows that had behavioural problems and cows that were too far into their lactation (more than 100 days in milk) were excluded. The dry cows in the herd were also reviewed and those cows that were close to calving were also included in the group that would be considered for selection for use in the trial.

The 4% fat corrected milk production (FCM = [0.4 * milk production] + [15 * milk production * BF%/100) of the three weeks prior to the start of the study, lactation number and days in milk (DIM) were used as criteria for blocking. Sixteen blocks were selected and cows were ranked according to block number. The cows within blocks were randomly allocated to the three different treatment groups. Tables A1 to A3 contains the three groups that received no supplemental lucerne (control), 1kg supplemental lucerne (LL) and 2 kgsupplemental lucerne (HL).

Table A1 The blocked cows that were allocated to the control group and received no supplemental lucerne

No.	Name	Lact. No.	DIM	Milk production	%BF	kgBF	FCM
1	AMSA 48	3	34	21.17	4.90	1.04	24.02
2	PAULET 11	3	57	26.79	3.85	1.03	26.19
3	WANDA 15	3	52	22.36	4.35	0.97	23.53
4	AMSA 64	2	78	24.08	4.24	1.02	24.95
5	ESME 2	6	44	20.46	5.46	1.12	24.94
6	AMSA 67	4	89	20.63	4.76	0.98	22.99
7	ARNA 3	6	144	18.51	4.93	0.91	21.10
8	LIZ 10	5	124	18.55	4.43	0.82	19.75
9	PAULET 12	3	67	24.86	4.25	1.06	25.80
10	ARNA 11	3	72	27.13	3.92	1.06	26.81
11	TES 2	6	51	26.56	4.35	1.16	27.96
12	PAULET 5	5	159	19.41	5.02	0.97	22.38
13	AMSA 68	2	22	17.45	4.89	0.85	19.78
14	BELLA 121	7	138	23.52	4.88	1.15	26.63
15	PAULET	9	6	17.67	4.62	0.82	19.31
16	MAX 14	6	3	23.10	4.84	1.12	26.02
AVE			71.25	22.02	4.61	1.01	23.88
SEM			48.35	3.28	0.43	0.11	2.76

DIM = days in milk
FCM = 4% fat corrected milk production
BF = butter fat
AVE = average
SEM = standard error of means

Table A2 The blocked cows that were allocated to the LL treatment and received 1 kg of supplemental lucerne per day

No.	Name	Lact. No.	DIM	Milk production	%BF	kgBF	FCM
1	LASS 7	3	42	23.56	4.16	0.98	24.13
2	PAULET 13	3	49	22.58	5.21	1.18	26.68
3	LUA 21	3	72	19.97	4.90	0.98	22.66
4	PANSY 5	3	84	21.91	4.71	1.03	24.25
5	BELLA 108	9	59	26.10	3.84	1.00	25.48
6	TES 6	3	85	22.99	4.13	0.95	23.44
7	BERTA 8	8	144	17.85	5.19	0.93	21.04
8	MAX 26	3	139	19.37	4.29	0.83	20.21
9	BERTA 36	5	52	23.12	4.90	1.13	26.24
10	AMSA 34	4	78	24.05	4.79	1.15	26.89
11	TES 3	5	85	23.54	4.97	1.17	26.96
12	AMSA 39	3	154	21.14	4.83	1.02	23.77
13	BERTA 67	3	13	16.20	4.93	0.80	18.46
14	SUSA 28	4	132	24.13	5.77	1.39	30.53
15	MAX 13	7	18	17.96	5.27	0.95	21.39
16	SUSA 23	5	3	22.74	4.54	1.03	24.58
AVE			75.56	21.70	4.78	1.03	24.17
SEM			47.24	2.72	0.49	0.15	3.02

DIM = days in milk
FCM = 4% fat corrected milk production
BF = butter fat
AVE = average
SEM = standard error of means

Table A3 The blocked cows that were allocated to the HL treatment and received 2 kg of supplemental lucerne per day

No.	Name	Lact. No.	DIM	Milk production	%BF	kgBF	FCM
1	SANTA 11	3	31	21.75	4.95	1.08	24.84
2	ESME 3	3	42	21.89	5.26	1.15	26.03
3	LUA 20	3	56	23.60	3.86	0.91	23.10
4	SUSA 37	3	79	23.60	4.20	0.99	24.31
5	TES	8	56	24.75	3.89	0.96	24.34
6	TES 7	3	94	24.67	3.51	0.87	22.85
7	ETNA	9	109	17.20	5.36	0.92	20.70
8	LUA 22	3	129	20.21	3.45	0.70	18.54
9	MELBA 1	4	74	23.13	4.60	1.06	25.21
10	ETNA 6	3	87	26.85	4.06	1.09	27.09
11	BERTA 29	5	63	24.60	4.91	1.21	27.96
12	BERTA 52	3	126	19.52	5.19	1.01	23.00
13	AMSA 57	3	22	16.54	4.97	0.82	18.95
14	MAX 19	4	157	20.00	6.60	1.32	27.81
15	ARNA 7	4	25	18.69	4.79	0.90	20.90
16	LIZ 13	4	14	22.31	5.32	1.19	26.73
AVE			72.75	21.83	4.68	1.01	23.90
SEM			42.24	2.92	0.82	0.16	2.97

DIM = days in milk
FCM = 4% fat corrected milk production
BF = butter fat
AVE = average
SEM = standard error of means