Fishmeal supplementation to high producing Jersey cows grazing ryegrass or kikuyu pasture

by

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Submitted in partial fulfilment of the requirements for the degree

MSc Agric (Animal Nutrition)

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University of Pretoria
February 2008

CONTENTS

DECLARATION

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

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February 2008

ACKNOWLEDGEMENTS

I would like to extend a huge thank you to all the following people who contributed to the success of this project and dissertation:

First of all to Dad and Mum for inspiring me and giving me the freedom to choose what I wanted to study, as well as for the love and support wherever I was and making sure I had whatever I needed to make my studies a success. And thank you Dad for helping proof-read my first draft and Mum for proof-reading the final draft.

My supervisor, Professor Lourens Erasmus, for the organization, advice, motivation, ideas and contributions throughout my years of study as well as for inspiring me. It has been a privilege to learn from someone with such a good reputation in the dairy world.

My co-supervisor, Professor Willem Van Niekerk, for the organization, advice, ideas and contributions.

Project leader, Doctor Robin Meeske for the inspiration behind this project, for organizing for me to go to George, teaching me so much and giving me such valuable support and advice throughout the project, as well as exposing me to the world of pasture-based dairy farming. I was extremely fortunate to end up in such a good research environment where I could learn a lot about the practical side of research while having the freedom to do things myself and take responsibility. And thank you for helping with some of the rumen sampling during the night.

Roelf Coertze for willingly helping so much with all the statistical analyses.

Gerrit Van der Merwe for being such a great help with everything to do with coordinating the practical side of the project; for helping select which cows to use for the project; for doing the condition scoring of the cows; for making sure that the milking machines and milk meters were in working order; for being there at all hours of the day and night to help take rumen samples, and for helping put the pH meters and data loggers on, even on weekends.

Pieter Cronjé for helping with pH meters and data loggers, even on a weekend, as well as helping take rumen samples at various hours of the day and night in the second trial. Pieter Du Plessis for helping take rumen samples at various hours of the night. Wesley Schaap for helping with the measurement of the pasture and checking on the cows on the pasture as well as helping with the taking and milling of pasture samples and milling the concentrate and dung samples. Marius Herman for helping with the feeding of the cows in the afternoons and helping take the

occasional samples. Ben Booysen for helping with the milk samples and also the measuring of the pasture when Wesley was on leave. Christopher Lottering for brining in the cows for milking and helping take milk samples. André February for helping feed the cows in the morning and bringing the cows in for milking. Isaac Wildschut for helping with the dung samples and for always being there when we needed to find certain cows. Hester De Klerk and Siena Smit for milking the cows and helping out with the feeding of the cows during milking. Jeffrey Van der Ross, Cupido Skippers and Edmund Douglas for coming in early in the mornings to feed the cows during the morning milking. Cyral Filander for bringing the cows in for milking in the beginning when Christopher was on leave. Abram Wildschut for coordinating the other staff and making sure the pasture was correctly allocated when Wesley was not there and for helping a bit with the rumen sampling. And to all the above-mentioned farm staff for helping with all the jobs that I have neglected to mention such as weighing the cows and doing the milking and feeding on weekends. Thank you for always being so friendly and willing to help. This project would not have been possible without you.

All the other people at the Outeniqua Experimental Farm for being so friendly and making my time there a memorable experience.

Theunis Van den Berg and the other staff from Bokomo (now Nova) Feeds, George, for the mixing of the feeds to our specifications and for giving such good customer service when we had to remix a batch as well as for storing the bags of feed that would not fit in our store room and for telling me the latest feed prices for economic calculations. And thanks to Bongani for showing me the feed mill while they were mixing the feed and making sure the feeds ended up in the right colour bags.

Elise Ferreira from Nutrilab for organizing and coordinating my laboratory analyses. Tilla Basson from Nutrilab for being such a huge help with most of my laboratory work. Truida Smit from Nutrilab for being such a huge help with a lot of my laboratory work. Gerda Kotze from Nutrilab for doing certain laboratory analyses for me. All the other staff of Nutrilab for your contributions, including Carol, Sonnika, Christina and Solly.

To all the other people of the Department of Animal and Wildlife Sciences at the University of Pretoria as well as my class mates for the friendly words of encouragement. Jacky Els and Liesl Els for organizing cannulated sheep for my *in vitro* analyses. Heather and Charles and all my friends for providing balance and inspiration in my life.

Kenneth Botha, Gert Cronjé and Natasha Snyman from AFGRI Animal Feeds for the input and help with post-examination changes and to Dr. Thomas Tylutki for the modelling tips.

Lactolab, Irene, as well as the Elsenburg Dairy Laboratory, for analyzing the milk samples.

Animal Production Research Trust for funding most of the project and my stay in George.

Western Cape Department of Agriculture for partially funding the project.

Protein Research Foundation for the bursary to support me during my studies.

And last, but certainly not least, a big thank you to Marius Botha for understanding my need to go to George for a few months. Your help, support and balanced outlook on life have been invaluable.

To anyone else that I may have neglected to mention, thank you.

SUMMARY

FISHMEAL SUPPLEMETATION TO HIGH PRODUCING JERSEY COWS GRAZING RYEGRASS OR KIKUYU PASTURE

by

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Rumen-undegradable protein might be the first limiting nutrient for high producing dairy cows receiving high levels of maize supplementation while grazing pasture. To test this hypothesis two trials were conducted using fishmeal as a high quality protein source rich in rumen-undegradable protein, Methionine and Lysine. In the first trial cows grazed annual ryegrass for two months in spring. In the second trial cows grazed kikuyu for two months in late summer. In addition to the pasture cows received 6 kg (as is) of a maize-based supplement, including minerals, fed in two equal portions in the milking parlour. A randomised complete block design was used. Three groups of 15 (ryegrass) or 14 (kikuyu trial) cows received control (no fishmeal), low fishmeal (4 % fishmeal replacing maize) or high fishmeal (8 % fishmeal replacing maize) treatments. Multiparous, high producing, Jersey cows in early to mid lactation were used. Milk production was measured and milk samples taken fortnightly. Simultaneous studies were conducted using eight rumen cannulated cows receiving the control and high fishmeal treatments in a cross over design experiment. Ruminal pH, ammonia-N and volatile fatty acid concentrations were measured.

In the ryegrass trial milk yield, 4 % fat-corrected milk yield and milk fat and protein percentages of cows on the low and high fishmeal treatments (21.9 and 22.1 kg milk/d, 24.1 and

24.2 kg 4 % fat-corrected milk/d, 4.73 and 4.67 % fat and 3.49 and 3.45 % protein) were significantly higher than the control (20.5 kg milk/d, 20.4 kg 4 % fat-corrected milk/d, 3.97 % fat and 3.25 % protein). There was no treatment effect on milk urea N (16.8, 17.4 and 17.9 mg/dl, for the control, low fishmeal and high fishmeal treatments, respectively). The ruminal ammonia-N concentration was significantly higher in the cows on the high fishmeal treatment than the control (16.67 vs. 14.16 mg/dl). Fishmeal supplementation to cows on ryegrass is profitable under any realistic price scenarios in South Africa.

In the kikuyu trial cows on the high fishmeal treatment produced significantly more milk (19.5 kg/d) than the cows on the control (18.2 kg/d), neither differing from the low fishmeal treatment (18.9 kg/d). The cows on the low fishmeal treatment had significantly higher milk fat percentage (4.18 %) than the control (3.71 %), neither differing from the high fishmeal treatment (3.91 %). The cows on the two fishmeal treatments produced significantly more 4 % fat-corrected milk than the control (19.4 and 19.2 vs. 17.3 kg 4 % fat-corrected milk/d). There was no treatment effect on milk protein percentage (3.30, 3.41 and 3.34 % for the control, low and high fishmeal treatments, respectively). Milk urea N was significantly higher for the high fishmeal treatment (10.80 mg/dl) than the control and low fishmeal treatments (9.09 and 9.44 mg/dl). Ruminal ammonia-N concentration was significantly higher in the cows on the high fishmeal treatment than the control (6.52 vs. 4.74 mg/dl). Fishmeal supplementation to cows on kikuyu could be profitable under certain price scenarios.

LIST OF ABBREVIATIONS

AA	amino acid
ADF	acid detergent fibre
ADIN	acid detergent insoluble nitrogen
ADIP	acid detergent insoluble protein
ADL	acid detergent lignin
ARC	agricultural research council
BCS	body condition score
BM	blood meal
BUN	blood urea nitrogen
BW	body weight
CNCPS	Cornell Net Carbohydrate and Protein System
CP	crude protein
Cr_2O_3	chromic oxide
DM	dry matter
DMI	dry matter intake
DOMD	digestible organic matter in dry matter
EAA	essential amino acids
EE	ether extract
ECM	energy-corrected milk
FCM	fat-corrected milk
FM	fishmeal
GE	gross energy
IVDMD	*in vitro* dry matter digestibility
IVOMD	*in vitro* organic matter digestibility
ME	metabolisable energy
MUN	milk urea nitrogen
MP	metabolisable protein
NAN	non-ammonia nitrogen
NANMN	non-ammonia non-microbial N

NDF	neutral detergent fibre
NDIN	neutral detergent insoluble nitrogen
NDIP	neutral detergent insoluble protein
NE	net energy
NE_l	net energy for lactation
NFC	non-fibre carbohydrates
NH_3	ammonia
NH_3-N	ammonia nitrogen
NPN	non-protein nitrogen
NRC	National Research Council
NSC	non-structural carbohydrate
OM	organic matter
PA	pasture allowance
PDMI	pasture dry matter intake
PUN	plasma urea nitrogen
RDP	rumen-degradable protein
RPM	rising plate meter
RSD	residual standard deviation
RUP	rumen-undegradable protein
SBM	soybean meal
SCA	Standing Committee on Agriculture
SD	standard deviation
SEM	standard error of mean
Sol CP	soluble crude protein
SR	substitution rate
TMR	total mixed ration
VFA	volatile fatty acids
WOL	week of lactation

LIST OF TABLES

Chapter 5

Appendix

LIST OF FIGURES

Page

Chapter 3

Chapter 5

Appendix

Chapter 1

INTRODUCTION AND MOTIVATION

Profitable milk production in the Southern Cape region of South Africa is based on pasture systems (Meeske *et al.*, 2006). Although cows on pasture do not perform as well as cows fed a total mixed ration (TMR), the fact that pasture farming is a lower input way of producing milk makes it an attractive option in areas of South Africa, including the Southern Cape, where the climate is suitable for producing high quality pastures. In certain feed cost and milk price scenarios a pasture system could be more profitable than TMR (Tozer *et al.*, 2003).

Lush pasture has a high content of crude protein (CP) that is highly degradable (Muller & Fales, 1998; McCormick *et al.* 1999; 2001b; 2003b; Bargo *et al.*, 2003a). Metabolisable energy (ME) is the first limiting nutrient for cows grazing high quality pasture (Muller & Fales, 1998; Kolver & Muller, 1998; Bargo *et al.*, 2002a; 2002b; Kolver, 2003), making it necessary to feed an energy rich grain if higher production is to be achieved from the cows. This, along with the fact that feeding excess rumen-degradable protein (RDP) can be detrimental to the animal (Muller, 2003b; Gehman *et al.*, 2006), means that many farmers in the Southern Cape have moved away from feeding a concentrate that is balanced for the needs of the cow to feeding only maize and minerals as this is seen to be the most economical. Maize is the most readily available grain for dairy cows in South Africa (Erasmus *et al.*, 2000) and is commonly used as a supplement in pasture-based systems (Delahoy *et al.*, 2003).

However, since many cows have a high genetic potential for milk production, and since high levels of maize are often fed, it is important to investigate whether nutrients other than energy could be limiting production and if so whether it is economical and practical to supplement these nutrients.

Although, when only high quality pasture is fed, milk production is usually limited by the supply of ME, at higher levels of supplementation (more than 200 g grain/kg of the diet) and when milk production is high, specific amino acids (AA), particularly Met and Lys, may be limiting milk production (Muller & Fales, 1998; Kolver *et al.*, 1998b; Kolver, 2003). Apart from the fact that the CP content of South African maize might not be high enough for high producing cows, the AA composition of maize is not ideal – Lys is low (NRC, 2001). Met and Lys were predicted to be first limiting when modelling maize grain supplementation with CNCPS (Kolver, 2003).

Once supplemental energy has been supplied to improve the utilisation of the high RDP from the pasture (Muller & Fales, 1998), the amount and profile of AA reaching the small

intestine can be improved by supplementation with a high quality rumen-undegradable protein (RUP) source (Schroeder & Gagliostro, 2000).

For the modern high yielding cow, especially in early lactation, a smaller proportion of the protein is generally supplied by the rumen microbes and more needs to escape rumen degradation than was the case a few decades ago when cows had a lower genetic potential (Santos *et al.*, 1998; Hongerholt *et al.*, 1998; Schroeder & Gagliostro, 2000).

Increasing RUP or replacing RDP sources with RUP sources in concentrates of pasture or TMR fed cows has not had a consistent effect on milk production or composition (Carruthers *et al.*, 1997; Santos *et al.*, 1998; Bargo *et al.*, 2003a; Muller, 2003b). Several studies have replaced RDP sources such as soybean meal (SBM), sunflower meal, urea or rapeseed meal with RUP sources such as animal protein blend, maize gluten meal, expeller SBM, blood meal (BM), feather meal, heat-treated rapeseed meal or fishmeal (FM) (Bargo *et al.*, 2003a). Pasture studies that reported an increase in milk production were those of Schroeder & Gagliostro (2000) and Schor & Gagliostro (2001) where the milk response was 6 and 18 % respectively. Menhaden FM was the RUP source that most frequently increased milk yield compared to SBM and is also ranked highest in essential AA (EAA) index (Santos *et al.*, 1998).

This trial was conducted at the Outeniqua Experimental Farm, near George in the Southern Cape, to investigate whether grazing cows that are receiving high levels of maize supplementation, with minerals included, would respond to the addition of a high quality protein source to their supplement. Fishmeal was used as the quality protein source since it is recognised as an excellent source of RUP, rich in Lys and Met which are probably the first and second limiting AA for milk yield and milk protein synthesis (Rulquin & Vérité, 1993; Santos *et al.*, 1998; Schroeder & Gagliostro, 2000; Bach *et al.*, 2000).

Positive responses to RUP supplementation, above that observed with energy, are most likely in early lactation cows, when pasture quality is poor and when high levels of concentrate grain is fed (Hongerholt & Muller, 1998; Schor & Gagliostro, 2001). Older research (mainly pre 1990) related to high RUP supplements for grazing dairy cows, done using relatively low yielding cows, found supplemental protein to have no effect on milk yield especially when the quality of the pasture was high (Hongerholt & Muller, 1998). The effects of RUP supply on milk production under grazing conditions has not been extensively investigated with high producing cows (Schor & Gagliostro, 2001) and high yielding cows are more likely to respond (Rogers *et*

al., 1980; Santos *et al.*, 1998; Hongerholt & Muller, 1998; Schor & Gagliostro, 2001; Muller, 2003a). Multiparous cows are also more likely to respond than primiparous cows (Holter *et al.*, 1992; Hongerholt & Muller, 1998). Therefore the higher producing, multiparous, cows of the herd in early to mid lactation were used for the trial.

Pastures of kikuyu (*Pennisetum clandestinum*) over-sown with annual ryegrass (*Lolium multiflorum*) are common in the Southern Cape. The former species, adapted to hot climates, is active in summer, complementing the latter which is used for winter grazing (Botha *et al.*, 2005; 2006).

Since ryegrass and kikuyu differ in nutritional value, the response, and hence economic benefit, of supplementing FM might differ for the two types of pasture. Thus the trial was conducted on annual ryegrass in spring and repeated on kikuyu pasture in late summer.

In each of the two trials a production study was done, investigating the response of the cows to FM supplementation in terms of milk yield and composition as well as body weight (BW) and body condition score (BCS) changes.

Insight into dietary inefficiency and imbalances can be gained by looking at indicators such as volatile fatty acid (VFA) concentrations and ratios, ammonia-N (NH_3-N) concentrations and ruminal fluid pH (Williams *et al.*, 2005). Rumen studies were conducted simultaneously to investigate the effect of the experimental treatments on these parameters.

An economic analysis was done and recommendations made that can be applied by the dairy farmers in the Southern Cape.

Chapter 2

LITERATURE REVIEW:

SUPPLEMENTATARY FEEDING OF DAIRY COWS ON PASTURE WITH PARTICULAR REFERENCE TO SUPPLEMENTATION OF QUALITY PROTEIN

2.1 INTRODUCTION

The nutrition of dairy cows on pasture varies from pasture-only to partial-TMR systems, with the most suitable system depending the situation at hand. Grazed grass can be an excellent quality feed when managed properly while it is a cheap source of nutrients, making the use of pasture for dairy cows a good low cost feeding system (Stakelum, 1986a; Dillon *et al.* 1997; Bargo *et al.*, 2003a). However, pasture on its own does not have an optimal nutrient composition for milk production. The continual changing of pasture quality and quantity and the difficulty in quantifying intake, make supplementation with a concentrate that complements the nutritional value and deficiencies of pasture and meeting the nutrient requirements of the cow, a big challenge (Jones-Endsley *et al.*, 1997; McCormick *et al.*, 2001a; Muller, 2001a; Bargo *et al.*, 2003a).

In order to formulate supplements for grazing cows there needs to be information on the nutrient requirements of the cow and its ruminal microbes, the nutrient composition of the pasture consumed, the expected pasture intake and interactions between the pasture and the supplement (Kellaway *et al.*, 1993; Paterson *et al.*, 1994; Bargo *et al.*, 2003a). In a pasture-based system, the supplement is accurately calculated after the pasture intake and quality are guessed (Fulkerson *et al.*, 2005). There needs to be an understanding of the supply of pasture nutrients and the order in which nutrients limit milk production (Kolver & Muller, 1998). The first limiting nutrient is usually ME (Muller & Fales, 1998; Kolver & Muller, 1998; Muller, 2001a; Bargo *et al.*, 2002a; 2002b; Muller, 2003a; Kolver, 2003) and hence it has gained the most attention. A few studies reported results on protein supplementation, particularly RUP, and these will be looked at in detail in this literature review.

Milk production from pasture depends on the amount of pasture available and its nutritional quality as well as the quantity and quality of supplement provided (Tesfa *et al.*, 1995; Dillon *et al.*, 1997). The economics of supplementation depends on the cost of the supplements versus the additional milk and milk solids produced. Many studies have been done on the responses to supplementation, looking at either the amount or the type of supplement.

2.2 NUTRIENT REQUIREMENTS OF THE COW

A cow's nutrient requirements depend on her milk production and composition, age, stage of lactation, BW and condition, the extent of body tissue mobilization, maintenance and pregnancy requirements and to some extent the quality of the diet (Stewart *et al.*, 1995; Hodgson & Brookes, 1999; Kolver, 2003). Feeding standards always refer to the average cow but it is only really practical to calculate requirements for whole groups of cows (Stewart *et al.*, 1995).

High yielding dairy cows in early lactation require diets that contain 16 to 19 % CP on a dry matter (DM) basis, and about 37 to 38 % of the total protein should be RUP (6.5 to 7.2 % RUP on a DM basis) to optimise milk production (Hongerholt & Muller, 1998; McCormick *et al.*, 2001a). Table 2.1 shows guidelines for the total diet nutrient concentrations for early lactation dairy cows as reported by Erasmus *et al.* (2000). In Table 14-2 of the NRC (2001) it is recommended that small breed cows producing 20 kg milk with fat and protein of 4.5 and 3.5 %, respectively, would require 1730 g RDP and 720 g RUP/day. The ratio of carbohydrates and protein should be such that microbial protein synthesis and flow of microbial N to the small intestine is optimised (Jones-Endsley *et al.*, 1997). The ratio of ruminally degraded N: organic matter (OM) should be approximately 19 to 25 g N/kg OM for optimal CP and ruminal NH_3-N utilisation (Hongerholt *et al.*, 1998; Reis & Combs, 2000).

Table 2.1 Guidelines for the total diet nutrient concentrations for early lactation cows[1]

Item	Recommended level (DM basis)
CP (%)	16-18
Sol CP (% CP)	30-35
RUP (% CP)	35-40
ME (MJ/kg)	11.3-11.5
ADF (%; minimum)	19
NDF (%; minimum)	28-32
Effective NDF (%)	20-24
NSC (%)	35-40
Fat (%)	5-7
Ca (%)	0.6-0.8
P (%)	0.38-0.42

DM – Dry matter, CP – Crude protein, Sol CP – Soluble CP, RUP – Rumen-undegradable protein, ME – Metabolisable energy, ADF – Acid detergent fibre, NDF – Neutral detergent fibre, NSC – Non-structural carbohydrate

[1]Erasmus *et al.*, 2000

Proteins with low degradability are especially valuable in ruminants with high protein requirements (Broderick *et al.*, 1988). Lactating and growing cattle with high MP requirements respond to supplementation with UDP even when the RDP is adequate (Klopfenstein *et al.*, 2001). As milk yield increases, a substantial amount of additional dietary protein from protein supplements needs to leave the rumen un-degraded to meet the protein requirements of the cow (Stern *et al.*, 1994).

Grazing cows require 10 to 30 % more energy over maintenance than non-grazing cows due to their higher level of activity (Muller & Fales, 1998; Muller, 2001a; 2003a). Most (88 %) of the difference in milk production between cows on TMR and those on pasture can be accounted for by the energy required for walking and grazing (Bargo *et al.*, 2002b).

The genetic merit of cows has increased such that cows on pasture only can produce more than 30 kg milk per day, making it a challenge to meet the energy requirements of these animals. Supplementation with concentrate supports the expression of this potential (Delaby *et al.*, 2001).

2.3 PASTURE COMPOSITION

The first step in balancing a pasture-based diet is to estimate the nutrient composition of the pasture and know how it changes over time (Fulkerson *et al.*, 1998). Predicting the nutrient intake from this pasture is complicated by the fact that the composition of the pasture on offer might not reflect that actually consumed by the cows due to the fact that cows tend to select grass of higher quality (leaf rather than stem and green rather than dead material) than that on offer (Kellaway *et al.*, 1993; Wales *et al.*, 1998; Dalley *et al.*, 1999; Hodgson & Brookes, 1999). It is almost impossible to mimic this selection when collecting pasture samples, unless samples are taken from the oesophagus and even then there is saliva contamination, affecting the protein and mineral levels (Kellaway *et al.*, 1993). Selection depends on the amount and type of pasture, pasture allowance (PA) and grazing pressure. At higher PA cows select higher quality leaf material and hence consume herbage of higher quality than when there is severe grazing pressure (Stakelum, 1986a; Dalley *et al.*, 1999; Peyraud & Delaby, 2001).

Kellaway *et al.* (1993) calculated the nutrient content of the pasture actually consumed by the animals as follows: $N_e = [(M_b)(N_b)- (M_a)(N_a)]/ (M_b-M_a)$ where N_e is the nutrient content of

the pasture eaten, M_b and M_a are the pasture mass before and after grazing and N_b and N_a are the nutrient content of the pasture before and after grazing, respectively. Fulkerson *et al.* (1998) also used this method to calculate the N in the pasture consumed by the animals. The selection differential, N_e/N_a was also calculated by Kellaway *et al.* (1993) and found to be greater than one, indicating selection by the cows especially for CP, Ca and ME. The cows in this trial of Kellaway *et al.* (1993) only consumed 38 % of the pasture so there was considerable opportunity for selection.

The quality of the pasture available depends on aspects such as species, cultivar, plant maturity, soil moisture, temperature and climate, stage of the growing season, fertilisation programme and management (Sheaffer *et al.*, 1998; Muller & Fales, 1998; McCormick *et al.*, 2001a). Pastures used for dairy cows are usually based on temperate species and are referred to as high quality or young and leafy (Bargo *et al.*, 2003a). The nutrient quality of the pasture is usually higher than the same plant material harvested as silage or hay, neutral detergent fibre (NDF) being lower and RDP higher (Hongerholt & Muller, 1998; Muller & Fales, 1998; Reis & Combs, 2000; Muller, 2003a).

Using tropical grasses, such as kikuyu (*Pennisetum clandestinum*, a summer active perennial pasture) with temperate grasses could be a way of maintaining higher digestibility and animal performance in the summer months, complementing annual pastures (Paterson *et al.*, 1994; McDowall *et al.*, 2003). Kikuyu is an important summer and autumn pasture in the main milk producing areas of the Eastern and Southern Cape as it is well adapted to the climate (Henning *et al.*, 1995; Botha *et al.*, 2005). Its low spring DM production can be overcome by incorporating a temperate species such as annual ryegrass (*Lolium multiflorum*) which is among the important crops planted for winter grazing in the Southern Cape (Botha *et al.*, 2005; 2006).

Temperate (cool-season, C_3) grasses, such as ryegrass, tend to be intrinsically higher in both protein (Hacker & Jank, 1998) and DM digestibility than tropical (warm-season, C_4) grasses such as kikuyu (Buxton & Fales, 1994; Merchen & Bourquin, 1994; Hacker & Jank, 1998). Temperate grass species also tend to have a higher ME and P content than tropical species, the latter being influenced by fertilizer application (Kellaway *et al.*, 1993).

Ryegrass usually has a lower NDF than many other cool season grasses (Muller, 2001a) and the highest values for effective degradability of CP and ruminally degraded NDF (Bargo *et al.*, 2003a).

The ME, CP, NDF, Ca, P and Na concentrations in pasture vary according to the time of year (Kellaway *et al.*, 1993; Doyle *et al.*, 2005). The estimated ME value of pasture ranges from 9 to 12 MJ/kg DM compared to a desirable level of 11 to 12 MJ/kg DM for dairy cows (Hodgson & Brookes, 1999). Well managed autumn to spring pastures can have 25 % CP and higher (even 33 %; higher than the desired level for dairy cows), with NDF concentrations of 30 to 50 % or less, with a high digestibility of 75 to 80 % digestible OM in dry matter (DOMD; Donaldson *et al.*, 1991; Muller & Fales, 1998; Hodgson & Brookes, 1999; McCormick *et al.*, 2001a; Muller, 2001a; 2003b). The non-fibre carbohydrates (NFC) and non-structural carbohydrates (NSC; 5 to 30 % of DM) concentration of cool-season pastures, a measure of ruminally available carbohydrate, are lower than the total ration needs (Carruthers & Neil, 1997; Muller & Fales, 1998; Muller, 2001a; 2003a). The ratio of CP (25 to 30 % DM) to soluble carbohydrates (10 to 15 % DM) is high (Hodgson & Brookes, 1999).

The CP of pasture has a high rate and extent of ruminal degradability with usually approximately 70 to 80 % of total protein being degradable in the rumen (Merchen & Bourquin, 1994; Carruthers *et al.*, 1997; Muller & Fales, 1998; McCormick *et al.* 1999; 2001b; Muller, 2001a; 2003a; 2003b). Holden *et al.* (1994a) found 60 to 80 % of CP in pasture to be RDP, this portion being higher at the times of year when the fibre content of the grass was lower.

The chemical composition of pasture changes continuously. In the trial of Meeske *et al.* (2006) the CP concentration of annual ryegrass varied from 13.6 to 31 % DM indicating the importance of regular analysis of grass samples. The quality of the pasture usually decreases with maturity in the warm summer months (Muller, 2001a; 2003a) with the protein content decreasing and the NDF content increasing (Muller, 2003b; Bargo *et al.*, 2003a) and the degradability of DM, OM, CP and NDF decreasing (Van Vuuren *et al.*, 1991; Merchen & Bourquin, 1994; Bargo *et al.*, 2003a).

The CP content of pasture is influenced by the time of fertilizer application (Kellaway *et al.*, 1993). Nitrogen fertilization can increase the total CP, soluble protein (Sol CP) and digestibility (*in sacco* degradability) of OM and CP of pastures (Van Vuuren *et al.*, 1991; Peyraud *et al.*, 1997; Hacker & Jank, 1998; Muller & Fales, 1998; Muller, 2001a; 2003a).

The ME content of pasture (or any feed) cannot be determined directly as with the other chemical components. Estimating the true ME value of a feed would require confinement of animals in respiration chambers to determine energy intake and excretion in faeces, urine and

methane. It needs to be estimated with an equation from the digestibility of the feed (Doyle *et al.*, 2005). Several equations could be used, all yielding similar estimates. For example the SCA (1990) suggested several equations for predicting ME/kg DM one of which is 0.18 DOMD % – 1.8. Kellaway *et al.* (1993) used the equation 0.16 × DOMD which was also suggested by Hodgson & Brookes (1999). The SCA (1990) equation for predicting ME from CP, ether extract (EE), crude fibre and ash would probably have a higher error than those predicted from digestibility. Robinson *et al.* (2004) found the equation ME (MJ/kg DM) = 0.82 × (GE × IVOMD) to be a good unified equation for any potential ruminant feedstuff.

The bottom line is that the nutrient intake from pasture depends on many factors such as the pasture type and management and the season. Lush pasture has a high ratio of CP: NSC and the protein is highly degradable.

2.4 NUTRITIONAL IMBALANCES IN PASTURE

Pasture alone does not meet the nutrient requirements of especially bigger, high producing dairy cows (Muller & Fales, 1998; Kolver & Muller, 1998; Delahoy *et al.*, 2003) and will probably result in partitioning of body energy reserves towards milk production (Muller & Fales, 1998). Peyraud & Delaby (2001) stated that a cow producing 40 kg of milk per day at turnout should be able to produce about 28 kg/d with no supplements on spring grazing.

Milk production might be limited by an imbalance of rumen fermentable carbohydrate and RDP (Reis & Combs, 2000) as pasture has high CP and NDF and low ME or NSC concentrations compared to diets recommended for high producing dairy cows (Carruthers *et al.*, 1997; Clark & Kanneganti, 1998). Nutrient, specifically protein, utilization by the rumen micro-organisms is not optimal with pasture alone as fermentable carbohydrate, the major source of energy for the rumen microbes, is lower in most pastures than required (Muller, 2001a; 2003a; 2003b). One of the challenges of utilising pasture is maximising ruminal N capture (Gehman *et al.*, 2006) as synthesis of microbial protein from RDP is energy dependent and energy supply is usually the main limiting factor (Carruthers *et al.*, 1997; Hodgson & Brookes, 1999).

Pastures containing up to 33 % CP are often considered to contain all the CP required (Donaldson *et al.*, 1991). In fact dairy cows consuming this pasture and receiving a grain

supplement could be receiving 20 to 30 % more CP and RDP than required for maximum performance (McCormick *et al.*, 1999; 2001a). There is an energy cost associated with overfeeding protein (McCormick *et al.*, 2001a). The high CP degradability of pasture and the asynchronous relationship between protein and energy availability for rumen microbial protein synthesis result in high ruminal ammonia (NH_3), the absorption of which requires energy, high blood urea N (BUN) and milk urea N (MUN), and excessive excretion of N in the urine (Carruthers *et al.*, 1997; Peyraud *et al.*, 1997; Kolver *et al.*, 1998a; Muller, 2003; Gehman *et al.*, 2006). The inefficient utilisation of the high protein and the energy needed to excrete this excess protein can cause losses in milk production (Muller, 2001a; 2003b). Thus the utilization of pasture protein is inefficient with less than 20 % of the dietary N appearing in the milk (Muller, 2003b). Apart from reduced milk production, feeding excess total rapidly degradable protein in pasture and supplementing inadequate fermentable carbohydrates can cause fast nutrient degradation and passage through the rumen, loose manure, reduced milk fat percentage and loss of body condition (Muller, 2003b). Feeding diets that contain excess dietary protein also impairs reproductive performance of dairy cows grazing ryegrass (McCormick *et al.*, 1999); BUN and MUN levels of greater than 20 mg/dl have been associated with low pregnancy rates (Gehman *et al.*, 2006). There is no advantage to feeding formulated levels of RDP or UDP above the proposed levels (Sloan *et al.*, 1988).

Although the CP of pasture is high, post-ruminal protein supply could be deficient as a significant portion of this protein does not reach the small intestine due to the high degradability in the rumen (Donaldson *et al.*, 1991). Metabolisable protein, both RUP and microbial protein, reaching the small intestine, may be inadequate for high producing cows in early lactation (MacDonald *et al.*, 1998; Muller, 2001a; 2003a; 2003b). See section 2.8 for more detail.

The "effective fibre" (fibre that stimulates rumination) may be too low in high quality pasture, which could result in low ruminal pH and reduced milk fat concentration (Hodgson & Brookes, 1999; Muller, 2001a). It is possible that only 40 to 50 % of the fibre in high quality pastures may be effective (De Veth & Kolver, 2001). The effective fibre of pasture ranges from 17 to 78 % with a mean of 43 % (Kolver & De Veth, 2002). Cows on only pasture could, on the other hand, have lower effective fibre requirements than recommended for mixed forage-concentrate diets (De Veth & Kolver, 2001).

Often minerals, including Ca, P, and Mg, S, Zn and salt are inadequate and K may be too high (Muller, 2001a; 2003a). Vitamins A and E are high and do not usually need to be supplemented (Muller, 2003a).

The bottom line is that, while pasture has a high RDP, energy is limited. Other nutrients such as RUP and effective fibre could also be sub-optimal.

2.5 SUPPLEMENTATION

Supplementation has been practised on both temperate and tropical grass pastures in an attempt to economically improve animal productivity by making up the deficiencies in the grass while at the same time maximising the utilisation of the pasture which, when grazed effectively, is the cheapest source of nutrients for dairy cows (Paterson *et al.*, 1994; Hacker & Jank, 1998; Peyraud & Delaby, 2001; Muller, 2001a; 2003a; Horan *et al.*, 2006). Feed supplements provide additional energy, protein and minerals when grazed forage falls short of the animal's nutrient requirements with the aim of providing the cow with a balanced diet to support production and maintain good health (Clark & Kanneganti, 1998; Doyle *et al.*, 2005).

High quality temperate pasture is adequate for cows producing up to 20 kg of milk a day (Fulkerson *et al.*, 1998). But for high genetic merit, high yielding diary cows in early lactation, providing complementary concentrates with high nutrient (specifically energy) concentrations is a necessary part of any grazing strategy in order for them to reach their genetic potential for milk production (Muller & Fales, 1998; Fulkerson *et al.*, 1998; Bargo *et al.*, 2002a; 2002b; Kolver, 2003).

The main objective of supplementing a grazing dairy cow is to increase the total DM and energy intakes and improve animal performance relative to a pasture-only diet and also to optimise profit per cow and per unit land (Peyraud & Delaby, 2001; Bargo *et al.*, 2003a). Reasons for supplementation include correction of a nutrient deficiency in the forage; increased milk production per cow; increasing milk protein content by energy supplementation; increasing the carrying capacity of the pasture (increased stocking rate and milk production per unit land area); providing a carrier for additives; maintaining a high BCS; helping prevent or treat potential health problems; and enhancing cattle management (Paterson *et al.*, 1994; Bargo *et al.*, 2003a).

Proper supplementation, in the form of ruminally available carbohydrates, maximises rumen fermentation and microbial protein synthesis, capturing the N from the pasture, which contributes to optimum milk production and profit (Muller & Fales, 1998; Muller, 2001a; 2003b). If needed, supplements can also contain protein and minerals (Clark & Kanneganti, 1998).

In order to develop appropriate supplemental feeding strategies there needs to be an understanding of the supply of pasture nutrients and the order in which they become first limiting to milk production (Kolver, 2003).

High levels of milk production can be achieved with high intakes of pasture DM and supplementation of grain at about 1 kg grain to 4 kg milk (Muller & Fales, 1998; Hongerholt & Muller, 1998; Bargo *et al.*, 2002b; Delahoy *et al.*, 2003). It is recommended not to supplement more than about 10 kg DM/d (or more than 50 % of the total diet dry matter intake (DMI)) in order to avoid metabolic health problems such as acidosis or sub-clinical acidosis (Bargo *et al.*, 2003a).

2.5.1 Types of supplements

2.5.1.1 Energy (grain) supplementation

Major sources of supplemental energy are carbohydrates from grains and concentrates (Muller & Fales, 1998; Muller, 2001a). Maize is a common supplement fed to grazing cows, providing supplemental energy and increasing the total DMI compared to pasture only (Delahoy *et al.*, 2003).

Grains ferment in the rumen at different rates which could be applied to match the rate of degradation of the pasture N with the rate of carbohydrate degradation from the supplement. Maize starch degrades at a slower rate than pasture N while barley has a faster starch degradation rate in the rumen and should theoretically improve microbial NH_3 capture (Gehman *et al.*, 2006).

Granzin (2004), on the other hand, found that feeding a maize-based rather than barley-based supplement resulted in greater milk fat and protein concentrations and milk fat yields for cows grazing ryegrass and prairie grass (29 % CP) and greater milk protein concentrations and yields for cows grazing kikuyu (20 % CP).

Peyraud & Delaby (2001) recommended not using highly fermentable carbohydrates when more than 5 kg of concentrate is offered, due to the risk of digestive disturbances.

Although processing (steam flaking or grinding) of grains is expected to improve performance (Muller, 2003a) there seems to be a lack of response, possibly because processing only changes the site of digestion (energy being available in the rumen rather than the post-ruminal tract) and not the total energy intake (Bargo *et al.*, 2003a; Delahoy *et al.*, 2003).

2.5.1.2 Protein supplementation

Lactating cows grazing high quality pasture are thought to require little supplementary protein (Schroeder & Gagliostro, 2000; Muller, 2001a). With high quality pasture that is high in protein, a concentrate containing 12 to 14 % CP should be adequate, providing a total ration with 16 to 18% CP (Muller, 2003a; 2003b). There would be no benefit in feeding extra RDP in the concentrate of cows grazing pasture containing more than 14 % CP (Schor & Gagliostro, 2001).

Since 70 to 80 % of the N in the pasture is degraded in the rumen, if supplemental protein is fed it should have low rumen degradability and be rich in limiting AA (Schroeder & Gagliostro, 2000). The addition of protein sources high in RUP should be considered with high producing cows in early lactation (Muller, 2001a). Examples of feedstuffs high in RUP that allow a high percentage of CP to flow to the abomasum are roasted soybeans, maize gluten meal, distillers dried grains, distillers dried grains with solubles, brewers dried grains, brewers wet grains, FM, meat and bone meal, feather meal, BM and specially processed soy protein (Donaldson *et al.*, 1991; Santos *et al.*, 1998; Muller, 2001a; 2003a). Formaldehyde treatment is a way of protecting protein from degradation in the rumen, increasing the supply of AA to the small intestine (Rogers *et al.*, 1980; Hamilton *et al.*, 1992).

The AA profile of the protein has a greater effect on production than the amount of CP in the diet (Bach *et al.*, 2000). The AA profile of protein sources is reflected in the profile of AA in the duodenal digesta, especially for protein sources of low degradability, emphasising the importance of careful selection of dietary protein supplements and combinations that will complement bacterial protein (Rulquin & Vérité, 1993; Erasmus *et al.*, 1994).

2.5.1.3 Forage supplementation

Conserved forage can be fed when pasture growth and availability is limited but not as a rule as the substitution rate is high (Muller & Fales, 1998; Peyraud & Delaby, 2001).

Feeding long hay will add some effective fibre to the diet and likely slow the rate of passage and help maintain feed intake and milk fat percentage (Muller, 2001a). It can benefit rumen fermentation since dietary fibre is often inadequate in high quality pastures (Muller & Fales, 1998). Hay supplementation has varying effects on total DMI and either a higher milk production or no response (Bargo *et al.*, 2003a).

Maize silage is a good supplemental forage to complement pasture because it is relatively high in energy and fibre and dilutes the high protein of spring pasture (it has a low CP of 8 – 10 % of DM). It is highly palatable, a good carrier for concentrate and can allow for lower amounts of grain to be fed (Hodgson & Brookes, 1999; Muller, 2001a).

2.5.1.4 Other supplements

The addition of non-forage fibre or fermentable fibre sources to the concentrate may be beneficial in providing fermentable fibre to the rumen. These include soy hulls, beet pulp, distillers grains, citrus pulp, wheat middlings, whole cottonseed, cottonseed hulls and some other by-products (Muller, 2001a; 2003a; Delahoy *et al.*, 2003). Supplementation with these non-forage fibre sources has sometimes increased pasture and total DMI (Delahoy *et al.*, 2003; Gehman *et al.*, 2006) and milk production (Delahoy *et al.*, 2003) as well as benefiting milk fat percentage (Muller, 2003a). A concentrate mixture that contains starch with some non-forage fibre that is finely ground, will provide a blend of rapidly and slowly fermentable carbohydrate and could improve the milk response (Muller, 2003a).

A few studies have indicated that pasture supplementation with fat generally does not affect DMI, increases milk production and fat and protein yield and has no effect on fat or protein percentage in the milk (Bargo *et al.*, 2003a).

2.5.2 Supplementation strategies

Supplements can be administered at a constant level to all the cows or computed as a function of cow potential: the higher the cow's potential the higher the concentrate allocation; the former method is feasible at least under grazing conditions where maximum grass intake is favoured (Peyraud & Delaby, 2001).

Grain is normally fed twice a day when the cows are milked, which could cause large fluctuations in rumen environment, compromising fibre digestion and microbial growth (Hongerholt *et al.*, 1997; 1998; Peyraud & Delaby, 2001; Muller, 2003a). Theoretically, increased frequency of feeding concentrates should result in less diurnal variation in rumen pH, which should increase the amount of grass the cow can consume and increase animal performance (Peyraud & Delaby, 2001). Increasing feeding frequency from two to four times a day reduced diurnal variation in ruminal pH in the continuous culture study of Holgerholt *et al.* (1998). However, increasing the frequency of concentrate meals has been found not to improve animal performance (Peyraud & Delaby, 2001). More frequent grain feeding in the study of Hongerholt *et al.* (1997) did not affect milk yield or composition.

Feeding more than 3 or 4 kg of grain at one feeding should be avoided (Muller, 2003a).

The bottom line is that concentrate feeding is used to supply limiting nutrients, especially energy, but also quality protein and other nutrients, in order to balance the diet to support higher milk production.

2.6 PASTURE INTAKE AND TOTAL DRY MATTER INTAKE

2.6.1 Pasture Intake

Under good management pasture intake is normally sufficient to meet the requirements of medium-sized cows but not larger cows producing high levels of milk, since larger cows have a greater milk production relative to intake capacity (Kolver, 2003). Low voluntary pasture DMI (PDMI) is a major factor limiting milk production from high producing cows under grazing

conditions (Dalley *et al.*, 1999; Reis & Combs, 2000; Bargo *et al.*, 2003a; Kolver, 2003; Kennedy *et al.*, 2003). The lower DMI of a cow consuming pasture only is due to physical constraints such as the capacity of the reticulo-rumen, the rate of forage removal from the rumen by digestion and passage, water consumption associated with pasture and grazing time (Hodgson & Brookes, 1999; Bargo *et al.*, 2003a; Kolver, 2003; Horan *et al.*, 2006). The level of intake of green forage is inversely related to the filling effect of the forage in the rumen, which depends on the fibre content (Journet & Demarquilly, 1979). The high *in vivo* digestibility of pasture NDF suggests that the upper limit to intake of high quality pasture could be related more to the constraints of grazing time and bite rate than to rumen fill (Kolver, 2003).

Dairy cows will typically consume approximately 3 % of their BW as DM when fed only high quality pasture (Kolver & Muller, 1998; Muller, 2003a). When there are no pasture quantity and quality restrictions, PDMI by large high producing dairy cows can reach 3.5 % of BW (Kolver, 2003). In New Zealand the DMI by cows consuming pasture, estimated using the difference technique, has been as much as 4.5 % of BW (Holmes, 1987).

High producing cows can increase their herbage intake according to their potential milk yield (Peyraud & Delaby, 2001). For each 1 kg increase in milk yield (in the range of 15 to 30 kg milk/d) cows will consume an extra 0.4 to 0.5 kg DM/d (Kolver, 2003). Since there is a close relationship between intake and digestibility, for high producing cows it is important to provide young, digestible forage to promote high intake (Journet & Demarquilly, 1979).

Forage intake depends on the quantity of forage present per hectare, its height and the quantity of refusals tolerated (Journet & Demarquilly, 1979). Pasture allowance (amount of pasture offered per cow in kg DM per cow per day) is an important factor affecting voluntary feed intake and production of dairy cows (Dalley *et al.*, 1999). Pasture DMI increases curvilinearly (at a declining rate) as the PA increases, associated with a decrease in pasture utilisation (pasture intake as a proportion of pre-grazing herbage mass) and increase in milk production (Dalley *et al.*, 1999; Bargo *et al.*, 2003a). Several studies (Wales *et al.*, 1998; 1999; Delaby *et al.*, 2001; Dalley, 2001; Williams *et al.*, 2005) found increased PDMI with increasing PA. Maximum PDMI is achieved when PA is 3 to 5 times the DMI (Bargo *et al.*, 2003a). Unrestricted pasture conditions (high PA) lead to low pasture utilisation (PDMI/PA less than 50 %) and the pasture quality deteriorates as the season progresses due to the increase in residual height (Dalley *et al.*, 1999; Peyraud & Delaby, 2001). Due to the deterioration of pasture quality

and low pasture utilisation at high PA, a practical recommendation is to provide a PA of two times the expected PDMI (Bargo, 2003a).

The intake of certain grass species is higher than others, promoting higher milk production. Intake is reduced as the plant matures. Later in the season intake is lower, possibly due to factors such as contamination by defaecation as well as decreased digestibility (Journet & Demarquilly, 1979; Hodgson & Brookes, 1999). Some, but not all, studies have found a greater voluntary intake of temperate than tropical forages (Merchen & Bourquin, 1994). High moisture content of some pastures could also restrict pasture intake (Hodgson & Brookes, 1999).

Apart from being affected by PA, PDMI is also affected by level of supplementation, interaction between PA and supplementation, fat-corrected milk (FCM), BW, change in BW, percentage legumes in the pasture and pasture NDF content. Most of the changes in DMI can be explained by variables related to the cow such as BW and milk yield (Vazquez & Smith, 2000).

The bottom line is that pasture intake is driven mainly by the requirements of the cow although it also depends on pasture management. High producing cows probably do not have the capacity to consume enough pasture to support production on pasture alone.

2.6.2 Total dry matter intake and substitution rate

When supplemental grain is fed, PDMI decreases as grain substitutes for pasture, but the total DM and energy intakes increase (Stakelum, 1986a; 1986b; Faverdin *et al.*, 1991; Muller & Fales, 1998; Muller, 2001b; Bargo *et al.*, 2003a). Increasing the concentrate supplementation linearly increases total DMI (Dillon *et al.*, 2002; Sairanen *et al.*, 2005); it helps overcome the physical limitations to pasture intake (Horan *et al.*, 2006).

The reduction in PDMI per kg supplement is known as substitution rate (SR) and is calculated as SR (kg/kg) = (PDMI in un-supplemented treatment – PDMI in supplemented treatment)/ supplement DMI. A SR of less than 1 kg/kg, which is normally the case, means that the total DMI on the supplemented treatment is higher than on pasture alone (Muller & Fales, 1998; Muller, 2001b; Bargo *et al.*, 2002a; 2003a). Substitution rate can vary from about 0.4 to 1.0 kg decrease in PDMI per kg concentrate fed (Journet & Demarquilly, 1979; Muller & Fales, 1998; Muller, 2001b).

Journet & Demarquilly (1979) stated that the higher the quantity of concentrate offered the greater the substitution. Faverdin *et al.* (1991) found that whatever the type of roughage used, the SR increases systematically as the amount of concentrate in the diet increases: SR increased 0.093 per kg of extra concentrate fed. Others (Jones-Endsley *et al.*, 1997; Dillon *et al.*, 1997; Peyraud & Delaby, 2001) found no consistent influence of amount of supplement on pasture intake. This is probably because high producing dairy cows seldom approach their maximum voluntary intake under grazing conditions (Peyraud & Delaby, 2001).

The higher the SR the lower the milk response per kg supplement and the lower the pasture utilisation (Clark & Kanneganti, 1998; Peyraud & Delaby, 2001; Bargo *et al.*, 2002a; 2003a; 2003b). Since grazed forage is the cheapest source of nutrients, the objective is to reduce the substitution effect while increasing the supplementation effect (Clark & Kanneganti, 1998).

Substitution rate and milk response are affected by pasture species, height, mass, allowance, intake and quality, amount and type of supplementation, as well as genetic merit, production level and stage of lactation of the cows (Stakelum, 1986b; Faverdin *et al.*, 1991; Dillon *et al.*, 1997; Bargo *et al.*, 2002a; 2003a).

Substitution rate generally increases as PA increases (Stakelum, 1986a; 1986b; Hodgson & Brookes, 1999; Peyraud & Delaby, 2001; Bargo *et al.*, 2002a; 2003a). Thus concentrate feeding increases the total DMI by a greater amount at lower PA (Stakelum, 1986a; Hodgson & Brookes, 1999; Bargo *et al.*, 2002a).

Substitution rate is also affected by the energy balance of the cow; it is low when energy intake is low compared to the cow's energy requirements (Peyraud & Delaby, 2001; Bargo *et al.*, 2002a). Thus a low SR and high milk response can be expected from high producing dairy cows because of the high genetic potential for intake and milk production and less partitioning of the energy for maintenance (Dillon *et al.*, 1997; Bargo *et al.*, 2002a). There is a higher milk response in cows in early lactation or with increasing grazing intensity (Peyraud & Delaby, 2001; Bargo *et al.*, 2002a). In practice energy balance differs according to grass intake which could explain why many studies have concluded that SR is positively related to PA (Peyraud & Delaby, 2001).

Substitution rate is also positively related to herbage digestibility (Paterson *et al.*, 1994; Peyraud & Delaby, 2001) as energy balance differs according to the quality of the grass, thus responses can increase during the grazing season when the grass quality and availability decrease

(Peyraud & Delaby, 2001). Substitution of kikuyu pasture (0.16 kg/kg) in the trial of Hamilton *et al.* (1992) was lower than that of temperate pastures.

The type of supplement influences SR and animal performance. Forage supplements decrease PDMI more than concentrates. However, if the pasture is highly degradable, adding a fibre-based supplement could result in a higher DMI by maintaining a higher pH in the rumen (Bargo *et al.*, 2003a). Fermentable carbohydrates, such as barley, reduce the ruminal pH, decreasing the activity of cellulolytic bacteria, reducing the rate of NDF digestion of the pasture and therefore the PDMI (Paterson *et al.*, 1994; Hodgson & Brookes, 1999; Bargo *et al.*, 2003a). When rapidly digestible fibre-based supplements were fed, the reductions in forage consumption were not as great as with starch-based supplements (Paterson *et al.*, 1994). Not all starch-based supplements consistently decrease forage intake. Small quantities of maize-based supplements can stimulate forage intake (Paterson *et al.*, 1994). Supplements with a slower fermentation rate would have a lower SR (Bargo *et al.*, 2003a).

To summarise: supplementation increases total DMI, especially when SR is low, in other words when PA is low and the requirements of the cow high.

2.6.3 Estimating dry matter intake in grazing cows

In grazing cows it is important, but difficult, to quantify pasture intake as it cannot be determined directly as with cows in confinement, and is one of the challenges when utilising pasture (Stockdale & King, 1983; Holden *et al.*, 1994a; Paterson *et al.*, 1994; Reeves *et al.*, 1996; Vazquez & Smith, 2000; Bargo *et al.*, 2003a). Estimations of pasture intake vary with the method used (Stockdale & King, 1983). With a group of cows it is very difficult to estimate what each individual cow consumes (Stewart *et al.*, 1995).

Dry matter intake of pasture can be estimated with animal- or pasture-based techniques (Stockdale & King, 1983). The disadvantage of the latter is that DMI is estimated as a group and not individually.

2.6.3.1 Animal-based techniques

The animal-based technique is based on the ratio between faecal production (estimated with markers such as chromium oxide and alkanes) and diet indigestibility (Stockdale & King, 1983; Bargo *et al.*, 2002a; 2003a). Chromic oxide (Cr_2O_3) is the most widely used marker for the determination of faecal output (Stockdale & King, 1983). Many grazing studies (including McCormick *et al.*, 2001a; Schor & Gagliostro, 2001; Bargo *et al.*, 2001; Delahoy *et al.*, 2003; Gehman *et al.*, 2006 and Soder *et al.*, 2006) determined DMI by dosing Cr_2O_3, an indigestible marker, and taking faecal grab samples. Faecal output (kg DM/cow/d) = daily dose of marker (g Cr/d)/ faecal concentration of marker (g Cr/kg faecal DM). Total DMI = faecal output/ (1 – *in vitro* DM digestibility (IVDMD)). Pasture DMI = total DMI – supplement DMI. Pasture DMI estimates were further refined through weighting IVDMD and recalculating PDMI. Chromic oxide as a faecal marker could overestimate DMI (Bargo *et al.*, 2002a; Gehman *et al.*, 2006).

Reeves *et al.* (1996), Dillon *et al.* (1997), Granzin (2004), Fulkerson *et al.* (2005) and Horan *et al.* (2006) estimated the intake of grazed grass by the cows using the n-alkane technique which uses the herbage C33 (or C31) to dosed C32 alkane ratio or C35 (high in kikuyu) to C36 alkane ratio (Reeves *et al.*, 1996). This method relies on the recovery rate of the different alkanes being the same and is generally more accurate and precise than using the rising plate meter (RPM; see section 2.6.3.2; Reeves *et al.*, 1996).

Dry matter intake could also be estimated by looking at aspects of ingestive behaviour such as grazing time, bite rate, bite mass and intake rate (Burns & Sollenberger, 2002).

2.6.3.2 Pasture-based techniques

The pasture technique for measuring PDMI involves measuring the pasture before and after grazing (Kellaway *et al.*, 1993). Stockdale & King (1983) suggested that this technique (difference method/ sward sampling method), based on pre- and post-grazing sampling has the greatest potential for providing valid estimates of pasture intake and is more likely to give reliable estimates of pasture intake of grazing dairy cows than the animal-based method, provided periods of grazing are short with high stocking densities and sampling is adequate. They (Stockdale & King, 1983) compared this method to the animal-based method (faecal output-indigestibility ratio using Cr_2O_3) and found that the former technique estimated higher DMI than

the latter. The pasture-based technique was considered more accurate as the animal productivity relative to DMI was closer to the expected values.

An advantage of the sward cutting technique for estimating intake is that it is unaffected by supplementation; it does not depend on estimating pasture digestibility which is affected by concentrate feeding (Stakelum, 1986a).

The amount of pasture available (pasture yield; kg DM/ha) can be measured directly with the quadrat technique which is based on randomly cutting small areas of grass, 0.1 to 1.0 m^2, to a certain height above the ground (Hodgson *et al.*, 1999). Earle & McGowan (1979) found a quadrat size of 0.2 m^2 to be most satisfactory, and most trials (including Kolver & Muller, 1998; Dalley *et al.*, 1999; Bargo *et al.*, 2002a; 2002b and Williams *et al.*; 2005) used an area similar to this. Due to the variability within the pasture, 10 to 20 quadrat areas should be cut at a time (Hodgson *et al.*, 1999) although the number has varied from 5 (Kolver & Mulller, 1998; Delahoy *et al.*, 2003) to 34 (Stockdale & King, 1983). Many trials (including Schor & Gagliostro, 2001; Bargo *et al.*, 2002a; 2002b; Delahoy *et al.*, 2003; Williams *et al.*; 2005 and Meeske *et al.*, 2006) cut the grass to ground level while others cut to 2.5 cm (McCormick *et al.*, 1999; 2001a), 3.5cm (Dillon *et al.*, 1997; 2002), 4 cm (Tesfa *et al.*, 1995) or 5cm (Hoden *et al.*, 1991; Fulkerson & Slack, 1993; Reeves *et al.*, 1996; Hongerholt & Muller, 1998; Delaby *et al.*, 2001) above ground level based on the assumption that cows would not graze below these levels. Cutting the quadrats to ground level means that no assumptions are made about the level to which the animals graze and is also the most repeatable (Kellaway *et al.*, 1993; Hodgson *et al.*, 1999). The DM content of the herbage samples is calculated after drying at temperatures ranging from 55°C (Kolver *et al.*, 1998a; Kolver & Muller, 1998; Bargo *et al.*, 2002a; 2002b) to 100°C (Earle & McGowan, 1979; Wales *et al.*, 1998; Dalley *et al.*, 1999; Williams *et al.*; 2005) for a period of 24 hours (Earle & McGowan, 1979; Wales *et al.*, 1998; Dalley *et al.*, 1999; Williams *et al.*; 2005) to 72 hours (Meeske *et al.*, 2006). Weight of DM can then be converted to yield in kg per ha (Earle & McGowan, 1979). Due to the variability within pastures, large numbers of samples must be cut which is physically limiting (Earle & McGowan, 1979).

Indirect techniques for measuring pasture include visual assessment, a sward stick, the rising or falling plate meter and the electronic capacitance probe (Gourley & McGowan, 1991; Fulkerson & Slack, 1993; Tesfa *et al.*, 1995; Hodgson *et al.*, 1999; Fulkerson *et al.*, 2005). The latter two are useful for obtaining herd estimates of pasture intake, are non-destructive and useful

in overcoming errors from variability within paddocks since many measurements can be conveniently obtained (Earle & McGowan, 1979; Reeves *et al.*, 1996).

The rising plate meter (RPM), such as the Ellinbank Pasture Meter, as described by Earle & McGowan (1979), measures compressed pasture height. It consists of a plate free to move up and down a central column. The pasture raises the level of the plate which rests on the pasture while the central column rests on the ground. The height at which the plate rests is recorded and a series of measurements accumulated on the counter. The height of the plate depends on a combination of pasture height, density and species and reflects the DM yield of the pasture (Earle & McGowan, 1979; Hodgson *et al.*, 1999).

Indirect techniques first need to be calibrated to herbage mass using direct cutting techniques. A calibration equation is used to relate the RPM reading to herbage mass (Stockdale & King 1983; Kolver *et al.*, 1998; Dalley *et al.*, 1999; Hodgson *et al.*, 1999). The weight per unit area of the plate affects the quantitative relation between the height of the plate and the yield of the pasture (Earle & McGowan, 1979). The RPM needs to be calibrated for a specific situation whenever it is used in a new environment or a new pasture type and for research (Earle & McGowan, 1979; Hodgson *et al.*, 1999). Sanderson *et al.* (2001) found high error levels of 26 to 33 % when universal calibration equations were used for the RPM, electronic capacitance meter and pasture ruler, indicating the importance of at least region specific calibration equations and preferable frequent calibration. Changes in the growth pattern of kikuyu mean that a few calibration equations are needed for different parts of the season (Reeves *et al*. 1996). Fulkerson & Slack (1993) found that separate regression equations were required for kikuyu in early (November to February) and late (March to May) season.

A standard linear regression is normally used: $Y = aX + b$ where Y = pasture mass (kg DM/ha) and X = RPM reading (Earle & McGowan, 1979; Hodgson *et al.*, 1999). How well the data fits the line can be represented with residual standard deviation (RSD) or R^2 (the latter should be 0.80 to 0.85; Hodgson *et al.*, 1999). Pairs of RPM readings and pasture mass, for a range of pasture masses (low, medium and high RPM readings), are used as data points to establish a linear equation by regression. At each site the RPM reading is taken and the pasture mass is measured at the same site, the latter involving cutting, washing (if samples are cut to ground level), drying and weighing pasture samples (Earle & McGowan, 1979; Fulkerson & Slack, 1993; Kellaway *et al.*, 1993; Hodgson *et al.*, 1999; Schroeder & Gagliostro, 2000; Schor

& Gagliostro, 2001). Accuracy of the regression established depends on the number of paired samples that were used to calculate it. It is recommended that at least 20 paired samples be used, although as few as five can be used (Hodgson *et al.*, 1999). Fulkerson & Slack (1993) used 36 or 100 per calibration equation. Data from calibrations on a similar pasture type can be pooled over time to develop one standard regression (Wales *et al.*, 1998; Hodgson *et al.*, 1999) with higher accuracy since more values have contributed (Earle & McGowan, 1979).

Pre- and post-grazing pasture yields can be measured with the RPM (Earle & McGowan, 1979; Kolver *et al.*, 1998a; Dalley *et al.*, 1999; Fulkerson *et al.*, 1998; 2005; Williams *et al.*, 2005) by taking many (25 to 200) readings per paddock (Hoden *et al.*, 1991; Kellaway *et al.*, 1993; Hodgson *et al.*, 1999; Delaby *et al.*, 2001; Williams *et al.*, 2005). Daily PDMI per cow is calculated from the difference between the estimated pasture yields pre- and post-grazing (kg DM/ha). PDMI = (pre-grazing pasture yield – post-grazing pasture mass)/ number of cows × area (Stockdale & King, 1983; Kellaway *et al.*, 1993; Reeves *et al.*, 1996; Delaby *et al.*, 2001). Reeves *et al.* (1996) and Fulkerson *et al.* (2005) corrected pasture mass for the growth between the times of measuring using the mean growth rate of the previous inter-grazing interval. Kellaway *et al.* (1993) ignored pasture growth because no more than 24 hours elapsed before measurements were made.

This technique is dependent on whether enough samples are taken to account for the inherent variability of the pasture and on correlation between height and yield (Stockdale & King, 1983). Earle & McGowan (1979) found that the variation in DM % of the pasture throughout the day only had small effects on the meter readings and that there was a high level of repeatability of readings within operators but there was a substantial degree of variation between operators. Fulkerson & Slack (1993), however, found between-operator variability to be small provided the correct operating procedures were followed.

Earle & McGowan (1979) stated that the Ellinbank Pasture Meter is “accurate enough for research purposes yet simple enough for use by farmers and their advisers as an aid to pasture management”. Gourley & McGowan (1991) found the RPM to have a similar ability to the direct plot-harvesting technique in detecting differences in herbage mass, with advantages in capital cost, time and efficiency.

Determining intake of tropical grass pasture is less accurate than temperate pastures due to higher DM on offer and a lower proportion being removed at each grazing (Fulkerson & Slack,

1993). Fulkerson & Slack (1993) found that for kikuyu calibrating the RPM against total DM (quadrats cut to ground level) gave low accuracy of estimating grass mass, even in well managed and highly utilised swards. The standard error of estimate was improved if the calibration was done against shoot DM (green leafy material). With tropical grasses there is a rapid build-up of senescent material. It is sensible to remove the stubble component when doing calibrations as cattle are unlikely to graze material below specified stubble heights and stubble DM below 5 cm increased as season progressed, increasing the slope of the equation (Fulkerson & Slack, 1993).

Stockdale & King (1983), Reeves *et al.* (1996), Wales *et al.* (1998) and Williams *et al.* (2005) obtained calibration equations for both pre- and post-grazing pasture. In the study by Reeves *et al.* (1996) pre-grazing calibration equations for the RPM differed from post-grazing calibrations thus, when pasture intake was determined as the difference between pre- and post-grazing pasture mass, separate equations were used.

The RPM could not detect differences in kikuyu pasture intake between cows receiving 3 and 6 kg concentrate/day, due to large errors with this technique (Reeves *et al.*, 1996).

An alternative to the RPM would be a drop disc method, based on the settled height of a light-weight disc or plate dropped onto the sward form a fixed height. The settled height is calibrated to herbage DM in the same way as for the RPM and is also rapid and non-destructive (Douglas & Crawford, 1994). Douglas & Crawford (1994) found a close linear relationship (R^2 = 0.829) between disc settlement height and DM mass up to 4 to 5 t/ha.

The electronic capacitance meter indirectly measures the herbage mass by measuring the electrical capacitance of the herbage (Hodgson *et al.*, 1999). It gives less accurate readings in wet conditions making the plate meter preferable in these conditions (Kellaway *et al.*, 1993; Hodgson *et al.*, 1999). A correction could be made for the moisture in the air by taking an air reading (Hodgson *et al.*, 1999). Kellaway *et al.* (1993) preferred the electronic meter as it was less subject to bias due to herbage with rigid stems. Virkjärvi (1999) found the disc meter to predict herbage mass more accurately than the capacitance meter.

2.6.3.3 Equations

Equations to predict DMI of grazing cows based on animal and pasture characteristics have been developed by Caird & Holmes (1986) and Vazquez & Smith (2000). NRC (2001)

predicts DMI with an equation using only FCM, BW and week of lactation (WOL) as follows: DMI (kg/d) = ((0.372)(4 % FCM) + (0.0968)($BW^{0.75}$))(1 – $e^{(-0.192 \times (WOL + 3.67))}$).

Caird & Holmes (1986) developed an equation for predicting total OM intake (TOMI; kg/d) based on validation of data from other experiments where cows were consuming 1.2 kg/d of concentrate and producing 21.5 kg milk/d. For rotationally grazed cows TOMI = 0.323 + (0.177)(MY) + (0.010)(LW) + (1.636)(C) – (1.008)(HM) + (0.540)(PA) – (0.006)(PA^2) – (0.048)(PA×C); R^2 = 0.677, RSD = 1.91, n = 165; where MY is milk yield (kg/d), LW is liveweight (kg), C is concentrate supplied (kg/d), HM is herbage mass (ton OM/ha), PA is pasture allowance (kg OM/cow/d).

Vazquez & Smith (2000) used data from 27 published grazing studies with mean 4 % FCM of 16.4 kg/d concentrate intake of 1.9 kg DM to obtain regression equations for predicting total and pasture DMI. DMI = 4.47 + (0.14)(4 % FCM) + (0.024)(BW) + (2.00)(CBW) + (0.04)(PA) + (0.022)(PASUP) + (0.10)(SUP) – (0.13)(NDFp) – (0.037)(LEG); R^2 = 0.95, SD = 0.90, n = 90; where CBW = change in BW (kg/d), PA = pasture allowance (kg DM/d), PASUP = interaction between PA and SUP, SUP = supplement offered (kg/d), NDFp = NDF content of pasture (%), LEG = % legume in pasture. Their equation for PDMI is the same except the SUP term is – 0.90 instead of 0.010; R^2 = 0.91, SD = 0.90, n = 90. The regression equations for predicting pasture intake are similar to those for predicting total DMI except for the supplementation term indicating the substitution effect (Vazquez & Smith, 2000).

Bargo *et al.* (2003a) used data from the study by Bargo *et al.*, (2002b) to compare intake measured using Cr_2O_3 as a faecal marker with intake estimated by the above three equations. The equations of Caird & Holmes (1986) and NRC (2001) were found to accurately predict DMI for that dataset with high producing dairy cows but the equation of Vazquez & Smith (2000) predicted a higher DMI than was measured.

Bargo *et al.* (2003a) used data from several studies to arrive at the equation PDMI = 7.79 (SE 1.49) + 0.26 (SE 0.06) PA – 0.0012 (SE 0.0007) PA^2; R^2 = 0.95 for cows producing 23.0 to 45.8 kg milk and grazing at a PA of 12.1 to 70 kg DM/cow/d.

Neutral detergent fibre is the best single chemical predictor of voluntary DMI because it ferments and passes from the rumen slowly (Allen, 1996). Dry matter intake tends to decline with increasing NDF concentration in diets when more than 25 % of the diet consists of NDF due to rumen fill (NRC, 2001). Pasture has a higher NDF content than TMR so in the study of

Kolver & Muller (1998) grazing cows consumed more NDF as % BW than TMR fed cows, an NDF intake of 1.5% BW compared to 1.2% for TMR fed cows. Bargo *et al.* (2002b) found NDF intake to be 1.3 % of BW for cows consuming 60 % pasture (of 50 % NDF) and 40 % concentrate (DM basis). Vazquez & Smith (2000) reported an average NDF intake of 1.51 % of BW on only pasture and 1.38 % when concentrate is fed. Intake of NDF can be a good predictor of DMI in Holstein cattle (Rayburn & Fox, 1993) and NDF is commonly used as a predictor of DMI (Kolver & Muller, 1998). However the high apparent digestibility of NDF in lush pasture suggests that the fibre might result in a low rumen fill, having a small effect on DMI (Kolver & Muller, 1998). Intake of NDF could even be as low as 0.9 % of BW as was found in the study of Hongerholt & Muller (1998) for Holstein cows weighing 568 kg, producing approximately 35 kg milk per day, consumed 11.3 kg pasture DM/d (42 % NDF) while receiving 9 kg concentrate/d. Including NDF in the model for predicting DMI increases accuracy and reduces bias (Rayburn & Fox, 1993). Rayburn & Fox (1993), however, found that using a constant NDF intake of 1.2 % of BW for predicting DMI, had a higher error than equations using FCM and BW. Neutral detergent fibre intake increases with increasing ration NDF, FCM and DIM (Rayburn & Fox, 1993).

Another way of estimating pasture intake is by using, in reverse, the accepted energy requirements for maintenance, production, liveweight change and physiological status (Reeves *et al.*, 1996). Tesfa *et al.* (1995) estimated the ME content of the herbage based on *in vitro* OM digestibility (IVOMD). They calculated the ME requirements for maintenance, liveweight change and milk production. The ME intake from concentrate and hay was known so the performance of the cows could be used to estimate the ME intake from the herbage and hence the DM intake of the herbage. This technique was found by Reeves *et al.* (1996) to under-predict intakes when high levels of concentrate (6 kg/cow/d) were fed.

2.7 RESPONSES TO SUPPLEMENTATION

It is difficult to predict quantitative responses of milk yield and composition to supplementation even if the factors affecting the efficiency have been identified and described (Delaby *et al.*, 2001). The cow's response to energy supplementation depends not only on the

production level but also the BCS, substitution effect, concentrate level, stage of lactation, genetic potential, quality and quantity of pasture and concentrate and season of the year (Dillon *et al.*, 1997; Muller & Fales, 1998; Walker *et al.*, 2001; Kennedy *et al.*, 2003). Substitution of concentrate for grazed grass makes responses lower than expected (Kennedy *et al.*, 2003). Lower SR (0.4 to 0.6 kg reduction in PDMI/kg increase in concentrate DMI) and higher production responses (>1 kg milk/kg concentrate DM) have been found in more recent studies with higher yielding cows than previously published for lower yielding cows (Kennedy *et al.*, 2003).

2.7.1 Milk yield response

Milk production increases as the level of concentrate feeding increases (Reis & Combs, 2000; Delaby *et al.*, 2001; Granzin, 2004; Meeske *et al.*, 2006). Responses in milk production to energy supplements are due partially to the increased total DMI as there is a positive relationship between milk production increase, concentrate DMI and total DMI increase (Muller & Fales, 1998; Bargo *et al.*, 2003a).

Some of the variation in milk response to supplementation may be explained by SR. There is a negative relationship between SR and milk response; milk response to supplements is higher if there is a lower SR because usually the larger the SR the smaller the increase in total DMI and hence the lower the milk response (Bargo *et al.*, 2003a).

Milk response to concentrates tends to decrease with increasing concentrate allowance (Muller & Fales, 1998; Peyraud & Delaby, 2001), that is the marginal milk response per unit concentrate fed follows the law of diminishing returns; the first units are the most profitable, with each extra unit giving lower returns (Muller, 2001b; Bargo *et al.*, 2003a). The milk response in the study by Meeske *et al.* (2006) was 1.25, 0.78 and 0.54 kg of FCM per kg of concentrate fed in cows fed an average of 2.4, 4.8 and 7.2 kg of concentrate per day, respectively. The highest margin over feed cost was obtained at the low level (2.4 kg/cow/d) of concentrate feeding. Dillon *et al.* (1997) and Peyraud & Delaby (2001), however, found responses in milk yield to increasing levels of concentrate to be linear, although highly variable, with the effect of diminishing marginal response being small in the latter study if the concentrate allowance was less than 6 kg/d. According to Bargo *et al.* (2003a) milk production increases linearly as the

amount of concentrate increases from 1.2 to 10 kg DM/d; above this the marginal milk response decreases.

The milk yield response to increased level of concentrate depends on PA (Delaby *et al.*, 2001). The diminishing returns from increasing the amount of concentrate, due largely to the substitution effect, are greater when PA is greater (Walker *et al.*, 2001). Response to concentrate is higher when the PA or grass height is low or when the stocking rate is very high and PDMI is restricted (Peyraud & Delaby, 2001; Bargo *et al.*, 2002a) while adequate pasture availability is usually associated with poorer responses (Stakelum, 1986a). Cowan & Davison (1978) found a milk response to concentrate feeding when pasture availability was limited but no response, although the average production was higher, when the pasture availability was higher. The milk response is linear up to 6 kg of concentrates at low PA and curvilinear with the response reaching a plateau after 4 kg of concentrates at high PA (Delaby *et al.*, 2001; Peyraud & Delaby, 2001). For cows producing less than 20 kg/d of milk responses have been found to be about 0.6 kg milk per kg concentrate when cows grazed restricted pasture and 0 kg milk per kg concentrate when cows grazed pasture *ad libitum* (Bargo *et al.*, 2002a).

Milk responses to feeding high energy supplements are influenced by characteristics of the herbage eaten in conjunction with the supplement (Stockdale, 1999). Concentrate is used more efficiently, that is greater responses have been obtained from an increased quantity of concentrate, later in the grazing season when the quality of the grass is poorer (Journet & Demarquilly, 1979). In the experiment by Stockdale (1999) the highest marginal responses to concentrate supplementation occurred in summer and early autumn when the pastures were low in energy. Milk production of cows grazing tropical pastures is consistently increased when highly digestible energy supplements such as grains are fed (Cowan & Lowe, 1998).

Milk responses to concentrate supplementation are generally high since cows rarely approach their potential intake at grazing. However, responses to concentrate would probably progressively decrease for low genetic merit cows at higher levels of concentrate when they reach their genetic potential (Peyraud & Delaby, 2001). Milk responses to concentrate supplementation are greater with higher yielding, high genetic merit cows, especially at higher stocking rates, due to higher energy deficits (Hoden *et al.*, 1991; Muller & Fales, 1998; Peyraud & Delaby, 2001; Bargo *et al.*, 2002a; Kolver, 2003). A few decades ago researchers found an average response of 0.4 to 0.6 kg milk per kg concentrate DM (Delaby *et al.*, 2001). Hoden *et al.* (1991) found a

mean efficiency of 0.6 kg FCM/kg supplement. With higher genetic merit this efficiency has reached close to or higher than 1 kg milk per kg concentrate DM (Delaby *et al.*, 2001; Bargo *et al.*, 2003a). For cows producing 23 to 27 kg milk per day on pasture only, milk response to grain feeding in high producing cows would be about 0.8 to 1.2 kg per kg grain fed (Muller & Fales, 1998). Dillon *et al.*, (1997) found milk responses to vary from 0.13 to 0.98 kg of milk per kg of concentrate and Delaby *et al.* (2001) found an average response of 1.04 kg milk per kg DM concentrate supplementation and that this response remained linear up to 4 to 6 kg concentrate. The higher milk response to concentrates in reports published after 1990 is probably due to the higher genetic merit of the cows (Peyraud & Delaby, 2001; Bargo *et al.*, 2003a) which partition more nutrients to milk production and lose more BW in early lactation than low genetic merit cows (Bargo *et al.*, 2003a). Cows also respond more to supplementation earlier in lactation (Bargo *et al.*, 2003a).

Response is consistent over longer periods although variable in the short term. There needs to be time for the rumen to adapt due to the differences in energy density of the grass and grain (Cowan & Lowe, 1998).

In summary: milk response to supplementation, due partly to increased total DMI, generally follows the law of diminishing returns above approximately 6 kg concentrate/cow/day. Response is higher when pasture quantity and quality are limited and when the cows have high genetic potential.

2.7.2 Milk composition

The effect of concentrate supplementation on milk composition varies (Peyraud & Delaby, 2001). Many studies have shown that increasing concentrate supplementation reduces milk fat content (Hamilton *et al.*, 1992; Berzaghi *et al.*, 1996; Carruthers *et al.*, 1997; Reis & Combs, 2000; Delaby *et al.*, 2001; Peyraud & Delaby, 2001; Walker *et al.*, 2001; Bargo *et al.*, 2002a; Granzin, 2004) while other studies found fat content of the milk to be unaffected by concentrate supplementation (Stakelum, 1986a; Hoden *et al.*, 1991; Carruthers & Neil, 1997; Dillon *et al.*, 1997; Meeske *et al.*, 2006). Milk would have a higher fat percentage with fibre-based than starch-based concentrates (Bargo *et al.*, 2003a).

Concentrate supplementation increased protein content in some studies (Stakelum, 1986a; Hoden *et al.*, 1991; Carruthers & Neil, 1997; Reis & Combs, 2000; Delaby *et al.*, 2001; Bargo *et al.*, 2002a; Granzin, 2004) while there was no change in others (Berzaghi *et al.*, 1996; Dillon *et al.*, 1997; Carruthers *et al.*, 1997; Meeske *et al.*, 2006).

Increased milk protein content usually accompanies responses in milk yield, indicating an improved energy status of the cows (Peyraud & Delaby, 2001).

With only pasture the diet would be high in RDP in relation to dietary carbohydrates and the MUN would often be high (Muller, 2003a). Cows fed concentrate supplements have lower MUN values than cows receiving pasture only (Muller, 2001a; 2003a; Bargo *et al.*, 2002a). Carruthers & Neil (1997) found lower milk urea for cows supplemented with NSC than grass only. Reis & Combs (2000) found a linear decrease in MUN as the level of concentrate supplementation increased.

2.7.3 Responses to protein supplementation

Apart from increasing the amount of supplement offered, increasing the CP concentration of the supplement may improve the supply and digestion of nutrients in grazing dairy cows (Jones-Endsley *et al.*, 1997). Cottonseed meal supplementation has been shown to improve animal performance more than an energy supplement alone (Paterson *et al.*, 1994).

Providing additional CP to cows stimulates forage intake if they are consuming low quality forages, rather than higher quality forages (Paterson *et al.*, 1994). The lower the protein content of the grass the higher the response to MP supplementation (Peyraud & Delaby, 2001). As the level of CP in the forage increases the magnitude of intake response becomes less evident. In the case of higher quality forages any response is likely to be due rather to changes in digestibility and efficiency of nutrient utilisation and the effect of RUP than to intake (Paterson *et al.*, 1994).

When energy is most limiting to production, protein supplementation provides little additional response (Muller & Fales, 1998). In most cases milk production is not limited by MP supply but in some cases the CP content of grass can decrease, such as when N fertilisation is low

or during summer grazing, and supplementation with MP could be beneficial (Peyraud & Delaby, 2001).

McCormick *et al.* (2001a) found that increasing the CP concentration in the supplement from 16.6 to 22.8 % of DM did not affect milk yield of early lactation Holstein cows grazing winter annual ryegrass-oat pastures although the fat and CP concentrations in the milk was higher for the cows receiving the higher protein supplement. Protein supplementation did not affect the pasture or total DMI.

The form in which N is supplied is important – N from protein is more valuable than non-protein N (NPN) sources (such as urea) probably because microbial requirements for NH_3 are better supplied by protein supplements that are degraded slower (MacDonald *et al.*, 1998). MacDonald *et al.* (1998) found that for cows grazing pasture and receiving maize silage supplementation, despite the diet being deficient in CP, supplementing urea had no effect on milk or milk solids yield, while supplementing FM or SBM increased production and liveweight gain. Soybean meal improved milk protein while FM improved both milk fat and protein. The response to FM was obtained in spring, summer and autumn while the response to SBM was only in autumn.

In the trial of McCormick *et al.* (1999) protein concentration in the diet did not affect FCM, while supplementation with RUP did.

The bottom line is that cows can respond to additional CP in their supplement especially if the CP of the pasture is low and/ or if the protein has a high RUP content.

2.7.4 Effects of supplementation on digestion and fermentation in the cow

2.7.4.1 Ruminal pH

Increasing the amount of concentrate supplementation decreases the ruminal pH (Carruthers & Neil, 1997; Carruthers *et al.*, 1997; Peyraud & Delaby, 2001; Bargo *et al.*, 2002a; 2003a; 2003b; Sairanen *et al.*,2005), although Reis & Combs (2000) found no effect. In some, but not all, studies the reduced ruminal pH was associated with a higher VFA concentration. There is an interaction between the amount and type of concentrate supplemented and pasture

DMI and quality, so there is not a simple relationship between amount of concentrate and ruminal pH (Bargo *et al.*, 2003a).

Increasing the amount of CP in the concentrate or the pasture does not affect ruminal pH (Jones-Endsley *et al.*,1997; Carruthers & Neil, 1997; Bargo *et al.*, 2003a).

2.7.4.2 Volatile fatty acids

Concentrate supplementation increases the total VFA concentration compared to pasture-only (Carruthers & Neil, 1997; Carruthers *et al.*, 1997; Bargo *et al.*, 2002b) although in the trial of Berzaghi *et al.* (1996) supplementing maize at 6.4 kg/cow/d did not affect total VFA concentration and Reis & Combs (2000) also found increasing levels of concentrate supplementation to have no effect on total VFA concentration.

Increasing the amount of concentrate supplementation decreases the acetate to propionate ratio (Jones-Endsley *et al.*, 1997; Peyraud & Delaby, 2001; Bargo *et al.*, 2002a; Sairanen *et al.*, 2005) in agreement with the lower milk fat percentage found in supplemented cows (Bargo *et al.*, 2002a). In the trial of Berzaghi *et al.* (1996) the decreased acetate to propionate ratio was due to increased propionate, a major end product of starch fermentation. Currthers & Neil (1997) on the other hand, found no difference in molar proportions of different VFA.

Bargo *et al.* (2002a) found that for cows grazing at a lower PA the total VFA concentration was increased by concentrate supplementation while there was no effect at higher PA. Propionate and butyrate concentrations were increased by concentrate supplementation, in agreement with the study by Reis & Combs (2000) and the study by Sairanen *et al.* (2005) where the molar proportion of butyrate increased as the concentrate increased (0, 3 or 6 kg/d).

Increasing the amount of CP in the concentrate does not usually affect ruminal VFA concentration (Jones-Endsley *et al.*,1997; Bargo *et al.*, 2003a).

In the study by Jones-Endsley *et al.* (1997) butyrate increased as CP in the supplement increased, possibly because of a lower concentration of NSC in the supplement.

The higher ruminal VFA levels in supplemented cows indicated that there was more fermentation in the rumen, hence more energy available.

In summary: concentrate supplementation increases total VFA in the rumen if PA is restricted, indicting that more material is fermented in the rumen. There is sometimes a change

in molar proportion of individual VFA, such as decreased acetate to propionate ratio, and sometimes no change.

2.7.4.3 Nitrogen capture and flow

In the studies by Berzaghi *et al.* (1996), Carruthers & Neil (1997), Stockdale (1997), Reis & Combs (2000), Vaughan *et al.* (2002), Bargo *et al.* (2002a; 2003b) and Sairanen *et al.* (2005) NH_3-N concentrations were decreased with concentrate (NSC), such as maize or barley, supplementation.

A reduction in ruminal NH_3-N concentration is the most consistent effect of concentrate supplementation on ruminal fermentation. This reduction could be due to a greater capture of NH_3-N from the highly degradable protein of the pasture for microbial protein synthesis and/or due to a reduction total CP intake because energy supplements usually have less CP than pasture (Reis & Combs, 2000; Bargo *et al.*, 2002a; 2003a).

The lower NH_3-N for cows receiving concentrate supplementation is consistent with the lower plasma urea N (PUN) and MUN (Bargo *et al.*, 2002a).

Bargo *et al.* (2002a) found that supplemented cows had a more constant NH_3-N pattern in the rumen than un-supplemented cows, indicating the improved utilisation of NH_3-N by the energy provided in the concentrate or a different diurnal pattern of grazing. Un-supplemented cows had a peak in ruminal NH_3-N at 1330 h, indicating rumen proteolysis after a period of high grazing activity (Bargo *et al.*, 2002a). Ruminal NH_3-N concentration peaks are not as high when more concentrate is fed, probably because microbial growth is stimulated (Berzaghi *et al.*, 1996).

Intake of N is usually lower in supplemented animals without affecting the flows of non-ammonia N (NAN), non-ammonia non-microbial N (NANMN) or microbial N (Bargo *et al.*, 2003a). Sairanen *et al.* (2005), however, found increasing the amount of supplements to increase microbial N synthesis and N flow to the omasum. Similarly Carruthers & Neil (1997) also found NSC supplementation to increase microbial protein synthesis and efficiency of synthesis on pasture containing 18 % CP. Non-ammonia N flow to the duodenum can be increased when cows consuming pastures are supplemented with NSC (Vaughan *et al.*, 2002).

Berzaghi *et al.* (1996) found that when pasture was supplemented with maize N losses were lower, associated with a lower NH_3-N concentration in the rumen, because additional N was being used for microbial protein production when energy was provided. Concentrate

supplementation increases the apparent use of dietary N for milk production, due to a lower N intake rather than improvements in N capture in the rumen (Sairanen *et al.*, 2005). In the study by Bargo *et al.* (2002a) concentrate supplementation increased the N in the faeces and milk while it decreased the N in the urine as proportions of N intake. This is in agreement with the study by Carruthers & Neil (1997) where NSC supplementation increased faecal N and decreased N in the urine while not affecting N retention in cows grazing ryegrass pasture.

In the continuous culture trial by Bargo *et al.*, (2003b) when SR was high (1 kg of pasture/kg of supplement), concentrate supplementation reduced NH_3-N concentration but did not increase the flow of bacterial N, due to reduced rumen-degradable N. At low SR (0.4 to 0.6 kg of pasture/kg of supplement, the typical SR found with high producing dairy cows), concentrate supplementation not only reduced NH_3-N concentration but also increased the flow of bacterial N, due to increased rumen-degradable OM. This helps explain why there would only be a milk response to supplementation when the SR is lower (Bargo *et al.*, 2003b).

In general, concentrate supplementation stimulates microbial production in the rumen, decreasing ruminal NH_3 levels and loss of N while increasing the flow of N to the duodenum.

2.7.4.4 Digestion

It is possible that when concentrates are fed there are negative associative effects with the digestion of the pasture decreasing (Bargo *et al.*, 2002a; Doyle *et al.*, 2005). Supplementation with energy concentrates, such as maize, reduces digestibility of NDF (Berzaghi *et al.*, 1996; Bargo *et al.*, 2002a; 2003a; Sairanen *et al.*, 2005). Cell wall digestion is depressed more by grains that ferment rapidly than by slowly degraded grains such as maize (Doyle *et al.*, 2005). It is possible that roughages with a high digestibility are less influenced by low ruminal pH than roughages with a low digestibility (De Veth & Kolver, 2001); digestibility of high quality fresh grass low in NDF is suppressed less by concentrate supplementation and low ruminal pH than that of high NDF forage is (De Veth & Kolver, 2001; Doyle *et al.*, 2005). Increasing levels of concentrate probably reduced the digestibility of kikuyu pasture in the trial of Reeves *et al.* (1996).

Concentrate (NSC) supplementation does not usually affect DM or OM digestibility (Carruthers & Neil, 1997; Bargo *et al.*, 2002a) although it caused a decrease in the trial of Berzaghi *et al.* (1996). The degradability of N linearly decreases with increasing concentrate level (Carruthers & Neil, 1997; Sairanen *et al.*, 2005).

Although the pasture fibre digestibility decreases with concentrate supplementation (Berzaghi *et al.*, 1996; Bargo *et al.*, 2003a), the total digestibility of the diet increases because the concentrates usually have a higher digestibility than the pasture (Reis & Combs, 2000; Bargo *et al.*, 2003a). Apparent digestibility of total DM is increased with supplementation (Bargo *et al.*, 2002a). The negative effects of increased concentrate on NDF digestion are overcome by the increased DMI, resulting in a linear increase in the total ME intake and milk production (Sairanen *et al.*, 2005).

Protein supplementation, on the other hand, increased digestibility of NDF and OM (Bargo *et al.*, 2003a) in agreement with the study by Jones-Endsley *et al.* (1997) where increasing the amount of CP in the supplement increased the ruminal and total tract digestion of NDF, possibly due to the lower NFC content. Positive associative effects could be possible if protein supplements (alleviating a protein deficiency) stimulate the growth of cellulolytic bacteria in the rumen (Doyle *et al.*, 2005).

A positive associative effect could also be possible if a supplement provides energy to assist in utilisation of excess protein from the pasture. In pasture fed cows positive associative effects are only likely to occur when supplements remove a limitation in an essential nutrient or when excess N is consumed from the pasture (Doyle *et al.*, 2005).

In general concentrate supplementation can decrease NDF digestion especially if the grain is highly fermentable or if the roughage has high NDF content. The total diet digestibility, however, is increased when a concentrate is fed.

2.7.5 Long term effects

Long term benefits of supplemental energy are usually greater than short term benefits (Muller & Fales, 1998). Supplementation with concentrate reduces BW loss or increases BW gain (Dillon *et al.*, 1997; Peyraud & Delaby, 2001). In the study of Hamilton *et al.* (1992) cows grazing kikuyu pasture lost weight while those receiving additional supplementation gained weight. Supplementation also reduces the number of services per conception (Dillon *et al.*, 1997). Meeske *et al.* (2006), however, found that increasing the level of concentrate feeding had no effect on intercalving period or change in liveweight, although cows fed no concentrate had a

shorter calving interval than cows fed a high level of concentrate. Condition score tended to improve as the level of concentrate increased. Stockdale (1997) also found supplementation to increase BCS.

2.8 SUPPLEMENTATION WITH RUMEN-UNDEGRADABLE PROTEIN

2.8.1 Protein degradability – a background

By measuring the amount of protein degraded in the rumen it is possible to estimate the amount of N available for the rumen microbes and the amount of protein made available for digestion in the small intestine (Zhao *et al.*, 1993). Estimates of protein degradability and the amount of protein escaping the rumen are very variable and cannot be ascribed fixed values (Batajoo & Shaver, 1998; Waters & Givens, 1992; Holtshausen & Cruywagen, 2000). A value for the rumen degradability of a protein depends on the feedstuff, the conditions in the rumen of the animal consuming the feed and the experimental procedure employed to arrive at the value. Many dietary and ruminal factors need to be considered when the value of a protein source is assessed (Zinn & Owens, 1983). Ruminal protein degradation of a feedstuff depends on factors such as microbial proteolytic activity, microbial access to the protein and ruminal retention time (Stern *et al.*, 2006).

Feed proteins are made up of fractions of different degradability (Broderick *et al.*, 1991). Proteins can be divided into an undegradable fraction, a potentially (slowly) degradable fraction and a rapidly degradable fraction. The degradability of a feed protein is determined by the fraction that is undegradable, while the degradation, or disappearance, of the protein is determined by the ratio of the rate of degradation and the rate of passage out of the rumen (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991).

Protein degradability varies between feeds, within feeds and with different chemical or physical treatments of the feed (Lindberg, 1985; Madsen & Hvelplund, 1987; Ørskov, 1992). There is no single degradability value for a protein source that applies to all feeding conditions (Siddons & Paradine, 1983; Miller & Ørskov, 1986; Kirkpatrick & Kennelly, 1987). The rate, as

opposed to just the extent, of degradability needs to be known to determine how much will be degraded at specific feeding conditions (Miller & Ørskov, 1986).

2.8.1.1 Some factors affecting protein degradability

The diet being fed, physical nature of the diet, level of feed intake, ruminal retention time and rate of passage of digesta, method and frequency of feeding, experimental animals used, rumen environment (such as ruminal pH) and microbial proteolytic activity have an effect on ruminal protein degradability (Tamminga, 1979; Stern *et al.*, 1994; Holtshausen & Cruywagen, 2000; NRC, 2001).

a) Characteristics of the protein

The extent of degradation of dietary protein and the *in situ* CP degradability values are affected by the characteristics of the feed in question such as solubility of the protein and structural differences caused by, for example, disulphide bridges and cross-linking of the protein (Tamminga, 1979; Lindberg, 1985; Kirkpatrick & Kennely, 1987; Zhao *et al.*, 1993). Protein structure affects the degradability of the protein in the rumen by influencing the accessibility to proteolytic enzymes (Leng & Nolan, 1984; Stern *et al.*, 1994). Some feeds are naturally resistant to ruminal microbial degradation (Stern *et al.*, 1994).

b) Feedstuff

Protein supplements of animal origin are generally broken down rapidly but incompletely and hence would have a low degradability over a range of retention times. Plant proteins are degraded more slowly but potentially completely hence the escape depends on the ruminal proteolytic activity and particle outflow rate that result from other components of the diet (Wallace, 1988).

Degradation of N is negatively correlated to the content of fibre in the feedstuff. Part of the nitrogenous compounds in many feedstuffs, including roughages or oilseed cakes, is protected from degradation by a fibrous structure that needs to be broken down by rumen micro-organisms before the N fraction can be potentially available for degradation (Lindberg, 1985).

c) Variation within feedstuffs

The composition of forages depends on species, maturity, fertilization level, season, soil type and weather conditions, and hence varies more than that of concentrates, making protein escape values for roughages based on nylon bag incubations more limited (Van Straalen & Tamminga, 1990). There are significant differences in degradability of CP between individual roughage samples from different farms hence the use of tabulated average values for forages in formulation equations could lead to inaccurate diet formulations since they might not reflect the particular forage being used on that farm (Von Keyserlingk *et al.*, 1996). The rumen degradable N content ranges from 41 to 60% in grasses and from 69 to 79% in legumes (Ibrahim *et al.*, 1995).

Degradability of CP in the rumen increases with increasing application of N fertilization. Nitrogen fertilization increases the CP content and the size of the rapidly degradable protein (NPN) fraction and the rate at which the slowly degradable fraction is degraded in the rumen, and decreases the undegradable fraction (Van Straalen & Tamminga, 1990; Van Vuuren *et al.,* 1991).

Large variation in ruminal degradation can also occur among and within different rendering by-products such as meat and bone meal, feather meal and BM (Howie *et al*., 1996). Preparation methods alter the ruminal degradation of FM protein (Yoon *et al.*, 1996). Yoon *et al.* (1996) evaluated FM samples from five processing plants and found ruminal degradation of the protein to range from 29 to 57%. It is important to evaluate individual batches of expensive protein sources like FM that could vary a lot due to processing method (Erasmus *et al.*, 1988).

d) Processing or treatment of the feedstuff

Protein degradability is decreased by treatment of the feedstuff with aldehydes such as formaldehyde and glutaraldehyde (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991; Ørskov, 1992) and strong acids such as formic acid and sulphuric acid (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991; Hristov & Sandev, 1998; Verbič *et al.*,1999).

Heat treatment decreases rumen protein degradability by reducing protein solubility and by blocking reactive sites for microbial proteolytic enzymes by denaturation and Maillard reactions, depending on the temperature reached, the processing time and the moisture content during processing (Broderick & Craig, 1980; Broderick *et al.*, 1991; Ørskov, 1992; Dakowski *et al.*, 1996; Goelema *et al.*, 1999). It increases both the undegradable and un-digestible protein

fractions (Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991; Ørskov, 1992). Many methods of processing feeds, such as pelleting, extrusion, expander treatment, pressure toasting and roasting of feedstuffs, either require or generate heat which can reduce protein degradability in the rumen (Stern *et al.*, 1985; Broderick *et al.*, 1991; Zaman *et al.*, 1995; Goelema *et al.*, 1999; Prestløkken, 1999a; Prestløkken, 1999b).

Protein can also be physically protected from degradation (Broderick *et al.*, 1991; Ørskov, 1992; Rossi *et al.*, 1999; Manterola *et al.*, 2001; Zahedifar *et al.*, 2002).

e) Animal variation

The rate of disappearance of protein supplements from nylon bags suspended in the rumen differs among animals and even within the same animal on different days (Lindberg, 1985; Broderick *et al.*, 1991). Different animals have different proteolytic microbial populations, even if they are on the same or similar diets (Broderick *et al.*, 1991).

f) Rumen retention time and frequency of feeding

Level of DM intake, residence time in the rumen and fractional outflow rate have a big effect on the degradability and extent of protein degradation in the rumen (Tamminga, 1979; Siddons & Paradine, 1983; Eliman & Ørskov, 1984; Lindberg, 1985; Miller & Ørskov, 1986; Erasmus *et al.*, 1988; Hvelplund & Madsen, 1990; Van Straalen & Tamminga, 1990; Zhao *et al.*, 1993). Protein degradability is lower with a higher level of intake and higher outflow rate (Tamminga, 1979; Erasmus *et al.*, 1988; Van Straalen & Tamminga, 1990; Zhao *et al.*, 1993). At a given degradation rate, the extent of degradation decreases as the passage rate increases (Broderick *et al.*, 1991). With greater fluid turnover in the rumen, soluble carbohydrates and proteins are more likely to escape rumen degradation (West *et al.*, 1987).

2.8.1.2 Techniques for estimating protein degradability

Methods of evaluating feed proteins include *in vivo* (sampling of digestive contents throughout digestion), *in situ* (incubation of feeds in bags suspended in the rumen) and *in vitro* (laboratory) techniques (Lindberg, 1985; Janicki & Stallings, 1988; Van Straalen & Tamminga, 1990; Broderick *et al.*, 1991). Solubility is a good way of estimating the rapidly degradable, or '*a*' fraction of the protein but not the total degradability (Crawford *et al.*, 1978; Cottrill, 1993).

Determination of solubility would only be adequate for protein sources where the degradable material consists mainly of the *a* fraction such as FM and silage (Ørskov, 1992).

The *in situ* procedure, as proposed by Ørskov & McDonald (1979), is probably the most widely used and reliable method to predict rumen degradability of protein (Broderick *et al.*, 1988; Negi *et al.*, 1988; Beckers *et al.*, 1995; Kohn & Allen, 1995). Routine *in situ* procedures should be standardised in terms of fineness of grinding, pore size of the bag material, washing procedure and so on (Batajoo & Shaver, 1998) in order to minimise differences in results. Size of the nylon bag, bag pore size, sample weight to bag surface area ratio, diet, feedstuff particle size, washing technique, microbial contamination, bag introduction sequence into the rumen, bag location in the rumen, animal and time variation, pre-ruminal incubation and pre-soaking are among the factors that affect *in situ* measurement of N disappearance (Weakly *et al.*,1983; Nocek, 1985; Chiou *et al.*, 1995; Vanzant *et al.*, 1998).

The data from *in situ* incubations is usually analysed with a first-order model (Ørskov & McDonald, 1979): the potentially degradable fraction = a + b $(1 - e^{-ct})$ where 'a' is the soluble fraction, 'b' the insoluble potentially degradable fraction, 'c' the fractional digestion rate constant and 't' is time (Bargo *et al.*, 2003).

The *in situ* procedure is used to measure the rate of disappearance of N in the rumen and this is combined with an estimate of the fractional outflow rate of the rumen contents to predict effective degradability – the proportion of dietary protein that will escape degradation in the rumen (Freer & Dove, 1984; Waters & Givens, 1992; Cottrill, 1993; Klopfenstein *et al.*, 2001). Thus to be able to predict degradability an estimate of the rumen retention time, or outflow rate of the feedstuffs from the rumen, is also required (Siddons *et al.*, 1985; Cottrill, 1993). In most cases, it is not measured but assumed or N loss at a specific incubation time or N loss relative to DM is taken as the index of degradability (Siddons *et al.*, 1985).

Feed protein can be divided into three fractions (as in the CNCPS model): A (NPN), B (true protein) and C (bound true protein). The true protein can be further sub-divided into B1, B2 and B3. Fractions A and B1 are soluble in buffer (Sol CP) and are degraded in the rumen. Fractions B3 and C (NDIP) can be considered un-degraded (the former being slowly degraded while the latter (ADIP) cannot be broken down by bacteria and does not supply AA post-ruminally either (Sniffen *et al.*, 1992). The degradation of the B2 fraction depends on passage

rate. Protein fraction B2 = 100 – (A + B1 + B3 +C) (Sniffen *et al.*, 1992), in other words 100 – (Sol CP + NDIP) (as % CP).

The percentage of CP degraded (effective degradability) = Sol CP (% CP) + (k_d/(k_d + k_p) × (B2 fraction; % CP) and the percentage of CP un-degraded = (k_p/(k_d + k_p) × (B2 fraction; % CP) + NDIP (% CP) where k_p is the passage rate and k_d is the rate of degradation of the insoluble potentially degraded fraction (determined by fitting data from residues of *in situ* bags after different times of incubation with a first-order degradation model) (Van Vuuren *et al.*, 1991; Sniffen *et al.*, 1992; Bargo *et al.*, 2003).

Ruminal passage rate per hour can be calculated based on duodenal flow and average ruminal contents (Van Vuuren *et al.*, 1992). Passage rates are affected by factors such as particle size, density and hydration rate and the level of intake (Sniffen *et al.*, 1992). Berzaghi *et al.* (1996) found the particle and liquid passage rates of cows grazing pasture and receiving maize supplementation (using Cr_2O_3 as a marker) to be 7.1 and 18.5 %/h, respectively. According to *in situ* data more than 90 % of N compounds in fresh grass are potentially degradable and degradation rate can vary from 10 to 20 %/h (Berzaghi *et al.*, 1996).

The total amount of digestible CP entering the small intestine can be calculated from this plus microbial protein (the latter depending on the amount of fermentable carbohydrate in the rumen) (Van Vuuren *et al.* 1991).

2.8.2 The pasture situation

Although the total protein in well managed pastures is high, it is highly degradable in the rumen to NH_3. Rather than being captured as microbial protein it may be lost from the rumen and converted to urea in the liver and eventually excreted in the urine. This costs energy, making the efficiency of N utilisation by the grazing dairy cow low (Muller & Fales, 1998; Schroeder & Gagliostro, 2000; Schor & Gagliostro, 2001; Kolver, 2003). Pre-duodenal loss of N occurs when there is more RDP than the microbes require (9 to 11 g CP per MJ ME consumed) when forage contains more than 16 % CP, which is the case in most cool-season grasses (Hodgson & Brookes, 1999; Muller & Fales, 1998).

This pre-duodenal loss of N due to the high degradability of the protein relative to the energy available means that high producing cows on pasture are deficient in protein and AA available for absorption from the small intestine for milk synthesis (Jones-Endsley *et al.*, 1997; Carruthers *et al.*, 1997; Schor & Gagliostro, 2001; Muller, 2001a; 2003a). Providing ruminally available energy in the form of fermentable carbohydrates improves the utilisation of the high RDP in pastures and optimises ruminal microbial protein synthesis (Jones-Endsley *et al.*, 1997; Muller & Fales, 1998). Supplementation with good quality RUP could be a way to improve the total amount and the profile of AA reaching the small intestine (Donaldson *et al.*, 1991; Jones-Endsley *et al.*, 1997; Schroeder & Gagliostro, 2000).

In most grazing situations, ME is the first limiting nutrient for milk production. Protein and AA are usually the second limiting nutrients. However, when more than 20 % of the diet consists of a grain supplement and the milk production is very high, specific AA, particularly Met and Lys, may become first limiting (Muller & Fales, 1998; Kolver *et al.*, 1998b; Kolver, 2003).

It has been suggested that, for dairy cows on high quality pasture, a milk yield of more than 25 kg/d is limited by absorbed AA (Beever & Siddons, 1986; Kolver & Muller, 1998; Kolver, 2003). The effects of RUP supply on milk production under grazing conditions needs to be better understood (Schroeder & Gagliostro, 2000).

2.8.3 The need for rumen-undegradable protein

For the modern high yielding cow a smaller proportion of the protein is supplied by the rumen microbes and more needs to escape rumen degradation than was the case a few decades ago when cows had a lower genetic potential (Santos *et al.*, 1998). In early lactation the MP requirements of high producing dairy cows are higher than can be supplied by the microbial and forage RUP, so body protein would be mobilised by the cow (Donaldson *et al.*, 1991; Hongerholt *et al.*, 1998; Muller & Fales, 1998; Schroeder & Gagliostro, 2000). Too much body protein mobilisation could have adverse effects on cow health (Schor & Gagliostro, 2001).

Sources of RUP can be used to increase the quantity of AA reaching the small intestine to complement the microbial protein and support the high requirements of early lactation cows,

improving lactational responses (Hongerholt & Muller, 1998; Schroeder & Gagliostro, 2000; Schor & Gagliostro, 2001). There is a positive relationship between milk yield and RUP intake; there is an average increase in milk production of 0.8 kg/d for each 100 g/d of RUP (Bargo *et al.*, 2003a). Supplementation with RUP could be beneficial because of fresh pasture having high ruminal CP degradability (greater than 70%) and therefore providing less RUP than a TMR diet would (Berzaghi *et al.*, 1996; Bargo *et al.*, 2003a).

2.8.4 Responses to rumen-undegradable protein supplementation

Although the balance of AA reaching the tissues is important, there has been little evidence that post-ruminal supplementation with protein protected against degradation and individual AA improves production in grazing animals, suggesting that the supply of microbial protein and RUP are sufficient to meet the AA requirements (Hodgson & Brookes, 1999). Increasing RUP or replacing RDP sources with RUP sources in concentrates of pasture or TMR fed cows has not had a consistent effect on milk production or composition (Carruthers *et al.*, 1997; Santos *et al.*, 1998; Bargo *et al.*, 2003a; Muller, 2003b).

Some research has indicated the benefit of including RUP to provide AA post-ruminally with high producing cows, while other researchers have not found differences in milk yield when cows were fed a concentrate ration with an increased amount of RUP (Muller, 2001a; 2003a). A Penn State study found small amounts of RUP increased milk protein yield in multiparous cows producing about 34 kg of milk a day and fed pasture as the main forage (Muller, 2003b).

In several studies RDP sources such as soybean meal, sunflower meal, urea or rapeseed meal have been replaced with RUP sources such as animal protein blend, maize gluten meal, expeller soybean meal, BM, feather meal, heat-treated rapeseed meal or FM. In most of the studies PDMI was not affected (Bargo *et al.*, 2003a). Studies that reported an increase in milk production were those of Schroeder & Gagliostro (2000) and Schor & Gagliostro (2001) where the milk response was 6 and 18 %, respectively, above the control. See section 2.8.4.2. Previous studies have shown enhanced milk and milk protein output in multiparous dairy cows grazing high N pasture when supplemented with protected casein, animal protein or BM suggesting that

metabolisable protein was inadequate for these multiparous cows to support high milk production (Schroeder & Gagliostro, 2000).

Santos *et al.* (1998) reviewed 88 lactation studies (127 comparisons) and found inconsistent results when protein supplements high in RUP replaced SBM. The type of RUP supplement (AA profile) seemed to be more important than the amount of RUP. Menhaden FM was the RUP source that most frequently increased milk yield compared to SBM controls and treated SBM was the next highest. Maize gluten meal caused more negative than positive effects on milk yield while other RUP supplements did not have consistent effects. Supplementation with FM has not always increased production (Carruthers *et al.*, 1997) and milk fat percentage was depressed by FM more than by other RUP supplements (Santos *et al.*, 1998).

Most of the studies found that the RUP content of the concentrate supplement did not affect the percentage of fat or protein in the milk (Bargo *et al.*, 2003a).

If the use of RUP does not decrease microbial protein flow and if the RUP supplement has an AA profile approaching that of milk then the quantity and quality of protein reaching the duodenum, and hence cow performance, is likely to improve (Schroeder & Gagliostro, 2000).

2.8.4.1 Effect on N flow

Although NH_3-N concentration increases with protein supplementation (due to increased N intake) it decreases if the protein supplement is of a low degradability (Jones-Endsley *et al.*, 1997; Hongerholt *et al.*, 1998; Bargo *et al.*, 2003a). When RDP sources such as sunflower meal or soybean meal in high CP concentrates are replaced by RUP sources such as feather meal, BM or maize gluten meal, rumen NH_3-N concentration is reduced (Erasmus *et al.*, 1994; Schor & Gagliostro, 2001; Bargo *et al.*, 2001; 2003a).

As CP in the supplement increased, N intake and flows of NAN, NANMN, AA and EAA, including Arg, His and Phe, to the duodenum tended to increase while the flow of microbial N and the efficiency of microbial synthesis were unaffected (Jones-Endsley *et al.*, 1997; Bargo *et al.*, 2003a). This was, however, not translated into increased milk production (Jones-Endsley *et al.*, 1997).

Bacterial protein decreases with RUP supplementation, as was found in the continuous culture experiment of Hongerholt *et al.* (1998) and in the trial of Erasmus *et al.* (1994) where

bacterial N flow was lower for cows receiving BM and/or maize gluten meal compared to sunflower meal.

Santos *et al.* (1998) in their review found that while replacing SBM with high RUP supplements decreased microbial protein synthesis and flow to the duodenum it increased the flow of NANMN so that there was little change in the total protein flow. The flows of Lys and Met to the duodenum were generally not increased by high RUP supplementation. Fishmeal consistently increased the proportion of Lys in the EAA flowing to the duodenum when supplied at greater then 4 % of diet DM but not if less than 4 %. Fishmeal brought the ratio of Lys to Met, as % EAA, at the duodenum close to the recommended levels.

2.8.4.2 Specific examples

Schroeder & Gagliostro (2000) reported results indicating the value of increasing RUP at the expense of RDP in diets based on high quality grazed pastures with excessive RDP supply. They used early lactation Holstein cows grazing a lucerne-based pasture containing red clover, orchardgrass and perennial ryegrass in the morning and a pasture of ryegrass, orchardgrass, lucerne and red clover in the afternoon, offered at an allowance of twice the expected maximum pasture DM requirement. The CP concentrations of the two pastures were 21.6 and 18.6 %, respectively. The cows received 5 kg of iso-energetic, iso-nitrogenous (19.5 to 19.7 % CP) concentrate a day. A high RDP source (sunflower meal) was replaced with one high in RUP (FM) so that the main difference between the concentrates was degradability and the quantity of CP. Milk yield (26.8 vs. 25.2 kg/d) and milk protein yield were improved while milk protein percentage (3.28 vs. 3.19 %) and milk fat percentage (3.32 vs. 3.22 %) remained similar. Milk fat yield and percentage and MUN (23.7 vs. 22.4 mg/dl) tended to be higher for the cows fed FM. The higher milk production with FM could be explained by the quantity and quality of absorbed protein (AA), higher glucose availability to the mammary gland and increased lipid (body fat) mobilisation.

Schor & Gagliostro (2001) found that a concentrate with a high RUP content increased milk and milk protein yields when spring pasture (perennial ryegrass, red and white clover and orchardgrass), offered at three times the pasture DM requirements, was the sole forage. They fed early lactation Holstein cows 6 kg concentrate DM per day containing either SBM or BM. The degradable fraction and the rate of disappearance of the CP were higher for SBM than for BM

(the effective degradability of SBM was 75 % and BM 44 %). The concentrates were formulated to be iso-energetic and iso-nitrogenous, so the effective degradability of the CP was the main difference between treatments. Dietary RUP of the 16 % CP diet was increased from 33.4 to 45.3 % by feeding the BM. Cows fed the BM concentrate produced more milk (29.3 vs. 24.9 kg/cow/d) and more milk protein than those fed SBM concentrate. Milk fat yield and percentages of milk fat, lactose and protein were not affected. These results suggested that there may have been an AA imbalance in the SBM but the AA composition was not measured. Forage DMI was higher in the cows receiving the BM. In this study the higher milk yield was more likely due to increased DMI than enhanced body lipid mobilisation. There was no difference in rumen pH or molar proportions of individual VFA between the two treatments. The ruminal NH_3-N concentrations were greater ($P<0.04$) in cows fed SBM than BM concentrate (25.3 vs. 21.2 mg/dl). Both of these were well above the range (5 to 10 mg/dl) proposed by Satter & Slyter (1974) as optimal for ruminal microbial growth.

Hamilton *et al.* (1992) found that cows grazing kikuyu pasture produced more milk with higher protein content if the sunflower meal in their concentrate (cracked barely mixed with sunflower meal) was treated with 0.5 % formaldehyde. Similarly Rogers *et al.* (1980) found formaldehyde treated casein to increase milk and milk protein yield of cows grazing high quality pasture.

Donaldson *et al.* (1991) fed one of three iso-energetic supplements to steers grazing high quality annual ryegrass pasture: high RUP, low RUP and maize which supplied an estimated 0.25, 0.125 and 0 kg of RUP/d in addition to that supplied by the maize. Fishmeal and distillers' dried grains with solubles were used as RUP sources. Feeding more RUP increased post-ruminal protein flow and more of the protein was digestible. Both total and forage DMI increased and fibre and DM digestion were not negatively affected. Donaldson *et al.* (1991) stated that RUP is superior to maize supplementation for improving forage intake and abomasal protein flow of growing steers on winter annual pastures.

Tesfa *et al.* (1995) evaluated supplements with different forms of N; a cereal by-product based dairy concentrate as control, the concentrate with urea, or with rapeseed meal or heat (expansion) treated concentrate with rapeseed meal. The heat treatment reduced the protein degradability of the concentrate. The pasture was a mixture of meadow fescue (*Festuca pratensa*), timothy (*Phleum pratense*) and red clover (*Trifolium protense*). They found no

difference between the treatments for energy-corrected milk (ECM) yield and fat content. Milk protein content tended to be higher with the rapeseed meal and heat treated concentrates than with the urea concentrate. Milk lactose was lower with the control concentrate alone than the other three treatments (Tesfa *et al.*, 1995).

In the trial of MacDonald *et al.* (1998), where cows grazing pasture and receiving maize silage supplementation were supplemented with urea, SBM, or FM, the response was greater and more consistent for the cows receiving FM than SBM and there was no response to urea. The RUP supply was also greater from the FM than the SBM and 0 from the urea, while the RDP supply was greatest from the SBM.

Hongerholt & Muller (1998) compared grain mixtures of a high or low RUP content given to grazing Holstein cows producing almost 40 kg of milk/d. Milk yield did not differ between the treatments. Milk fat percentage tended to be lower and milk protein yield tended to be higher for the cows fed the high RUP concentrate. Plasma urea N was unaffected by treatments. The protein in the high RUP concentrate was supplied by maize gluten meal and an animal protein blend (containing meat and bone meal, BM, feather meal, poultry by-product meal and FM) while the protein for the low RUP concentrate was supplied by SBM. The total CP in the high and low RUP concentrates was 13.7 and 14.7 %, respectively, and the RUP 62.3 and 47.0 % of CP, respectively. The RUP in the pasture was 15.8 % of CP, bringing the RUP in the total diets to 29.1 and 26.2 % of CP for the high and low RUP treatments, respectively. The total DMI was 20.9 and 19.9 kg per day for the cows fed the high and low RUP diets, respectively. Pasture was the sole forage and consisted of orchardgrass, Kentucky bluegrass and smooth bromegrass. The CP in the total diets was higher than required by the cows, mainly due to the high CP of the pasture (25.6 % of DM).

McCormick *et al.* (2001a) found that supplementation of RUP in the form of maize gluten meal and BM did not improve overall lactational performance even though the ryegrass-oat pastures contained low concentrations of RUP.

2.8.5 Factors affecting response to rumen-undegradable protein supplementation

The variable responses reported in the literature of grazing dairy cows to RUP supplementation probably reflect the changing nature of the first limiting nutrient for a given feeding and production scenario (Kolver, 2003). Increased protein flow post-ruminally can increase performance if protein is a limiting nutrient (Donaldson *et al.*, 1991).

Positive responses to RUP supplementation, above that observed with energy, are most likely in early lactation cows, when pasture quality is poor and when a high level of concentrate grain is fed (Hongerholt & Muller, 1998; Schor & Gagliostro, 2001).

2.8.5.1 Metabolisable energy first limiting

Milk responses to an increased supply of AA would only be likely if additional ME were supplied either by dietary supplementation or increased tissue mobilization (Kolver, 2003). The inconsistent milk response of cows consuming high quality pasture and supplemented with RUP could be because the deficiency in ME would first need to be corrected (Kolver & Muller, 1998; Kolver, 2003).

The study by Tesfa *et al.* (1995) demonstrated that, for dairy cows grazing pasture, additional protein feeding is not economical in terms of milk protein yield and content, since the microbial protein synthesised in the rumen seems to be adequate if there is enough energy available. Energy seemed to be the limiting factor. The lack of benefit from supplementing additional RUP in the study by McCormick *et al.* (2001a) indicated that an energy shortage may have been the major nutritional constraint for high producing dairy cows grazing lush pasture. In the trial by Jones-Endsley *et al.* (1997) the yield of FCM and concentrations of fat and protein in milk were unaffected by changing the CP concentration of the supplement from 12 to 16 % (the latter having a greater proportion of RUP than former supplement). The cows were in a negative energy balance and there was no milk response to the improved AA flow to the duodenum, indicating that milk synthesis in these grazing dairy cows was more limited by the supply of ME than by CP.

Hongerholt & Muller (1998) and Kolver & Muller (1998) did evaluations using the CNCPS model, which suggested that energy and not protein may be first-limiting to high yielding cows on grass pasture.

2.8.5.2 Rumen-degradable protein limiting

Santos *et al.* (1998) reviewed data from various studies and concluded that replacing SBM with protein supplements high in RUP results in decreased microbial protein flow to the duodenum if RDP is insufficient to meet the of needs the rumen microbes. There will be less circulating AA for milk and milk protein synthesis if there is less microbial protein synthesis (Tesfa *et al.*, 1995). The goal is to maximise microbial protein synthesis after which RUP should be supplied for high producing cows (Stern *et al.*, 2006). There will not be an increase in total protein, EAA, or Lys and Met flows to the duodenum if microbial synthesis is limited by low RDP and high RUP. It is not logical to increase RUP at the expense of RDP unless RDP is excessive, especially since microbial protein is the best source of protein for milk synthesis (Santos *et al.*, 1998; Stern *et al.*, 2006). Increasing RUP at the expense of RDP in the concentrate could be logical in diets where RDP is excessive as would be the case with rapidly degraded pasture protein (Schor & Gagliostro, 2001). The adequacy of RDP and RUP in the diets for lactating dairy cows should be considered independently (Santos *et al.*, 1998).

2.8.5.3 Diet and pasture type

If the control diet already has sufficient RUP there will be no response to supplementing RUP (Santos *et al.*, 1998).

Many of the trials that have shown inconsistent responses have been conducted in confinement. Grazing cows consume forage with a high CP content and the ruminal CP is rapidly degraded (Schor & Gagliostro, 2001).

Pasture species have a big impact on the amount of RUP that the cows receive since the protein escaping the rumen depends on the pasture DMI and its RUP content and the supplement DMI and its RUP content (Bargo *et al.*, 2003a).

If there is adequate pasture available a response to increased RUP is not as likely (Tesfa *et al.*, 1995). In the study by Donaldson *et al.* (1991) most of the increased CP in the diets of the

RUP treatments was due to increased forage intake rather than from the CP provided by the supplements.

2.8.5.4 Cows

Older research (mostly pre 1990), related to high RUP supplements for grazing dairy cows done using relatively low yielding cows, found supplemental protein to have no effect on milk yield especially when the quality of the pasture was high (Hongerholt & Muller, 1998). The effects of RUP supply on milk production under grazing conditions has not been very extensively investigated with high producing cows (Schor & Gagliostro, 2001).

Bargo *et al.* (2001) suggested that RUP was not limiting for cows on pasture producing less than 22 kg of milk. Addition of high RUP sources is most likely to be beneficial when the cows are producing more than 30 to 36 kg of milk a day and these cows will respond more favourably to FM supplementation than lower yielding cows (Santos *et al.*, 1998; Muller, 2003a). Rogers *et al.* (1980) found higher producing cows to respond more favourably to formaldehyde treated casein compared to casein supplementation only. In high yielding (40 kg milk/d) dairy cows in early lactation, a supplemental grain mixture with a high RUP content tended to increase milk protein yield when a grass pasture was the sole forage source (Hongerholt & Muller, 1998; Schor & Gagliostro, 2001).

When Lys and Met are supplemented in early lactation increases in milk yield are most likely, while in mid-lactation mainly milk protein increases (Rulquin & Vérité, 1993; Schroeder & Gagliostro, 2000). McCormick *et al.* (1999) found that early lactation, but not later lactation, cows receiving supplemental RUP in the form of maize gluten meal and BM produced more 3.5 % FCM. Similarly Broderick (1992) found no advantage of supplementing FM vs. SBM in mid-lactation cows while there was a response in early lactation cows.

Multiparous cows respond more than primiparous cows (Holter *et al.*, 1992; Hongerholt & Muller, 1998).

2.8.5.5 Amino acid composition of rumen-undegradable protein

The inconsistent effects of high RUP supplements on milk protein percentage could be due to differences in AA composition of the RUP sources (Santos *et al.*, 1998; Bargo *et al.*,

2003a). For a RUP supplement to improve performance it needs to have an AA profile that would complement that of microbial protein (Santos *et al.*, 1998).

If a protein source of low rumen degradability is supplemented it will not have an effect on milk yield or composition if the quality is poor (low Lys and Met) even if rumen NH_3-N is decreased (Santos *et al.*, 1998; Schroeder & Gagliostro, 2000). Many high RUP protein sources are inferior to microbial protein in terms of EAA index and Lys and Met concentration (low quality) including feather meal, maize gluten meal and distillers' grain (Santos *et al.*, 1998). Bargo *et al.* (2001) found no effect of feather meal supplementation on milk yield and Holter *et al.* (1992) found small, sometimes even negative, effects of RUP supplementation on milk yield when maize gluten meal was used, emphasising that AA adequacy may be more important than un-degradability.

Santos *et al.* (1998), in their review, found that the high RUP sources that most consistently benefited lactational performance were FM and treated SBM. These also ranked highest in the EAA index when compared with milk protein. Fishmeal is recognised as an excellent source of RUP as it is rich in Lys and Met which are probably the first and second limiting AA for milk yield and milk protein synthesis (Rulquin & Vérité, 1993; Santos *et al.*, 1998; Schroeder & Gagliostro, 2000; Bach *et al.*, 2000). Fishmeal led to the greatest increase in protein yields especially when the maize was used (Rulquin & Vérité, 1993).

Blood meal is also high in Lys but low in Met, Ile and His, while maize gluten meal is a good source of Met but is low in Lys (Rulquin & Vérité, 1993; Santos *et al.*, 1998). These protein sources with unbalanced AA composition would be less efficient for milk protein synthesis than soybeans or FM (Rulquin & Vérité, 1993).

It has been suggested that the EAA in the duodenal digesta should contain 15 % Lys and 5 % Met to maximise milk and milk protein yields (Santos *et al.*, 1998). The second limiting AA (after Lys) could also be Ile in early lactation while it is Met in late lactation (Holter *et al.*, 1992).

Cows did not respond to ruminally protected Lys when Lys was not the first limiting nutrient (Robinson *et al.*, 1998).

2.8.5.6 Digestibility of rumen-undegradable protein source

The intestinal digestibility of feedstuffs varies (Stern *et al.*, 2006). Another reason for lack of response to increased RUP is if the RUP source is of poor digestibility in the small

intestine (Santos *et al.*, 1998). Milk production is higher for cows fed RUP sources of higher digestibility (Chalupa & Sniffen, 2006). Feather meal, BM and meat and bone meal have lower intestinal digestibility than SBM (less than 80 % vs. greater than 90 %).

2.8.6 Economics

Milk response, in the short term, determines whether supplementation is profitable or not, depending on milk and concentrate prices (Bargo *et al.*, 2003a). The cost of RUP supplementation must be considered (Muller, 2003b) as the use of supplements is only economical when the value of the extra milk exceeds the supplement cost (Clark & Kanneganti, 1998). If the milk to feed ratio approaches one or less, then concentrate feeding becomes unprofitable, except perhaps for early lactation, high genetic merit cows (Muller, 2001b).

The economic benefits of including protein meals in concentrates is not as clear as concentrates as a whole. Present evidence suggests that, although the level and type of protein is critical to the cost of milk production, there is little or no economic benefit to specific addition of protein of low degradability (Cowan & Lowe, 1998).

2.8.7 Conclusion

Response to RUP supplementation has been inconsistent, depending to a large extent on the quality (AA composition) of the supplement, with FM, as well as BM (high in Lys), giving the most consistent positive response. Supplementation with RUP is only beneficial if its AA composition is superior to that of microbial protein. There is no response if ME or RDP are limiting or if RUP is more than adequate. Higher producing and early lactation cows respond more favourably.

2.9 THE USE OF MODELS TO AID IN FORMULATING SUPPLEMENTS

Nutrition models such as CNCPS and CPM Dairy are promising tools to better understand the limiting nutrients in a grazing system and develop feeding programmes that provide limiting nutrients through supplemental feeding (Muller & Fales, 1998; Kolver & Muller, 1998). Reformulating supplements taking into account the identified nutrient deficiencies allows potentially more milk to be produced from the same intake of pasture and supplement (Kolver, 2003).

The CNCPS and CPM Dairy models evolved together, incorporating the same equations and ideas, and hence are similar to each other and might even merge in the future (Sniffen, 2006). These two models are routinely used by nutritional consultants and feed companies as well as to design and interpret experimental results (Chalupa & Boston, 2006).

The CNCPS model can be used to give relatively realistic predictions of ME and MP supplies and subsequent milk production when cows are grazing medium to high quality pasture as well as responses to changing inputs such as DMI, NDF, lignin, NDF degradation and CP (Kolver & Muller, 1998; Kolver *et al.*, 1998b; Kolver, 2003). The model can predict efficiencies of microbial protein synthesis comparable with efficiencies reported for high quality pasture (Kolver & Muller, 1998).

Hongerholt & Muller (1998), for example, used the CNCPS model to compare the results of their trial with those predicted by the model assuming similar DMI. The model predicted that ME and MP were equally limiting for cows fed the low RUP concentrate (see section 2.8.4.2 above) and actual milk yield was comparable with that predicted. For the cows fed the high RUP concentrate ME, not MP or AA, was the most limiting nutrient. Actual milk yield was similar to the predicted ME allowable milk while the predicted MP allowable milk was higher. This helped explain why there was a lack of response in this trial to increasing the RUP content of the concentrate as ME was most limiting, suggesting that the MP in the diet would have been adequate to support higher milk production if more ME was supplied. Amino acids were predicted to be limiting at higher levels of concentrate supplementation and at higher milk production (Kolver & Muller, 1998).

The CNCPS model can take into account differences in DMI, activity, cost of excreting urea, milk composition, and BW of grazing cows vs. TMR fed cows, predicting a lower milk production in the former (Kolver & Muller, 1998).

2.10 CONCLUSION

From the literature reviewed it is clear that there first need to be adequate rumen fermentable carbohydrates in the rumen for microbes to be able to utilise the highly degradable pasture CP. Once this has been supplied, RUP can be added to the diet to improve the flow of AA to the small intestine, supplying the demands of high producing cows. Although responses to RUP supplementation have been inconsistent, it has been positive in studies where protein source with a good AA profile, such as FM and BM, have been used.

High producing, early lactation cows receiving high levels of grain are most likely to respond to RUP supplementation.

This leads to the hypothesis that high producing, early lactating dairy cows receiving high levels of maize supplementation while grazing lush pasture could respond to supplementation with a protein source high in RUP with a good AA composition, such as FM.

Chapter 3

FISHMEAL SUPPLEMENTATION TO HIGH PRODUCING JERSEY COWS GRAZING RYEGRASS PASTURE

3.1 MATERIALS AND METHODS

3.1.1 Location and duration of the project

The project was conducted at the Outeniqua Experimental farm, George (Longitude 22º25', latitude 33º57', altitude 190 m). The long term (39 years) average rainfall in this area is 725 mm per annum. The mean daily maximum and minimum temperatures during the experimental period of the trial were 21 and 11°C, respectively. See appendix A for more details on the climate during the trial.

The cows were grazing on 8.5 hectares of land, with estcourt soil type (Soil Classification Working Group, 1991), with a pasture of kikuyu (*Pennisetum clandestinum*) over-sown with annual ryegrass (*Lolium multiflorum var. westerwoldicum*, cv Energa), fertilised with 56 kg N (LAN, limestone ammonium nitrate)/ha after each grazing. In this trial (late winter/ spring) the kikuyu was dormant so the pasture was predominantly ryegrass.

This trial took place from 26 August to 4 November 2005. The selection of the cows was done on 26 August 2005 and they were weighed on 31 August and 1 September 2005. The cows were on the experimental treatments from 8 September to 4 November 2005. Measurements were only taken from 20 September 2005, after an adaptation period.

A study using rumen cannulated cows was conducted simultaneously. This was divided into two periods: period A which was from 8 September to 6 October 2005 and period B which was from 7 October to 4 November 2005.

3.1.2 Production study

3.1.2.1 Cows and experimental treatments

3.1.2.1.1 Cows

Forty-five high producing multiparous Jersey cows [BW, 331 ± 29.9 kg; milk yield, 21.4 ± 1.65 kg/d; parity, 4.1 ± 1.53; days into lactation, 73 ± 28.3; (mean ± SD)] from the Outeniqua

Experimental Farm were used. The average milk production of the herd of 326 cows in lactation from which the cows were selected was 17.0 kg/d in August 2005.

A randomised complete block design was used. Just before the experimental period (26 August 2005) the cows were blocked according to milk production (of the previous 25 days) and days into lactation, and within each block were randomly divided into three groups. These three groups were randomly allocated to the three experimental treatments (see section 3.1.2.1.3 below for the treatments). Appendix B shows the selection and grouping of the cows.

The milk production of the cows in the three experimental groups (control, low FM and high FM) were 21.5 ± 1.56, 21.4 ± 1.85 and 21.4 ± 1.63 (mean ± SD) kg/d respectively, at the beginning of the trial. The mean days into lactation on the day of selection of the cows (26 August 2005) was 73 ± 24.1, 73 ± 29.8 and 75 ± 32.2 days for the control, low FM and high FM groups respectively and the mean lactation number 4 ± 1.9, 4 ± 1.3 and 4 ± 1.5, respectively.

3.1.2.1.2 Management

The cows strip grazed the ryegrass pasture and were moved to a new strip twice a day, after each milking. The cows were milked at 0600 and 1430 h. The average walking distance from the pasture to the milking parlour was 0.9 km (range 0.55 to 1.18 km).

The cows grazed 24 hours a day (except for the milking times) and clean water was available *ad libitum*.

All the cows were grazed together as a single herd to ensure equal pasture allocation. The mean PA was 11 kg DM/cow/d above 3 cm pasture height.

The cows in the three groups each received a different concentrate in the milking parlour (see section 3.1.2.1.3 below). Since the cows grazed together they needed to be separated just before milking so that the three groups could be milked, and thus fed their respective concentrates, separately. To facilitate this each cow was marked with a coloured tag hanging around her neck: yellow for the control group, blue for the low FM group and red for the high FM group. To ensure that no mistakes were made regarding feed allocation to the three groups of cows, the feed was bagged (at Bokomo Feeds, George) with colours corresponding to the colours of the tags of the cows. The concentrates were offered individually to the cows in the milking parlour. Half of the daily allowance (3 ± 0.45 kg as is) of the pellets was measured with a bucket and poured into the feed cribs in the milking parlour before the cows went in to be milked and the

cows were only allowed out of the milking parlour when they had all finished consuming their pellets. In the unlikely event that a cow did not finish her pellets, it was a small amount left over.

3.1.2.1.3 Experimental treatments

Each of the three groups received a different supplement (experimental treatment). The cows received the supplement twice a day in the milking parlour.

The three experimental treatments were:

1. Control treatment: grazed ryegrass pasture plus 5.5 kg DM (6 kg as is) a day of pellets containing no fishmeal (FM).
2. Low FM treatment: grazed pasture plus 5.5 kg DM a day of pellets containing 4 % FM (220 g FM DM/d).
3. High FM treatment: grazed pasture plus 5.5 kg DM a day of pellets containing 8 % FM (440 g FM DM/d).

The cows received their supplement in two equal portions, that is 3kg (as is) at each milking, so that they were not consuming too much concentrate at any one time which could be detrimental to rumen health.

The cows adapted to their new diets for 12 days before any samples were taken.

3.1.2.1.4 Experimental diets

The concentrates were mixed, pelleted and bagged at Bokomo (now Nova) Feeds, George (Saagmeul St., George Industria, P.O. Box 1351, George, 6530). Table 3.1 shows the raw materials that were used as well as the chemical composition of the three concentrates based on analyses done at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria).

The diets were formulated to be iso-energetic. The Megalac (a rumen-protected fat; Church & Dwight Co., Inc., 469 N. Harrison St., Princeton, NJ 08543-5297) was added to the latter two experimental treatments to bring the energy to the same level in all three. The CP concentration of the diets differed since it was the effect of additional protein that needed to be investigated. The molasses was added to facilitate pelleting which was done to increase the palatability.

Table 3.1 Ingredient and chemical composition of the concentrate pellets used in the ryegrass trial (n = 1)

Parameter	Experimental treatment		
	Control	**Low FM**	**High FM**
Ingredient composition, % DM			
Maize meal	88.75	84.1	78.5
Fishmeal (FM)	0	4.0	8
Megalac[1]	0	0.65	1.3
Molasses	6.8	6.8	6.8
MonoCaP	1.3	1.3	1.3
Feed lime	1.8	1.8	1.8
Salt	0.5	0.5	0.5
MgO	0.5	0.5	0.5
Premix[2]	0.35	0.35	0.35
Chemical composition			
DM %	91.9	91.5	91.4
ME MJ/kg DM[3]	12.9	13.3	12.9
% DM			
OM %	93.2	91.2	90.4
CP %	8.2	11.2	14.6
NDF %	11.2	11.8	12.7
ADF %	3.7	3.7	4.0
IVOMD %	92.4	95.1	91.3
Ca %	1.55	2.03	2.30
P %	0.56	0.75	0.87
Ca: P	2.77	2.71	2.64

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)

[2]Premix (Lactating Cow (Organic); DSM Nutritional Products South Africa (Pty) Ltd.) contained 7.23 % Mn, 7.50 % Zn, 1.83 % Cu, 0.11 % Co, 0.14 % I, 0.03 % Se (1 %), 1.28 % organic Mn, 2.00 % organic Zn, 0.32 % organic Cu, 0.01 % organic Se, 5 % Rumensin (20 %), 3.5 % Stafac 500 and provided 96,250 IU of vitamin A, 28,875 IU of vitamin D3, and 577.5 mg of vitamin E/cow/d

[3]ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

The mean chemical composition of the ryegrass pasture grazed during the trial is shown in Table 3.2 (see Tables 3.5 and 3.6 in section 3.2.1.1.2 for the chemical composition of the ryegrass on a weekly and three-weekly basis).

Table 3.3 shows the composition of the total diets consumed by the cows based on an intake of 5.5 kg DM/cow/d of the concentrates with composition as shown in Table 3.1 and a mean intake of 8.6 kg DM/cow/d of ryegrass pasture with an mean composition as shown in Table 3.2. See section 3.2.1.1.1 for the estimation of the pasture intake.

Table 3.2 Chemical composition (mean ± SD) of the ryegrass pasture grazed by the cows during the ryegrass trial

Nutrient	Mean composition
DM (%)	13.7 ± 3.60[1]
ME (MJ/kg DM)[3]	11.3 ± 0.42[2]
OM (% DM)	86.6 ± 1.44[1]
CP (% DM)	26.2 ± 3.23[1]
NDF(% DM)	46.3 ± 3.23[1]
ADF(% DM)	25.6 ± 1.46[1]
IVOMD (% DM)	80.2 ± 3.34[1]
Ca (% DM)	0.52 ± 0.103[2]
P (% DM)	0.41 ± 0.026[2]
Ca: P	1.28 ± 0.214[2]

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility
[1]n = 9, [2]n = 3
[3]ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

Table 3.3 Mean chemical composition of the total diets (8.6 kg ryegrass DM and 5.5 kg supplement DM/cow/d) consumed by the cows in the ryegrass trial

Nutrient	Experimental treatment[1]		
	Control	Low FM	High FM
ME (MJ/kgDM)[2]	11.9	12.1	11.9
OM (%DM)	89.1	88.4	88.1
CP (% DM)	19.2	20.3	21.6
NDF(%DM)	32.6	32.8	33.2
ADF(%DM)	17.0	17.0	17.1
IVOMD(%DM)	84.9	86.0	84.5
Ca (% DM)	0.92	1.11	1.22
P (%DM)	0.47	0.54	0.59
Ca: P	1.97	2.05	2.06

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility
[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

3.1.2.2 Experimental parameters and sample analyses

3.1.2.2.1 Pasture

a) Calibration of the rising plate meter

To calibrate the rising plate meter (RPM; Filip's folding plate pasture meter, Jenquip, Rd 5, Fielding, New Zealand) that was used to estimate pasture intake, three low, medium and high pasture heights were selected, the pasture height was measured with the RPM, and the grass under the plate was cut at a height of 3 cm above the ground. Each sample was weighed and dried at 60°C for 72 hours for determination of the amount of DM present. Since the area under the plate was known to be 0.0985 m^2, this could be extrapolated to kg DM/ha. Each RPM reading was paired with its corresponding pasture mass. This was done every week and all the data composited.

A linear regression equation [Y = aH + b, where Y = pasture mass in kg DM/ha and H = RPM reading] was fitted to the data using the LINEST function in Microsoft® Excel. This regression equation could then be used to estimate the DM yield at a given RPM reading. After doing this procedure every week from 23 August to 24 October 2005, the average equation obtained was Y = 62H – 57 (R^2 = 0.4; n = 90).

Since this was done during the experiment the equation could only be used afterwards to estimate what the pasture allowance and intake of the cows was. For the purpose of pasture allocation during the trial the standard equation for the area and time of year, Y = 52H (Meeske, R., personal communication, robinm@elsenburg.com), was used. Once the yield/ha had been estimated, the area needed for a PA of approximately 10 kg DM/cow/d could be calculated. The cows were allowed to graze on half this area per grazing (a grazing being the period between two milking times) so that after every milking they would have access to fresh pasture.

b) Estimating pasture allowance and intake using the rising plate meter

Pasture height was measured before and after grazing with the RPM. This was used to estimate the average pasture intake for all the cows. The pasture height was estimated by taking 100 RPM readings on each strip to determine the average pasture height prior to and after grazing. The pasture yield (kg DM available at a given RPM reading) was calculated with the

calibration equations above. The difference between the DM available before and after grazing was assumed to be the intake of the cows. This was divided by the number of cows to get the pasture intake per cow per day.

c) Estimating pasture intake of the three treatment groups separately using the rising plate meter

The mean pasture intake of the cows in each of the three experimental treatment groups was estimated by separating the groups for three consecutive days and grazing them each on their own strip (the strip was divided with cross wires so that each of the three groups still got the same PA per cow). The pre- and post-grazing pasture mass was measured with the RPM as above. This was done on 12 to 15 September and 10 to 13 October 2005.

d) Estimating pasture intake using equations

Various equations for estimating PDMI were compared. The expected PDMI (kg)/cow/d was calculated based on the assumption that a cow consumes 1.3 % of BW (kg) as NDF (Bargo *et al.*, 2002b; Meeske, R., personal communication, robinm@elsenburg.com) or on the assumption that if the cow were consuming only pasture she would be able to consume 1.5 % of BW as NDF (Kolver & Muller, 1998) and then correcting for the substitution of pasture for concentrate at a rate of 0.093 × kg concentrate DM/cow/d (Faverdin, *et al.*, 1991). The PDMI was also back calculated from the ME required for the performance of the cow, ME consumed from the concentrate and ME content of the pasture (Tesfa *et al.*, 1995).

e) Estimating pasture intake using the CPM Dairy model

The CPM Dairy model (Version 3.0.7a; Cornell University, Ithaca, NY, University of Pennsylvania, Philadelphia, PA; Willam H. Miner Agricultural Institute, Chazy, NY) reports a predicted DMI for an animal to be able achieve a certain level of production. The production results of the trial were put into the model (see chapter 4) and the concentrate intake subtracted from the model-predicted DMI to estimate the pasture intake.

f) Ryegrass pasture samples

Once a week (total of 9 times) a sample of the pasture was taken. These samples were taken every Monday from 5 September to 31 October 2005. The pasture samples were taken at approximately midday (between 1200 and 1400 h) so that the sugar content would not be too

extreme. Samples were taken from four random places by blindly throwing a ring 35.4 cm in diameter and cutting the area in the ring, where it happened to land, to a height of 3 cm above the ground. These samples were dried in paper bags at 60°C for 72 hours (Wales *et al.*, 1998) to determine the % DM. The four dried samples were composited and milled through a 1mm screen with a Retsch GmbH5657 laboratory mill (Retsch GmbH 5657 Haan, West Germany) and stored in airtight containers to be analysed at Nutrilab. Some of the results of these analyses are given in Table 3.2.

3.1.2.2.2 Concentrate samples

Once every week (every Monday from 12 September to 31 October 2005) representative samples of the concentrate pellets were taken. These were dried at 60°C for 72 hours (Wales *et al.*, 1998) to determine the % DM and then milled through a 1mm screen (Retsch GmbH5657 laboratory mill) and stored in airtight plastic containers to be analysed at Nutrilab. These samples were composited so that there was one sample per experimental treatment. Some of the results of these analyses are given in Table 3.1.

3.1.2.2.3 Milk production and composition

The cows were milked in a 20 point Dairy Master (Total Pipeline Industries, 33 Van Riebeeck St. P.O. Box 252, Heidelberg, 6665) swing over milking machine with weigh-all electronic milk meters. The daily milk production of the cows was measured in the milking parlour and automatically recorded. The mean milk production for the experimental period (20 September to 4 November 2005, after an adaptation period) was calculated.

The experimental period was also divided into four sub-periods and the mean milk production for each of these periods calculated, to detect any treatment × time interactions.

Composite milk samples (ratio 9 ml: 15 ml, afternoon: morning milking) were taken every 14 days and preserved with bronopol to be analysed for fat, protein, lactose and MUN. Milk samples were taken on 21 September, 4/5 October, 18/19 October and 1/2 November 2005. These samples were sent to Lactolab Pty (Ltd) (ARC, Main rd., Irene, 0062) to be analysed using the Milkoscan FT 6000 (Foss Electric, Denmark).

3.1.2.2.4 Body weight and body condition score

The cows were weighed just before milking on two consecutive days at both the beginning and end of the trial. This was done on 31 August and 1 September and 3 and 4 November 2005. They were weighed twice because the BW can vary depending on when the cow last drank water, urinated or defecated. The average BW between these two days was used for analysis.

On the first of these two consecutive days the BCS of the cows was also determined by palpitation of the back and hind quarter area and a score of 1 to 5 was given, where 1 is thin and 5 is fat (Wildman *et al.*, 1982). The condition scoring was done each time by the same person (Gerrit Van der Merwe, the Research Technician at the Outeniqua Experimental Farm).

3.1.2.2.5 Faecal samples

Faecal rectal samples were taken from the cows of three randomly chosen blocks: block 6 (cows RC6, RL6 and RH6), block 9 (cows RC9, RL9 and RH9) and block 16 (cows RC16, RL16 and RH16). Samples were taken three times at two week intervals on days 23, 37 and 49 (30 September and 14 and 26 October 2005) and composited so that in the end there was one sample per cow. These were analysed for starch at Nutrilab as an indication of rumen health and efficiency of rumen fermentation.

3.1.2.2.6 Laboratory analyses

The nine ryegrass pasture samples as well as the composite concentrate samples were analysed for DM (AOAC 2000, procedure 934.01), ash (AOAC 2000, procedure 942.05), CP (N was determined using a Leco N analyser, model FP-428, Leco Corporation, St Joseph, MI, USA and CP was calculated as N × 6.25), NDF (Robertson & Van Soest, 1981), acid detergent fibre (ADF; Goering & Van Soest, 1970), and IVOMD (Tilley & Terry, 1963; Engels & Van der Merwe, 1967; using rumen fluid from a rumen cannulated sheep on lucerne). Every three samples were composited and analysed for gross energy (GE; MC – 1000 Modular Calorimeter, Operators Manual), EE (crude fat; AOAC 2000, procedure 920.39), Ca (AOAC 2000, procedure 965.09), P (AOAC 2000, procedure 965.17), starch (MacRae & Armstromg, 1968; Faichney & White, 1983; AOAC 1984), acid detergent lignin (ADL; Goering & Van Soest, 1970), non-protein N (NPN; Faichney & White, 1983) and sol CP, neutral detergent insoluble protein

(NDIP) and acid detergent insoluble protein (ADIP; Krishnamoorthy *et al.*, 1982) at Nutrilab. Sub-samples were sent to the department of biochemistry of the University of Pretoria where they were analysed for AA composition with the PICOTag method (Bidlingmeyer *et al.*, 1984) using a Waters HPLC with two Model 510 pumps, UV protector Model 440, autosampler Model 712 and Waters Millennium 32 software. ME was calculated with the following formula: ME (MJ/kg DM) = 0.82 × GE × IVOMD (Robinson *et al.*, 2004). The following formula was used to calculate NFC: NFC = [100 – (NDF + ash + CP + EE)] (NRC, 2001). Trace minerals and vitamins were not measured in this trial; they were assumed to be adequate as an appropriate premix was added to the concentrates.

A representative sample of the FM that was used in the concentrates was analysed for DM, ash, OM, CP, EE and AA with the same methods as above.

The faecal samples were analysed for starch (MacRae & Armstromg, 1968; Faichney & White, 1983; AOAC 1984) at Nutrilab.

3.1.3.2.7 Soil and climate

The minimum and maximum temperatures during the experiment as well as the rainfall were measured daily at a weather station on the same experimental farm. A tensiometer was used to monitor the moisture content of the soil and irrigation applied when the tensiometer reading was greater than –25 kPa. (Tensiometer readings were kept between –10 and –25 kPa).

No soil sample was taken during the ryegrass trial.

3.1.2.3 Statistical analyses

An analysis of variance with the ANOVA model (Statistical Analysis Systems, 2001) was used to determine the difference between the experimental treatments in milk production and composition, FCM, ECM, change in BW and BCS, difference in pasture intake and starch concentration of the faeces. Significance of difference was determined using Duncan's test (Samuels, 1989).

An analysis of variance was also done with the GLM model (Statistical Analysis Systems, 2001) to determine the difference between the experimental treatments for milk composition

using the initial values as covariates if there was a significant covariate effect. Significance of difference was determined using Fischer's test (Samuels, 1989).

The experimental period was also divided into four sub-periods and the difference in milk production and composition between the treatments analysed with Proc GLM Repeated Measures Analysis of Variance (Statistical Analysis Systems, 2001).

Difference was considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. Tendency was indicated at $P \leq 0.1$.

3.1.3 Rumen study

3.1.3.1 Cows and experimental treatments

3.1.3.1.1 Cows and management

Eight multiparous Jersey cows from the Outeniqua Experimental Farm, George, were used. The cows had been fitted with ruminal cannulae (with rolled inner flange 10 cm in diameter; Bar Diamond, Inc., P.O. Box 60, Parma, Idaho, USA). These cows were each given a number preceded by the letters Ru (for rumen cannula).

These cows grazed, were milked and received concentrate with the cows of the production study.

Four of these cows (Ru2, Ru3, Ru4 and Ru6, chosen at random) received the control treatment and four of them (Ru1, Ru5, Ru7 and Ru8) received the high FM treatment. There were not enough cannulated cows on the farm to include the low FM treatment in the rumen study. Since it was expected that the results of the low FM treatment would be between the other two, it was decided to only compare the two extreme treatments.

A cross-over design was used: each animal received both treatments in different periods of the trial.

Since there were 16 cows in each group for the production study, the control and high FM groups had 20 cows each when the cannulated cows were included. Four "filler" cows were used for the low FM group so that each group had 20 cows, which fitted in with the milking system.

When samples of rumen fluid were taken (see section 3.1.3.1.2) the cows were restrained on the pasture with temporary gates and halters so as to minimise disturbance to their grazing. The rumen fluid samples were taken by inserting a tube though a hole in the cannula so that the rumen environment (and pH monitoring) was not disturbed by opening the cannula.

3.1.3.1.2 Experimental treatments

a) Period A

The cows were allowed to adapt to the diet from days 1 to 19 of the trial (8 to 26 September 2005).

On days 20 to 28 (27 September to 5 October 2005) the cows were fitted with automated pH meters with data loggers (WTW pH 340i pH meter/ data logger with a WTW SenTix 41 pH electrode) so that the ruminal pH at 10 minute intervals throughout the day could be monitored. The electrode was placed in the rumen through the cannula and connected to the data logger that was strapped on like a saddle (Figure 3.1). Two cows from each experimental treatment were monitored for two days and then the pH meters changed over to the other cows for two days. This was repeated so that each cow was monitored for a total of four days with a two day rest in the middle.

On days 26 to 29 (3 to 6 October 2005), samples of rumen fluid were taken to be analysed for ruminal NH_3-N, VFA and pH. The samples were taken at 2000 h on 3 October, 0800 h on 4 October, at 0400, 1200 and 0000 h (12 midnight) on 5 October and at 1600 h on 6 October. The sampling times were chosen so that in the end there were samples representing every four hours of the day.

b) Period B

On day 30 (7 October 2005) the cows were switched to the opposite experimental treatment (i.e. those that were on the control treatment moved to the high FM treatment and vice versa) so that cows Ru1, Ru5, Ru7 and Ru8 received the control treatment and cows Ru2, Ru3, Ru4 and Ru6 received the high FM treatment. Thus in the end each cow received both treatments and so there were eight cows per treatment.

The cows adapted to their new diets from days 30 to 47 (7 to 24 October 2005).

On days 48 to 56 (25 October to 2 November 2005) the cows were fitted with automated pH meters with data loggers to monitor pH throughout the day. Two cows from each experimental treatment were monitored for two days and then the pH meters changed over to the other cows for two days, back to the first cows for two days and back to the latter cows for two days so that each cow was monitored for a total of four days with a two day rest in the middle.

On days 49 to 51 (26 to 28 October 2005), samples of rumen fluid were taken to be analysed for NH_3-N, VFA and pH. The samples were taken at 2000 h on 26 October, 0400, 1200 and 0000 h (12 midnight) on 27 October and at 0800 and 1600 h on 28 October.

3.1.3.2 Experimental measures and sample analyses

The rumen pH was recorded every 10 minutes with the data loggers for a total of four days per cow for each of the two periods of the trial. The mean rumen pH for each half hour was calculated for each cow on each experimental treatment.

Rumen fluid was collected into a plastic container with a lid. The pH of the rumen fluid was measured immediately with a pH meter (WTW pH 340i pH meter with a WTW SenTix 41 pH electrode). The rumen fluid was then filtered through a layer of mutton cloth. From each sample 30 ml of rumen filtrate was preserved with 5 ml 50 % H_2SO_4 and frozen for NH_3-N analysis (De Bruin, 1995) and 20 ml of rumen filtrate was preserved with 4 ml of 25 % H_3PO_4 and frozen for VFA analysis (Beauchemin *et al.*, 2003).

These samples were analysed at Nutrilab for rumen NH_3-N (Broderick & Kang, 1980) and VFA (acetic, propionic, butyric, iso butyric and valeric acids; Webb, 1994, with modifications).

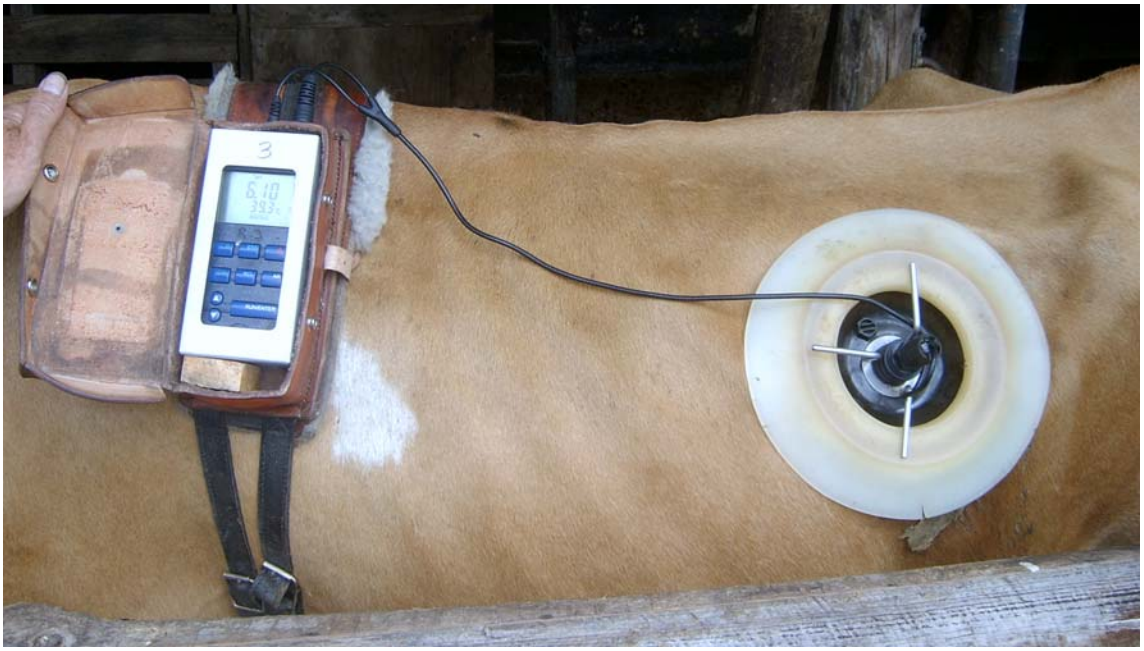

Figure 3.1 Ruminal pH was recorded at 10 minute intervals on a data logger (WTW pH 340i pH meter/ data logger) connected to an electrode (WTW SenTix 41 pH electrode) placed in the rumen via the cannula

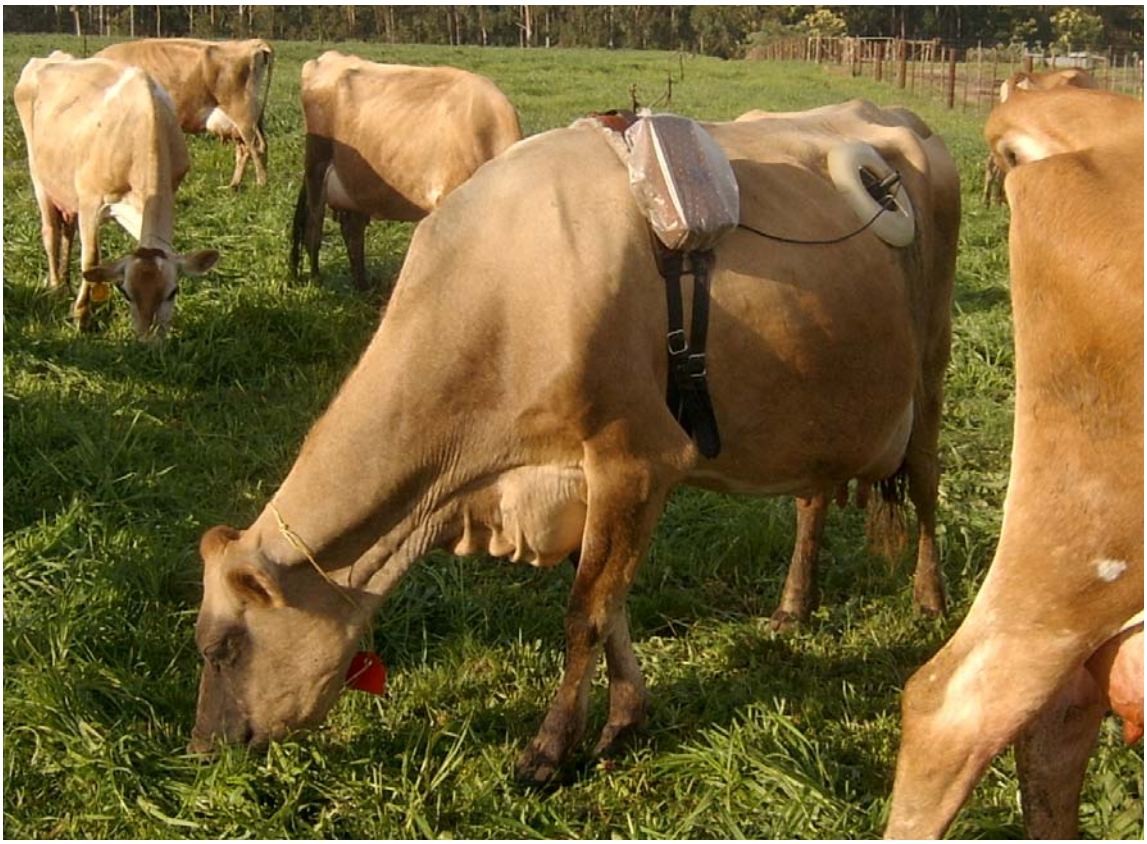

Figure 3.2 The cows of the rumen study were grazed with the cows of the production study

Figure 3.3 Samples of ruminal fluid were taken at times representing every four hours of the day

3.1.3.3 Statistical analyses

Proc GLM Repeated Measures Analysis of Variance (Statistical Analysis Systems, 2001) was used to determine the difference between the experimental treatments for the rumen parameters measured at six times of the day.

The daily mean values were calculated and analysed with the ANOVA model (Statistical Analysis Systems, 2001) to determine differences between experimental treatments. Significance of difference was determined using Duncan's test (Samuels, 1989).

Difference was considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. Tendency was indicated at $P \leq 0.1$.

3.2 RESULTS

3.2.1 Production study

3.2.1.1 Pasture

3.2.1.1.1 Pasture allowance and intake

a) Pasture allowance and intake estimated using the rising plate meter

The mean RPM readings (in half cm increments) for the duration of the trial (8 September to 4 November 2005) were 30 (± 5.8) before grazing and 11 (± 2.5) after grazing. Using the standard calibration equation Y = 52H, it was calculated that there was, on average, 1548 (± 300.5) kg pasture DM available/ha before grazing and 585 (± 127.4) kg pasture DM/ha left after grazing. Thus the cows removed on average 963 kg DM/ha off the pasture.

The area allocated to the cows was adjusted every day to keep the daily PA as close as possible to 10 kg pasture DM/cow, but due to management constraints, it was not possible to be precise. The grass was examined after grazing and the allowance adjusted to ensure that the cows grazed the grass down to the correct level to ensure a good quality growth of grass for the next grazing cycle. Thus the PA fluctuated at times (Figure 3.4). The mean PA was 9.6 (± 1.47) kg DM/cow/d and the mean PDMI was 6.4 (± 1.37) kg DM/cow/d. Gaps in the graph are due to missing data from the days that the three treatment groups were separated or due to post grazing pasture height not being measured because of management constraints.

At the end of the trial, when the calibration equation had been obtained for the ryegrass that was grazed during the trial, the equation Y = 62H – 57 could be applied to the same RPM readings as with the above. Due to the higher value for "a", the equation predicted greater DM yields at the higher RPM readings (Figure 3.5). It was found that there was actually on average 1789 (± 358.3) kg pasture DM/ha available before grazing and 641 (± 151.9) kg pasture DM/ha after grazing. Thus the cows removed 1148 kg DM/ha off the pasture. The mean PA was 11.1 (± 1.75) kg DM/cow/d and the mean intake was 7.6 (± 1.64) kg DM/cow/d.

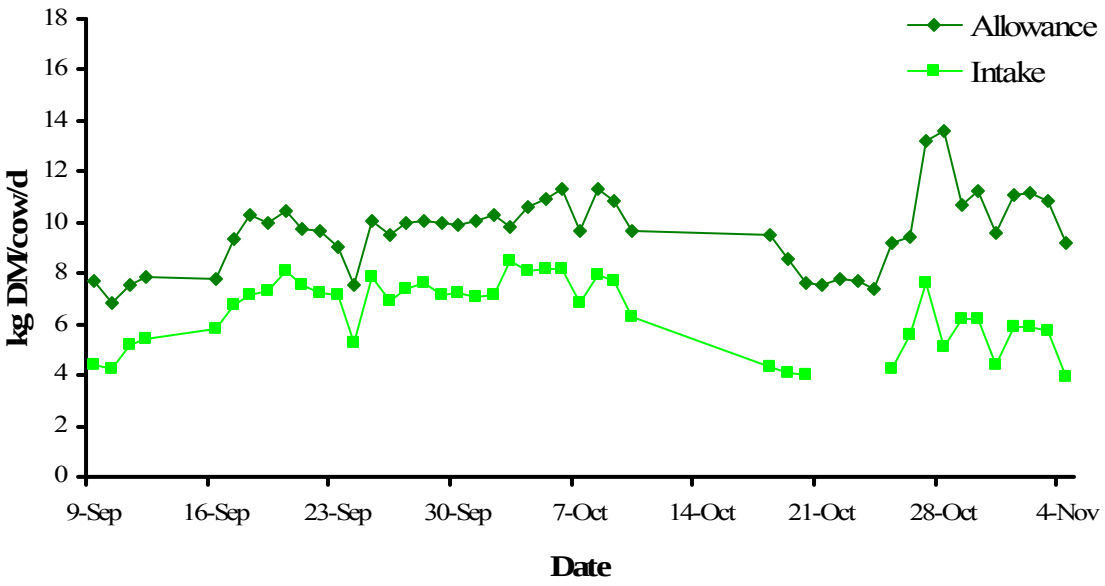

Figure 3.4 Ryegrass pasture allowance and intake estimated with a rising plate meter (RPM) based on the calibration equation Y = 52 H where Y is pasture yield (kg DM/ha) and H is the average RPM reading

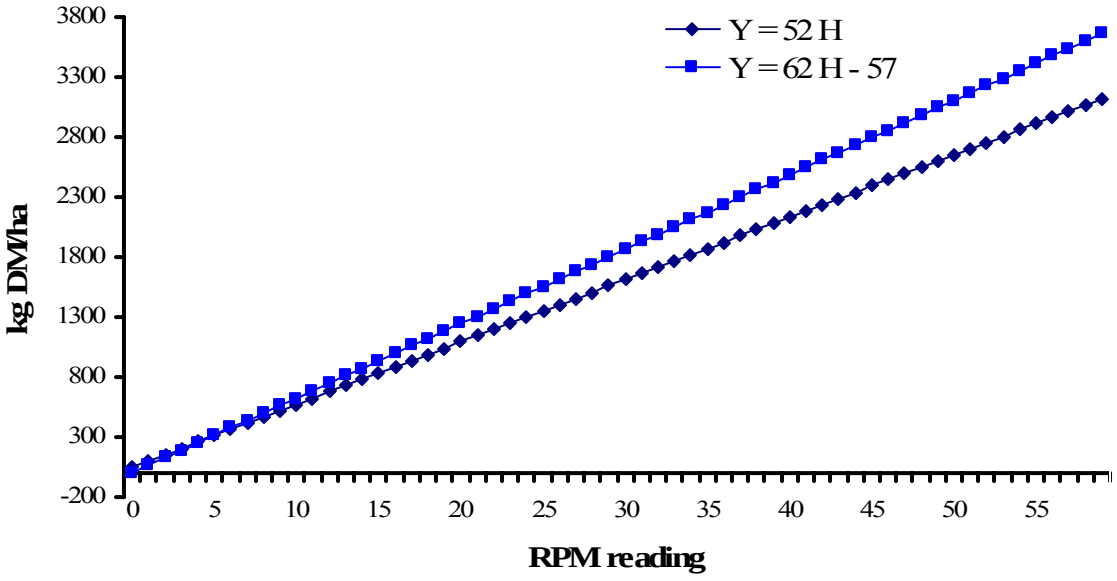

Figure 3.5 Relationship between rising plate meter (RPM) reading and pasture yield (kg DM/ha) with the standard calibration equation Y = 52 H and the equation Y = 62 H – 57 (R^2 = 0.4; n = 90) obtained during the trial

Figure 3.6 shows the mean pasture allowance and intake per cow over time using the equation Y = 62H – 57. Since this is the equation that was determined by cutting samples of grass from the actual pasture used in the trial (see section 3.1.2.2.1a), this should be a more accurate estimate of the pasture allowance and intake of the cows during this trial. However, due to the limited number of samples taken, this equation would have been more accurate if it could have been repeated over a few years. The low R^2 (0.4) indicates that the accuracy with which the equation predicts the pasture yield from the RPM reading is low.

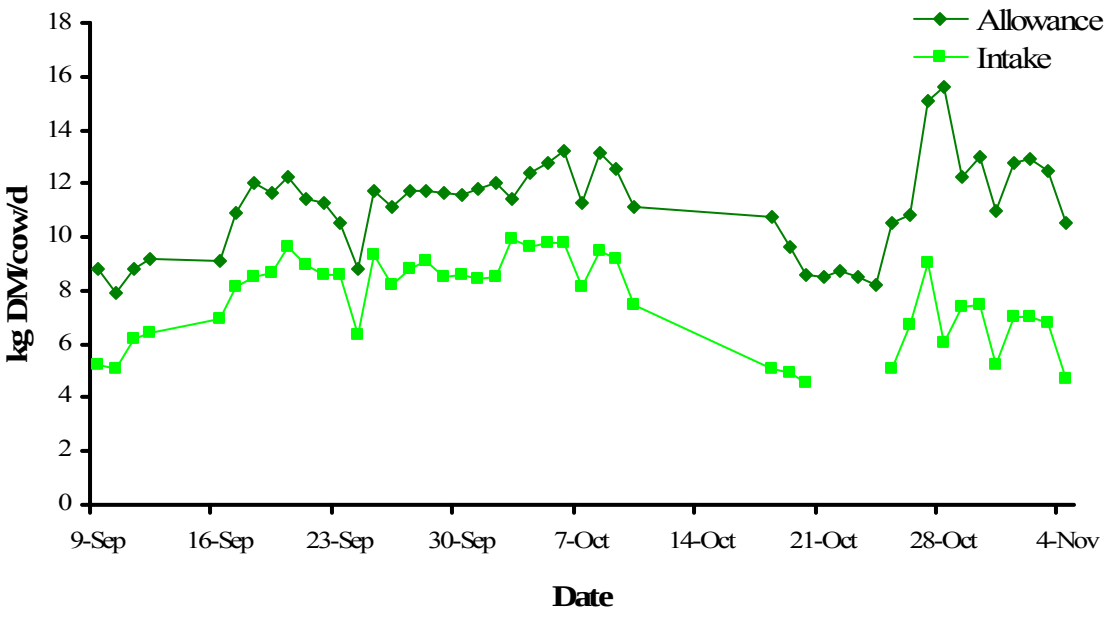

Figure 3.6 Ryegrass pasture allowance and intake estimated with a rising plate meter (RPM) based on the calibration equation Y = 56 H – 57 where Y is pasture yield (kg DM/ha) and H is the average RPM reading

The cows went through approximately two grazing cycles during the experiment. The average growth rate of the pasture between the two grazing cycles was 38 kg DM/ha.

b) Estimation of pasture intake of the three treatment groups separately using the rising plate meter

The mean pasture allowance and intake of the cows in the three experimental treatment groups of the six measurement days (12 to 15 September and 10 to 13 October 2005) is reported

in Table 3.4. The calibration equation Y = 62H – 57 was used. There was no difference in pasture allowance or intake between the three experimental groups (P > 0.1).

Table 3.4 Mean daily pasture allowance and intake of the three experimental treatment groups grazing ryegrass pasture (n = 6)

Pasture (kg DM/cow/d)	Experimental treatment group[1]			SEM
	Control	Low FM	High FM	
Allowance	11.5	11.4	11.3	0.40
Intake	7.7	7.8	7.4	0.34

[1]Control: cows receiving maize-based supplement containing no fishmeal (FM); Low FM: cows receiving maize-based supplement containing 4 % FM; High FM: cows receiving maize-based supplement containing 8 % FM

c) Estimation of pasture intake using equations

The average BWs of all 60 cows at the beginning and end of the trial were 332 and 377 kg, respectively. Thus the average BW during the trial would have been 355 kg with a mean increase in BW of 0.7 kg/d.

The assumption was made that the PDMI of the cows on the three treatments was the same. The various equations for predicting pasture intake (see section 2.6.3.3 above) were used and the results compared.

The first method is to assume that the cows can consume 1.3 % of BW per day as NDF (Meeske, R., personal communication, robinm@elsenburg.com; Bargo *et al.*, 2002b). It is expected that each cow would have been able to consume 4.6 kg NDF/d (1.3 % of 355 kg). The mean NDF concentration of the pasture was 46.3 % (Table 3.2), the NDF of the concentrate 11.9 % (Table 3.1) and the concentrate intake 5.5 kg DM/d. Pasture DMI was calculated as follows: 46.3% of PDMI + 11.9 % of 5.5 = 4.6, therefore PDMI = 8.5 kg.

The second method would be to assume the cows would have been able to consume 1.5 % of BW as NDF if consuming pasture only (Kolver & Muller, 1998) and then to use the equation of Faverdin *et al.* (1991; SR = 0.093 per kg concentrate fed) to account for the effect of concentrate substitution on PDMI. Each cow would have been able to consume 5.3 kg NDF or 11.5 kg pasture/d (46.3 % NDF; Table 3.2) on pasture only. Since each cow was receiving 5.5 kg concentrate DM, the SR would have been 0.51 (0.093 × 5.5) thus pasture intake would have dropped by 2.8 kg (0.51 × 5.5) to 8.7 kg pasture DM/d. This is similar to the above 8.5 kg. The average of the two would be 8.6 kg ryegrass DM/cow/d.

The other way of estimating pasture intake would be the method used by Tesfa *et al.* (1995) where pasture intake is calculated backwards if the ME requirement of the cow and the ME intake from the concentrate are known. The mean estimated ME concentration of the ryegrass pasture was 11.3 MJ/kg DM (see section 3.2.1.1.2 below). For the levels of production obtained in the trial the mean ME requirement of the cows on the two FM treatments was 182.9 MJ ME/d (see Appendix C). If these cows consumed 5.5 kg concentrate with a mean ME concentration of 13.1 MJ ME/kg DM (section 3.2.1.2), 72.1 MJ ME/d would have been supplied by the concentrate. The remaining 110.8 MJ ME would have been supplied by the pasture. For this to be the case the cows would have had to consume 9.8 kg ryegrass DM/d.

The equation of Caird & Holmes (1986) grossly over-predicted DMI and will not be mentioned. The equation of Vazquez & Smith (2000) predicted the PDMI to be 7.2 kg/cow/d which appears to be an underestimation. The NRC (2001) equation predicted the PDMI to be 11.1 kg/cow/d which appears to be an overestimation. The equation of Bargo *et al.* (2003a) was not used as the PA was lower than 12.1 kg DM/cow/d. (See section 2.6.3.3 for these equations.)

So the question is: which value is correct? The RPM predicted 7.6 kg, the NDF as % BW method predicted 8.6 kg and the energy balance method predicted 9.8 kg. The latter appears to be an overestimate as it would be highly unlikely that the cows would have utilised as much as 88 % of the 11.1 kg pasture DM allocated/cow/d. A safe estimate would be to use the middle value of 8.6 kg. This value was used in subsequent sections as the assumed pasture intake for calculating the nutrient composition of the total diet. A precise estimate of pasture intake will not affect the discussion of the outcome of the experiment as it was assumed that all the cows had the same average pasture intake and the only difference in their diets was the concentrate fed.

d) Estimation of pasture intake using the CPM Dairy model

The CPM Dairy model (see chapter 4) predicted the DMI of the cows on the control, low FM and high FM treatments to be 12.6, 14.1 and 13.8 kg DM/cow/d, hence pasture intake of the cows on the three treatments would have been 7.1, 8.6 and 8.3 kg DM/cow/d, respectively. The average of these three is 8.0, slightly lower than calculated in section 3.2.1.1.1 c). If the pasture intake is adjusted so that ME allowable milk production is equal to the actual milk production observed then the pasture intake of the cows on the three treatments would have been 7.2, 9.3 and

9.1 kg DM/cow/d, respectively, averaging 8.5 kg for all three groups, close to that calculated in section 3.2.1.1.1 c).

3.2.1.1.2 Pasture composition

The chemical composition of the ryegrass pasture and how it changed over time is reported in Table 3.5. For a more extensive analyses the samples of every three weeks were composited (Table 3.6). Figure 3.7 presents the changes in CP, NDF and IVOMD over time.

Table 3.5 Chemical composition on a weekly basis of the ryegrass pasture grazed during the trial

Parameter	Sampling date								
	05/09	**12/09**	**19/09**	**26/09**	**03/10**	**10/10**	**17/10**	**24/10**	**31/10**
DM %	11.3	10.4	12.0	10.7	13.2	13.7	13.1	21.4	17.7
OM (%DM)	84.9	85.7	85.9	84.4	87.1	87.0	87.8	87.7	88.7
CP (%DM)	26.6	24.3	33.5	27.0	22.7	25.3	26.9	26.6	22.6
NDF (%DM)	43.2	46.6	41.9	43.2	48.8	46.1	46.4	48.3	52.2
ADF (%DM)	24.0	27.3	23.6	25.7	28.0	25.2	25.1	24.7	26.6
IVOMD (%DM)	85.4	82.8	82.4	82.4	80.2	77.3	78.5	74.8	77.8

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility

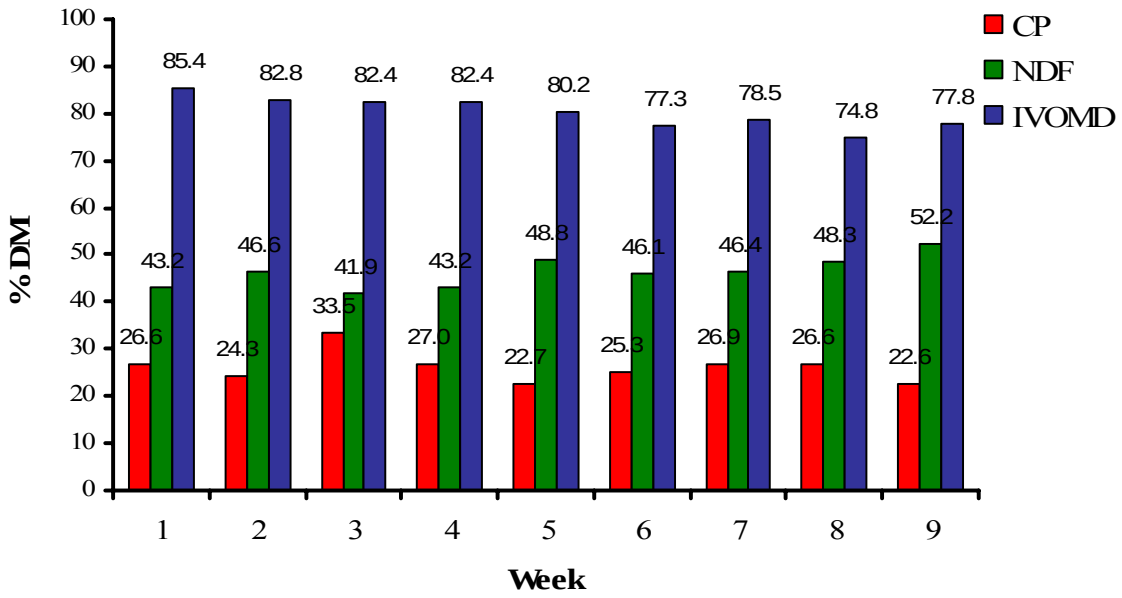

Figure 3.7 Crude protein (CP), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD) on a weekly basis of the ryegrass pasture grazed during the trial. Week 1 = 5 September 2005, week 9 = 31 October 2005

Table 3.6 Chemical composition on a three-weekly basis of the ryegrass pasture grazed during the trial

Parameter	Sampling dates		
	05/09 – 19/09	**26/09 – 10/10**	**17/10 – 31/10**
DM %[1]	11.3	12.5	17.4
Ash (%DM)	14.5	13.0	12.0
OM (%DM)[2]	85.5	87.0	88.0
CP (%DM)	28.1	25.4	25.9
NDF (%DM)	43.1	44.5	48.0
ADF (%DM)	25.2	27.5	25.4
IVOMD (%DM)[1]	83.6	80.0	77.1
GE (MJ/kg DM)	17.2	17.0	17.3
ME (MJ/kgDM[3]	11.7	11.2	10.9
EE (%DM)	3.4	3.6	2.7
Ca (%DM)	0.44	0.49	0.64
P (%DM)	0.42	0.38	0.43
Ca: P	1.06	1.28	1.49
Lignin (%NDF)	10.3	7.2	6.9
NFC (% DM)[4]	10.1	11.6	11.0
Starch (%DM)	0.0	0.3	0.3
NDIP (%CP)	23.0	21.3	28.3
ADIP (%CP)	4.1	5.0	5.8
Sol CP (%CP)	45.0	42.3	40.6
NPN (%Sol CP)	48.8	48.7	43.9

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility; GE – Gross energy; ME – Metabolisable energy; EE – Ether extract; NFC – Non-fibre carbohydrates; NDIP – Neutral detergent insoluble protein; ADIP – Acid detergent insoluble protein; Sol CP – Soluble CP; NPN – Non-protein N

[1] Average for the three weeks was calculated as analysis on the composite sample was not done

[2] OM = 100 - ash

[3] ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

[4] NFC = 100 – (CP + NDF + EE + ash)

Table 3.7 Essential amino acid (AA) composition of the ryegrass pasture grazed during the trial (n = 3)

AA	Mean (g/100 g AA) ± SD
Met	0.64 ± 0.144
Lys	6.35 ± 0.263
Arg	5.27 ± 0.175
Thr	4.84 ± 0.116
Leu	9.56 ± 0.276
Ile	5.26 ± 0.107
Val	6.73 ± 0.137
His	2.02 ± 0.057
Phe	6.23 ± 0.106
Total EAA[1]	46.9
Total NEAA[2]	53.1

[1]Essential AA (EAA): Met, Lys, Arg, Thr, Leu, Ile, Val, His and Phe (Jones-Endsley *et al.*, 1997)

[2]Nonessential AA (NEAA): Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr (Jones-Endsley *et al.*, 1997)

The mean composition of EAA in the ryegrass pasture, expressed as g/100 g AA, is reported in Table 3.7. The Lys and Met concentrations in the ryegrass pasture DM were 0.95 and 0.10 % DM, respectively.

3.2.1.2 Concentrate composition

Table 3.8 Chemical composition of the control, low FM and high FM concentrate pellets fed in the ryegrass trial (n = 1)

Parameter	Experimental treatment[1]		
	Control	Low FM	High FM
DM %	91.9	91.5	91.4
Ash (%DM)	6.8	8.8	9.9
OM (%DM)	93.2	91.2	90.4
CP (%DM)	8.2	11.2	14.6
NDF (%DM)	11.2	11.8	12.7
ADF (%DM)	3.7	3.7	4.0
IVOMD (%DM)	92.4	95.1	91.3
GE (MJ/kg DM)	17.0	17.0	17.3
ME (MJ/kg DM)[2]	12.9	13.3	12.9
EE (%DM)	1.7	2.3	3.0
Ca (%DM)	1.55	2.03	2.30
P (%DM)	0.56	0.75	0.87
Ca: P	2.77	2.71	2.64
Lignin (%NDF)	9.7	10.1	17.9
NFC (% DM)[3]	72.1	65.9	59.8
Starch (%DM)	57.7	54.6	48.3
NDIP (%CP)	12.4	23.6	29.7
ADIP (%CP)	23.8	21.3	17.6
Sol CP (%CP)	29.1	34.6	31.2
NPN (%Sol CP)	37.3	33.1	18.7

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility; GE – Gross energy; ME – Metabolisable energy; EE – Ether extract; NFC – Non-fibre carbohydrates; NDIP – Neutral detergent insoluble protein; ADIP – Acid detergent insoluble protein; Sol CP – Soluble CP; NPN – Non-protein N

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM

[2]ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

[3]NFC = 100 – (CP + NDF + EE + ash)

The CP of the control, low FM and high FM concentrates were 8.2, 11.2 and 14.6 % DM respectively (Table 3.8), as the CP concentration of the FM was 70.3 % DM (Table 3.9).

Table 3.9 Chemical composition of the fishmeal used in the concentrate pellets for the ryegrass trial (n = 1)

Parameter	Percentage
DM (%)	90.8
Ash (% DM)	22.0
OM (% DM)	78.0
CP (% DM)	70.3
EE (% DM)	8.5

DM – Dry matter; OM – Organic matter; CP – Crude protein; EE – Ether extract

Table 3.10 reports the EAA composition of the three concentrates expressed as g/100 g of AA. The Lys concentration in the concentrate DM was 0.22, 0.41 and 0.52 % DM and the Met concentration 0.04, 0.14 and 0.30 % DM for the control, low FM and high FM treatments, respectively. The increased levels of these two AAs with increasing FM levels in the concentrate is to be expected since the Lys and Met concentration of the FM that was used was 4.78 and 1.54 % DM, respectively.

Table 3.10 Essential amino acid (AA) composition of the control, low FM and high FM concentrate pellets fed in the ryegrass trial (n = 1)

AA (g/100 g AA)	Experimental treatment[1]		
	Control	Low FM	High FM
Met	0.69	1.84	3.03
Lys	3.79	5.67	5.21
Arg	5.34	6.06	6.66
Thr	3.97	4.61	4.60
Leu	10.86	10.94	8.72
Ile	3.79	4.48	4.36
Val	5.52	5.67	5.81
His	2.76	2.64	2.78
Phe	5.17	4.87	4.60
Total EAA[2]	41.9	46.8	45.8
Total NEAA[3]	58.1	53.2	54.2

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Essential AA (EAA): Met, Lys, Arg, Thr, Leu, Ile, Val, His and Phe (Jones-Endsley *et al.*, 1997)
[3]Nonessential AA (NEAA): Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr (Jones-Endsley *et al.*, 1997)

3.2.1.3 Total diet composition

The cows each consumed 5.5 kg concentrate DM/d. If it is assumed that the ryegrass pasture intake was 8.6 kg/cow/d (section 3.2.1.1.1), the total diet composition would be as partly shown in Table 3.3.

The total diet of a cow consuming 8.6 kg ryegrass DM with AA composition as in Table 3.7, and 5.5 kg concentrate with AA composition as in Table 3.10, would contain 0.67 % Lys and 0.07 % Met (94 g Lys and 10 g Met/d) for the control treatment, 0.74 % Lys and 0.11 % Met (105 g Lys and 16 g Met/d) for the low FM treatment and 0.78 % Lys and 0.18 % Met (111 g Lys and 25 g Met/d) for the high FM treatment. Both Lys and Met increased as the level of FM in the concentrate increased, especially Met as the ratio of Lys to Met in the total diet was 9.0, 6.7 and 4.4 for the control, low FM and high FM treatments, respectively. The high FM treatment comes closest to the ideal Lys: Met ratio of 3.0: 1 (NRC, 2001).

3.2.1.4 Milk production and composition

3.2.1.4.1 Mean for the whole experimental period

a) Milk yield

The mean milk yield of the 15 cows on each treatment is shown on a daily basis in Figure 3.8 with the mean for the whole experimental period (20 September to 4 November 2005) being reported in Table 3.11.

The mean milk yields of the cows on the control, low FM and high FM treatments were 20.5, 21.9 and 22.1 kg milk/cow/d, respectively. Thus the milk production of the cows in the control group dropped by 1.0 kg and that of the low and high FM groups went up by 0.5 and 0.7 kg from initial values 21.5 of 21.4 and 21.4 kg/cow/d, respectively.

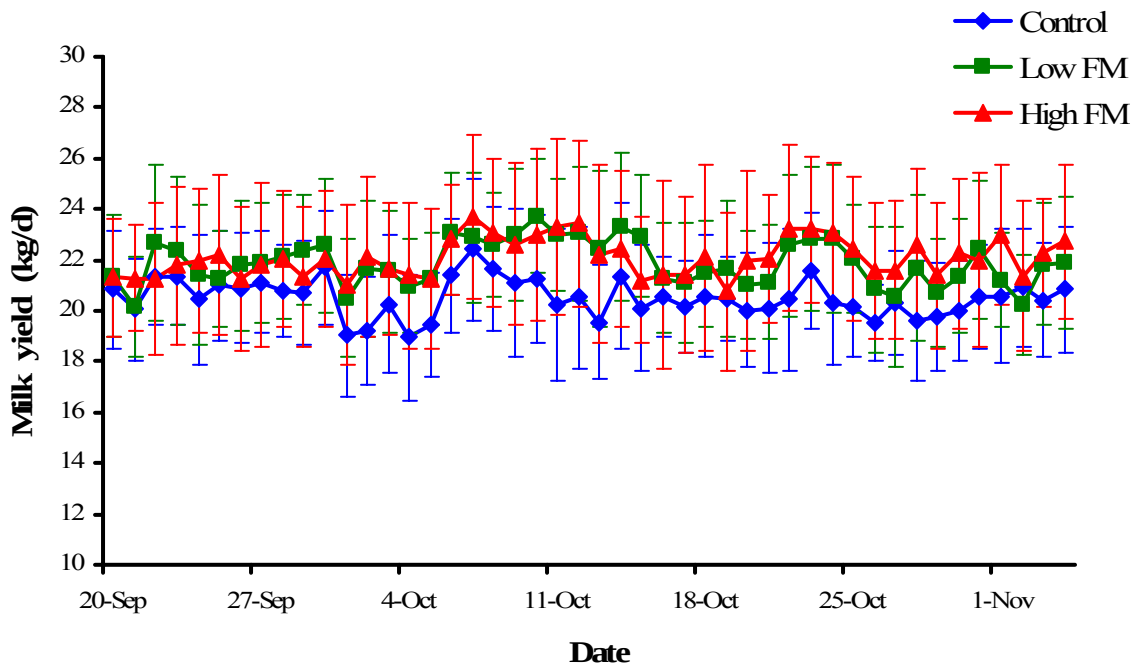

Figure 3.8 Mean daily milk yield of Jersey cows grazing ryegrass and receiving 5.5 kg DM/cow/d of supplement containing either no fishmeal (FM; Control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 15

The mean milk yields of the cows on the low and high FM treatments were 7 and 8 % higher than the mean milk yield of the cows on the control treatment ($P < 0.01$), while the milk yields of the cows on the low FM and high FM treatments did not differ from each other ($P > 0.1$).

Table 3.11 Effect of fishmeal (FM) supplementation on mean milk yield (kg/d) of cows grazing ryegrass (n = 15)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk yield (kg/d)	20.5[a]	21.9[b]	22.1[b]	0.34

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b] Means in the same row with different superscripts differ ($P < 0.01$)

b) Milk composition

The mean milk fat percentages of the cows on the low and high FM treatments (4.73 and 4.67 %, respectively), were higher than the mean milk fat percentage of the cows on the control treatment (3.97 %; $P < 0.01$). There was no difference in milk fat percentage between the cows on the two FM treatments ($P > 0.1$; Table 3.12).

Table 3.12 Effect of fishmeal (FM) supplementation on mean milk composition of cows grazing ryegrass (n = 15)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Fat (%)	3.97[a]	4.73[b]	4.67[b]	0.132
Protein (%)	3.25[a]	3.49[b]	3.45[b]	0.051
Lactose (%)	4.59[a]	4.78[b]	4.79[b]	0.019
Milk urea N (mg/dl)	16.80	17.43	17.93	0.440

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.01$)

The mean milk protein percentages of the cows on the low and high FM treatments (3.49 and 3.45 %, respectively), were higher than the mean milk protein percentage of the cows on the control treatment (3.25 %; $P < 0.01$). There was no difference in milk protein % between cows on the two FM treatments ($P > 0.1$).

The fat and protein yields (calculated from the milk yield and fat and protein percentages) were 0.81 and 0.67 kg/d for the control and 1.03 and 0.76 kg/d for the two FM treatments (Table 3.31). The fat and protein yields of the two FM treatments were higher than the control ($P < 0.01$).

The mean lactose percentages of the cows on the low and high FM treatments (4.78 and 4.79%, respectively) were higher than the mean milk lactose percentage of the cows on the control treatment (4.59 %; $P < 0.01$). There was no difference between the two FM treatments in terms of lactose % in the milk ($P > 0.1$).

The MUN values of the cows on the control, low FM and high FM treatments (16.8, 17.43 and 17.93 mg/dl, respectively) did not differ from each other ($P > 0.1$).

c) Covariate adjusted milk composition

In order to ensure that the difference in milk composition was due to treatment effects and not due to the natural variation between the cows, the initial milk composition was used as a covariate if there was a covariate effect. Table 3.13 shows the milk composition of the cows during the last milk recording of the whole herd before the trial started (29 August 2005). There was no difference in these initial values between the three experimental treatments for any of the parameters ($P > 0.1$). There was no covariate effect for milk fat, protein and lactose percentages ($P > 0.1$). However, the initial MUN values did influence the final MUN values as covariates ($P < 0.05$) so these initial values were used as covariates. The covariate adjusted MUN values are reported in Table 3.14. The three treatments still did not differ from each other ($P > 0.1$).

Table 3.13 Mean milk composition of the experimental cows at the time of the last milk recording before the ryegrass trial started (n = 15)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Fat (%)	4.73	4.96	4.94	0.183
Protein (%)	3.44	3.57	3.46	0.077
Lactose (%)	4.74	4.74	4.79	0.035
Milk urea N (mg/dl)	13.39	12.39	14.51	0.874

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

Table 3.14 Effect of fishmeal (FM) supplementation on covariate adjusted milk urea N of cows grazing ryegrass (n = 15)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk urea N (mg/dl)	16.81	17.70	17.64	0.389

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

d) Fat- and energy-corrected milk yield

The cows on the two FM treatments produced 18 and 19 % more 4 % FCM (24.1 and 24.2 kg/d) than the cows on the control treatment (20.4 kg/d; $P < 0.01$). The 4 % FCM of the cows on the two FM treatments did not differ from each other ($P > 0.1$; Table 3.15).

The cows on the two FM treatments produced 18 % more ECM (both 25.7 kg/d) than the cows on the control treatment (21.8 kg/d; P < 0.01; Table 3.15).

Table 3.15 Effect of fishmeal (FM) supplementation on mean 4% fat-corrected milk (FCM) yield and energy-corrected milk (ECM) yield (kg/d) of cows grazing ryegrass (n = 15)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
4 % FCM (kg/d)[3]	20.4[a]	24.1[b]	24.2[b]	0.470
ECM (kg/d)[4]	21.8 [a]	25.7 [b]	25.7 [b]	0.483

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[3]4% FCM (kg) = 0.4 × kg of milk + 15 × kg of milk fat (Erasmus *et al.*, 2000; NRC, 2001)
[4]ECM (kg) = 0.3246 × kg of milk + 12.86 × kg of milk fat + 7.04 × kg of protein (Gehman *et al.*, 2006)
[a,b]Means in the same row with different superscripts differ (P < 0.01)

3.2.1.4.2 Milk production and composition of four sub-experimental periods

The experimental period was divided into four sub-periods: period 1: the first 12 days of the trial (milk production from 18 to 29 September 2005 and milk composition from the milk recording done on 21 September 2005); period 2: the second 12 days of the trial (milk production from 30 September to 11 October and composition on 5 October 2005); period 3: third 12 days of the trial (milk production of 12 to 23 October and composition on 19 October 2005); and period 4: the last 12 days of the trial (milk production from 24 October to 4 November and composition on 2 November 2005).

There was no difference in milk production between the three experimental treatments in the first period (P > 0.1; Table 3.16). Thereafter the milk production of the cows on the two FM treatments were higher than the control (P < 0.01) while the two FM treatments did not differ from each other (P > 0.1).

There tended to be an effect of period on the overall mean milk production (P = 0.06): it tended to increase from periods 1 to 2 (P = 0.06) and decrease from periods 3 to 4 (P = 0.08). There was also a period × treatment interaction between the first and second periods (P < 0.01).

Table 3.16 Effect of time and fishmeal (FM) supplementation on mean milk yield (kg/d) of cows grazing ryegrass (n = 15)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	21.0	21.8	21.6	0.35
2	20.6[a]	22.2[b]	22.3[b]	0.41
3	20.5[a]	22.1[b]	22.1[b]	0.37
4	20.2[a]	21.5[b]	22.2[b]	0.36

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.01)

In the first, third and fourth periods the fat percentages in the milk of the cows on the two FM treatments were higher than the control treatment (P < 0.01 for periods 1 and 3; P < 0.05 for period 4) not differing between the two FM treatments (P > 0.1; Table 3.17). In the second period there was no difference in milk fat concentration between any of the three treatment groups although the cows on the low FM treatment tended to have more fat in their milk than the cows on the control treatment (P = 0.07).

There was no effect of period on overall mean milk fat percentage (P > 0.1). There was, however, a period × treatment interaction between the first and second period (P < 0.05).

Table 3.17 Effect of time and fishmeal (FM) supplementation on mean milk fat percentage of cows grazing ryegrass

Period[1]	Experimental treatment[2]			SEM[5]
	Control[3]	Low FM[4]	High FM[3]	
1	3.87[a]	4.64[b]	4.83[b]	0.142
2	4.08	4.59	4.33	0.190
3	3.89[a]	4.83[b]	4.57[b]	0.145
4	4.01[a]	4.86[b]	4.55[b]	0.157

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]n = 14, [4]n = 15
[5]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

In the first and fourth periods the milk protein percentage of the cows on the two FM treatments was higher than the control treatment (P < 0.01) and did not differ between the two FM treatments (P > 0.1; Table 3.18). In the second period the milk protein percentage of the

cows on the low FM treatment was higher than the cows on the control ($P < 0.01$). The milk protein percentage of cows on the high FM treatment tended to be higher than the control treatment ($P = 0.06$) and the low FM tended to be higher than the high FM treatment ($P = 0.09$). In the third period the milk protein percentage of the cows on the low FM treatment was higher than the control ($P < 0.05$) with the high FM treatment not differing from the other two ($P > 0.1$).

There was an effect of period on overall mean milk protein percentage ($P < 0.01$): it increased between the first and second period and decreased between the second and third period ($P < 0.01$). There tended to be a period × treatment interaction between all the periods ($P < 0.1$).

Table 3.18 Effect of time and fishmeal (FM) supplementation on milk protein percentage of cows grazing ryegrass

Period[1]	Experimental treatment[2]			SEM[5]
	Control[3]	Low FM[4]	High FM[3]	
1	3.09[a]	3.39[b]	3.31[b]	0.052
2	3.34[a]	3.61[b]	3.48[ab]	0.052
3	3.30[a]	3.49[b]	3.40[ab]	0.059
4	3.20[a]	3.48[b]	3.42[b]	0.050

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]n = 14, [4]n = 15
[5]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.05$)

A graphic illustration of the effects of FM supplementation over time on milk production, milk fat percentage and milk protein percentage is shown if Figure 3.9.

In all four periods the milk lactose percentage of the cows on the two FM treatments was higher than the control treatment ($P < 0.01$) and did not differ between the two FM treatments ($P > 0.1$; Table 3.19). There was an effect of period on overall mean milk lactose percentage ($P < 0.01$): it increased between the first and second period ($P < 0.01$), tended to decrease between the second and third period ($P = 0.09$) and increased between the third and fourth period ($P < 0.01$). There was a period × treatment interaction between the third and fourth period ($P < 0.5$).

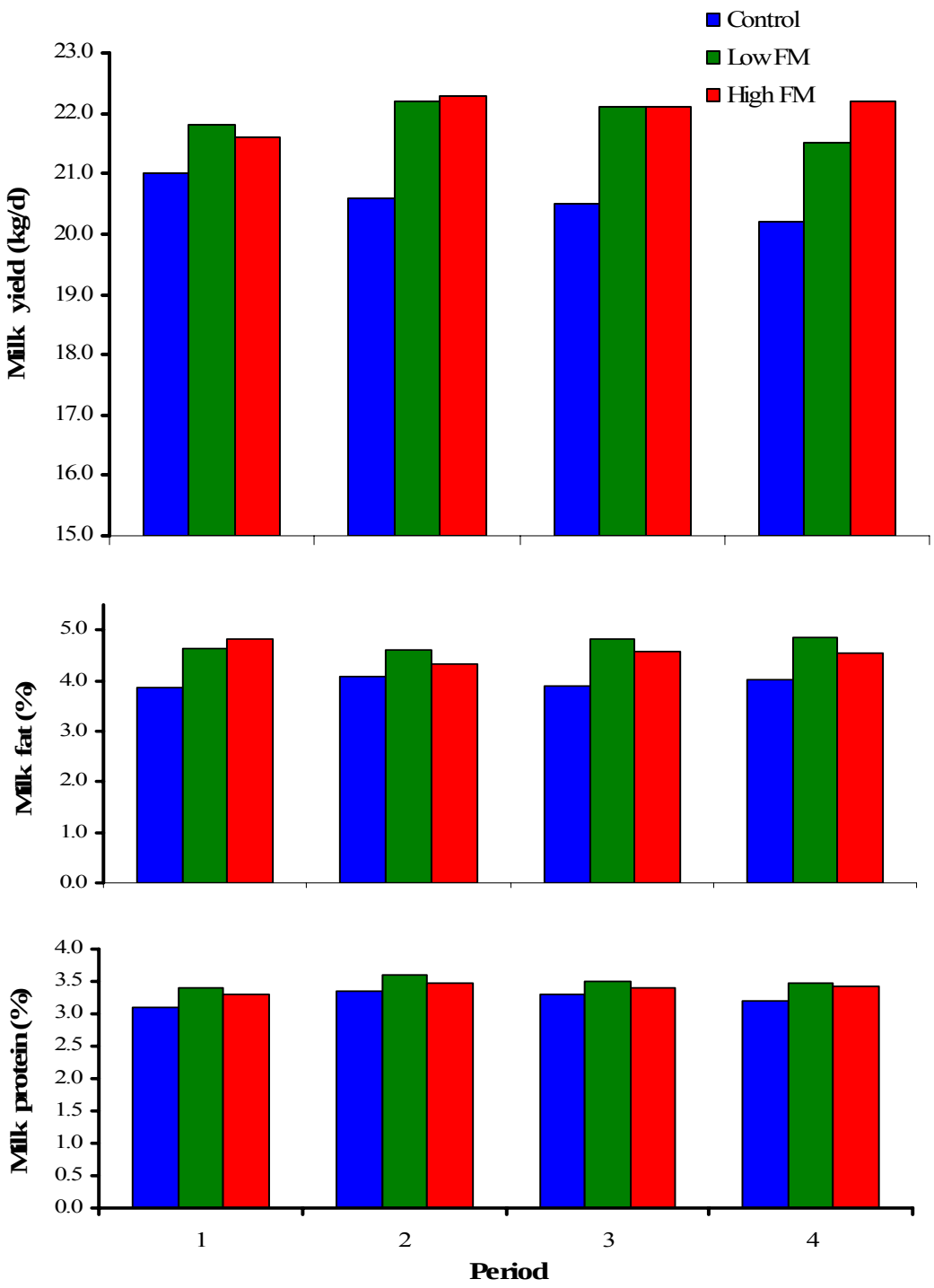

Figure 3.9 The effect of time and fishmeal (FM) supplementation on mean milk yield (kg/cow/d) and milk fat and protein percentage of cows grazing ryegrass and receiving 5.5 kg DM/cow/d of supplement containing either no FM (Control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment). Period 1 = 18 to 29 September, period 2 = 30 September to 11 October, period 3 = 12 to 23 October and period 4 = 24 October to 4 November 2005

Table 3.19 Effect of time and fishmeal (FM) supplementation on mean milk lactose percentage of cows grazing ryegrass

Period[1]	Experimental treatment[2]			SEM[5]
	Control[3]	Low FM[4]	High FM[3]	
1	4.49[a]	4.75[b]	4.72[b]	0.024
2	4.62[a]	4.79[b]	4.79[b]	0.032
3	4.61[a]	4.74[b]	4.77[b]	0.027
4	4.60[a]	4.84[b]	4.87[b]	0.028

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]n = 14, [4]n = 15
[5]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.01)

The MUN concentration of the milk did not differ between any of the three experimental treatments (P > 0.1) in any of the periods (Table 3.20). There was an effect of period on overall mean MUN concentration (P < 0.01): it decreased between the first and second period, increased between the second and third period and decreased between the third and fourth period (P < 0.01). There was no period × treatment interaction (P > 0.1). The overall mean MUN concentration differed greatly between periods varying from 14.54 mg/dl in the second period to 20.52 mg/dl in the third period, with no consistent trend over time.

Table 3.20 Effect of time and fishmeal (FM) supplementation on mean milk urea N (mg/dl) of cows grazing ryegrass

Period[1]	Experimental treatment[2]			SEM[5]
	Control[3]	Low FM[4]	High FM[3]	
1	18.14	18.41	18.24	0.477
2	14.22	14.74	14.67	0.432
3	19.86	20.60	21.09	0.586
4	15.39	15.96	16.26	0.421

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]n = 14, [4]n = 15
[5]Standard error of mean

To summarise: there was only a milk response to FM supplementation from the second period onwards, after the cows had been on the experimental treatments for three weeks. There was no consistent effect of period on milk fat and protein concentrations, indicating the importance of obtaining an average of several milk samples as there is variation between

different milk recording sessions. There was a consistent response in lactose concentrations of the milk in all four periods and there was consistently no difference between the three treatments for MUN.

The variations in overall mean milk production and composition over time emphasise the importance of taking many measurements over a long period of time in order to obtain accurate mean values.

3.2.1.4.3 Milk production and composition of early and mid lactation cows

Positive responses to RUP supplementation are most likely in early lactation cows (Muller & Fales, 1998; McCormick *et al.*, 1999; Schor & Gagliostro, 2001). In order to investigate whether the cows in early lactation responded more to FM supplementation than the cows in mid lactation, the results of the cows that were less than 70 days into lactation (early lactation) were separated from those of the cows that were between 70 and 120 days into lactation (mid lactation) at the beginning of the trial.

Table 3.21 shows the mean milk production and milk composition of the cows that were less than 70 days into lactation at the beginning of the trial (blocks 3, 5, 8, 9, 12 and 15). Despite a large numerical difference in milk production (20.6, 21.7 and 22.8 kg/d), there was not a difference in milk production between the treatments ($P > 0.1$) due to insufficient degrees of freedom. Similarly there was no significant difference in milk fat (4.08, 4.70 and 4.47 %), protein (3.25, 3.50 and 3.35 %,) and MUN (16.85, 17.69 and 17.69 mg/dl) concentrations in the milk, between the three treatments ($P > 0.1$). The lactose percentages in the milk of the cows on the two FM treatments (4.77 and 4.78 %) were higher than that of the control treatment (4.64 %; $P < 0.01$) while not differing between the two FM treatments ($P > 0.1$).

Table 3.22 shows the mean milk production and composition of the cows that were more than 70 days into lactation (blocks 2, 4, 6, 7, 13, 14 and 16). The milk production (20.2, 21.8 and 21.7 kg/d) and milk fat (3.92, 4.73 and 4.73 %), protein (3.25, 3.47 and 3.53 %) and lactose (4.57, 4.78 and 4.81 %) percentages in the milk of the cows on the two FM treatments were higher than the control treatment ($P < 0.05$) but did not differ between the two FM treatments ($P > 0.1$). The MUN concentration of the milk (16.98, 17.19 and 18.22 mg/dl) did not differ between the three treatments ($P > 0.1$).

Table 3.21 Effect of fishmeal (FM) supplementation on mean milk yield and composition of early lactation cows grazing ryegrass (n = 6)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk (kg/d)	20.6	21.7	22.8	0.67
Fat (%)	4.08	4.70	4.47	0.236
Protein (%)	3.25	3.50	3.35	0.092
Lactose (%)	4.64[a]	4.77[b]	4.77[b]	0.027
Milk urea N (mg/dl)	16.85	17.69	17.69	0.898

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

Table 3.22 Effect of fishmeal (FM) supplementation on mean milk yield and composition of mid lactation cows grazing ryegrass (n = 7)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk (kg/d)	20.2[a]	21.8[b]	21.7[b]	0.40
Fat (%)	3.92[a]	4.73[b]	4.73[b]	0.196
Protein (%)	3.25[a]	3.47[b]	3.53[b]	0.074
Lactose (%)	4.57[a]	4.78[b]	4.81[b]	0.026
MUN (mg/dl)	16.98	17.19	18.22	0.574

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

3.2.1.5 Body weight and body condition score

Table 3.23 summarises the mean BW and BCS of the cows on the three treatments at the beginning and end of the trial. There was no difference between the three treatments in change in BW or BW before or after (P > 0.1). There was also no difference between treatments in BCS before and after, although the cows on the control treatment did put on more condition than those on the high FM treatment (P < 0.05).

Table 3.23 Effect of fishmeal (FM) supplementation on body weight (BW) and body condition score (BCS)[1] of cows grazing ryegrass (n = 15)

Parameter	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
BW (kg)				
Beginning	327	338	327	6.2
End	371	387	369	7.6
Change	44	49	42	3.7
BCS				
Beginning	2.1	2.1	2.2	0.06
End	2.5	2.3	2.4	0.07
Change	0.4[a]	0.2[ab]	0.2[b]	0.06

[1]Five-point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982)
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same column with different superscripts differ (P < 0.05)

3.2.1.6 Faeces

Table 3.24 shows the mean starch concentration in the faeces of the three cows on each experimental treatment. There was no difference in the starch concentration of the faeces of the cows on the three experimental treatments (P > 0.1). The mean starch concentrations (% DM) in the faeces of the cows on the control, low FM and high FM treatments were 1.05, 0.93 and 0.53 %, respectively. The starch concentrations in the total diets consumed by these animals were 23.78, 22.51 and 20.04 %, respectively.

Table 3.24 Effect of fishmeal (FM) supplementation on starch concentration in the faeces of cows grazing ryegrass (n = 3)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Starch in faeces (% DM)	1.05	0.93	0.53	0.299

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

3.2.2 Rumen study

3.2.2.1 Ruminal pH

3.2.2.1.1 Results from data loggers

The data loggers recorded the ruminal pH every 10 minutes for a total of four days per cow. The mean pH for every half hour was calculated. The pH at 0800 h is the mean of the three readings between 0800 and 0829 h; the same principle applies to all the times. The mean for the four days was calculated.

The mean of all eight cows on each treatment was calculated and is shown in Figure 3.10. Each point on this graph is the mean of 96 readings. The SD (n = 8) is shown with the bars on this graph.

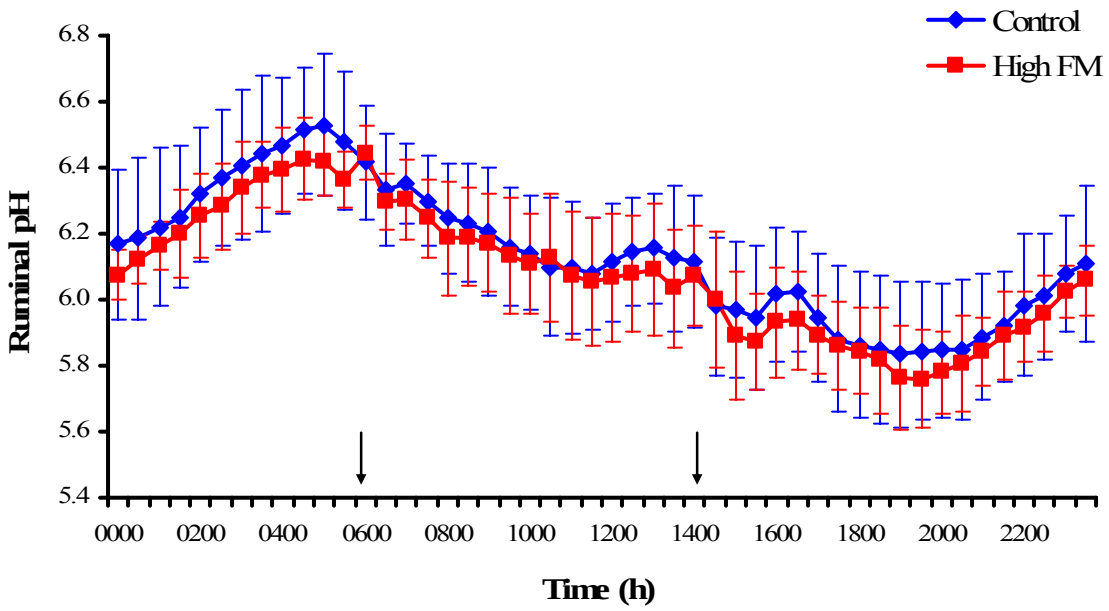

Figure 3.10 Ruminal pH of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean pH per four hour period was calculated to get a mean ruminal pH for each cow for the following times: 0000, 0400, 0800, 1200, 1600 and 2000 h. The value for 0800 h is the

mean of the values from 0600 to 0930 h and so on for all six times. These values were analysed with Proc GLM Repeated Measures Analysis of Variance (Statistical Analysis Systems, 2001; see section 3.1.3.3).

The mean for all eight cows on each treatment is reported in Table 3.25 for the six times of the day. The ruminal pH did not differ between treatments ($P > 0.1$) for any of the times except 0400 h where the ruminal pH of the cows on the control treatment tended to be higher than the cows on the high FM treatment ($P = 0.07$).

The mean daily ruminal pH of the cows on the control and high FM treatments were 6.14 and 6.08, respectively, and did not differ from each other ($P > 0.1$).

Table 3.25 Effect of time of day and fishmeal (FM) supplementation on mean ruminal pH of cows grazing ryegrass (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	6.13	6.06	0.21	0.031
0400	6.44	6.36	0.07	0.028
0800	6.28	6.25	0.36	0.024
1200	6.12	6.08	0.40	0.032
1600	5.98	5.93	0.22	0.027
2000	5.86	5.81	0.19	0.023

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

These results show how the pH in the rumen changes throughout the day, being consistently the highest early in the morning (0400 h) and lowest in the evening (2000 h). The ruminal pH started dropping after the morning feeding and onset of grazing. It dropped even further after the afternoon feeding (Figure 3.10). There was an effect of time of day on ruminal pH ($P < 0.01$): the overall mean ruminal pH increased from 2000 to 0000 h and from 0000 to 0400 h and then decreased from 0400 to 0800 h and for all the times of day thereafter. There was no treatment × time interaction ($P > 0.1$).

3.2.2.1.2 Results from the manual recording of ruminal pH

Figure 3.11 shows the ruminal pH that was measured when the samples of rumen fluid were taken. Although these values were not used in the statistical analysis they do give a good

indication of whether the rumen samples were representative of the whole rumen fluid. The mean pH from the manual recording never deviated more than 5 % from the mean ruminal pH measured with the data loggers. Although not as refined, Figure 3.11 shows the same general trends in ruminal pH changes throughout the day as Figure 3.10.

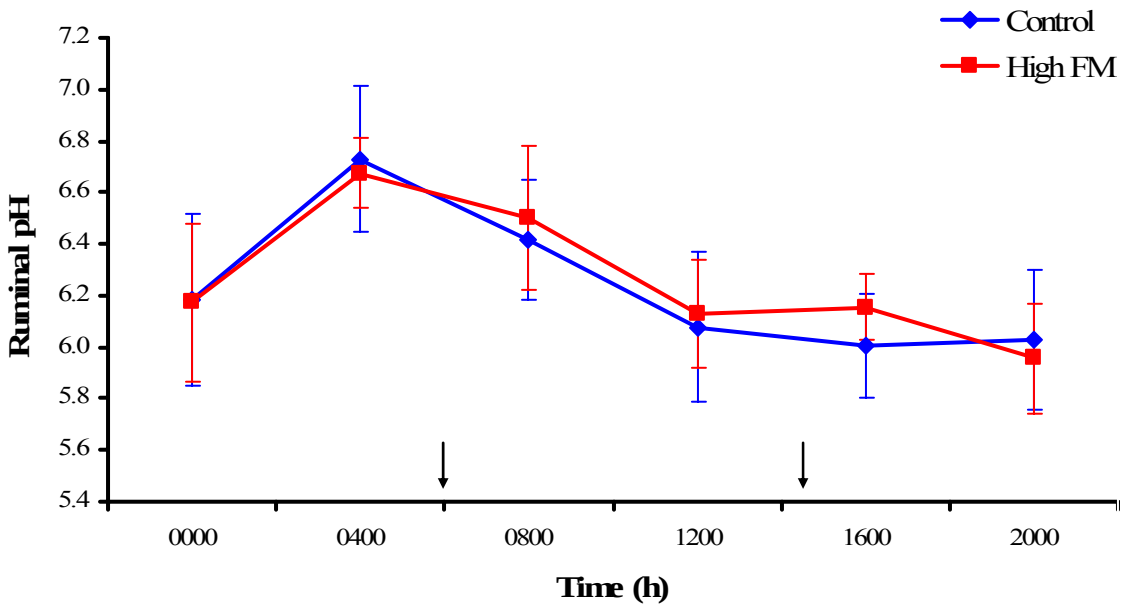

Figure 3.11 Ruminal pH, measured manually at the six sampling times, of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

3.2.2.2 Ruminal ammonia

The mean ruminal NH_3-N concentration (mg/dl) for the eight cows on each treatment was calculated for each of the six times of the day and is shown in Table 3.26 and Figure 3.12.

At 0000, 0800, 1600 and 2000 h the ruminal ammonia concentration of the cows on the two treatments did not differ from each other ($P > 0.1$). At 0400 and 1200 h the ruminal NH_3-N concentration was higher for the cows on the high FM treatment than the cows on the control treatment ($P < 0.01$).

Table 3.26 Effect of time of day and fishmeal (FM) supplementation on mean ruminal ammonia-N concentration (mg/dl) in the rumen fluid of cows grazing ryegrass (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	8.65	11.00	0.18	0.915
0400	5.93	7.46	<0.01	0.201
0800	14.31	14.09	0.90	0.971
1200	12.79	22.70	<0.01	1.210
1600	18.70	18.89	0.95	1.550
2000	24.54	25.86	0.62	1.527

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

The mean daily ruminal NH_3-N concentration of the cows on the control and high FM treatments were 14.16 and 16.67 mg/dl, respectively, higher for the high FM treatment than the control ($P < 0.05$).

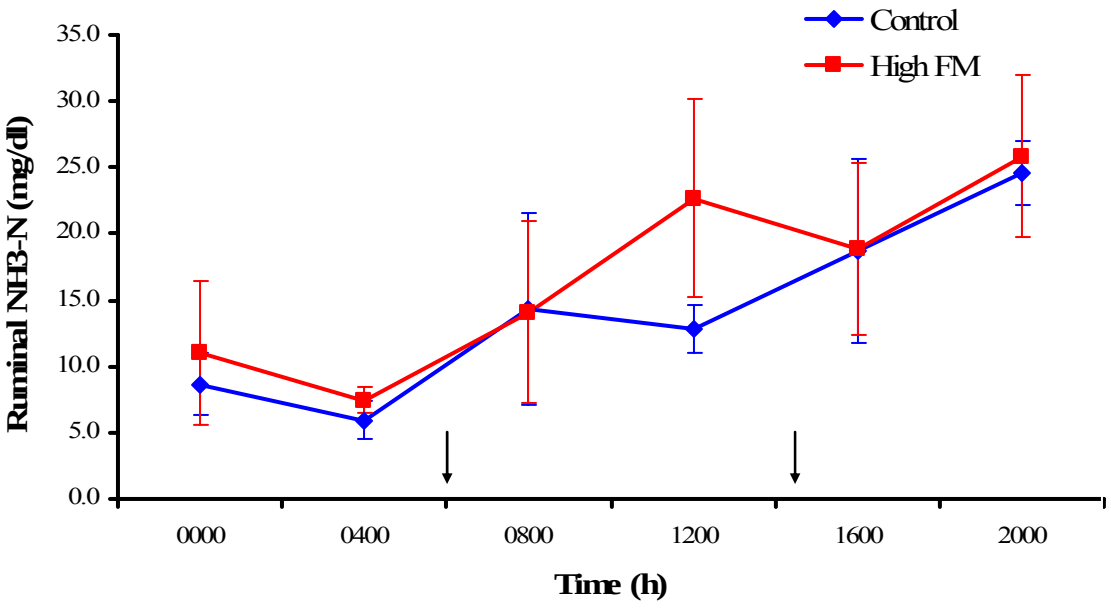

Figure 3.12 Ruminal concentration of ammonia-N (NH_3-N; mg/dl) of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

Time of day affected the mean ruminal NH_3-N concentration ($P < 0.01$). The overall mean NH_3-N concentration decreased from 2000 to 0000 h and from 0000 to 0400 h ($P < 0.01$), increased from 0400 to 0800 h ($P < 0.01$), tended to increase from 0800 to 1200 h ($P = 0.05$) and increased from 1600 to 2000 h ($P < 0.05$). The ruminal NH_3-N concentration was lowest at 0400 h after the night, when the NH_3 would have been absorbed from the rumen. It started rising again after the cows received fresh grazing after the morning milking at 0600 h, peaking at 2000 h.

There was a treatment × time interaction between 0800 and 1200 h ($P > 0.05$) and tended to be a treatment × time interaction between 1200 and 1600 h ($P > 0.1$), indicating that the daily variations in ruminal NH_3-N concentration differed between treatments.

3.2.2.3 Volatile fatty acids

The concentration of total ruminal VFA (mmol/L), including acetic, propionic, butyric, iso butyric and valeric acids, averaged for the eight cows on each treatment, was calculated for each of the six times of the day and is reported in Table 3.27 and shown in Figure 3.13.

At 0000, 0400 and 1200 h the total VFA concentration of the cows on the two treatments did not differ from each other ($P > 0.1$). At 0800 and 1600 h the total VFA concentration was higher for the cows on the control treatment than for the cows on the high FM treatment ($P = 0.01$) and at 2000 h it tended to be higher for the cows on the control treatment than the high FM treatment ($P = 0.05$).

Table 3.27 Effect of time of day and fishmeal (FM) supplementation on mean total volatile fatty acid (VFA) concentration (mmol/L) in the rumen fluid of cows grazing ryegrass (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	120.0	121.5	0.82	1.50
0400	103.5	99.7	0.66	1.81
0800	112.2	106.5	0.01	0.91
1200	122.9	120.9	0.72	0.37
1600	130.8	111.4	0.01	1.12
2000	140.9	131.8	0.05	0.83

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

The mean daily total VFA concentration in the rumen fluid of the cows on the control and high FM treatments were 123.4 and 115.3 mmol/L, respectively (Table 3.32), lower for the cows on the high FM treatment than the control ($P < 0.05$).

There was an effect of time of day on VFA concentration ($P < 0.01$). The mean concentration of total VFA decreased from 2000 to 0000 h and from 0000 to 0400 h ($P < 0.05$), increased from 0400 to 0800 h ($P < 0.05$), corresponding to the time of feeding, tended to increase from 0800 to 1200 h ($P = 0.09$) and increased from 1600 to 2000 h ($P < 0.05$) as the cows digested their food.

There was a treatment × time interaction between 1200 and 1600 h ($P < 0.05$) indicating that the daily variations in ruminal total VFA concentration differed among treatments.

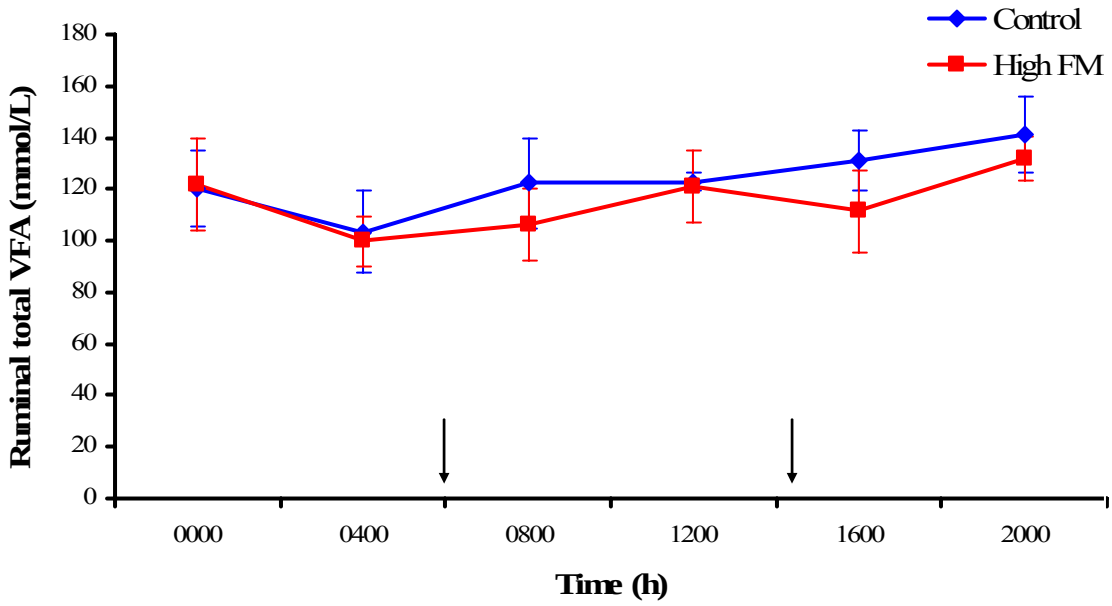

Figure 3.13 Ruminal concentration of total volatile fatty acids (VFA; mmol/L) of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean concentration of ruminal acetic acid (mmol/L) for the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 3.28 and shown in Figure 3.14. Table 3.28 also reports acetic acid as a proportion of total VFA.

Table 3.28 Effect of time of day and fishmeal (FM) supplementation on mean acetic acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing ryegrass (n = 8)

Time (h)	Acetic acid (mmol/L)				Acetic acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	80.3	80.7	0.92	0.89	67.0	66.5	0.49	0.52
0400	71.0	66.5	0.41	1.13	68.7	66.7	0.13	0.80
0800	81.0	69.9	<0.01	0.59	66.4	65.6	0.18	0.35
1200	79.6	78.1	0.69	0.77	64.8	64.6	0.77	0.56
1600	85.2	72.4	0.01	0.75	65.1	65.0	0.92	0.51
2000	92.0	84.8	0.05	0.67	65.3	64.3	0.21	0.52

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

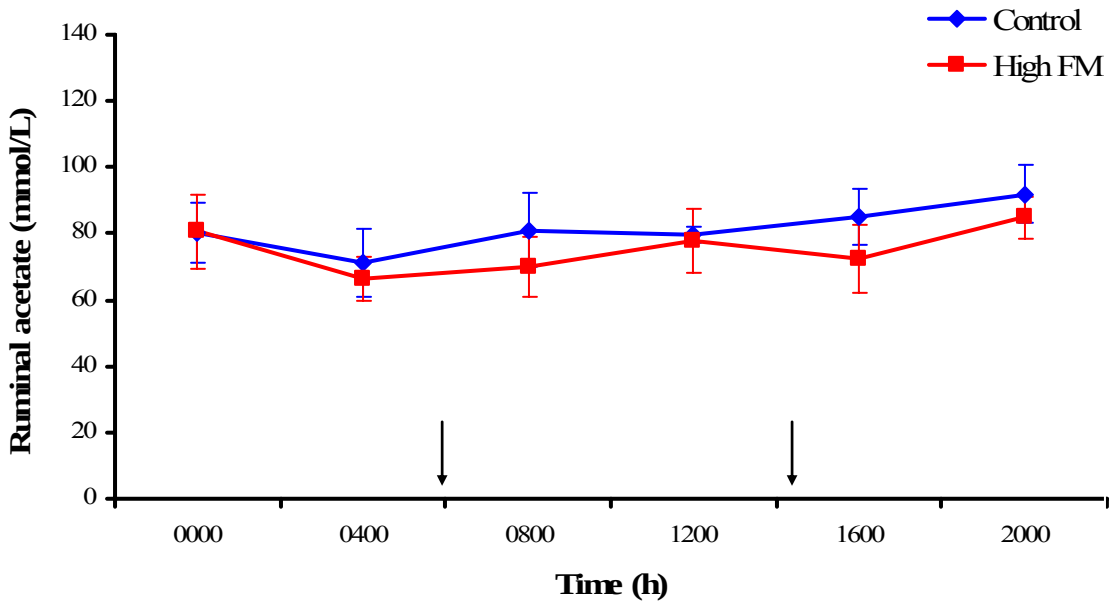

Figure 3.14 Ruminal concentration of acetic acid (mmol/L) of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The acetic acid concentration of the cows on the two treatments did not differ from each other at 0000, 0400 and 1200 h (P > 0.1). At 0800 and 1600 h the acetic acid concentration was higher for the cows on the control treatment than for the cows on the high FM treatment (P < 0.01) and at 2000 h it tended to be higher for the cows on the control treatment than the high FM

treatment (P = 0.05). The molar proportion of acetic acid did not differ between treatments (P < 0.1).

The mean daily acetic acid concentrations in the rumen fluid of the cows on the control and high FM treatments were 81.5 and 75.4 mmol/L, lower for the cows on the high FM treatment than the control (P < 0.05). The molar proportions were 66.1 and 65.4 mol/100 mol VFA for the control and high FM treatments respectively (Table 3.32), not differing between treatments (P > 0.1).

The mean concentration of ruminal propionic acid (mmol/L) for the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 3.29 and shown in Figure 3.15. Table 3.29 also reports propionic acid as a proportion of total VFA.

The propionic acid concentrations of the cows on the two treatments did not differ from each other at 0000, 0400 and 1200 h (P > 0.1) and was higher for the cows on the control treatment than for the cows on the high FM treatment at 0800, 1600 and 2000 h (P < 0.01). There was no treatment effect on the molar proportions of propionic acid (P > 0.1).

The mean daily concentrations of propionic acid in the rumen fluid of cows on the control and high FM treatments were 22.8 and 21.3 mmol/L, respectively. It tended to be lower for high FM treatment than the control (P = 0.09). The molar proportion of propionic acid was 18.5 mol/100 mol VFA in both treatments.

Table 3.29 Effect of time of day and fishmeal (FM) supplementation on mean propionic acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing ryegrass (n = 8)

Time (h)	Propionic acid (mmol/L)				Propionic acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	22.4	22.6	0.89	0.40	18.5	18.5	0.93	0.45
0400	16.7	17.3	0.81	0.50	16.1	17.3	0.35	0.79
0800	20.8	18.1	0.03	0.21	17.0	17.0	0.97	0.30
1200	23.7	22.5	0.26	0.21	19.3	18.7	0.53	0.55
1600	25.6	21.2	0.03	0.33	19.5	19.0	0.47	0.48
2000	27.5	26.0	0.03	0.12	19.5	19.7	0.62	0.34

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

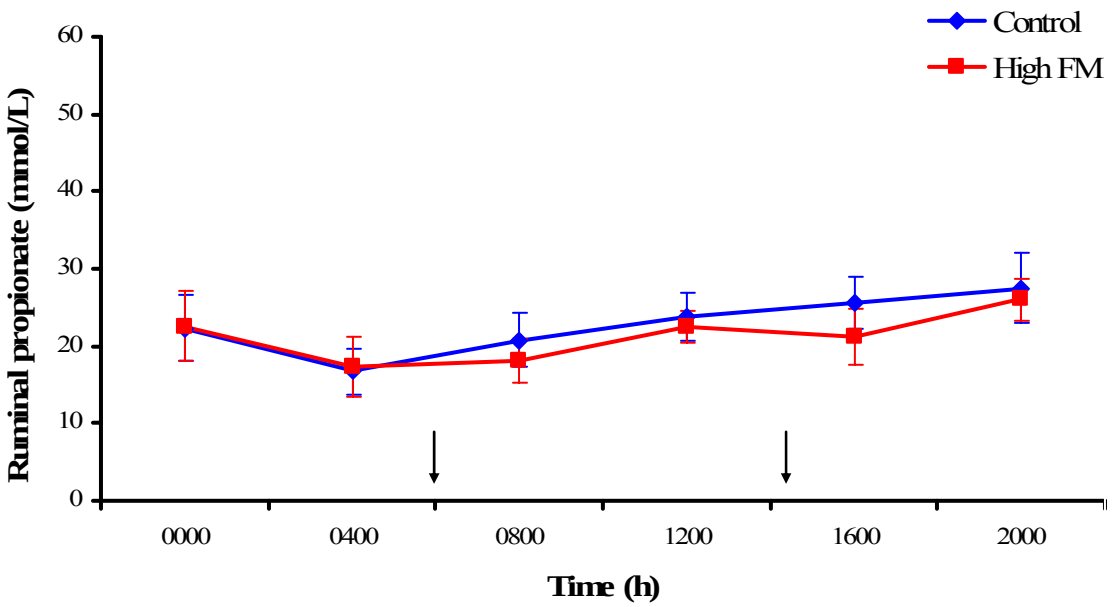

Figure 3.15 Ruminal concentration of propionic acid (mmol/L) of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean acetate: propionate ratios for the cows on the control and high FM treatments were 3.61 and 3.56, respectively. There was no difference between the treatments ($P > 0.1$).

The mean concentration of ruminal butyric acid (mmol/L) for the eight cows on each treatment was calculated for each of the 6 times of the day and is reported in Table 3.30 and shown in Figure 3.16. Table 3.30 also reports butyric acid as a proportion of total VFA.

The butyric acid concentrations in the rumen fluid of the cows on the two treatments did not differ from each other at 0000, 0400, 1200 and 2000 h ($P > 0.1$) and was higher for the cows on the control treatment than the high FM treatment at 0800 and 1600 h ($P < 0.05$). The molar proportion of butyrate, however, tended to be higher for the high FM treatment than the control at 0800 and 1600 ($P < 0.1$).

The mean daily butyric acid concentration in the rumens of the cows on the control and high FM treatments were 16.7 and 16.3 mmol/L, respectively, not differing between treatments ($P > 0.1$). The mean daily molar proportion of butyrate (13.5 and 14.1 mol/100 mol VFA, respectively; Table 3.32) tended to be higher for the high FM treatment ($P = 0.07$).

Table 3.30 Effect of time of day and fishmeal (FM) supplementation on mean butyric acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing ryegrass (n = 8)

Time (h)	Butyric acid (mmol/L)				Butyric acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	15.2	16.0	0.45	0.21	12.7	13.2	0.19	0.24
0400	14.0	14.1	0.98	0.22	13.5	14.2	0.24	0.39
0800	17.9	16.3	0.05	0.15	14.6	15.3	0.09	0.25
1200	17.0	17.4	0.74	0.32	13.9	14.4	0.39	0.44
1600	17.7	15.6	0.01	0.12	13.5	14.0	0.06	0.15
2000	18.4	18.1	0.70	0.16	13.1	13.8	0.10	0.27

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

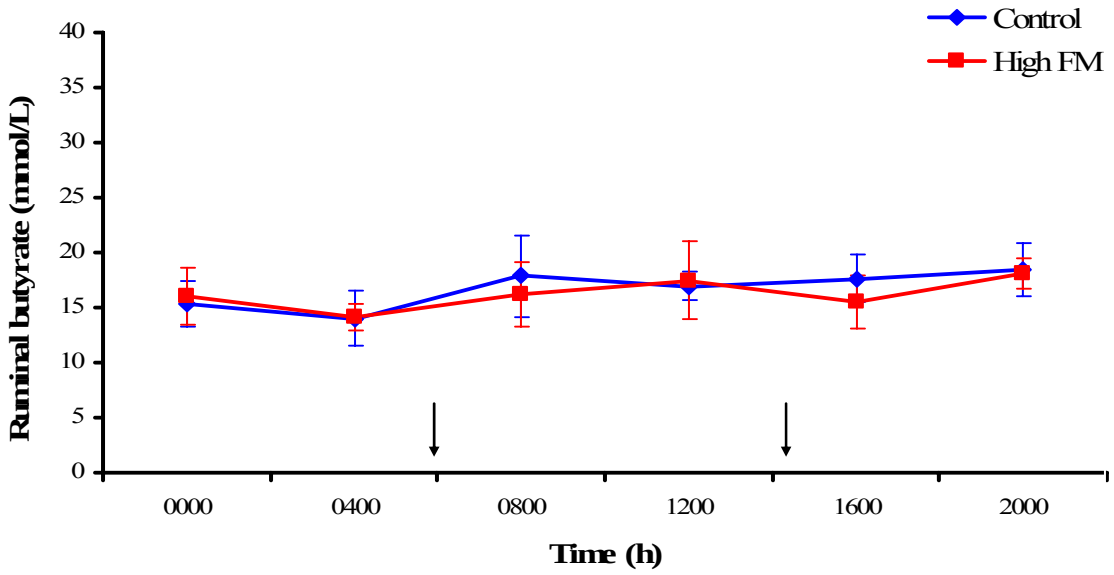

Figure 3.16 Ruminal concentration of butyric acid (mmol/L) of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

To summarise: at 0000, 0400 and 1200 h there was no difference between the two treatments for any of the three VFA. At 0800 and 1600 h the concentrations of all three were higher for the cows on the control treatment than for the cows on the high FM treatment. At 2000 h the concentration of propionic acid was higher and acetic acid tended to be higher for the

cows on the control treatment while there was no difference in butyric acid concentration between the two treatments. Overall the mean daily concentrations of total VFA and acetate were higher in the control treatment ($P < 0.05$). The reason for this is not clear.

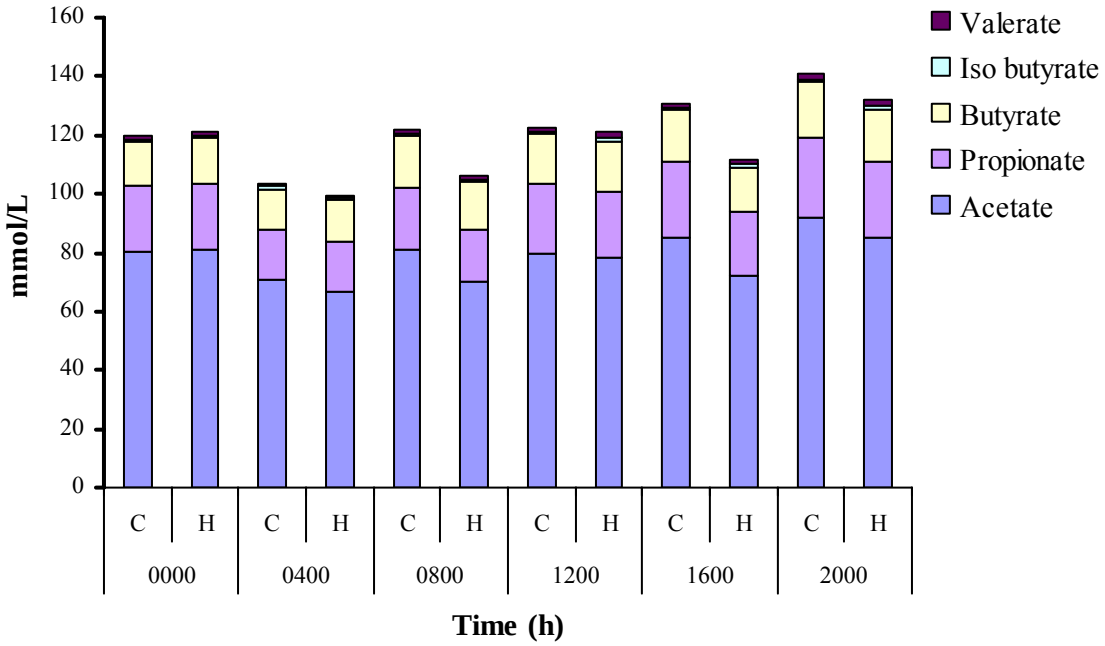

Figure 3.17 Concentrations of individual volatile fatty acids (VFA) making up the total VFA in the rumen fluid of cows grazing ryegrass and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment; C) or 8 % FM (High FM treatment; H)

3.2.3 Summary of results

The cows were allowed 11.1 kg DM/cow/d of the annual ryegrass pasture. The mean intake of this pasture was approximately 8.6 kg DM/cow/d. The chemical composition of the pasture was in the range expected for annual ryegrass: the mean CP, NDF, ADF, IVOMD and ME were 26.2, 46.3, 25.6, 80.2 % DM and 11.3 MJ/kg DM, respectively.

The main difference between the three experimental treatments was the CP content of the supplements: 8.2, 11.2 and 14.6 % for the control, low FM and high FM treatments, respectively. Although the EE rose slightly with the inclusion of FM, the ME of the three concentrates was similar.

The total diets of the cows on all three treatments were adequate in all the main nutrients. There was enough ME to support 24 kg of 4 % FCM/d. The CP (19.2, 20.3 and 21.6 % DM for the control, low FM and high FM diets, respectively) was adequate in all three diets. Including FM in the concentrate increased both RDP and RUP as well as increasing the Met and Lys levels of the diet.

The cows on the low FM treatment responded by producing 7 % more milk, 28 % more milk fat (yield), 13 % more milk protein (yield) and 18 % more 4 % FCM and ECM than the cows on the control treatment ($P < 0.01$). There was no additional benefit to the higher level of FM (Table 3.31).

There was no effect on change in BW ($P > 0.1$). The cows on the control treatment put on more condition than the cows on the high FM treatment ($P < 0.05$).

The starch content of the faeces was low, indicating efficient and extensive digestion of starch.

The ruminal pH did not differ between treatments ($P > 0.1$) and, although it varied throughout the day, was never suboptimal (below the 5.8). The ruminal NH_3-N concentration was higher for the cows on the high FM treatment than the control ($P < 0.05$), both well above the minimum level of 5 mg/dl for maximum microbial protein synthesis (Satter & Slyter, 1974) and within the range expected for cows on pasture concentrate. The concentrations of total VFA in the ruminal fluid were higher in the control treatment ($P < 0.05$; Table 3.32).

Table 3.31 Effect of fishmeal (FM) supplementation on mean milk yield, milk composition, body weight (BW) and body condition score (BSC)[1] of cows grazing ryegrass pasture and receiving 5.5 kg concentrate supplement DM/d (n = 15)

Parameter	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
Milk yield (kg/d)	20.5[a]	21.9[b]	22.1[b]	0.34
4 % FCM (kg/d)	20.4[a]	24.1[b]	24.2[b]	0.47
Fat (%)	3.97[a]	4.73[b]	4.67[b]	0.132
Fat yield (kg/d)	0.81[a]	1.03[b]	1.03[b]	0.028
Protein (%)	3.25[a]	3.49[b]	3.45[b]	0.051
Protein yield (kg/d)	0.67[a]	0.76[b]	0.76[b]	0.014
Lactose (%)	4.59[a]	4.78[b]	4.79[b]	0.019
MUN (mg/dl)	16.80	17.43	17.93	0.440
BW beginning (kg)	327	338	327	6.2
BW end (kg)	371	387	369	7.6
BW change (kg)	+44	+49	+42	3.7
BCS beginning	2.1	2.1	2.2	0.06
BCS end	2.5	2.3	2.4	0.07
BCS change	+0.4[a]	+0.2[ab]	+0.2[b]	0.06

FCM – fat-corrected milk; MUN – Milk urea N
[1]Five-point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982)
[2]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.05$)

Table 3.32 Effect of fishmeal (FM) supplementation on mean daily ruminal pH, ammonia-N (NH_3-N) and volatile fatty acid (VFA) concentrations of cows grazing ryegrass pasture and receiving 5.5 kg concentrate supplement DM/d (n = 8)

Parameter	Experimental treatment[1]		SEM[2]
	Control	High FM	
pH	6.14	6.08	0.022
NH_3-N (mg/dl)	14.16[a]	16.67[b]	0.405
Total VFA (mmol/L)	123.4[b]	115.3[a]	0.67
Acetate (mol/100 mol)	66.1	65.4	0.377
Propionate (mol/100 mol)	18.5	18.5	0.308
Butyrate (mol/100 mol)	13.5	14.2	0.183
Acetate: propionate	3.61	3.56	0.080

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a, b]Means in the same row with different superscripts differ ($P < 0.05$)

3.3 DISCUSSION

3.3.1 Production study

3.3.1.1 Pasture

3.3.1.1.1 Pasture allowance and intake

a) Pasture allowance and intake estimated using the rising plate meter

The height of the pasture after grazing was in keeping with the desired post grazing stubble height of 5 to 6 cm (Fulkerson *et al.*, 1998). The amount of DM removed off the pasture was similar to values reported by Meeske & Van der Merwe (2006a; 2006b) for the same pasture type.

The difference between the equation obtained during the trial and the standard calibration equation (Figure 3.5) emphasises the importance, when using a RPM, of determining a calibration equation for each unique combination of pasture/area/season, rather than using an overall average equation for the area.

The growth rate of the pasture was lower than the growth rate of 49 kg DM/ha/d reported by Botha *et al.* (2006) for the same type of pasture in October.

Pasture intake is related to pasture on offer (Fulkerson *et al.*, 2005). Pasture utilisation is pasture intake expressed as a proportion of pre-grazing pasture mass (Dalley *et al.*, 1999). The pasture utilisation in this study (68 %) was higher than that reported by Dalley *et al.* (1999; 54 and 26 % for PA of 11.2 and 18.7, respectively) which is to be expected since the PA was lower in this study and herbage utilisation decreases as herbage allowance increases (Dalley *et al.*, 1999).

It is possible that the intake estimated with the RPM meter could be slightly underestimated since in this trial values were not corrected for growth of the pasture between measurements as in the trials by Reeves *et al.* (1996) and Fulkerson *et al.* (2005). This should not have had a major effect since the post-grazing pasture height was usually measured immediately or within a day after the cows finished grazing that strip, in which time no

significant pasture growth would have occurred, although there were times when three days elapsed before measurements were taken.

Another potential cause for inaccuracies could be the fact that only one calibration equation was used both pre- and post-grazing. Reeves *et al.* (1996) used different calibration equations for pre- and post-grazing pasture as these equations differed from each other.

b) Estimation of pasture intake of the three treatment groups separately using the rising plate meter

The RPM would not have been a very accurate way of estimating differences in pasture intake with the cows grazing such a small area at a time and with so few days (repetitions) in which to measure it. Even though 100 RPM readings were taken each time, the average reading appeared to vary, according to factors such as the time of day and how flat the grass was lying in the heat or wind. The readings from the whole strip did not correlate with the readings from the fractions of the same strip. It was concluded that the RPM is not accurate enough to determine differences in pasture intake between the cows in the three groups. Reeves *et al.* (1996) also found that the RPM was not accurate enough to detect differences in pasture intake of cows fed different levels of concentrate.

If there were any differences they would have been more likely to be detected if more days were used for measurement but, since the aim of the experiment required all the cows to be grazing the same pasture, it was considered important not to separate the cows for longer than necessary, as the pasture can vary within a paddock.

c) Estimation of pasture intake using equations

If the cows consumed 8.6 kg ryegrass DM/d, the total DMI would have been 14.1 kg/cow/d which is 4.0 % of BW. This is similar to the DMI of 3.58 % of BW reported by Bargo *et al.* (2002b) for cows on pasture-concentrate (60 % pasture, 40 % concentrate) and 4.1 % of BW found by Gehman *et al.* (2006) where the total DMI was 25 kg/d for 606 kg Holstein cows grazing annual ryegrass and receiving 9.2 kg maize-based concentrate. They (Gehman *et al.*, 2006) stated that this might be a slight over-estimation as the Cr_2O_3 method was used to estimate intake. Fulkerson *et al.* (2005) obtained a slightly lower value (the DMI was 3.6 % of BW for cows grazing ryegrass and receiving 4.75 kg pellets/d, measured by using the alkane method),

which is to be expected since the concentrate level was lower in their study and total DMI increases as the level of concentrate increases (Sairanen *et al.*, 2005).

d) Estimation of pasture intake using the CPM Dairy model

The model predicts pasture intake to be higher in the cows on the two fishmeal treatments than the control cows. According the NRC (2001) cows appear to consume feed to meet their energy needs. The NRC equation for predicting DMI is based on milk production and BW. The higher production of the cows on the two fishmeal treatments could have driven the cows to consume more pasture. The slightly higher intake of the cows on the low FM treatment is related to the greater average BW of these cows.

The higher pasture intake of cows receiving FM agrees with some other studies. According to Paterson *et al.* (1994) CP stimulates intake of pasture, although more so for pasture with low CP, while there was no effect in the study of McCormick *et al.* (2001a). Bargo *et al.* (2001; 2003a) stated that pasture and total DMI are usually not affected by level and source of protein in the supplement. In general, replacing SBM with a high RUP source does not affect DMI (Santos *et al.*, 1998). On the other hand Donaldson *et al.* (1991) found RUP supplementation (in the form of FM and dried distillers grains and solubles) to increase forage and total DMI in growing steers and in the study of Schor & Gagliostro (2001) forage DMI was higher in cows receiving concentrate with higher RUP (in the form of BM).

3.3.1.1.2 Pasture composition

The mean DM concentration of 13.7 ± 3.60 % (mean ± SD; n = 9) is in keeping with the range of DM concentrations of 11.6 to 16.2 % reported in the studies of McCormick *et al.* (2001b), Marais *et al.* (2003), Meeske *et al.* (2006) and Meeske & Van der Merwe (2006a; 2006b) for annual ryegrass.

The mean ash concentration of 13.4 ± 1.44 % DM (n = 9) is higher than the values of 10.0 ± 1.65 and 10.5 % reported by Meeske *et al.* (2006) and McCormick *et al.* (2001b) for annual ryegrass and the range of 8 to 9 % of DM reported by Muller Fales (1998) for cool season grass pasture in spring. The mean OM concentration (100 – ash) of 86.6 ± 1.44 % DM is lower than the 93.0 % reported by Gehmen *et al.* (2006). This indicates the possibility of slight soil contamination of the samples as they were not rinsed before being dried and milled.

The mean IVOMD of 80.2 ± 3.34 % DM (n = 9) is similar to that reported by Botha *et al.* (2006) for the same type of pasture: mean 78.5 % with a general decline as the season progressed. Fulkerson *et al.* (2005) reported a slightly lower OM digestibility of 75.6 % DM for annual ryegrass during September to November in Australia.

The ME concentration, calculated as 0.82 × GE × IVOMD (Robinson *et al.*, 2004), averaged 11.3 ± 0.42 MJ/kg DM (n = 3). This is within the expected range for annual ryegrass. Fulkerson *et al.* (1998; 2005), Granzin (2004), Meeske *et al.* (2006) and Meeske & Van der Merwe (2006b; 2006b) reported ME values for annual ryegrass ranging form 10.3 MJ ME/kg DM (Fulkerson *et al.*, 2005) to 12.2 MJ ME/kg DM (Meeske & Van der Merwe, 2006b). Differences in ME values could be due to different equations being used to calculate it. Fulkerson *et al.* (1998; 2005) used the equation ME = OMD × 0.16 – 1.8 which yielded a similar ME value to the equation ME = 18.4 × IVOMD × 0.81 used by Meeske *et al.* (2006).

The CP concentration of the pasture used in this trial was as expected for annual ryegrass. The CP concentration averaged 26.2 ± 3.23 % DM (n = 9) and varied from 22.6 to 33.5 %. The latter value (during the week of 19 September) is unusually high compared with the rest of the samples. This value was, however, confirmed with a second CP analysis done at a later stage on the same sample. There is a possibility that this sample was not representative of the whole pasture due to variation within the paddock, even though samples from four places were composited. McCormick *et al.* (1999; 2001b), Marais *et al.* (2003), Granzin (2004), Fulkerson *et al.* (2005), Gehman *et al.* (2006), Meeske *et al.* (2006), Meeske & Van der Merwe (2006a; 2006b) and Botha *et al.* (2006) reported mean CP values for annual ryegrass ranging from 16.5 % DM (Gehman *et al.*, 2006) to 29.2 % DM (Granzin, 2004), also with a decrease as the season progressed. Gehman *et al.* (2006), upon trying to explain the low CP of their pasture, stated that climatic factors such as rainfall and temperature at the time of fertilisation could have affected N volatilization, leaching and growth rate and hence that CP content of the pasture.

On average 42.7 % of this CP was soluble. This is lower than the mean sol CP value of 60.1 ± 3.53 % CP reported by Gehman *et al.* (2006) and higher than the 28.5 % of CP in the trial of McCormick *et al.* (1999). On average 47.1 % of this sol CP was NPN.

The mean NDF concentration of 46.3 ± 3.23 % DM (n = 9), ranging from 41.9 to 52.2 % with a general increase as the season progressed, is as expected. All but the last value fall within the range of 40 to 50 % DM reported by Muller & Fales (1998) as the mean NDF concentration

of cool season grass pasture in spring. Fulkerson *et al.* (1998), McCormick *et al.* (1999; 2001b), Granzin (2004), Fulkerson *et al.* (2005), Gehman *et al.* (2006), Meeske *et al.* (2006) and Meeske & Van der Merwe (2006a; 2006b) reported NDF values for annual ryegrass ranging from 37 % DM (Fulkerson *et al.*, 1998) to 52.7 % DM (Gehman *et al.*, 2006). The lignin concentration of the pasture averaged 8.1 ± 1.88 % of NDF (n = 3) which is higher than the average of 2.84 ± 0.568 (n = 19) for South African ryegrass samples tested for the AFRGI Animal feeds database (Cronjé, G., personal communication, gert.cronje@afgri.co.za).

The mean ADF concentration of 25.6 ± 1.46 % DM (n = 9) is within the range of 24 to 28 % DM for cool season grass pasture in spring (Muller & Fales, 1998). Fulkerson *et al.* (1998; 2005), McCormick *et al.* (1999; 2001b), Granzin (2004), Gehman *et al.* (2006) and Meeske *et al.* (2006) reported ADF values for annual ryegrass ranging from 17 % DM (Fulkerson *et al.*, 1998) to 28 % (Meeske *et al.*, 2006).

Neutral detergent insoluble N (NDIN) includes N associated with the cell wall that is insoluble in neutral detergent solution while acid detergent insoluble N (ADIN) is that fraction which is insoluble in acid detergent solution and includes lignified N and Maillard products and is largely unavailable to the animal (Krishnamoorthy *et al.*, 1982). The N fraction in NDIN varies between samples (Krishnamoorthy *et al.*, 1982). The mean NDIP in this trial was 24.7 ± 3.68 % of CP and ADIP 5.1 ± 0.90 % of CP (n = 3).

The mean EE concentration of 3.2 ± 0.45 % DM (n = 3) is within the range of 3 to 4 % DM reported by Muller & Fales (1998) as the average for cool season grass pasture in spring, although it is slightly lower than the 3.8 % of Granzin (2004) and lower than the mean value of 4.6 ± 1.23 % found by Gehman *et al.* (2006).

The mean calculated NFC concentration of 10.9 ± 0.75 % DM (n = 3) is the same as the 10.9 % reported by Granzin (2004) for annual ryegrass although it is lower than the range of 15 to 31 % (Muller & Fales, 1998; McCormick *et al.*, 1999; 2001b; Gehman *et al.*, 2006) and did not follow the same trend of decreasing with time. The mean starch concentration of 0.2 ± 0.17 % DM (n = 3) is lower than the 1.8 % found by Gehman *et al.* (2006) for annual ryegrass and 1.3 % DM found by Williams *et al.* (2005) for perennial ryegrass.

The mean Ca concentration was 0.52 ± 0.103 % DM (n = 3) and the mean P concentration was 0.41 ± 0.026 % (n = 3). The mean Ca to P ratio was 1.28 ± 0.214. Fulkerson *et al.* (1998), Granzin (2004), Gehman *et al.* (2006), Meeske *et al.* (2006) and Meeske & Van der Merwe

(2006a; 2006b) reported Ca values for annual ryegrass ranging from 0.45 % DM (Meeske & Van der Merwe, 2006a) to 0.72 % DM (Granzin, 2004) and P values ranging from 0.31 % DM (Fulkerson *et al.*, 1998; Gehman *et al.*, 2006) to 0.52 % DM (Meeske & Van der Merwe, 2006a), hence the Ca and P in the pasture used for this trial are within the expected range.

There is a possibility that the samples taken were not entirely representative of the pasture actually consumed by the cows. Cows tend to select pasture of higher quality than that on offer, especially at high PA (Wales *et al.*, 1998; Dalley *et al.*, 1999; Peyraud & Delaby, 2001). Wales *et al.* (1998) and Williams *et al.*, (2005) took pasture samples pre- and post-grazing and used these, along with the pasture mass pre- and post-grazing, to calculate the nutrient composition of the pasture actually consumed by the cows. In this study the pasture samples were cut at a level of 3 cm above ground level and the cows grazed the pasture down to almost this level. The PA was low (intake is restricted if stubble height is below 8 to 10 cm (Stakelum, 1986a)), so it can be assumed that the composition of the pasture on offer did not differ much from that actually consumed by the cows.

The AA levels (Table 3.7) are in agreement with those in pasture in the study of Jones-Endsley *et al.* (1997).

3.3.1.2 Concentrate composition

The chemical composition of the concentrates (Table 3.8) are in line with the maize-based concentrate used by Granzin (2004) except that the EE in the present study is lower than the fat concentration of 3 to 4 % DM in the study of Granzin (2004).

The drop in OM and rise in EE and CP as the level of FM increased is due to the high ash, EE and CP concentration of the FM (Table 3.9). The higher EE in the two FM concentrates is also due to the Megalac.

It is not clear why the IVOMD is higher for the low FM than for the other two concentrates. The higher ME in the low FM concentrate is due to the higher IVOMD which was used in the equation to calculate the ME.

The ADIP is higher than the NDIP in the control concentrate which should be impossible. Krishnamoorthy *et al.*, (1982) also found ADIN exceeding NDIN (in maize gluten meal and SBM) and stated that this could be due to interfering substances in the analysis.

Soluble CP, ADIP, NDIP and lignin were higher and NPN and EE were lower than what would be expected from the same concentrates based average South African raw materials (see Table 4.3 in section 4.1).

Only one composite concentrate sample was analysed per treatment so there is no indication of the variation in composition in the whole batch of feed.

3.3.1.3 Total diet composition

The total diet composition (Table 3.3) can be compared to the recommendations of Erasmus *et al.* (2000) for early lactation cows reported in Table 2.1 of the literature review. The ME concentration of all three diets (11.9, 12.1 and 11.9 MJ ME/kg) was adequate as they were above the recommended level of 11.3 to 11.5 MJ ME/kg DM. However, due to the fact that grazing cows require 10 to 30 % more ME due to the energy requirements of grazing and walking (Muller & Fales, 1998), the ME concentrations of these diets could be inadequate. At a total DMI of 14.1 kg the total diet would have supplied approximately 169 MJ ME/d.

The NDF (32.6, 32.8 and 33.2 % DM) was close to and slightly above the minimum recommendation of 28 to 32 % while ADF (17.0, 17.0 and 17.1 % DM) was below the minimum recommendation of 19 %. The EE (2.6, 2.8 and 3.1 % DM) was higher for the FM treatments but still below the recommended 5 to 7 %. Calcium (0.92, 1.11 and 1.22 % DM) and P (0.47, 0.54 and 0.59 % DM) were adequate compared to the recommendations of 0.6 to 0.8 % and 0.38 to 0.42 %, respectively.

The CP concentration (19.2, 20.3 and 21.6 % DM) increased with the level of FM in the diet although it was not excessively high even in the high FM diet; all three were above the recommended 16 to 18 %. The soluble CP (43.6, 42.5 and 41.9 % CP) was above the recommended 30 to 35 % of CP, and was highest for the control treatment, as would be expected since the FM is high in RUP.

Unfortunately the rumen degradability of the protein was not measured. It can, however, be estimated based on literature values of potential degradability and passage rate.

Berzaghi *et al.* (1996) found the particle passage rate of cows grazing pasture and receiving maize supplementation to be 7.1 %/h. They also stated that according to *in situ* data more than 90 % of N compounds in fresh grass are potentially degradable (D) and degradation rate can vary from 10 to 20 %/h. If the equation $k_p/(k_d + k_p) \times D$ (Van Vuuren *et al.*, 1991) is used with k_p = 7.1 %/h, k_d = 15 %/h (mid-way between 10 and 20 %/h) and D = 90% then the amount of CP from the pasture escaping degradation would be 29 %, and 71 % of the pasture protein would be degraded. This is in line with other data for protein degradability of pasture since 60 to 80 % of pasture CP is degradable (Holden *et al.*, 1994a). Berzaghi *et al.* (1996) found the NANMN to be 25.5 % of N intake (for cows on pasture with maize supplementation) indicating that about 74 % of the pasture protein was degraded in the rumen. This is in line with results of Beever *et al.* (1986) who found a mean N degradation of 75 % for perennial ryegrass in steers grazing perennial ryegrass. Ryegrass pasture containing 26.46 % CP of which 71 % is degraded, at a PDMI of 8.6 kg, would have supplied the cow with 1434 g RDP and 586 g RUP.

Animal proteins are degraded rapidly and incompletely (Wallace, 1988) and hence degradability is not as dependant on passage rate as for plant proteins. Hence it would be safe to use a book value for protein degradability of FM. According to Table 15-2a of NRC (2001) FM has an RUP value of 65.8 % of CP. This is a crude estimate since protein degradability of FM can vary greatly depending on the processing method (Yoon *et al.*, 1996).

The degradability of plant proteins depends more on passage rate (Wallace, 1988). The B1 fraction of maize protein would be 100 – (Sol CP + NDIP) = 100 – (11 + 15) (see Table 4.3) = 74 % of CP. Assuming a passage rate constant for maize meal of 6 %/h and a digestion rate constant of 7.5 %/h (6 to 9 %/h; Sniffen *et al.*, 1992) the amount of protein degraded would be Sol CP (% CP) + $k_d/(k_d + k_p) \times$ (B1 fraction; % CP) = 11 + 7.5/(7.5 + 6) × 74 = 52 % of CP and 48 % of the protein would be un-degraded (see section 2.8.1.2).

Assuming the maize contained 9.27 % CP (Table 4.3) with a degradability of 52 %, the molasses contained 4.5 % protein that was 100 % soluble (Table 4.3) and the FM contained 73.77 % CP (Table 4.3) with a degradability of 34 % (NRC, 2001), the control, low FM and high FM concentrates would have supplied RDP and RUP as shown in Table 3.33. Each successive FM treatment added almost 100 g RUP to the diet.

Table 3.33 Approximate daily supply of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) from the three experimental diets of cows grazing ryegrass, calculated based on estimates of ruminal passage rate and protein degradation rate

	Experimental treatments[1]		
	Control	**Low FM**	**High FM**
Concentrate			
RDP (g/d)	252	295	335
RUP (g/d)	217	313	410
Total diet			
RDP (g/d)	1686	1729	1769
RUP (g/d)	803	899	996
RDP (% CP)	68	66	64
RUP (% CP)	32	34	36

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM

Including FM in the diet increased both RDP and RUP supply, the latter more so as a greater proportion of the CP was RUP (Figure 3.18). The estimated RDP and RUP supplied by the total diets (Table 3.33) were greater for all three experimental treatments than the recommended requirements of 1730 g RDP and 720 g RUP/d (NRC, 2001; see section 2.2 in literature review) except for the RDP in the control treatment being lower than required. This could be partly related to the fast passage rate allowing less time for protein degradability.

The RDP and RUP concentrations (% CP) in the total diets are very close to those predicted by the CPM Dairy model (Table 4.6) of 69, 66 and 63 % RDP for the control, low FM and high FM treatments, respectively.

Of the protein that was degraded it cannot be certain how much of it flowed to the small intestine as microbial protein. Nitrogen can be lost across the rumen wall without being incorporated into microbial protein, which is why cows on pasture have been observed to have proportionally less N flowing to the duodenum (Holden *et al.*, 1994b). Similarly, of the protein that was un-degraded, not all of it necessarily supplied AA as some protein is bound and cannot be broken down by bacteria and does not supply AA post-ruminally (Sniffen *et al.*, 1992). To be able to determine what AA were available to the animal from the small intestine it would have been necessary to take samples of the duodenal contents, which was beyond the scope of this experiment.

The CPM Dairy model predicted MP from bacteria to be 925, 979 and 932 g/d and MP from RUP to be 550, 770 and 849 g/d for the cows on the control, low FM and high FM treatments, respectively (Table 4.6).

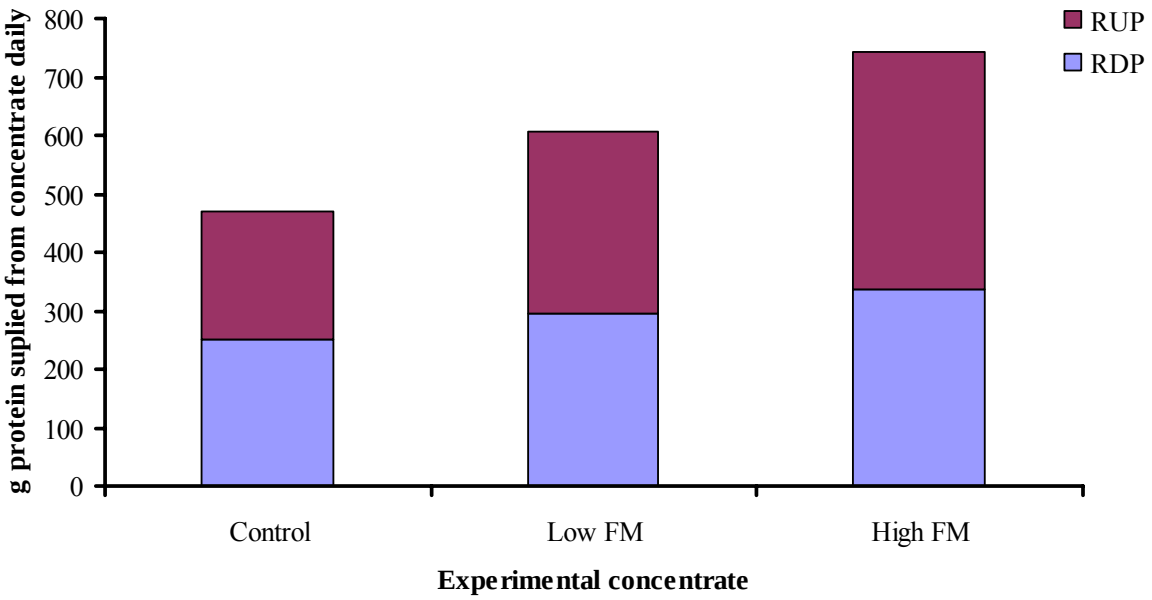

Figure 3.18 Estimation of the amount of rumen degradable protein (RDP) and rumen-undegradable protein (RUP) supplied by the control, low fishmeal (FM) and high FM concentrates based on composition of average South African raw materials, calculated based on estimates of ruminal passage rate and protein degradation rate

3.3.1.4 Milk production and composition

3.3.1.4.1 Mean for the whole experimental period

a) Milk yield

Since the same pasture was consumed by all the cows and the same amount of ME supplied by the respective concentrates, the cows on the three experimental treatments must have had the same total ME supply (unless pasture intake differed). The ME must have been adequate

for the higher level of production. Hence ME was not limiting for the cows on the control treatment (unless PDMI was lower).

The milk response of the cows on the two FM treatments over the control could be due to the increased CP *per se* or due to the increased supply of EAA to the small intestine. The RDP, RUP and Met and Lys concentrations of the diet increased with the inclusion of FM (section 3.2.1.3). The shortage in RDP of the control diet (Table 3.33) indicates that the production response could be partly related to increased RDP supply.

Santos *et al.* (1998) stated that FM consistently increased the proportion of Lys in the EAA flowing to the duodenum when supplied at greater than 4 % of diet DM but not if less than 4 % and brought the ratio of Lys to Met, as % EAA, at the duodenum close to the recommended level. Xu *et al.* (1998) found increased milk production (39 vs. 34 kg/d) when cows were fed a blend of BM, FM and meat and bone meal as an AA source or ruminally protected Lys and Met compared to maize distillers' grains as a control. Robinson *et al.* (1995) and Wu *et al.* (1997) also found increased milk yield with supplementation of ruminally stable Met and Lys while there have also been small and inconsistent responses (Rulquin *et al.*, 1993). Supplementing FM (vs. SBM) increased milk yield in the trial of Broderick (1992). In the trial of MacDonald *et al.* (1998), where cows grazing pasture and receiving maize silage supplementation were supplemented with urea, SBM, or FM, the response was greater and more consistent for the cows receiving FM than SBM and there was no response to urea. The RUP supply was also greater from the FM than the SBM and 0 from the urea, while the RDP supply was greatest from the SBM.

The lack of response to the higher level of FM was probably because ME once again became the first limiting nutrient.

Bargo *et al.* (2003a) in their review found that there is an average increase in milk production of 0.8 kg/d for each 100 g/d of RUP. Since the low FM diet supplied an additional 100 g RUP/d, the milk response was 1.4 kg milk per 100 g additional RUP supplementation, almost double the level reported by Bargo *et al.* (2003a). This could be related to the good AA profile of FM since the AA profile of the RUP supplement is more important than the amount of RUP (Santos *et al.*, 1998).

There is the possibility that factors other than RDP, RUP and AA could have contributed to the milk yield response. For example EE increased with the FM treatments. Fat is normally

added to increase the energy density of the diet (NRC, 2001) as was done in this study. Since the three diets were iso-energetic, the fat content of the diets should not have had an effect on the outcome of the experiment. It cannot, however, be ruled out as the fatty acid composition was not measured.

There is also the possibility that the cows on the FM treatments could have consumed more pasture and hence had more energy for milk production (this higher pasture intake being driven by higher energy demands due to higher milk production).

The Ca and P concentration also increased with FM inclusion. Levels were, however adequate in all three diets and should not have limited production.

b) Milk composition

The mean milk fat percentage for all registered Jerseys in South Africa was 4.65 % in 2005/2006 (National milk recording scheme, South Africa, Annual Report, 2006, Volume 26, ARC, Livestock Business Division, Animal Production, Irene, 0062). Thus the value of 3.97% for the cows on the control treatment is unusually low. There does not seem to be an obvious explanation as to why there was this drop in milk fat for the control group during the trial since dietary fibre, ruminal pH and acetate: propionate ratio did not differ from the other two treatments. In fact acetate levels in the rumen were higher than in the high FM treatment (Table 3.32).

The protein values are slightly lower than the mean protein concentration of 3.75 % in the milk of registered Jerseys in South Africa in 2005/2006 (National milk recording scheme, South Africa, Annual Report, 2006, Volume 26, ARC, Livestock Business Division, Animal Production, Irene, 0062).

The response in milk fat and protein percentage may be partly due to the increased CP in the diet. In the study by McCormick *et al.* (2001a) milk fat (3.34 vs. 3.11 %) and protein (3.42 vs. 3.27 %) percentages were increased when Holstein cows grazing annual ryegrass-oat pastures were fed a high CP supplement (22.8 % CP) vs. a moderate CP supplement (16.6 % CP), while RUP (maize gluten meal-BM mixture) had no effect. The response could also be attributed to the increased flow of EAA to the small intestine as this is known to increase milk protein yield (Rulquin & Vérité, 1993). Supplementation with a protein source rich in EAA increases milk protein yield especially when maize (low in Lys) is fed and even with pasture of high N content

(Rulquin & Vérité, 1993). Robinson *et al.* (1995) and Xu *et al.* (1998) found supplementing rumen protected Lys and Met to increase milk fat and protein percentage and yield. Increased milk protein concentration and yield has been the most consistent response to supplementing ruminally protected Met and Lys (Rulquin *et al.*, 1993; Robinson *et al.*, 1995; 1998) and was also found by Wu *et al.* (1997) and Robinson *et al.* (1999). Supplementing FM (vs. SBM) increased milk protein percentage in the trial of Broderick (1992). Some studies have shown no effect on milk fat production (Robinson *et al.*, 1999) or even a tendency to decrease (Rulquin *et al.*, 1993). Schor & Gagliostro (2001) found no effect of BM supplementation on milk fat concentration. Feeding FM could even reduce milk fat percentage, mainly due to high concentrations of unsaturated long-chain fatty acids in FM or a reduction in acetate to propionate ratio in ruminal fluid negatively affecting milk fat (Schroeder & Gagliostro, 2000). There was however no difference in acetate: propionate ratio between the control and high FM treatments in this study (section 3.2.2.3). The fatty acid composition of the diets was not measured.

The lactose response is in agreement with the results of Tesfa *et al.* (1995) where the milk lactose was lower in cows supplemented with a cereal by-product based concentrate (12.4 % CP) than in cows given additional N, in the form of urea or rapeseed meal (non-heat treated or heat treated), in their concentrates (15.0 to 15.6 % CP). Robinson *et al.* (1995) found increased milk lactose when ruminally protected Lys and Met were fed. There is, however, no biological explanation for the difference in milk lactose reported in this study.

Milk urea N testing can help monitor the efficiency of protein utilisation and the adequacy of dietary fermentable carbohydrates; a value of above 16 mg/dl indicates excess dietary protein in relation to dietary carbohydrates (Muller, 2003b). The MUN values of the cows in this trial were slightly above the target values of 10 to 16 mg/dl suggested by Jonker *et al.* (1999) but still within the range of 12 to 18 mg/dl suggested by Linn & Olsen (1995) and De Villiers *et al.* (2000) as indicative of a balanced ration and still below 20 mg/dl where reproductive performance of the cow could start being negatively affected (De Villiers *et al.*, 2000). Although MUN values can be excessive when cows graze pasture only, when they receive supplements the levels are acceptable (Muller, 2003b). Previous research (Khalili & Sairanen, 2000; Bargo *et al.*, 2002b; Delahoy *et al.*, 2003; Liebenberg *et al.*, 2005; Gehman *et al.*, 2006) reported MUN values of supplemented cows on pasture ranging from 10 mg/dl (Gehman *et al.*, 2006) to 38 mg/dl (Khalili & Sairanen, 2000). Higher MUN could be due to higher RDP intake (Schroeder &

Gagliostro, 2000). There is a high seasonal variation in MUN (ranging from 4 to 32 mg/dl) with the highest values being in early spring and the lowest in late summer, correlating with the CP content of the pasture (Bargo *et al.*, 2002b). Milk urea N is closely correlated to BUN (Broderick & Clayton, 1997). Blood urea N and MUN can be used as indicators of rumen N capture as they are positively associated with ruminal NH_3-N concentrations (Broderick & Clayton, 1997; Gehman *et al.*, 2006). Milk urea N is also closely correlated to dietary CP intake and excess N intake (Baker *et al.*, 1995; Broderick & Clayton, 1997). Although there was no significant difference in MUN in this study there was a difference in ruminal NH_3-N concentration (Table 3.32).

c) Fat- and energy-corrected milk yield

The FCM response is in agreement with the results of Broderick (1992) where supplementing FM (vs. SBM) increased 3.5 % FCM yield.

The responses in FCM and ECM would have been due to increased RDP, RUP and Lys and Met (increased EAA flow) as discussed above for milk yield and composition responses.

3.3.1.4.2 Milk production and composition of early and mid lactation cows

From the results in section 3.2.1.4.3 it appears that the cows in early lactation did not respond while those in mid lactation did, even though there was an apparently big numerical response in the former. It, however, has to be borne in mind that there were less cows in the early lactation comparison and hence less degrees of freedom when doing the statistical analysis. This emphasises the importance of having enough cows in each experimental treatment group to be able to find a significant difference between the treatments.

3.3.1.5 Body weight and body condition score

The fact that the cows on the control treatment put on more condition than those on the high FM treatment could be because absorbed protein may induce mobilisation of body fat (Schor & Gagliostro, 2001). In the study of Schroeder & Gagliostro (2000) body fat mobilisation

was possibly enhanced by RUP feeding. There was no difference between treatments in changes in BCS or BW in the study by Jones-Endsley *et al.* (1997) where the amount of CP in the concentrate was increased or in the study by Hongerholt & Muller (1998) where the RUP in the concentrate was increased.

Due to the short duration of the experiment the effect of FM supplementation on long term factors such as reproductive efficiency could not be measured.

3.3.1.6 Faeces

The levels of starch in the faeces were much lower than those reported by Granzin (2004) who found faecal starch levels of 5.7 and 9.5 % DM for cows grazing annual ryegrass and prairie grass (PDMI 13.1 and 11.5 kg DM/cow/d) and receiving 4.5 and 8.1 kg barley-based concentrate, respectively, and faecal starch of 7.8 and 16.0 % DM for cows grazing the same pasture (PDMI 13.5 and 11.0 kg DM/cow/d) and receiving 4.5 and 8.1 kg maize-based concentrate, respectively.

Hagg, F. (personal communication, fhagg@kkan.com) found that in TMR fed cows on a 60 % concentrate diet (25 % starch) and a 70 % concentrate diet (30 % starch) the starch concentration of the faeces was 3.98 and 4.34 %, respectively. The ratio of % starch in faeces to % starch in feed was 0.16 and 0.15, respectively, compared to 0.04, 0.04 and 0.03 for the control, low FM and high FM treatments of this trial, respectively. Thus it is clear that starch was digested efficiently and extensively in all three of the experimental treatments.

3.3.1.7 Economics

In order to determine if the inclusion of FM in the supplement would be economical (increase profit) the additional revenue from the milk response would have to be greater than the additional cost since FM is an expensive protein source.

In the following example the low FM treatment will be compared to the control since there was no additional response to the higher FM level.

Replacing 280 g maize (at R1990/ton) with 240g FM (at R6369/ton) and 40 g Megalac (at R5468/ton) would cost an additional R1.19/cow/d.

Since milk solids affect price the price the farmer receives for milk, a more direct comparison can be made if FCM is used rather than milk yield *per se*. The cows on the low FM treatment produced 3.7 kg 4 % FCM/d more than the cows on the control treatment. Assuming a milk price of R3.00/kg (for milk with 4 % fat) this would bring an extra income of R11.10/cow/d which would lead to an additional profit of R9.91/cow/d.

Even if the higher FM level was used and additional feed cost doubled (R2.38/cow/d) there would still be additional profit of R8.27/cow/d.

The amount of additional profit made would depend on the relative prices of milk, maize and FM. The maize price can vary a lot. If the farmer produces his own maize the price would be the lowest. In Table 3.34 the FM, Megalac and milk prices are kept constant at R6000/ton, R5500/ton and R3.00/kg, respectively. As the maize price increases the additional profit made from FM supplementation increases due the fact that replacing some of the maize with FM causes a smaller increase in feed cost than if the maize price were lower.

In addition to FCM response, the milk yield response is included in Tables 3.34 to 3.36 to show that increased profit can even be made if the farmer does not receive a higher price for milk with a higher fat content.

Table 3.34 Effect of changing maize price on additional profit made by replacing 280 g maize in the supplement with 240 g fishmeal (FM) and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing ryegrass, assuming a constant FM price of R6000/ton, Megalac price of R5500/ton and milk price of R3.00/kg

Maize price (R/ton)	Additional cost of low FM diet over control (R/cow/d)	Additional profit from 3.7 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 1.4 kg milk yield/cow/d response (R/cow/d)
500	1.52	9.58	2.68
1000	1.38	9.72	2.82
1500	1.24	9.86	2.96
2000	1.10	10.00	3.10
2500	0.96	10.14	3.24

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

In Table 3.35 the maize, Megalac and milk prices are kept constant at R2000/ton, R5500/ton and R3.00/kg, respectively. As the FM price increases the additional profit made

from FM supplementation decreases due the fact that replacing some of the maize with FM causes a greater increase in feed cost than if the FM price were lower.

Table 3.35 Effect of changing fishmeal (FM) price on additional profit made by replacing 280 g maize in the supplement with 240 g FM and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing ryegrass, assuming a constant maize price of R2000/ton, Megalac price of R5500/ton and milk price of R3.00/kg

FM price (R/ton)	Additional cost of low FM diet over control (R/cow/d)	Additional profit from 3.7 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 1.4 kg milk yield/cow/d response (R/cow/d)
4000	0.62	10.48	3.58
5000	0.86	10.24	3.34
6000	1.10	10.00	3.10
7000	1.34	9.76	2.86

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

In Table 3.36 the feed prices are kept constant and the effect of changing milk price on the profitability of FM supplementation examined. Even if the milk price is low (R1.80/kg) FM supplementation is profitable.

Table 3.36 Effect of changing milk price on additional profit made by replacing 280 g maize in the supplement with 240 g fishmeal (FM) and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing ryegrass, assuming constant maize, FM and Megalac prices of R2000, R6000 and R5500/ton, respectively

Milk price (R/kg)	Additional income from 3.7 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 3.7 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional income from 1.4 kg milk yield/cow/d response (R/cow/d)	Additional profit from 1.4 kg milk yield/cow/d response (R/cow/d)
1.80	6.66	5.56	2.52	1.42
2.00	7.40	6.30	2.80	1.70
2.20	8.14	7.04	3.08	1.98
2.40	8.88	7.78	3.36	2.26
2.60	9.62	8.52	3.64	2.54
2.80	10.36	9.26	3.92	2.82
3.00	11.10	10.00	4.20	3.10
3.20	11.84	10.74	4.48	3.38
3.40	12.58	11.48	4.76	3.66
3.60	13.32	12.22	5.04	3.94
3.80	14.06	12.96	5.32	4.22

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

It is clear that under any realistic price scenario in South Africa the milk response to FM supplementation is large enough for profit to be increased. This proves that increased profit is made by including FM in the maize-based supplement of high producing cows in early to mid lactation grazing ryegrass.

A major factor affecting the economics is the magnitude of the production response. This can vary depending on things like the genetic potential of the cow, level of milk production, stage of lactation, concentrate level and quality, and quantity of the pasture and season of the year (Dillon *et al.*, 1997; Muller & Fales, 1998; Walker *et al.*, 2001; Kennedy *et al.*, 2003).

3.3.2 Rumen study

3.3.2.1 Ruminal pH

The ruminal pH was not expected to differ between the treatments. Increasing the amount of CP in the concentrate or the pasture does not affect ruminal pH (Jones-Endsley *et al.*, 1997; Carruthers & Neil, 1997; Bargo *et al.*, 2003a). Schor & Gagliostro (2001) found no difference in ruminal pH of cows fed concentrate with SBM or BM (differing in RUP concentration), consistent with the lack of response in total VFA concentration, which is to be expected when protein sources of differing ruminal protein degradability are compared.

The mean daily ruminal pH values are higher than the mean ruminal pH of 5.89 reported by Bargo *et al.* (2002c) for a pasture concentrate system and lower than the 6.2 and 6.27 reported by Berzaghi *et al.* (1996) and Bargo *et al.* (2002a), respectively, for cows on pasture receiving a maize-based concentrate.

These results are consistent with those of Bargo *et al.* (2001) where differing the level and source of protein in the supplement did not affect the ruminal pH or its variation throughout the day.

The daily trend in ruminal pH is similar to those reported by Carruthers & Neil (1997), Carruthers *et al.* (1997), Graf *et al.* (2005) and Bargo & Muller (2005): the pH was highest in the morning and dropped as the day progressed. Similarly Bargo *et al.* (2001; 2002a; 2002c) found the highest rumen pH values after periods of lower grazing activity while the pH dropped after

the concentrate was fed and the cows started grazing, related to increased fermentable substrates being available after feeding concentrates and grazing pasture. Similar trends were found by Bargo *et al.* (2003b) where the pH in continuous culture, where only pasture or pasture and concentrate were fed, peaked before feeding and was lowest a couple of hours after feeding. In the trial of Williams *et al.* (2005) with cows on perennial ryegrass the rumen pH was below 6.0 during the day and was higher at night and there was a lag time between the onset of grazing and the starting of the drop in pH. The drop in pH as the day progresses could be due to the increasing sugar content of the grass. The total sugar content of herbage increases during the day with the highest concentrations shortly before sunset (Van Vuuren *et al.*, 1986).

The pH was below 6 from 1430 to 2200 h and from 1430 to 2230 h for the cows on the control and high FM treatments, respectively, in other words for seven and a half to eight hours of the day. Cows grazing perennial ryegrass had a rumen pH of less than 6.0 for at least 15 hours of the day in the trial of Williams *et al.* (2005). Carruthers *et al.* (1997) also found the ruminal pH of cows on spring pasture to be below 6 for much of the day and Graf *et al.* (2005) even found the ruminal pH of cows grazing full time to be below 5.8 for almost 2 hours of the day. Cows experience sub-clinical acidosis if the ruminal pH is below 5.8 (Graf *et al.*, 2005). The ruminal pH in this trial was, however, almost never below 5.8.

The low pH often measured for cows on high quality pasture could be due to the low effective fibre, rapidly fermentable NDF and low buffering capacity of the forage, and is associated with a high (90 to 120 mmol/L) VFA concentration (Carruthers *et al.*, 1997; Bargo *et al.*, 2001; Kolver & De Veth, 2002). The reduction in rumen pH on pasture diets results from VFA production rather than lactate (De Veth & Kolver, 2001).

It is generally accepted that the ideal ruminal fluid pH for fibre digestion is greater than 6.0 (Mould *et al.*, 1983; Williams *et al.*, 2005). Models such as CNCPS regard pH 6.2 as the critical value below which fibre digestion is impaired. The ruminal pH of cows fed high quality pasture is often below this (5.8 to 6.2) due to rapid digestion of high quality pasture (De Veth & Kolver, 2001). The threshold pH below which fibre digestibility of high quality pasture (in sole-pasture diets) is reduced was found by De Veth & Kolver (2001) to be 5.8, lower than the previously reported 6.0 and the 6.2 used by the CNCPS model for mixed forage-concentrate diets. Performance of cows consuming high quality pasture is not affected when the ruminal pH

decreases to 5.8 (Kolver & De Veth, 2002). Hence fibre digestion and performance should not have been impaired in this trial where the pH was not below 5.8.

3.3.2.2 Ruminal ammonia

The mean daily ruminal NH_3-N concentrations were within the range of 8.7 to 32.2 mg/dl reported by Bargo *et al.* (2003a) for cows on pasture-concentrate and well above the minimum recommended level of 5 mg/dl for maximum microbial protein synthesis (Satter & Slyter, 1974). Even the lowest value (5.93 mg/dl) was above this level. The mean ruminal NH_3-N concentration was 8.9 mg/dl in the study by Bargo *et al.* (2002a) where cows on pasture with 20 % CP were supplemented with a maize-based concentrate, as well as in the study of McCormick *et al.* (2001b) where cows grazing annual ryegrass were supplemented with maize-based concentrate. This is lower than the NH_3-N concentrations of 19.9 mg/dl when the CP of the pasture was 26% (Bargo *et al.*, 2002c) and 17.1 mg/dl for cows grazing fescue and receiving 6.4 kg/d of maize supplementation (Berzaghi *et al.*, 1996). The NH_3-N concentration in the trial of Bargo *et al.* (2002c) was highest in the month when the CP of the pasture was the highest (28.8 mg/dl; CP of pasture 29.5 % of DM) and lowest (10.3 mg/dl) when the CP of the pasture was 25.5 %, the latter being the closest to the situation in this trial. The high NH_3-N concentrations (>19 mg/dl) found by Bargo *et al.* (2001) reflect the high CP degradability of the pasture. These values are all well below 100 mg/dl where NH_3 toxicity could start occurring (Owens & Zinn, 1988).

The higher ruminal NH_3-N concentration for the cows on the high FM treatment is to be expected due to the higher CP content of the diet. Jones-Endsley *et al.* (1997) found the NH_3-N concentration to be 16.55 and 20.21 mg/dl when the supplements contained 12 and 16% CP, respectively. In the study of Carruthers *et al.* (1997) the NH_3-N concentration was higher for cows on an energy-protein concentrate than those on an energy concentrate. Bargo *et al.* (2001) found higher ruminal NH_3-N concentrations at 1200, 1500 and 2000 h ($P < 0.05$) for cows supplemented with high protein sunflower meal than cows supplemented with low protein sunflower meal or high protein feather meal (RUP) concentrates, likely related to the higher CP concentration of the former. In the study of Schor & Gagliostro (2001) where cows received iso-

nitrogenous concentrates with different protein degradability (BM vs. SBM) the ruminal NH_3-N concentrations were lower ($P < 0.04$) in the cows fed the diet of higher RUP (BM).

Despite the difference in ruminal NH_3-N concentrations between the two treatments there was no difference in MUN concentration of the milk (Table 3.12).

The daily trends in ruminal NH_3-N concentration were similar to the trials of Bargo *et al.* (2001) and Carruthers *et al.* (1997). Ruminal NH_3-N concentrations peaked in the late afternoon in the trial of Bargo & Muller (2005). The peaks were close to the 25.5 and 25.8 mg/dl found by Kolver *et al.* (1998a) and Bargo *et al.* (2002c) for cows on pasture-concentrate. The ruminal NH_3-N concentration rises and peaks after supplementation and the onset of grazing, reflecting the occurrence of ruminal proteolysis (Kolver *et al.*, 1998a; Bargo *et al.*, 2001; 2002c).

3.3.2.3 Volatile fatty acids

The mean daily total VFA concentrations were within the range of 90 to 151 mmol/L reported by Bargo *et al.* (2003a) for cows on pasture-concentrate and is in agreement with the results of other pasture-concentrate studies where the mean total VFA concentrations were 116 (Kolver *et al.*, 1998a; Schor & Gagliostro, 2001), 130 (Bargo *et al.*, 2002a), 141 (Bargo *et al.*, 2002c) and 148 mmol/L (Berzaghi *et al.*, 1996). Bargo *et al.* (2001) found no effect of differing level and source of protein on total VFA or molar proportions of individual VFA. Schor & Gagliostro (2001) found no difference in total ruminal VFA or molar proportions of individual VFA of cows receiving concentrate with SBM or BM (differing in RUP concentration). Broderick (1992) reported no difference in total VFA when cows were supplemented with FM vs. SBM (133.8 vs. 122.2 mmol/L).

Volatile fatty acid concentration follows the inverse pattern of that of pH (Bargo *et al.*, 2003b). Carruthers & Neil (1997) also found the VFA concentration to rise as the day progressed. Williams *et al.* (2005) found a logarithmic relationship between rumen fluid pH and total VFA concentration with the pH declining as the VFA concentration increased.

The molar proportions and concentrations of acetate, propionate and butyrate are within the expected range for highly digestible pasture (65 to 68 % acetate, 18 to 25 % propionate and 8 to 15 % butyrate; Doyle *et al.*, 2005).

The mean acetate concentration for cows on pasture concentrate was 67 mmol/L (57.9 mol/100 mol) in the study of Schor & Gagliostro (2001), 91.4 mmol/L (64.9 mol/100 mol) in the study of Bargo *et al.* (2002c), 85 mmol/L in the study of Bargo *et al.* (2002a) and 92.3 mmol/L (62.4 mol/100 mol) in the study of Berzaghi *et al.* (1996) where cows grazing fescue received 6.4kg/cow/d of maize supplementation. It was 64.4 and 63 mol/100 mol in the studies of Jones-Endsley *et al.* (1997) and McCormick *et al.* (2001b), respectively, and an average of 55.8 mol/100 mol in the study of Bargo *et al.* (2001).

In the study of Bargo *et al.* (2002c) the mean propionate concentration of cows on pasture-concentrate was 27.4 mmol/L (19.4 mol/100 mol), in the study of Bargo *et al.* (2002a) it was 26 mmol/L, in the study of Berzaghi *et al.* (1996) it was 28.2 mmol/L (19.1 mol/ 100 mol) and in the study of Schor & Gagliostro (2001) it was 28 mmol/L (24.6 mol/100 mol). It was 21 mol/100 mol in the study of Jones-Endsley *et al.* (1997) and an average of 23.5 mol/100 mol in the study of Bargo *et al.* (2001) and McCormick *et al.* (2001b).

The acetate: propionate ratios in this trial are well above the level of 2.2: 1 where milk starts to be depressed (Emery, 1976). Since the acetate: propionate ratio was not lower for the control cows, this does not help explain the low fat percentage in the control cows (section 3.2.1.4.1 b), Table 3.12). Other pasture-concentrate studies reported the mean acetate to propionate ratio to be 2.41 (Schor & Gagliostro, 2001), 2.42 (Bargo *et al.*, 2001), 3.3 (Berzaghi *et al.*, 1996; McCormick *et al.*, 2001b) and 3.35 (Bargo *et al.*, 2002a; 2002c). Broderick (1992) reported acetate: propionate ratios of 3.96 and 3.69 for receiving FM and SBM respectively, tending to be higher for the former (P = 0.011).

In the study by Bargo *et al.* (2002c) the mean butyrate concentration for cows on pasture-concentrate was 16.0 mmol/L (11.6 mol/100 mol); it was 15 mmol/L in the study of Bargo *et al.* (2002a), 15 mmol/L (13.1 mol/100 mol) in the study of Schor & Gagliostro (2001), 20 mmol/L (13.5 mol/100mol) in the study of Berzaghi *et al.* (1996) and 9.8 and 12 mol/100 mol in the studies of McCormick *et al.* (2001b) and Jones-Endsley *et al.* (1997), respectively.

The results are in agreement with the study of Broderick (1992) where molar proportions of acetate (65.4 vs. 65.1 mol/100 mol) and butyrate (11.8 vs. 11.2 mol/100 mol) did not differ between cows supplemented with FM vs. SBM while propionate was lower for cows supplemented with FM (16.6 vs. 17.7 mol/100 mol). Erasmus *et al.* (1994) found that BM (vs. sunflower meal) decreased the molar percentage of propionate.

Differences in diet composition, DMI and starch intake were probably too small to elicit an effect on rumen parameters. Furthermore the extent of natural variation that exists between cows would have masked any relatively small effects that the experimental treatments could have induced.

3.4 CONCLUSIONS

High producing, multiparous Jersey cows in early to mid lactation grazing annual ryegrass pasture while receiving 6 kg (as is) a day of maize-based supplement, respond to addition of FM in their supplement up to 240 g (as is) FM per day above which there is no additional response. The cows on the low FM and high FM treatments produced 18 and 19 % more 4 % FCM than the cows on the control (24.1 and 24.2 vs. 20.4 kg 4 % FCM/d). This response was probably due to increased RDP and RUP especially Met and Lys levels in the diet. Higher levels of FM were not beneficial as milk production was limited by ME to 24 kg of 4 % FCM/d.

The magnitude of the production response is great enough that under any realistic maize/ FM/ milk price scenario in South Africa, FM supplementation would increase profit.

It appears that there is not enough protein in maize to support maximum milk production. Additional protein needs to be included in the supplement, preferably of high quality (low degradability and good AA composition) to complement the highly degradable protein of the pasture.

Future research could look at other levels of FM, in the region of 240 g FM per day, to establish the optimal level. Research with larger breeds, such as Holsteins, would also be useful as the response might be different.

Chapter 4

MODELING OF THE RYEGRASS TRIAL

4.1 MATERIALS AND METHODS

The CPM Dairy nutrition model was developed, evaluated and validated with data mainly from TMR fed cows. It has been suggested that CPM Dairy predictions are less accurate on pasture-based systems and more validation is needed. It was therefore decided to use data from this study to evaluate the usefulness of the CPM Dairy model on pasture-based systems.

Milk yields of the cows on the control, low FM and high FM treatments were compared with what was predicted by the CPM Dairy model (Version 3.0.7a; Cornell University, Ithaca, NY, University of Pennsylvania, Philadelphia, PA; Willam H. Miner Agricultural Institute, Chazy, NY).

Predictions were based on the average cow of each treatment (Table 4.1). The same environmental and management inputs were used for each of the three treatments (Table 4.2).

Table 4.1 Animal inputs used in the CPM Dairy model for the cows on the ryegrass control, low FM and high FM treatments

Animal Input	Experimental treatment[1]		
	Control	Low FM	High FM
Lactation	4	4	4
Current age (mo)	67	67	67
First calving age (mo)	24	24	24
Calving interval (mo)	13	13	13
Current weight (kg)	349	363	348
Mature weight (kg)	349	363	348
Calf birth weight (kg)	25	25	25
Days pregnant	34	34	34
BCS	2.3	2.2	2.3
Production (kg)	20.5	21.9	22.1
Fat (%)	3.97	4.73	4.67
Days in milk	124	124	124
Crude Protein (%)	3.25	3.49	3.45

BCS – Body condition score
[1]Ryegrass pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)

The first calving age and calving interval were assumed and used to estimate the average age of the cows. Calf birth weight and days pregnant were also assumed. Relative humidity, wind speed and hours in sunlight were assumed. The value used for temperature was ½

minimum + ½ maximum temperatures (Tylutki, T., personal communication, tom-_tylutki@mac.com). The cows were on continuous grazing and would have been exposed to any storms. There was no mud on their coats. The model's default values were used for hair depth, time standing and body position changes. Although the distance walked depended on which area of the pasture the cows were grazing on and how many times a cow would walk to water, an average distance of 5000 m a day was used. It was assumed that this was all flat as the slope was gentle.

Table 4.2 Inputs used in the CPM Dairy model for environment and management variables for the cows in the ryegrass trial

Environment	
Current temperature (°C)	16
Current RH	85
Previous temperature (°C)	16
Previous RH	85
Wind speed (mps)	0
Hours in sunlight	12
Storm exposure	Yes
Min night temperature (°C)	11
Mud depth (cm)	0
Hair depth (tenths of cm)	0.63
Hair coat	No mud
Management	
Activity	Continuous grazing
Time standing (h/d)	18
Body position changes	6
Distance walked flat (m)	5000
Distance walked sloped (m)	0

Feeds from the 2005 AFGRI Animal Feeds CPM feed library (Cronjé, G., personal communication, gert.cronje@afgri.co.za), representing average South African raw materials, were used for maize, FM and molasses since raw materials making up the concentrates were not individually analysed. Megalac and the other smaller ingredients were obtained from the CPM feed library. The default values from the CPM library were used for AA of all the raw materials. The composition of these raw materials is shown in Table 4.3. The composition of the experimental concentrates based on these raw materials (Table 4.3) can be compared to the laboratory results of the concentrates used (Table 3.8). Soluble CP, ADIP, NDIP and lignin were

lower and NPN and EE were higher in the composite in Table 4.3 than what was found from the laboratory analyses done on the concentrate samples. Since the latter was based on one sample, the long term average was considered more realistic.

Feeds ControlConcR, LowFMConcR and HighFMConcR (Table 4.4) were created using CornGrainGrndFin from the CPM feed library and modifying the nutrients to results of the laboratory analyses (section 3.2.1.2). Soluble CP, NPN, ADIP, NDIP, lignin and EE were modified to be closer to what would be expected from these concentrates based on average South African raw materials. The AA concentration was converted from DM basis to % RUP based on the CP content (Table 3.8) and estimated degradability (Table 3.33).

Table 4.3 Chemical composition of the raw materials used in the experimental concentrates based on average South African raw materials and the experimental concentrates[1] based on these raw materials

Parameter	Concentration in raw material				Concentration in Experimental Concentrate		
	Maize	FM	Molasses	Megalac	Control[2]	Low FM[2]	High FM[2]
DM (%)	89	90.62	78	97	88	89	92
CP (% DM)	9.27	73.77	5.49	0	8.54	11.06	13.87
SolCP (% CP)	11	21	100	0	16.56	16.89	17.85
NPN (% SolCP)	70	84	92	0	68.93	69.03	71.54
ADIP (% CP)	5	1	0	0	4.44	4.25	4.20
NDIP (% CP)	15	24.93	0	0	13.31	13.61	14.36
ADF (% DM)	4	1	0	0	3.55	3.4	3.38
NDF (% DM)	13	20.6	0.5	0	11.54	11.76	12.37
peNDF (% NDF)	25	10	0	0	22.19	21.43	21.41
Lignin (% NDF)	2.22	0	0	0	1.97	1.87	1.83
Ash (% DM)	1.02	15.33	12.12	15.5	6.33	7.00	7.93
EE (% DM)	3.65	12.47	0.1	84.5	3.33	4.21	5.20
Ca (% DM)	0.13	3.77	0.95	9	1.04	1.24	1.49
P (% DM)	0.22	2.33	0.16	0	0.49	0.57	0.67
Met (% RUP)	1.12	2.84	0	0	0.99	1.07	1.13
Lys (% RUP)	1.65	7.13	0	0	1.46	1.46	2.15
Arg (% RUP)	1.82	7.19	0	0	1.62	1.62	2.14
Thr (% RUP)	2.80	4.17	0	0	2.49	2.49	2.67
Leu (% RUP)	10.73	7.01	0	0	9.52	9.52	9.23
Ile (% RUP)	2.69	4.53	0	0	2.39	2.39	2.61
Val (% RUP)	3.75	4.81	0	0	3.33	3.33	3.52
His (% RUP)	2.06	2.30	0	0	1.83	1.83	1.97
Phe (% RUP)	3.65	4.33	0	0	3.24	3.30	3.33

[1]Ingredient composition is shown in Table 3.1
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM

A feed Ryegrass was created using GrssP24Cp40Ndf6Lndf from the CPM feed library and inserting the values from Table 4.4 (based on laboratory analyses – see section 3.2.1.1.2). The model defaults values of GrssP24Cp40Ndf6Lndf were used for AA and nutrients not shown in this table as well as the rates of carbohydrate fermentation in the rumen and protein degradation. The analysed lignin content was higher than the average for ryegrass in the AFGRI Animal Feeds database (Cronjé, G., personal communication, gert.cronje@afgri.co.za). A value closer to the latter was used instead.

Table 4.4 Chemical composition of the feeds Ryegrass, ControlConcR, LowFMConcR and HighFMConcR used in the CPM Dairy model

Parameter	**Concentration in raw material**			
	Ryegrass	**ControlConcR[1]**	**LowFMConcR[2]**	**HighFMConcR[3]**
DM (%)	13.72	91.93	91.54	91.35
CP (% DM)	26.46	8.19	11.16	14.56
SolCP (% CP)	42.66	16	17	18
NPN (% SolCP)	47.12	69	70	71
ADIP (% CP)	4.96	4	4	4
NDIP (% CP)	24.22	13	13.5	14
ADF (% DM)	25.24	3.68	3.73	3.98
NDF (% DM)	45.86	11.22	11.77	12.74
Lignin (% NDF)	3	2	2	2
Ash (% DM)	13.16	6.83	8.78	9.62
EE (% DM)	3.22	3	4	5
Ca (% DM)	0.52	1.55	2.03	2.30
P (% DM)	0.41	0.56	0.75	0.87
Met (% RUP)	0.67	1.10	2.50	3.36
Lys (% RUP)	2.83	6.05	7.67	5.79
Arg (% RUP)	2.83	8.52	8.20	7.40
Thr (% RUP)	2.83	6.32	6.24	5.11
Leu (% RUP)	5.49	17.32	14.80	9.69
Ile (% RUP)	2.83	6.05	6.06	4.84
Val (% RUP)	3.83	8.80	7.67	6.46
His (% RUP)	1.00	4.40	3.57	3.09
Phe (% RUP)	3.50	8.25	6.60	5.11

DM – Dry matter; CP – Crude protein, Sol CP – Soluble CP; NPN – Non-protein N; ADIP – Acid detergent insoluble protein; NDIP – Neutral detergent insoluble protein; ADF – Acid detergent fibre; NDF – Neutral detergent fibre; peNDF – physically effective NDF; EE – Ether extract;
[1]Control concentrate (no fishmeal)
[2]Low fishmeal concentrate (4 % fishmeal)
[3]High fishmeal concentrate (8 % fishmeal)

Concentrate intake was set at 5.5 kg DM and the pasture intake adjusted so that the actual and predicted total DMI were the same. Then the concentrate was replaced with the individual raw materials (from Table 4.3) in the correct proportions in order to determine if it is necessary to use each individual raw material or if it would be adequate to only analyse the concentrate and use that as a raw material.

4.2 RESULTS

Table 4.5 shows the predictions of the CPM Dairy model (Version 3.0.7a) for the cows on the control, low FM and high FM treatments based on the concentrates from Table 4.4. When the concentrate was replaced with the individual raw materials (from Table 4.3) the model predictions were as shown in Table 4.6.

Table 4.5 The CPM Dairy model predicted outputs from the control, low FM and high FM diets[1] in the ryegrass trial with the analysed concentrates used as raw materials

Parameter	Control	Low FM	High FM
Target Milk (kg/d)	20.5	21.9	22.1
ME allowed milk (kg/d)	20.1	20.2	19.9
MP allowed milk (kg/d)	23.5	25.0	25.6
AA allowed milk (kg/d)	21.5	24.2	22.9
DMI predicted (kg/d)	12.6	14.1	13.8
DMI actual (kg/d)	12.6	14.1	13.8
Pasture DMI (kg/d)	7.1	8.6	8.3
Diet RDP (% CP)	69.1	67.3	66.1
MP from bacteria (g/d)	925	949	849
MP from RUP	550	734	800
Diet CP (% DM)	18.5	20.5	21.7
Diet ME (MJ/kg DM)	11.66	11.36	11.37
Days to lose 1 CS	670	153[2]	116[3]
Weight change due to reserves (kg/d)	-0.08	-0.34	-0.43
Predicted MUN (mg %)	10	13	15

ME – Metabolisable energy; MP – Metabolisable protein; DMI – Dry matter intake; RDP – Rumen-degradable protein; CP – Crude protein; RUP – Rumen-undegradable protein; MUN – Milk urea N; CS – Condition score
[1]Ryegrass pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)
[2]Or decrease milk production -1 kg/d
[3]Or decrease milk production -2 kg/d

Table 4.6 The CPM Dairy model predicted outputs from the control, low FM and high FM diets[1] in the ryegrass trial with individual raw materials used to make up the concentrates

Parameter	Control	Low FM	High FM
Target Milk (kg/d)	20.5	21.9	22.1
ME allowed milk (kg/d)	20.3	20.8	20.8
MP allowed milk (kg/d)	24.0	26.4	27.7
AA allowed milk (kg/d)	20.7	22.4	23.5
DMI predicted (kg/d)	12.6	14.1	13.8
DMI actual (kg/d)	12.6	14.1	13.8
Pasture DMI (kg/d)	7.1	8.6	8.3
Diet RDP (% CP)	68.7	65.9	63.8
MP from bacteria (g/d)	937	979	938
MP from RUP (g/d)	561	770	853
Diet CP (% DM)	18.7	20.5	21.3
Diet ME (MJ/kg DM)	11.76	11.58	11.67
Days to lose 1 CS	1653	243[2]	191[2]
Weight change due to reserves (kg/d)	-0.03	-0.21	-0.25
Predicted MUN (mg %)	10	13	14

ME – Metabolisable energy; MP – Metabolisable protein; DMI – Dry matter intake; RDP – Rumen-degradable protein; CP – Crude protein; RUP – Rumen-undegradable protein; MUN – Milk urea N; CS – Condition score
[1]Ryegrass pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)
[2]Or decrease milk production -1 kg/d

4.3 DISCUSSION

The results shown in Tables 4.5 are similar to those in Table 4.6, indicating that a concentrate can be used as a raw material for modelling purposes as long as the concentrate is accurately analysed. The AA allowed milk production is higher is Table 4.5 than 4.6 for the control and low FM diets as the analysed AA concentration of these concentrates is higher than expected from the long term average raw material composition. The inconsistent trend in AA allowed milk in Table 4.5 could be due to inaccurate AA analyses. The rest of this discussion will be based on modelling with individual raw materials rather than composite concentrates.

The model predicted that AA were more limiting to milk production than MP strengthening the argument that the response was likely due to an increase in AA rather than CP *per se* (see section 3.3.1.4). Metabolisable energy was predicted to be limiting in all three diets and in the two fishmeal scenarios was lower than the actual production achieved.

The model predicted the cows to be in a negative energy balance, using body reserves and losing condition, especially those on the two FM treatments. However the BW of the cows increased during the trial and there was no decrease in BCS observed in the short duration of the trial (Table 3.23).

It appears that the model over-predicts the energy requirement for the activity of grazing cows. If the daily distance walked is changed from 5000 to 1000 m then the ME allowed milk production for the cows on the control, low FM and high FM treatments is 21.5, 21.9 and 21.9 kg/d, respectively. If this were the case then the model predicts that milk production of the cows on the control treatment was limited by AA, and the two FM treatments limited by ME.

Alternatively the model under-predicts DMI. If pasture DMI is adjusted until milk production observed is equal to ME, MP or AA allowable milk production (whichever is lowest) in other words ME allowable milk is equal to observed milk production (20.5, 21.9 and 22.1 kg/d for the three treatments, respectively) then the pasture DMI would have been 7.2, 9.3 and 9.1 kg/cow/day for the cows on the control, low FM and high FM treatments, respectively. The DMI actual is then higher than DMI predicted. The average PDMI of the three groups (8.5 kg/cow/day) is close to that calculated in section 3.2.1.1.1 c).

This indicates that ME was limiting in all three treatments and that the response to the FM treatments could have been due to higher ME intake from the additional pasture consumed. The higher pasture intake would have been driven by the higher milk production of these cows (cows appear to consume feed to meet their energy needs (NRC, 2001)). The higher milk production must have been driven by the FM in the concentrates as discussed in section 3.3.1.4.

The predicted dietary RDP concentration is close to that calculated and shown in Table 3.33.

Predicted MUN is lower than what was observed (Table 3.14).

Kolver *et al.* (1998b) found that the predicted milk production using the CNCPS model was particularly sensitive to changes in pasture lignin content, effective fibre, rate of fibre digestion and AA composition of ruminal microbes. The fact that the lignin value of the pasture was adjusted from the analysed value to be closer to the long term average alleviated some of the model predicted shortage in ME.

The model can be used to estimate under what circumstances milk production is limited by AA. For example the milk production of the cows on the control treatment is limited by AA if

the distance walked is less than 4000 m a day, in other words the cows have adequate energy. The greater the distance walked by the cows the more ME becomes limiting making it less likely for AA to be limiting production. Also the lower the pasture intake the more ME limits production.

The model can also predict the effect of changes in pasture composition. For example if the CP of the pasture is lower and the NDF higher (all other factors staying the same) both the ME and AA allowed production drop, the latter more so as MP from RUP decreases. If the CP of the pasture is higher and the NDF lower the ME and AA allowed milk remain similar. Predicted MP from bacteria decreases while MP from RUP increases. This indicates that cows are more likely to respond to AA supplementation if the pasture quality is poorer, provided pasture intake remains high (which is unlikely since DMI tends to decline as NDF increases (NRC, 2001)).

According to the model AA still limit production if the cow is in first lactation. On the other hand if the cow is at the end of her lactation and well into her gestation period both ME and AA allowed milk decrease, the former more so, ME becoming the first limiting nutrient. If the cow inputs are changed to represent a bigger (e.g. Holstein) cow, the DMI increases and so do ME and AA allowed milk production. Amino acids still limit production if the milk fat content is below 3.0. If the milk fat is higher then ME becomes limiting.

4.4 CONCLUSION

Apart from possibly under predicting DMI or over-predicting the amount of energy required for grazing activity the CPM Dairy model can predict milk production to within 0.5 kg/d of that actually observed. The model is useful for predicting pasture DMI as well as for predicting under what circumstances AA vs. ME limit milk production. Cows on ryegrass are most likely to respond to AA supplementation in early to mid lactation, if the pasture quality has lower CP and higher NDF and if the distance walked is not too high.

Chapter 5

FISHMEAL SUPPLEMENTATION TO HIGH PRODUCING JERSEY COWS GRAZING KIKUYU PASTURE

5.1 MATERIALS AND METHODS

5.1.1 Location and duration of the project

This trial was conducted on the same area of land as the first trial (section 3.1.1). The average daily maximum and minimum temperatures during the experimental period of the trial were 25 and 16°C, respectively. See appendix A for more details on the climate during the trial as well as the soil on which the pasture was grown.

With the change of season (summer) the pasture had changed to being dominated by kikuyu (*Pennisetum clandestinum*).

This trial took place from 19 January to 20 March 2006. The selection of the cows was done on 16 January 2006 and they were weighed and condition scored on 19 and 20 January and 16 and 17 March 2006. The cows were on the experimental treatments from 20 January to 20 March 2006 but measurements were only taken from 30 January 2006 after an adaptation period.

Period A of the rumen study took place from 20 January to 17 February 2006 and period B from 18 February to 20 March 2006.

5.1.2 Production study

5.1.2.1 Cows and experimental treatments

5.1.2.1.1 Cows

Forty two high producing multiparous Jersey cows [BW, 363 ± 29.2 kg; milk yield, 22.0 ± 1.35 kg/d; parity, 4.2 ± 1.59; days into lactation, 65 ± 21.7; (mean ± SD)] from the Outeniqua Experimental Farm were used. The average milk production of the herd of 345 cows in milk from which the cows were selected was 16.7 kg/d in January 2006.

A randomised complete block design was used. Just before the experimental period (16 January 2006) the cows were blocked according to milk production (of the previous 21 days) and days into lactation, and within each block were randomly divided into three groups. These three

groups were randomly allocated to the three experimental treatments. See appendix B for details on the selection and grouping of the cows.

The mean milk production of the cows in the three experimental groups (control, low FM and high FM) were 21.9 ± 1.37, 21.9 ± 1.38 and 22.0 ± 1.39 kg/d respectively, at the beginning of the trial. The mean days into lactation on the day of selection of the cows (16 January 2006) was 65 ± 22.3, 64 ± 20.7 and 64 ± 23.6 days for the control, low FM and high FM groups, respectively, and the mean lactation number 4 ± 1.3, 5 ± 1.6 and 3 ± 1.3 respectively.

5.1.2.1.2 Management

The grazing, feeding and milking of the cows was the same as for the ryegrass trial (see section 3.1.2.1.2). The average PA was 13 kg DM/cow/d above 3 cm pasture height.

5.1.2.1.3 Experimental treatments

The three experimental treatments were the same as for the ryegrass trial (see section 3.1.2.1.3) except that the cows grazed kikuyu pasture instead of ryegrass.

The cows adapted to their new diets for 10 days before any samples or measurements were taken.

5.1.2.1 .4 Experimental diets

Table 5.1 shows the ingredients that were used in the three concentrates as well as the chemical composition of the three concentrates based on analyses done at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria). The same diet formulation as in the ryegrass trial was used. Any differences in nutrient composition were due to differences in the composition of the raw materials used at the time.

Table 5.2 shows the mean chemical composition of the kikuyu pasture grazed during the trial (see Table 5.4 in section 5.2.1.1.2 for the chemical composition of the kikuyu on a weekly basis).

Table 5.3 shows the composition of the total diets consumed by the cows based on an intake of 5.5 kg DM/cow/d of the concentrates with composition as shown in Table 5.1 and a mean intake of 6.8 kg DM/cow/d of kikuyu pasture with a mean composition as shown in Table 5.2. See section 5.2.1.1.1 for the estimation of the pasture intake.

Table 5.1 Ingredient and chemical composition of the concentrate pellets used in the kikuyu trial (n = 1)

Parameter	Experimental treatment		
	Control	**Low FM**	**High FM**
Ingredient composition, % DM			
Maize meal	88.75	84.1	78.5
Fishmeal (FM)	0	4.0	8
Megalac[1]	0	0.65	1.3
Molasses	6.8	6.8	6.8
MonoCaP	1.3	1.3	1.3
Feed lime	1.8	1.8	1.8
Salt	0.5	0.5	0.5
MgO	0.5	0.5	0.5
Premix[2]	0.35	0.35	0.35
Chemical composition			
DM %	92.4	91.4	91.5
ME MJ/kg DM	13.6	13.8	13.6
% DM			
OM%	94.0	92.1	91.4
CP%	7.7	10.1	12.7
NDF%	13.9	14.9	17.5
ADF%	3.6	3.4	3.6
IVOMD%	95.8	95.8	94.1
Ca %	1.23	1.53	2.02
P %	0.53	0.63	0.81
Ca: P	2.30	2.43	2.48

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility
[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Premix (Lactating Cow (Organic); DSM Nutritional Products South Africa (Pty) Ltd.) contained 7.23 % Mn, 7.50 % Zn, 1.83 % Cu, 0.11 % Co, 0.14 % I, 0.03 % Se (1 %), 1.28 % organic Mn, 2.00 % organic Zn, 0.32 % organic Cu, 0.01 % organic Se, 5 % Rumensin (20 %), 3.5 % Stafac 500 and provided 96,250 IU of vitamin A, 28,875 IU of vitamin D3, and 577.5 mg of vitamin E/cow/d

Table 5.2 Chemical composition (mean ± SD) of the kikuyu pasture grazed by the cows during the kikuyu trial

Nutrient	Mean composition
DM (%)	15.7 ± 2.62[1]
ME (MJ/kg DM)	10.0 ± 0.28[2]
OM (%DM)	88.2 ± 1.58[1]
CP (% DM)	22.1 ± 3.07[1]
NDF(%DM)	60.3 ± 4.51[1]
ADF(%DM)	30.5 ± 3.50[1]
IVOMD (% DM)	69.9 ± 4.53[1]
Ca (% DM)	0.37 ± 0.032[2]
P (% DM)	0.35 ± 0.027[2]
Ca: P	1.08 ± 0.054[2]

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility
[1]n = 8, [2]n =

Table 5.3 Mean chemical composition of the total diets (6.8 kg kikuyu DM and 5.5 kg supplement DM/cow/d) consumed by the cows in the kikuyu trial

Nutrient	Experimental treatment[1]		
	Control	Low FM	High FM
ME (MJ/kg DM)	11.6	11.7	11.6
OM (%DM)	90.8	89.9	89.6
CP (% DM)	15.6	16.7	17.9
NDF(%DM)	39.5	40.0	41.1
ADF(%DM)	18.4	18.3	18.4
IVOMD	81.5	81.4	80.7
Ca (% DM)	0.76	0.90	1.11
P (%DM)	0.43	0.47	0.56
Ca: P	1.76	1.88	1.99

DM – Dry matter; ME – Metabolisable energy; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility
[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; Hhigh FM: maize-based supplement containing 8 % FM

5.1.2.2 Experimental measures and sample analyses

5.1.2.2.1 Pasture

a) Calibration of the rising plate meter

The same method for calibration of the RPM as described in section 3.1.2.2.1 a) was used. The data from the eight weeks from 23 January to 29 March 2006 were composited. The average equation obtained was Y = 54H + 764 (R^2 = 0.4; n = 72).

Since this was done during the experiment the equation could only be used afterwards to estimate what the pasture allowance and intake of the cows was. For the purpose of pasture allocation during the trial the standard equation for the area and time of year, Y = 60H (Meeske, R., personal communication, robinm@elsenburg.com), was used. The cows were allocated approximately 11.5 kg DM/cow/d with fresh grazing being available after every milking.

b) Estimating pasture allowance and intake using the rising plate meter

The same method as described in section 3.1.2.2.1 b) was used.

Estimation of the pasture intake of the three experimental treatment groups separately was not done in the kikuyu trial as it was felt, after looking at the results of the ryegrass trial, that the

RPM was not accurate enough to measure any difference in intake between the three groups in only a few days. The three groups were rather allocated the same pasture.

c) Estimating pasture intake using equations and the CPM Dairy model

The same equations as in section 3.1.2.2.1 d) were used. The average intake of the three treatment groups was also estimated with the CPM Dairy model as in section 3.1.2.2.1 e).

d) Kikuyu pasture samples

Once a week (total of eight times) a sample of the kikuyu pasture was taken. These samples were taken every Monday from 23 January to 13 March 2006 at approximately midday. The samples were taken, dried and milled as described in section 3.1.2.2.1 f) and stored in airtight containers to be analysed at Nutrilab, University of Pretoria. Some of the results of these analyses are given in Table 5.2.

5.1.2.2.2 Concentrate samples

Once every week (every Monday from 23 January to 13 March 2006) samples of the concentrate pellets were taken. These were dried, milled and composited as described in section 3.1.2.2.2 and stored in airtight containers to be analysed at Nutrilab. Some of the results of these analyses are given in Table 5.1.

5.1.2.2.3 Milk production and composition

The daily milk production of the cows was measured and recorded in the milking parlour as in section 3.1.2.2.3. The mean milk production for the experimental period (30 January to 20 March 2006) was calculated.

Composite milk samples (ratio 9ml: 15ml afternoon: morning milking) were taken on a). 31 January/ 1 February, b) 14/15 February, c) 28 February/ 1 March and d) 14/15 March 2006. These were preserved with bronopol and then analysed for milk fat, protein, lactose and MUN at Lactolab Pty (ltd) (ARC, Main rd. Irene, 0062) using the Milkoscan FT 6000 (Foss Electric, Denmark). In addition to this, composite milk samples from a) 21/22 February, b) 28 February/ 1 March and c) 14/15 March were sent to the Elsenburg ARC Dairy Laboratory (P.O. Box 65, Elsenburg, 7607) to be analysed for fat, protein and lactose. Milk samples of the whole herd were taken during only afternoon milkings on 30 January and 6 March and analysed at Lactolab

for fat, protein, lactose and MUN. The results for the cows in the trial were used, along with all the results of the composite milk samples that were taken on the above dates, and the overall mean milk composition for each cow during the experimental period calculated.

5.1.2.2.4 Body weight and body condition score

The cows were weighed just before milking on two consecutive days at both the beginning and end of the trial. This was done on 19 and 20 January and 16 and 17 March 2006. The cows were weighed twice and the average BW between these two days was used for analysis.

On the first of these two consecutive days the BCS of the cows was also determined as described in section 3.1.2.2.4.

5.1.2.2.5 Faecal samples

Faecal rectal samples were taken from the cows of three randomly chosen blocks: block 2 (cows KC2, KL2 and KH2), block 3 (cows KC3, KL3 and KH3) and block 11 (cows KC11, KL11 and KH11). Samples were taken three times at two week intervals on days 21, 34 and 49 (9 and 22 February and 9 March 2006) and composited so that in the end there was one sample per cow. These were analysed for starch at Nutrilab as an indication of rumen health and efficiency of rumen fermentation.

5.1.2.2.6 Laboratory analyses

The kikuyu pasture, concentrate, FM and faecal samples were analysed as described in section 3.1.2.2.6.

5.1.3.2.7 Soil and climate

The minimum and maximum temperatures during the experiment as well as the rainfall were measured daily at a weather station on the same experimental farm. A tensiometer was used to monitor the moisture content of the soil and irrigation applied when necessary.

Soil samples were taken from all the strips and composited into one sample and sent to Elsenburg Production Technology Laboratory (Department Agriculture, Western Cape, Private Bag X1, Elsenburg, 7607) to be analysed.

5.1.2.3 Statistical analyses

The same statistical analyses as in section 3.1.2.3 were done.

5.1.3 Rumen study

5.1.3.1 Cows and experimental treatments

5.1.3.1.1 Cows and management

Eight Jersey cows fitted with ruminal cannulae from the Outeniqua Experimental Farm, George, were used. The same cows as in the ryegrass trial were used except two cows, Ru6 and Ru7, were no longer lactating and had to be replaced with two other cannulated cows: Ru9 and Ru10.

These cows grazed with the cows of the production study as in the ryegrass trial.

A cross-over design was used as described in section 3.1.3.1.1.

5.1.3.1.2 Experimental treatments

a) Period A

Four of these cows (Ru1, Ru5, Ru8 and Ru10), chosen at random, received the control treatment and four of them (Ru2, Ru3, Ru4 and Ru9) received the high FM treatment.

The cows were allowed to adapt to the diet from days 1 to 14 of the trial (20 January to 2 February 2006).

On days 15 to 25 (3 to 13 February 2006) the cows were fitted with automated pH meters with data loggers so that the ruminal pH at 10 minute intervals throughout the day, for a total of five days per cow, could be monitored, as in the ryegrass trial.

On days 27 and 28 (15 and 16 February), samples of rumen fluid were taken to be analysed for NH_3-N, VFA and pH. The samples were taken at 0400, 1200 and 2000 h on 15 February and 0800, 1600 and 0000 h (12 midnight) on 16 February 2006.

b) Period B

On day 29 (17 February 2006) the cows were switched to the opposite experimental treatment (i.e. those that were on the control treatment moved to the high FM treatment and vice versa) so that cows Ru2, Ru3, Ru4 and Ru9 received the control treatment and cows Ru1, Ru5, Ru8 and Ru10 received the high FM treatment.

The cows adapted to their new diets from days 30 to 41 (18 February to 1 March 2006).

On days 42 to 50 (2 to 10 March 2006) the cows were fitted with automated pH meters with data loggers to monitor the pH throughout the day for a total of four days per cow.

On days 53 to 55 (13 to 14 March 2006), samples of rumen fluid were taken to be analysed for NH_3-N, VFA and pH. The samples were taken at 0000 h (12 midnight) on 13 March, at 0800 and 1600 h on 14 March and at 0400, 1200 and 2000 h on 15 March 2006.

5.1.3.2 Experimental measures and sample analyses

The rumen pH was measured and rumen samples taken, preserved and analysed as described in section 3.1.3.2.

The same analytical methods as described in section 3.1.3.2.1 were used to analyse the rumen samples for NH_3-N and VFA.

5.1.3.3 Statistical analyses

The same statistical procedures were followed as described in section 3.1.3.3.

5.2 RESULTS

5.2.1 Production study

5.2.1.1 Pasture

5.2.1.1.1 Pasture allowance and intake

a) Pasture allowance and intake estimated using the rising plate meter

The mean RPM reading (in half centimetre increments) for the duration of the trial (19 January to 20 March 2006) was 52 (± 8.9) before grazing and 22 (± 3.9) after grazing. Using the standard calibration equation Y = 60H, it was calculated that there was, on average, 3118 (± 532.7) kg pasture DM available/ha before grazing and 1322 (± 235.9) kg pasture DM/ha left after grazing. Thus the cows removed 1796 kg DM/ha off the pasture.

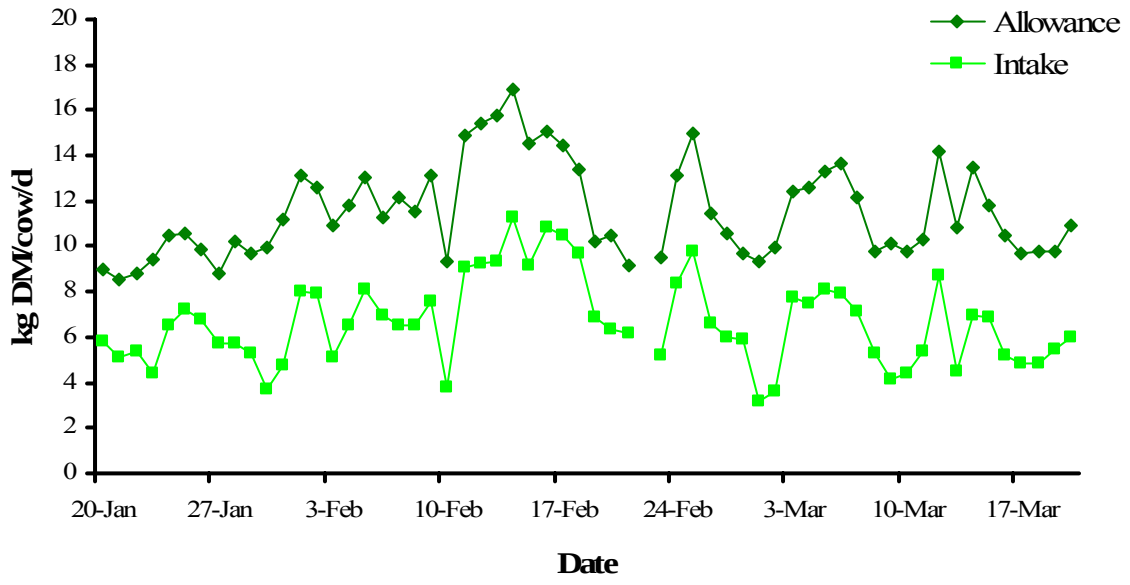

Figure 5.1 Kikuyu pasture allowance and intake estimated with a rising plate meter (RPM) based on the calibration equation Y = 60 H where Y is pasture yield (kg DM/ha) and H is the average RPM reading

Due to the higher pasture DM available/ha, the cows were allocated a smaller area per grazing than in the ryegrass trial. The PA (Figure 5.1) fluctuated based on DM availability and management constraints. The mean PA was 11.5 (± 1.68) kg DM/cow/d and the mean PDMI was 6.6 (± 1.67) kg DM/cow/d.

For kikuyu different calibration equations might be needed for different parts of the season (Fulkerson & Slack, 1993; Reeves *et al*., 1996). At the end of the trial the regression equations that had been obtained for the kikuyu that was grazed during the trial were Y = 49 H + 899 (R^2 = 0.4; n = 36) for January and the first half of February, Y = 58 H + 605 (R^2 = 0.4; n = 36) for the second half of February and March. These two equations yielded similar DM yield to the combined equation of Y = 54H + 764 (R^2 = 0.4; n = 72) for the whole experimental period (Figure 5.2). The latter equation was applied to the same RPM readings as with the above. It was found that there was actually on average 3571 (± 479.4) kg pasture DM/ha before grazing and 1954 (± 212.3) kg pasture DM/ha after grazing. Thus the cows removed 1617 kg DM/ha off the pasture.

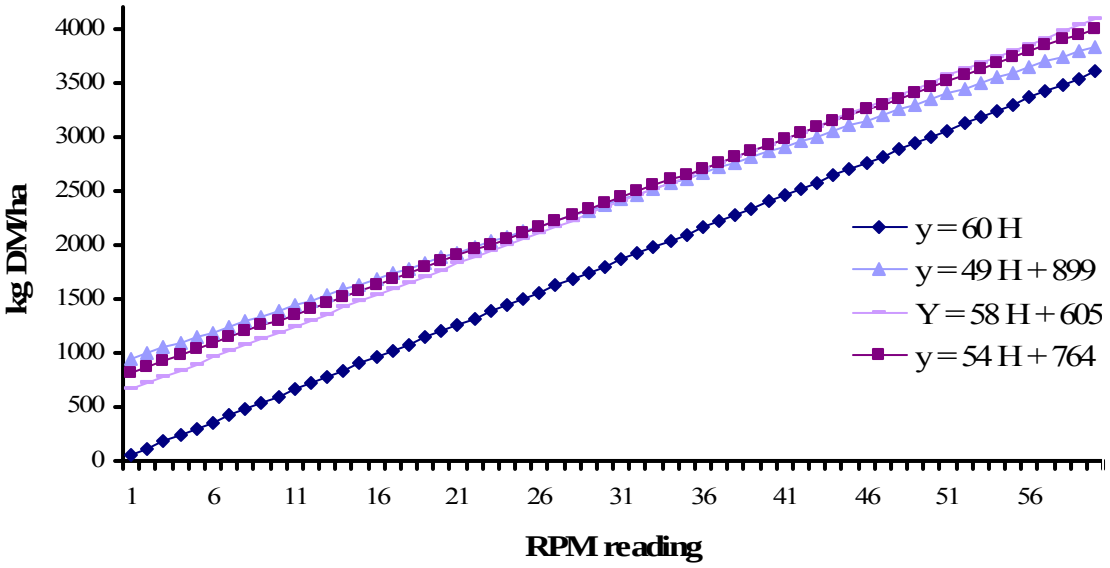

Figure 5.2 Relationship between rising plate meter (RPM) reading and pasture yield (kg DM/ha) with the standard calibration equation Y = 60 H and the equations obtained during the trial: Y = 49 H + 899 (R^2 = 0.4; n = 36) for January and the first half of February, Y = 58 H + 605 (R^2 = 0.4; n = 36) for the second half of February and March and Y = 54 H + 764 (R^2 = 0.4; n = 72) for the whole duration of the trial

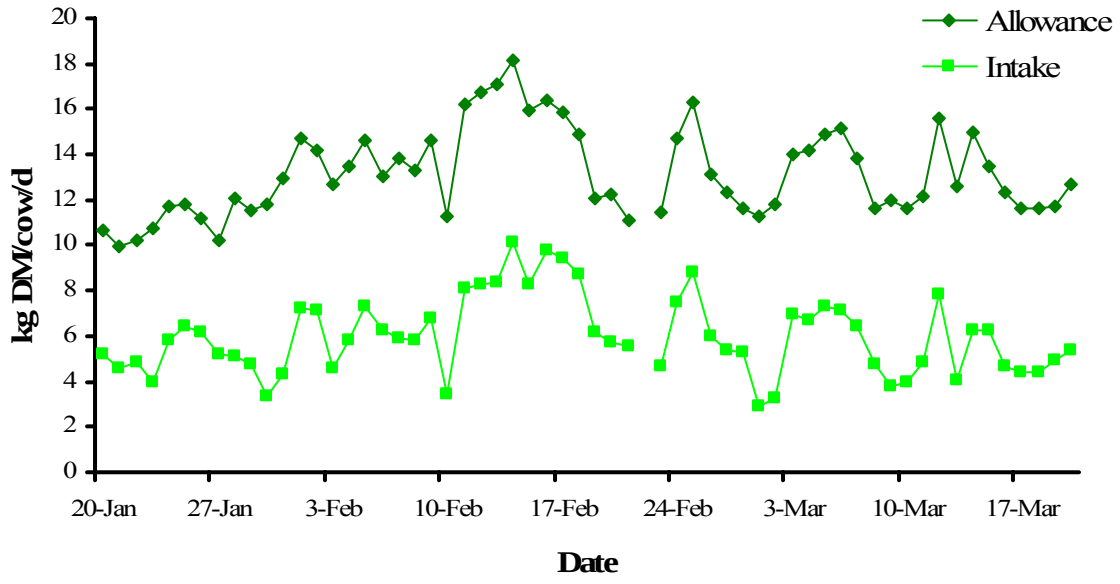

Figure 5.3 Kikuyu pasture allowance and intake estimated with a rising plate meter (RPM) based on the calibration equation Y = 54 H + 764 where Y is pasture yield (kg DM/ha) and H is the average RPM reading

The mean PA was 13.2 (± 1.51) kg DM/cow/d and the mean PDMI was 6.0 (± 1.51) kg DM/cow/d. The cows went through approximately two grazing cycles during the experiment and the average growth rate of the pasture between the two grazing cycles was 52 kg DM/ha.

b) Estimation of pasture intake using equations

The average BWs of all 60 cows at the beginning and end of the trial were 367 and 377 kg, respectively. Thus the average BW during the trial would have been 372 kg with a mean increase in BW of 0.18 kg/d.

Once again the various equations for predicting pasture intake (see section 2.6.3.3 above) were used and the results compared.

If the cows were to consume 1.3 % of 372 kg it is expected that each cow would have been able to consume 4.8 kg NDF/d. The mean NDF concentration of the pasture was 60.3 % (Table 5.2), the NDF of the concentrate 15.4 % (Table 5.1) and the concentrate intake 5.5 kg DM/d. Pasture DMI was calculated as follows: 60.3% of PDMI + 15.4 % of 5.5 = 4.8, therefore PDMI = 6.6 kg.

If the cows were consuming pasture only they would have been able to consume 1.5 % of 372 kg or 5.6 kg NDF/d. Since the mean NDF percentage of the kikuyu was 60.3 %, this would have been 9.3 kg of pasture DM/d. Since each cow was receiving 5.5 kg concentrate DM the SR was assumed to be 0.51 (0.093 x 5.5; Faverdin, *et al*., 1991) thus pasture intake would have dropped by 2.8 kg (0.51 x 5.5) to 6.5 kg pasture DM/d. This is similar to the 6.6 kg calculated above. The average of the two would be 6.6 kg kikuyu DM/cow /d.

The method used by Tesfa *et al.* (2005; see section 2.6.3.3 above) for estimating pasture intake, based on the energy requirements of the cow vs. energy from the diet, was also used for comparison. The mean estimated ME concentration of the kikuyu pasture was 10.0 MJ/kg DM (see section 5.2.1.1.2 below). For the levels of production obtained in the trial the mean ME requirement of the cows on the two FM treatments was 151.9 MJ ME/d (see Appendix C). If these cows consumed 5.5 kg concentrate with a mean ME concentration of 13.7 MJ ME/kg DM (section 5.2.1.2), 75.4 MJ ME/d would have been supplied by the concentrate. The remaining 76.5 MJ ME would have been supplied by the pasture. For this to be the case the cows would have had to consume 7.7 kg kikuyu DM/d. It is possible that the cows selected more nutritious material, rejecting the stalks as is common with kikuyu (Fulkerson & Slack, 1993), making this an overestimation of intake as the samples taken might have had a lower nutritious value than the material actually consumed by the cows.

The equation of Vazquez & Smith (2000) predicted the PDMI to be 4.2 kg/cow/d a clear underestimation. The NRC (2001) equation predicted the PDMI to be 9.2 kg/cow/d which appears to be an overestimation.

Once again the question is which value is correct? The RPM predicted 6.0 kg, the NDF as % BW method predicted 6.6 kg and the energy balance method predicted 7.7 kg. A safe assumption is to take the average of the three: 6.8 kg. This value was used in subsequent sections as the assumed pasture intake for calculating the nutrient composition of the total diet. This value is close to that predicted by the method of NDF as % BW, which was also the most accurate method for estimating ryegrass intake.

c) Estimation of pasture intake using the CPM model

The CPM Dairy model (see chapter 6) predicted the DMI of the cows on the control, low FM and high FM treatments to be 12.0, 12.8 and 12.3 kg DM/cow/d hence pasture intake of the

cows on the three treatments would have been 6.5, 7.3 and 6.8 kg DM/cow/d, respectively. The average of these three is 6.9, close to that calculated in section 5.2.1.1.1 b).

5.2.1.1.2 Pasture composition

The chemical composition of the kikuyu pasture and how it changed over time is reported in Table 5.4. For a more extensive analyses the samples of every two or three weeks were composited (Table 5.5). Figure 5.4 presents the changes in CP, NDF and IVOMD over time.

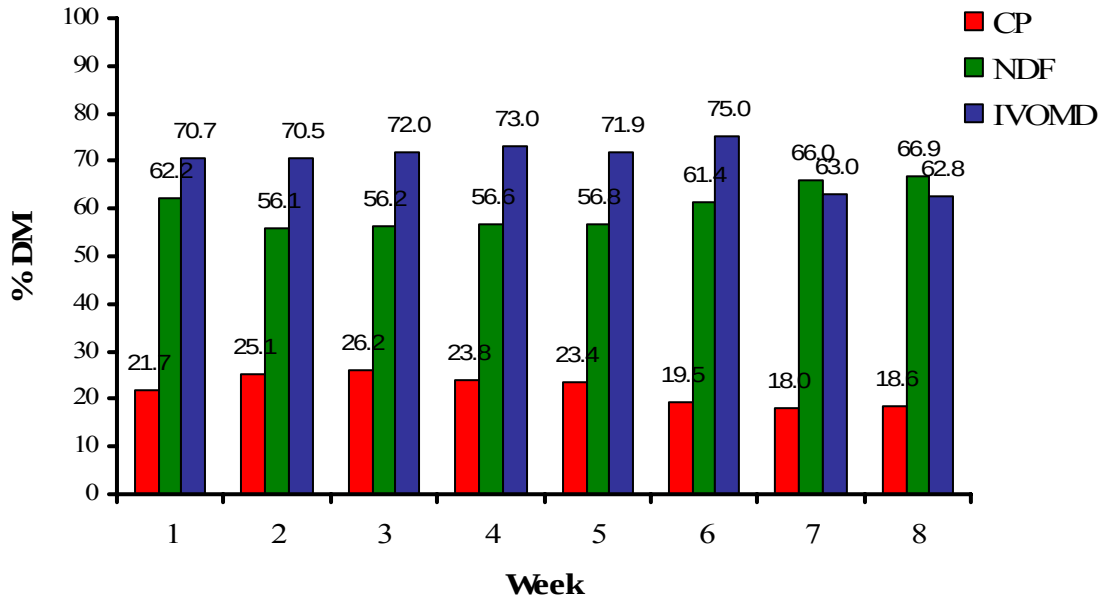

Figure 5.4 Crude protein (CP), neutral detergent fibre (NDF) and *In vitro* organic matter digestibility (IVOMD) on a weekly basis of the kikuyu pasture grazed during the trial. Week 1 = 23 January, week 8 = 13 March 2006

Table 5.4 Chemical composition on a weekly basis of the kikuyu pasture grazed during the trial

Parameter	Sampling date							
	23/01	**30/01**	**06/02**	**13/02**	**20/02**	**27/02**	**06/03**	**13/03**
DM %	14.6	14.7	12.7	15.2	17.2	12.6	19.7	18.6
OM (% DM)	88.6	86.7	87.0	87.1	86.9	89.1	89.2	91.2
CP (% DM)	21.8	25.1	26.2	23.8	23.4	19.5	18.0	18.6
NDF (% DM)	62.2	56.1	56.2	56.6	56.8	61.4	66.0	66.9
ADF (% DM)	29.6	26.1	27.3	29.2	28.4	32.6	35.8	34.7
IVOMD (% DM)	70.7	70.5	72.0	73.0	71.9	75.0	63.0	62.8

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility

Table 5.5 Chemical composition on a two- to three-weekly basis of the kikuyu pasture grazed during the trial

Parameter	Sampling dates		
	023/01 – 30/01	**06/02 – 20/02**	**27/20 – 13/03**
DM %[1]	14.6	15.0	16.9
Ash (% DM)	12.2	13.1	10.6
OM (% DM)[2]	87.8	86.9	89.9
CP (% DM)	23.6	24.6	18.5
NDF (% DM)	59.6	57.1	64.9
ADF (% DM)	31.4	30.6	32.0
IVOMD (% DM)[1]	71.0	72.1	66.8
GE (MJ/kg DM)	17.5	17.3	17.7
ME (MJ/kg DM)[3]	10.1	10.2	9.7
EE (% DM)	2.1	2.4	1.9
Ca (% DM)	0.34	0.40	0.39
P (% DM)	0.33	0.38	0.34
Ca: P	1.04	1.05	1.14
Lignin (% NDF)	9.9	10.7	9.6
NFC (% DM)[4]	3.0	3.7	4.4
Starch (% DM)	0.3	0.3	0.3
NDIP (% CP)	38.2	36.1	41.2
ADIP (% CP)	10.8	12.3	12.3
Sol CP (% CP)	40.8	42.4	40.9
NPN (% Sol CP)	53.5	52.1	43.8

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility; GE – Gross energy; ME – Metabolisable energy; EE – Ether extract; NFC – Non-fibre carbohydrates; NDIP – Neutral detergent insoluble protein; ADIP – Acid detergent insoluble protein; Sol CP – Soluble CP; NPN – Non-protein N

[1] Average for the three weeks was calculated as analysis on the composite sample was not done

[2] OM = 100 - ash

[3] ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

[4] NFC = 100 – (CP + NDF + EE + ash)

Table 5.6 Essential amino acid (AA) composition of the kikuyu pasture grazed during the trial (n = 3)

AA	Mean (g/100 g AA) ± SD
Met	1.68 ± 0.179
Lys	6.15 ± 0.419
Arg	5.99 ± 0.204
Thr	4.93 ± 0.334
Leu	8.27 ± 0.196
Ile	4.81 ± 0.170
Val	6.76 ± 0.123
His	2.12 ± 0.104
Phe	5.78 ± 0.084
Total EAA[1]	46.5
Total NEAA[2]	53.5

[1]Essential AA (EAA): Met, Lys, Arg, Thr, Leu, Ile, Val, His and Phe (Jones-Endsley *et al.*, 1997)

[2]Nonessential AA (NEAA): Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr (Jones-Endsley *et al.*, 1997)

The mean composition of EAA in the kikuyu, expressed as g/100 g AA, is reported in Table 5.6. The Lys and Met concentrations in the kikuyu pasture DM were 0.80 and 0.22 % DM, respectively.

5.2.1.2 Concentrate composition

The CP of the control, low FM and high FM concentrates were 7.7, 10.1 and 12.7 % DM, respectively (Table 5.7) and the CP concentration of the FM was 65.7 % DM (Table 5.8).

Table 5.7 Chemical composition of the control, low FM and high FM concentrate pellets fed in the kikuyu trial (n = 1)

Parameter	Experimental treatment[1]		
	Control	Low FM	High FM
DM %	92.4	91.4	91.5
Ash (%DM)	6.0	7.9	8.7
OM (%DM)	94.0	92.1	91.4
CP (%DM)	7.7	10.1	12.7
NDF (%DM)	13.9	14.9	17.5
ADF (%DM)	3.6	3.4	3.6
IVOMD (%DM)	95.8	95.8	94.1
GE (MJ/kg DM)	17.3	17.5	17.6
ME (MJ/kgDM[2]	13.6	13.8	13.6
EE (%DM)	2.3	2.7	3.0
Ca (%DM)	1.23	1.53	2.02
P (%DM)	0.53	0.63	0.81
Ca: P	2.30	2.43	2.48
Lignin (%NDF)	6.1	6.4	6.0
NFC (% DM)[3]	70.1	64.4	58.1
Starch (%DM)	59.4	54.6	52.3
NDIP (%CP)	19.3	30.0	39.3
ADIP (%CP)	23.9	22.1	20.7
Sol CP (%CP)	27.1	29.0	28.6
NPN (%Sol CP)	11.9	10.8	14.4

DM – Dry matter; OM – Organic matter; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; IVOMD – *In vitro* organic matter digestibility; GE – Gross energy; ME – Metabolisable energy; EE – Ether extract; NFC – Non-fibre carbohydrates; NDIP – Neutral detergent insoluble protein; ADIP – Acid detergent insoluble protein; Sol CP – Soluble CP; NPN – Non-protein N

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM

[2]ME = 0.82 x GE x IVOMD (Robinson *et al.*, 2004)

[3]NFC = 100 – (CP + NDF + EE + ash)

Table 5.8 Chemical composition of the fishmeal used in the concentrate pellets for the kikuyu trial (n = 1)

Parameter	Percentage
DM (%)	92.4
Ash (% DM)	22.9
OM (% DM)	77.1
CP (% DM)	65.7
EE (% DM)	10.4

DM – Dry matter; OM – Organic matter; CP – Crude protein; EE – Ether extract

Table 5.9 reports the EAA composition of the three concentrates expressed as g/100 g of AA. The Lys concentration in the concentrate DM was 0.17, 0.34 and 0.49 % DM and the Met concentration 0.10, 0.16 and 0.21 % DM for the control, low FM and high FM treatments, respectively. The increased levels of these two AA with increasing FM levels in the concentrate is to be expected since the Lys and Met concentration of the FM that was used was 4.63 and 1.34 % DM, respectively.

Table 5.9 Essential amino acid (AA) composition of the control, low FM and high FM concentrate pellets fed in the kikuyu trial (n = 1)

AA (g/100 g AA)	Experimental treatment[1]		
	Control	Low FM	High FM
Met	1.84	2.21	2.33
Lys	3.01	4.70	5.41
Arg	6.02	6.63	6.68
Thr	4.01	4.83	4.24
Leu	11.54	10.22	9.65
Ile	3.68	3.87	4.24
Val	5.52	5.52	5.73
His	2.34	2.76	2.76
Phe	4.85	4.56	4.77
Total EAA[2]	42.8	45.3	45.8
Total NEAA[3]	57.2	54.7	54.2

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Essential AA (EAA): Met, Lys, Arg, Thr, Leu, Ile, Val, His and Phe (Jones-Endsley *et al.*, 1997)
[3]Nonessential AA (NEAA): Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr (Jones-Endsley *et al.*, 1997)

5.2.1.3 Total diet composition

The cows each consumed 5.5 kg concentrate DM/d. If it is assumed that the kikuyu pasture intake was 6.8 kg/cow/d (section 5.2.1.1.1), the total diet composition would be as partly shown in Table 5.3.

The total diet of a cow consuming 6.8 kg kikuyu DM with AA composition as in Table 5.6, and 5.5 kg concentrate with AA composition as in Table 5.9, would contain 0.52 % Lys and 0.17 % Met (63 g Lys and 21 g Met/d) for the control treatment, 0.59 % Lys and 0.19 % Met (73 g Lys and 24 g Met/d) for the low FM treatment and 0.66 % Lys and 0.22 % Met (81 g Lys and 26 g Met/d) for the high FM treatment. The ratio of Lys to Met in the total diet was 3.1 for all three treatments, with the levels of both these AA increasing as the level of FM in the concentrate increased, and was close to the ideal ratio of 3.0 (NRC, 2001).

5.2.1.4 Milk production and composition

5.2.1.4.1 Mean for the whole experimental period

a) Milk yield

The mean daily milk production of the 14 cows on each treatment (Figure 5.5) decreased as the trial progressed, as did the average production of the whole herd. This is consistent with the autumn slump, or drop in milk yield after February, on kikuyu reported by Henning *et al.* (1995). As the production decreased, the magnitude of the difference between the treatments appeared to increase. The gap in Figure 5.5 from 19 to 23 February is due to missing data due to power cuts.

The mean milk production for the experimental period (30 January to 20 March 2006) is reported in Table 5.10. The mean milk yield of the cows on the control, low FM and high FM treatments were 18.2, 18.9 and 19.5 kg milk/cow/d, respectively. It was 7 % higher for the cows on the high FM treatments than the cows on the control treatment ($P < 0.05$). The milk yield of the cows on the low FM treatment did not differ from the control or the high FM treatments ($P > 0.1$).

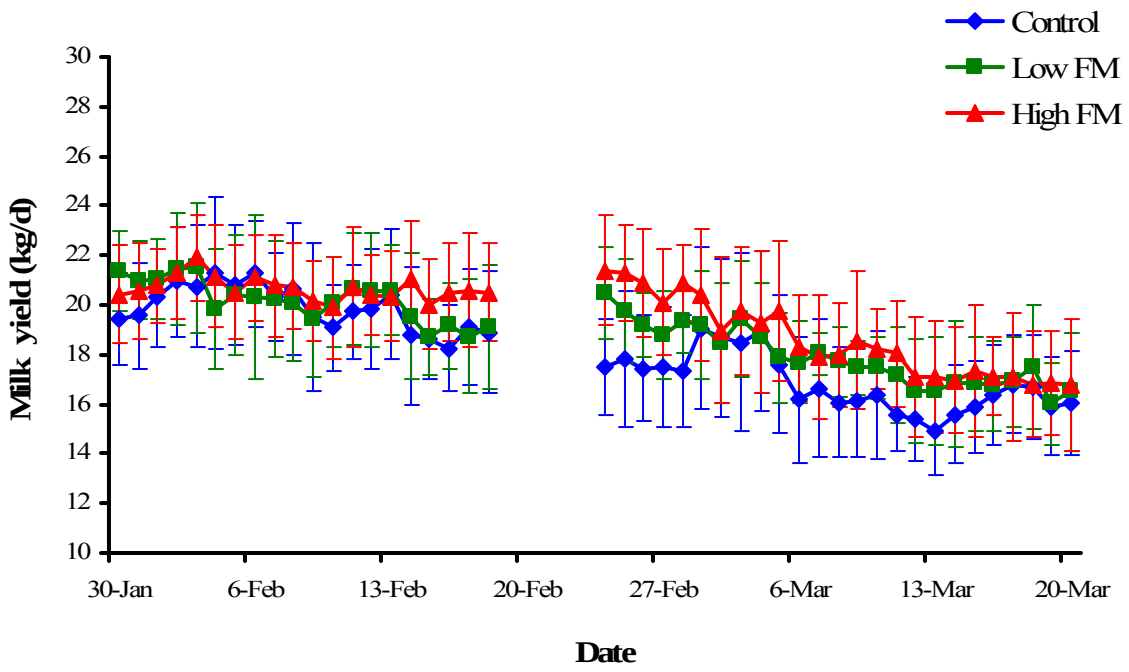

Figure 5.5 Mean daily milk yield of Jersey cows grazing kikuyu and receiving 5.5 kg DM/cow/d of supplement containing either no fishmeal (FM; Control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 14

Table 5.10 Effect of fishmeal (FM) supplementation on mean milk yield (kg/d) of cows grazing kikuyu (n = 14)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk yield (kg/d)	18.2[a]	18.9[ab]	19.5[b]	0.30

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b] Means in the same row with different superscripts differ (P < 0.05)

b) Milk composition

Table 5.11 summarises the mean milk composition of the cows on the three experimental treatments. The values reported for fat, protein and lactose are the average from the nine milk

samples per cow while the values reported for MUN are the average from the six milk samples per cow that were analysed at Lactolab.

The fat percentage in the milk of the cows on the low FM treatment (4.18 %) was higher than that of the cows on the control treatment (3.71 %; $P < 0.05$) while the fat percentage in the milk of the cows on the high FM treatment (3.91 %) did not differ from either of the other two treatments ($P > 0.1$).

There was no difference in the protein percentage in the milk of the cows in any of the three treatments (3.30, 3.41 and 3.34%; $P > 0.1$).

The fat and protein yields (calculated from the milk yield and fat and protein percentages) were 0.67 and 0.60 kg/d for the control, 0.79 and 0.64 kg/d for the low FM and 0.76 and 0.65 kg/d for the high FM treatment. The fat and protein yields of the two FM treatments were higher than the control ($P < 0.01$).

Table 5.11 Effect of fishmeal (FM) supplementation on mean milk composition of cows grazing kikuyu (n = 14)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Fat (%)	3.71[a]	4.18[b]	3.91[ab]	0.101
Protein (%)	3.30	3.41	3.34	0.042
Lactose (%)	4.43[a]	4.60[b]	4.63[b]	0.038
Milk urea N (mg/dl)	9.09[a]	9.44[a]	10.8 [b]	0.260

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b] Means in the same row with different superscripts differ ($P < 0.05$)

The lactose percentages in the milk of the cows on the low and high FM treatments (4.60 and 4.63 %, respectively) were higher than the control treatment (4.43 %; $P < 0.01$). The two FM treatments did not differ from each other ($P > 0.1$).

The mean MUN concentration in the milk of the cows on the high FM treatments (10.80 mg/dl) was higher than the control and low FM treatments (9.09 and 9.44 mg/dl, respectively; $P < 0.01$). The control and low FM treatments did not differ from each other ($P > 0.1$).

c) Covariate adjusted milk composition

In order to ensure that the difference in milk composition was due to treatment effects and not due to the natural variation between the cows, the initial milk composition was used as a covariate if there was a covariate effect. Table 5.12 shows the milk composition of the cows during the last milk recording that included the whole herd before the trial started (12 December 2005). There was no difference in these initial values between the three experimental treatments for any of the parameters ($P > 0.1$). There was no covariate effect for milk protein and lactose percentages and MUN ($P > 0.1$). However, the initial milk fat values tended to influence the final milk fat values as covariates ($P < 0.1$ for fat) so these initial values were used as covariates. The covariate adjusted milk fat percentages are reported in Table 5.13.

As with the unadjusted, values the cows on the low FM treatment had higher fat in their milk than the cows on the control treatment ($P < 0.05$). The cows on the low FM treatment also tended to have a higher milk fat concentration than the cows on the high FM treatment ($P = 0.09$).

Table 5.12 Mean milk composition of the experimental cows at the time of the last milk recording before the kikuyu trial started

Parameter	Experimental treatment[1]			SEM[5]
	Control[2]	Low FM[3]	High FM[4]	
Fat (%)	4.58	4.35	4.63	0.113
Protein (%)	3.32	3.31	3.29	0.061
Lactose (%)	4.67	4.64	4.70	0.036
Milk urea N (mg/dl)	14.45	14.23	14.18	0.545

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]n = 11, [3]n = 13, [4]n = 12
[5]Standard error of mean

Table 5.13 Effect of fishmeal (FM) supplementation on covariate adjusted milk fat percentage of the cows grazing kikuyu

Parameter	Experimental treatment[1]			SEM[5]
	Control[2]	Low FM[3]	High FM[4]	
Fat (%)	3.66[a]	4.20[b]	3.92[ab]	0.110

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]n = 11, [3]n = 13, [4]n = 12
[5]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.01$)

d) Fat- and energy-corrected milk yield

The cows on the two FM treatments produced 12 and 11 % more 4 % FCM (19.4 and 19.2 kg/d) than the cows on the control treatment (17.3 kg/d; $P < 0.01$). The two FM treatments did not differ from each other ($P > 0.1$; Table 5.14).

Table 5.14 Effect of fishmeal (FM) supplementation on mean 4% fat-corrected milk (FCM) yield and energy-corrected milk (ECM) yield (kg/d) of cows grazing kikuyu (n = 14)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
4 % FCM (kg/d)[3]	17.3[a]	19.4[b]	19.2[b]	0.30
ECM (kg/d)[4]	18.7 [a]	20.8 [b]	20.7 [b]	0.31

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[3]4% FCM (kg) = 0.4 × kg of milk + 15 × kg of milk fat (Erasmus *et al.*, 2000; NRC, 2001)
[4]ECM (kg) = 0.3246 × kg of milk + 12.86 × kg of milk fat + 7.04 × kg of protein (Gehman *et al.*, 2006)
[a,b] Means in the same row with different superscripts differ ($P < 0.01$)

The cows on the two FM treatments produced 11 % more ECM (20.8 and 20.7 kg/d) than the cows on the control treatment (18.7 kg/d; $P < 0.01$) and the two FM treatments did not differ from each other ($P > 0.1$; Table 5.14).

The FCM and ECM response was due to increased milk fat percentage for the low FM treatment and due to increased milk yield for the high FM treatment.

5.2.1.4.2 Milk production and composition of four sub-experimental periods

The experimental period was divided into four sub-periods: period 1: the first 12 days of the trial (milk production from 30 January to 10 February 2006 and average milk composition from the milk recordings done on 30 January and 1 February 2006); period 2: the second 14 days of the trial (milk production from 11 to 24 February and the average composition from the milk recordings done on 15 and 22 February 2005); period 3: third 12 days of the trial (milk production from 25 February to 8 March and average composition from the two milk samples taken on 28 February and the one on 6 March); and period 4: the last 12 days of the trial (milk production from 9 to 20 March and the average composition from the two milk samples from 15 March 2006).

There was no difference in milk production between the three experimental treatments in the first period (P > 0.1; Table 5.15). In the second period the cows on the high FM treatment produced more milk than the cows on the other two treatments (P < 0.05) while there was no difference between the control and low FM treatment (P > 0.1). In the third and fourth periods the cows on the high FM treatment produced more milk than the cows on the control treatment (P < 0.01 for period 3; P < 0.05 for period 4). The cows on the low FM treatment tended to produce more milk than the cows on the control (P = 0.08).

There was an effect of period on the overall mean milk production (P < 0.01): it decreased between each successive period (P < 0.01). There was also a period × treatment interaction between the first and second period (P < 0.01).

Table 5.15 Effect of time and fishmeal (FM) supplementation on mean milk yield (kg/d) of cows grazing kikuyu (n = 14)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	20.3	20.6	20.8	0.35
2	19.0[a]	19.7[a]	20.6[b]	0.30
3	17.7[a]	18.7[ab]	19.6[b]	0.40
4	16.0[a]	16.9[ab]	17.3[b]	0.35

[1]Periods 1, 3 and 4 = first, third and fourth 12 days and period 2 = second 14 days of the experimental period
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

In the first period there was no difference in milk fat percentage between any of the three treatments (P > 0.1; Table 5.16). Thereafter the cows on the low FM had higher milk fat percentage than the cows on the control treatment (P < 0.05). In the third period this difference was highly significant (P < 0.01) and there also tended to be higher milk fat percentage for the cows on the high FM treatment than the control (P = 0.06).

There was an effect of period on overall mean milk fat percentage (P < 0.01): it increased from the second to the third period (P < 0.01). There was a period × treatment interaction between the first and second period (P < 0.05).

Table 5.16 Effect of time and fishmeal (FM) supplementation on mean milk fat percentage of cows grazing kikuyu (n = 14)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	3.79	3.87	3.64	0.109
2	3.51[a]	4.01[b]	3.77[ab]	0.130
3	3.70[a]	4.25[b]	4.03[ab]	0.116
4	3.79[a]	4.53[b]	4.08[ab]	0.202

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

In the first and fourth periods milk protein percentage did not differ between any of the three experimental treatments (P > 0.1; Table 5.17). Milk protein percentage of the cows on the low FM treatment was higher than the control (P < 0.05) in the second and third periods.

There was an effect of period on overall mean milk protein percentage (P < 0.01): it increased between the second and third period and between the third and fourth period (P < 0.01). There was no period × treatment interaction (P > 0.1).

Table 5.17 Effect of time and fishmeal (FM) supplementation on mean milk protein percentage of cows grazing kikuyu (n = 14)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	3.26	3.30	3.19	0.047
2	3.21[a]	3.34[b]	3.25[ab]	0.041
3	3.25[a]	3.41[ab]	3.35[b]	0.046
4	3.48	3.60	3.54	0.062

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

A graphic illustration of the effects of FM supplementation over time on milk production, milk fat percentage and milk protein percentage is shown if Figure 5.6.

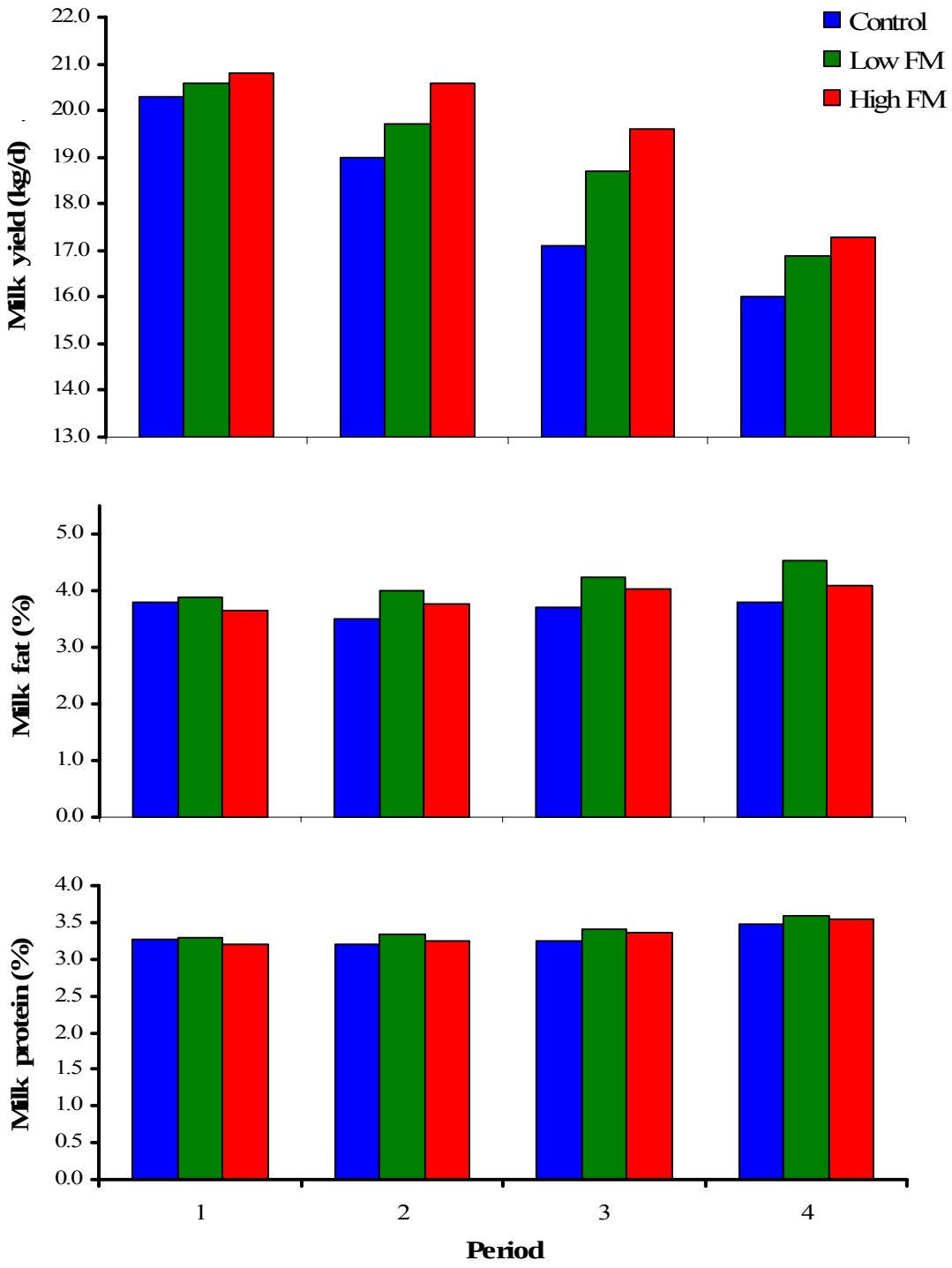

Figure 5.6 The effect of time and fishmeal (FM) supplementation on mean milk yield (kg/cow/d) and milk fat and protein percentage of cows grazing kikuyu and receiving 5.5 kg DM/cow/d of supplement containing either no FM (Control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment). Period 1 = 30 January to 10 February, period 2 = 11 to 24 February, period 3 = 25 February to 8 March and period 4 = 9 to 20 March 2006

In the first, second and fourth periods the milk lactose percentages of the cows on the two FM treatments were higher than the control ($P < 0.05$) while the two FM treatments did not differ from each other ($P > 0.1$; Table 5.18). In the third period the cows on the high FM treatment had higher a milk lactose percentage than the cows on the control ($P < 0.01$) while the cows on the low FM treatment tended to have a higher milk lactose percentage than the control ($P = 0.06$).

There was an effect of period on overall mean milk lactose percentage ($P < 0.01$): it increased between the first and second period ($P < 0.01$) and decreased between the second and third period ($P < 0.05$) and between the third and fourth period ($P < 0.01$). There was no period × treatment interaction ($P > 0.1$).

Table 5.18 Effect of time and fishmeal (FM) supplementation on mean milk lactose percentage of cows grazing kikuyu (n = 14)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	4.58[a]	4.71[b]	4.72[b]	0.033
2	4.64[a]	4.80[b]	4.80[b]	0.041
3	4.48[a]	4.69[ab]	4.79[b]	0.074
4	4.01[a]	4.17[b]	4.16[b]	0.043

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.05$)

Milk urea N was higher for the cows on the high FM treatment than the other two ($P < 0.05$) in the first and third periods (Table 5.19). In the second period it tended to be higher for the high FM treatment than the control ($P = 0.07$). In the fourth period there was no difference in MUN between any of the three treatments ($P > 0.1$). These differences are probably biologically insignificant.

There was an effect of period on overall mean MUN concentration ($P < 0.01$): it increased between the first and second periods and decreased between the second and third periods and between the third and fourth periods ($P < 0.01$). There was a period × treatment interaction between the third and fourth periods ($P < 0.01$).

Table 5.19 Effect of time and fishmeal (FM) supplementation on mean milk urea N (mg/dl) of cows grazing kikuyu (n = 14)

Period[1]	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
1	8.06[a]	8.60[a]	10.19[b]	0.414
2	10.78	11.03	11.98	0.452
3	9.12[a]	9.67[a]	11.57[b]	0.322
4	9.15	9.00	9.21	0.326

[1]Periods 1 to 4 = first, second, third and fourth 12 days of the experimental period
[2] Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

To summarise: the difference in milk production between the treatments only started becoming evident from the second period when the cows had been on the experimental treatments for four weeks. The milk fat percentage was higher in the low FM treatment from the second period onwards. There was no consistent trend in milk protein response. The milk lactose percentage of the cows on the high FM treatment was consistently higher then for the cows on the control. The MUN was higher in the milk of the cows on the high FM treatment in two of the periods.

Once again these variations in milk production and composition over time emphasise the importance of taking many measurements over a long period of time in order to obtain accurate mean values.

5.2.1.4.3 Milk production and composition of early and mid lactation cows

Table 5.20 shows the mean milk production and composition of cows that were less than 70 days into lactation at the beginning of the trial (blocks 3, 5, 6, 7 10, 14 and 16). Despite a numerical difference in milk production of 1.1 kg milk/d between the FM treatments and the control (19.5 vs. 18.4 kg/d), there was not a significant difference in milk production or protein percentage (3.19, 3.30 and 3.28 %) between the treatments (P > 0.1) probably due to too few degrees of freedom. The milk fat percentage of the cows on the low FM treatment (4.23 %) tended to be higher than that of the control treatment (3.65 %; P = 0.09). The lactose percentage in the milk was higher for the cows on the high FM (4.63 %) treatment than the cows on the control (4.39 %; P < 0.05). The MUN in the milk was higher for the cows on the high FM treatment (11.05 mg/dl) than the other two treatments (9.08 and 9.58 %; P < 0.01).

Table 5.20 Effect of fishmeal (FM) supplementation on mean milk yield and composition of early lactation cows grazing kikuyu (n = 7)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk (kg/d)	18.4	19.5	19.5	0.51
Fat (%)	3.65	4.23	3.89	0.168
Protein (%)	3.19	3.30	3.28	0.050
Lactose (%)	4.39[a]	4.54[ab]	4.63[b]	0.055
Milk urea N (mg/dl)	9.08[a]	9.58[a]	11.05[b]	0.303

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

Table 5.21 shows the milk production and composition of cows that were more than 70 days into lactation at the beginning of the trial (blocks 2, 4, 8, 9, 12, 13, and 15). The milk production of the cows on the high FM treatment (19.6 kg/d) was higher than the other two treatments (18.0 and 18.4 %; P < 0.01). There was no difference in milk fat (3.78, 4.12 and 3.94%) or protein (3.40, 3.52 and 3.40%) percentage in the milk of any of the three treatments (P > 0.1). The lactose percentages in the milk of the cows on the two FM treatments (4.67 and 4.63%) were higher than the control treatment (4.46 %; P < 0.05). The MUN value tended to be higher for the cows on the higher FM treatment (10.55 mg/dl) than the cows on the control (9.10 mg/dl; P = 0.08).

Table 5.21 Effect of fishmeal (FM) supplementation on mean milk yield and composition of mid lactation cows grazing kikuyu (n = 7)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Milk (kg/d)	18.0[a]	18.4[a]	19.6[b]	0.29
Fat (%)	3.77	4.12	3.94	0.121
Protein (%)	3.40	3.52	3.40	0.069
Lactose (%)	4.46[a]	4.67[b]	4.63[b]	0.053
MUN (mg/dl)	9.10	9.31	10.55	0.442

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.05)

5.2.1.5 Body weight and body condition score

Table 5.22 summarises the mean BW and BCS of the cows on the three treatments at the beginning and end of the trial. There was no difference in BW or BCS before or after or change in BW or BCS between any of the three experimental treatments (P > 0.1).

Table 5.22 Effect of fishmeal (FM) supplementation on body weight (BW) and body condition score (BCS)[1] of cows grazing kikuyu (n = 14)

Parameter	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
BW (kg)				
Beginning	364	374	352	7.3
End	376	384	360	7.9
Change	12	10	8	3.0
BCS				
Beginning	2.2	2.3	2.3	0.08
End	2.2	2.2	2.3	0.05
Change	0	-0.1	0	0.08

[1]Five-point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982)
[2]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[3]Standard error of mean

5.2.1.6 Faeces

Table 5.23 shows the starch concentration in the faeces of the three cows on each experimental treatment. The starch concentration in the faeces of the cows on the low FM treatment (1.82 % DM) was higher than the control and high FM treatments (0.77 and 0.88 % DM; P < 0.01). These differences, although statistically significant, are of such small magnitude that it is biologically insignificant.

Table 5.23 Effect of fishmeal (FM) supplementation on starch concentration in the faeces of cows grazing kikuyu (n = 3)

Parameter	Experimental treatment[1]			SEM[2]
	Control	Low FM	High FM	
Starch in faeces (% DM)	0.77[a]	1.82[b]	0.88[a]	0.124

[1]Control: maize-based supplement containing no FM; Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean
[a,b]Means in the same row with different superscripts differ (P < 0.01)

5.2.2 Rumen study

5.2.2.1 Ruminal pH

5.2.2.1.1 Results from data loggers

The mean ruminal pH for the eight cows on each treatment every half hour was calculated as in section 3.2.2.1.1 and is shown in Figure 5.7 with the standard deviation bars (n = 8).

The mean pH per 4 hour period was calculated to get a mean ruminal pH for each cow for the following times 0000, 0400, 0800, 1200, 1600 and 2000 h. The value for 0800 h is the mean of the values from 0600 to 0930 h and so on for all six times. These values were analysed with Proc GLM Repeated Measures Analysis of Variance (Statistical Analysis Systems, 2001; see section 3.1.3.3).

Table 5.24 Effect of time of day and fishmeal (FM) supplementation on mean ruminal pH of cows grazing kikuyu (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	6.23	6.14	0.1042	0.035
0400	6.50	6.44	0.1685	0.025
0800	6.25	6.29	0.3184	0.027
1200	6.13	6.14	0.8429	0.034
1600	5.90	5.99	0.1944	0.043
2000	5.87	5.88	0.9581	0.042

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

The mean for all eight cows on each treatment for the six times of the day is reported in Table 5.24. The ruminal pH did not differ between treatments ($P > 0.1$).

The mean daily ruminal pH for the cows on the control and high FM treatments were both 6.15 and did not differ from each other ($P > 0.1$).

Time of day affected the ruminal pH ($P < 0.01$). The trends in ruminal pH throughout the day were similar to the cows on ryegrass being the highest at 0400 h and lowest at 2000 h. The overall mean ruminal pH increased from 2000 to 0000 h and from 0000 to 0400 h ($P < 0.01$), decreased from 0400 to 0800 h ($P < 0.05$), from to 0800, 1200 to 1600 h ($P < 0.01$) and between 1600 and 2000 h ($P < 0.05$).

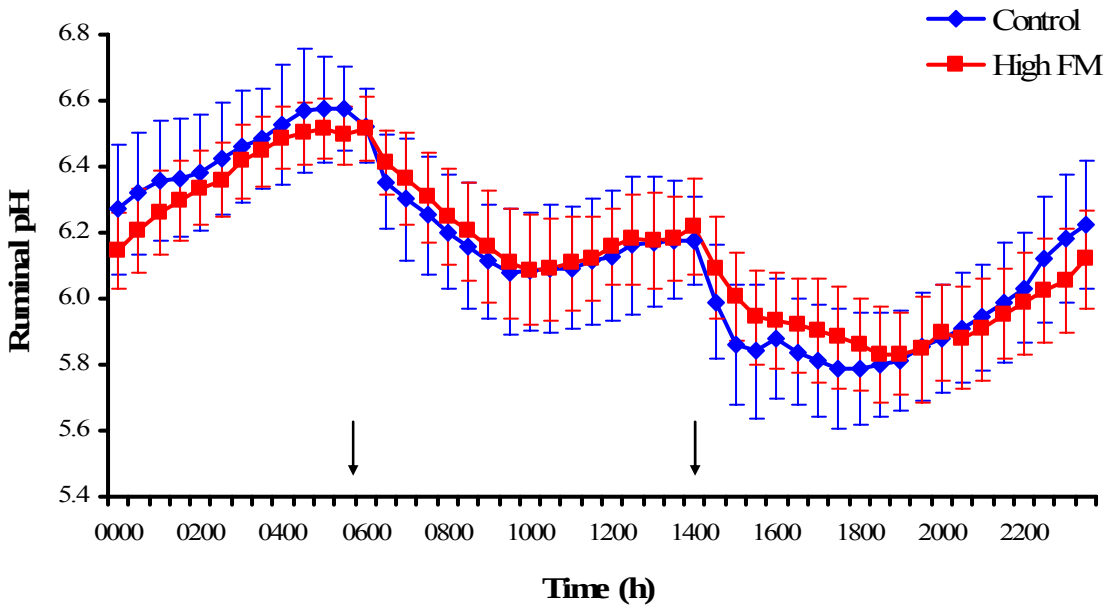

Figure 5.7 Ruminal pH of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

There were treatment × time interactions between 2000 and 0000 h ($P < 0.01$), 0400 and 0800 h ($P < 0.05$) and tended to be treatment × time interactions between 1200 and 1600 h ($P = 0.08$) and 1600 and 2000 h ($P = 0.08$), indicating that the daily trend in ruminal pH differed slightly between the treatments.

5.2.2.1.2 Results from the manual recording of ruminal pH

Figure 5.8 shows the ruminal pH that was measured when the samples of rumen fluid were taken. Although these values were not used in the statistical analysis they do give a good indication of whether the rumen samples were representative of the whole rumen fluid. The mean pH from the manual recording never deviated more than 6 % from the mean ruminal pH measured with the data loggers. Although not as refined, Figure 5.8 shows the same general trends in ruminal pH changes throughout the day as Figure 5.7.

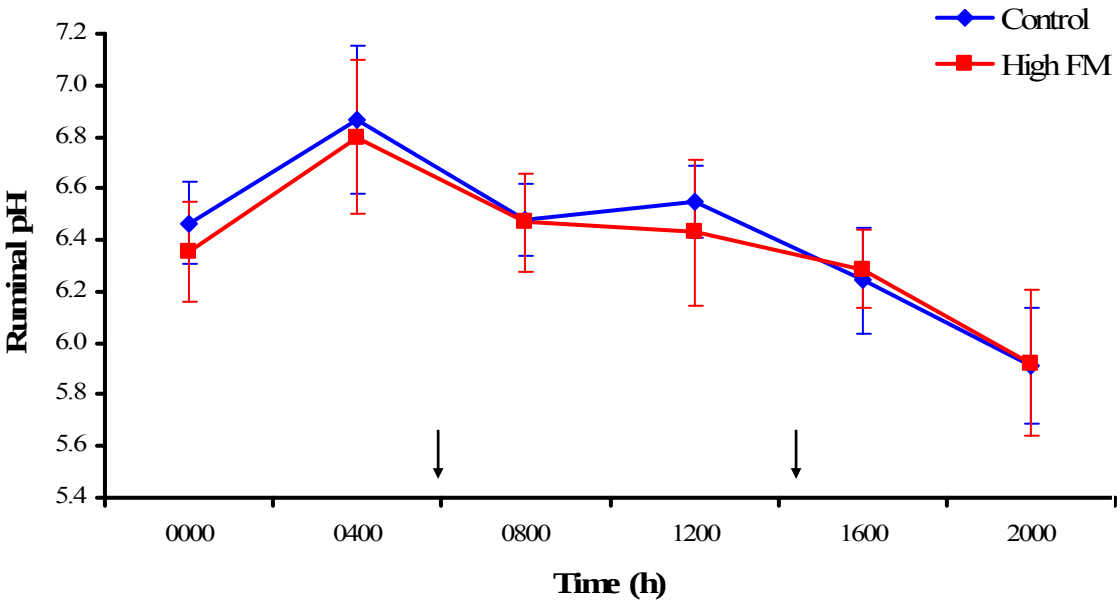

Figure 5.8 Ruminal pH, measured manually at the six sampling times, of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

5.2.2.2 Ruminal ammonia

The mean ruminal NH_3-N concentration (mg/dl) for the eight cows on each treatment was calculated for each of the six times of the day and is shown in Table 5.25 and Figure 5.9.

At 0000 h there was no difference between the two treatments ($P > 0.1$). At 0400 h the ruminal NH_3-N concentration tended to be higher for the cows on the high FM treatment (P =

0.07) and at 0800, 1200, 1600 and 2000 h the cows on the high FM treatment had a higher ruminal NH_3-N concentration than the cows on the control treatment ($P < 0.05$). The difference at 1600 h was highly significant ($P < 0.01$).

Table 5.25 Effect of time of day and fishmeal (FM) supplementation on mean ruminal ammonia-N concentration (mg/dl) in the rumen fluid of cows grazing kikuyu (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	6.18	6.43	0.75	0.452
0400	5.31	6.71	0.07	0.373
0800	4.36	6.65	0.02	0.448
1200	2.22	4.32	0.01	0.356
1600	4.88	7.23	<0.01	0.282
2000	5.47	7.81	0.02	0.453

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

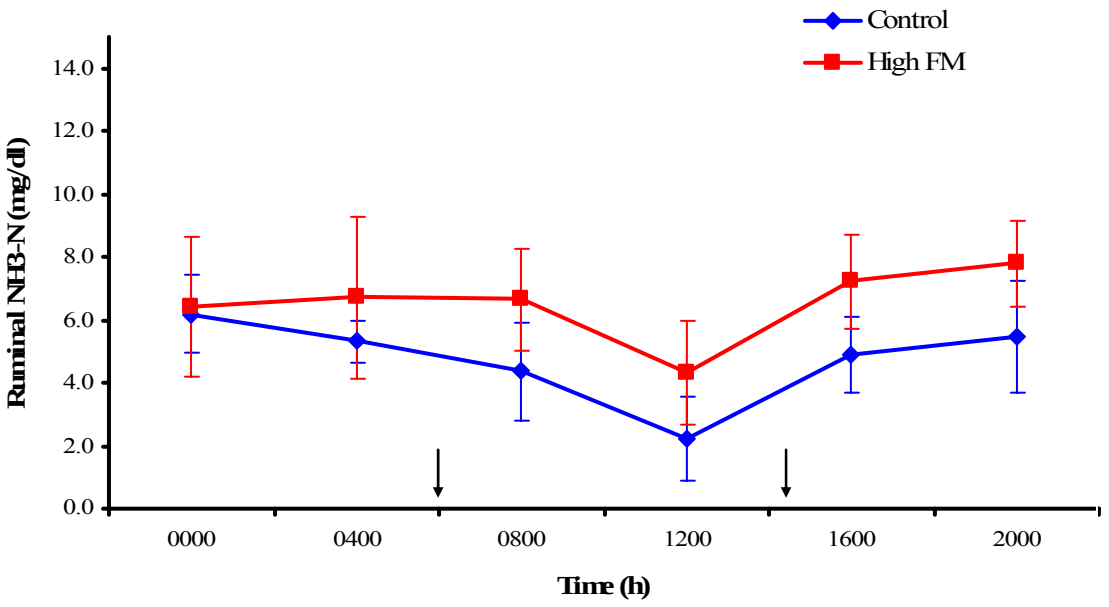

Figure 5.9 Ruminal concentration of ammonia-N (NH_3-N; mg/dl) of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean daily ruminal NH_3-N concentrations of the cows on the control and high FM treatments were 4.74 and 6.52 mg/dl, respectively, higher for the cows on the high FM treatments ($P = 0.01$).

There was an effect of time on ruminal NH_3-N concentration ($P < 0.01$). The overall mean NH_3-N concentration decreased from 0800 to 1200 h and increased from 1200 to 1600 h ($P < 0.01$). It was lowest at 1200 h, indicating that the rumen microbes had enough carbohydrates from the morning concentrate feeding to utilise the NH_3-N in the rumen.

There were no treatment × time interactions ($P > 0.1$).

5.2.2.3 Volatile fatty acids

The concentration of total ruminal VFA (mmol/L), including acetic, propionic, butyric, iso butyric and valeric acids, averaged for the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 5.26 and shown in Figure 5.10.

There was no difference in the total VFA concentration in the rumens of the cows on the two treatments at any of the six times of day ($P > 0.1$).

The mean daily total VFA concentration in the rumen fluid of cows on the control and high FM treatments were 118.6 and 118.5 mmol/L, respectively, not differing between treatments ($P > 0.1$).

Table 5.26 Effect of time of day and fishmeal (FM) supplementation on mean total volatile fatty acid (VFA) concentration (mmol/L) in the rumen fluid of cows grazing kikuyu (n = 8)

Time (h)	Experimental treatment[1]		P =	SEM[2]
	Control	High FM		
0000	123.5	125.2	0.71	1.01
0400	100.6	102.3	0.79	1.34
0800	119.4	116.4	0.74	1.91
1200	113.5	111.8	0.78	1.36
1600	118.1	113.1	0.20	0.77
2000	136.4	142.3	0.64	2.70

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

Time of day affected the total VFA concentration ($P < 0.01$). The overall mean total VFA concentration tended to decrease from 2000 to 0000 h ($P = 0.06$), decreased from 0000 to 0400 h ($P < 0.01$), increased from 0400 to 0800 h ($P < 0.05$) and from 1600 to 2000 h ($P < 0.01$). The daily trend in VFA concentration was the inverse of ruminal pH as in the studies of Carruthers & Neil (1997), Bargo *et al.* (2003b) and Williams *et al.* (2005). There were no treatment × time interactions for total VFA concentrations ($P > 0.1$).

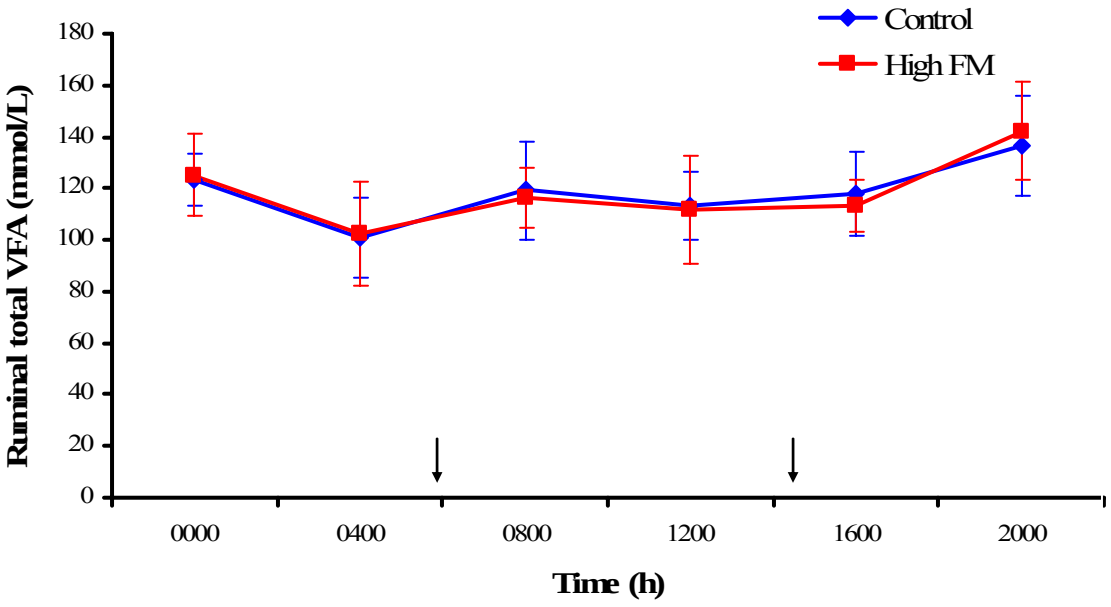

Figure 5.10 Ruminal concentration of total volatile fatty acids (VFA; mmol/L) of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean concentration of acetic acid (mmol/L) in the ruminal fluid of the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 5.27 and shown in Figure 5.11. Table 5.27 also reports acetic acid as a proportion of total VFA.

There was no treatment effect on ruminal acetic acid concentration ($P > 0.1$). The molar proportion of acetic acid tended to be higher for the high FM treatment at 0400 h ($P = 0.06$) and was higher for the high FM treatment than the control at 1200, 1600 and 2000 h ($P < 0.01$).

Table 5.27 Effect of time of day and fishmeal (FM) supplementation on mean acetic acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing kikuyu (n = 8)

Time (h)	Acetic acid (mmol/L)				Acetic acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	83.1	85.3	0.52	0.72	67.2	68.1	0.13	0.34
0400	66.9	7008	0.43	1.02	66.2	69.1	0.06	0.90
0800	79.2	78.0	0.83	1.21	66.4	67.1	0.12	0.26
1200	74.6	75.8	0.78	0.93	65.7	67.9	<0.01	0.32
1600	77.2	75.7	0.51	0.48	65.5	67.0	<0.01	0.26
2000	87.2	94.9	0.31	1.56	64.1	66.8	<0.01	0.50

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

The mean daily ruminal acetic acid concentrations of the cows on the control and high FM treatments were 78.0 and 80.1 mmol/L not differing between treatments (P > 0.1). The mean molar proportions of acetate were 65.8 and 67.6 mol/100 mol VFA, respectively (Table 5.31), higher for the high FM treatment than the control (P < 0.05).

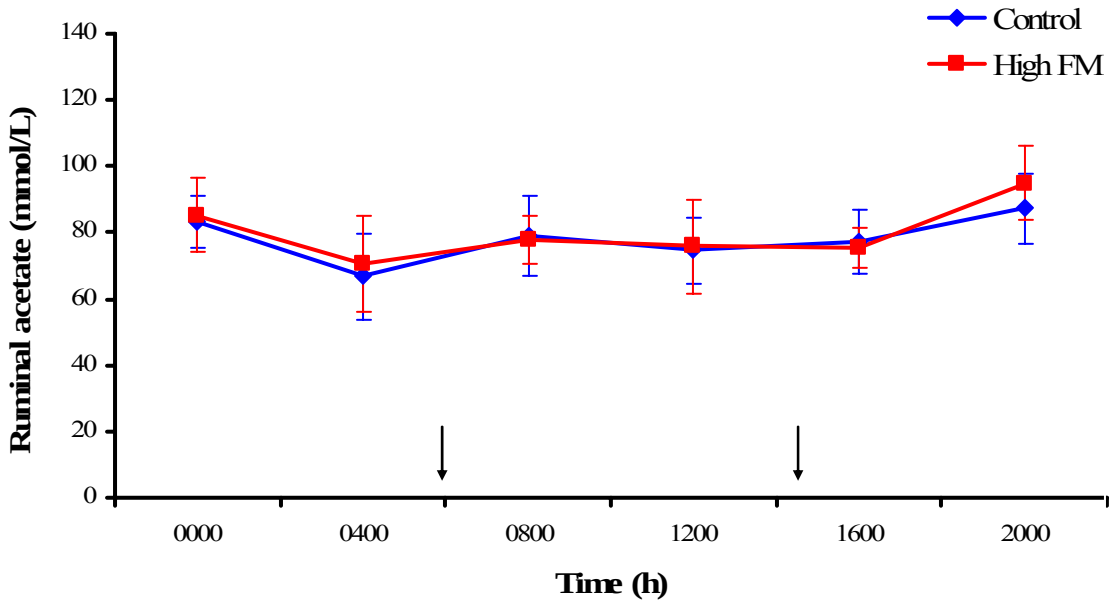

Figure 5.11 Ruminal concentration of acetic acid (mmol/L) of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean concentration of propionic acid (mmol/L) in the ruminal fluid of the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 5.28 and shown in Figure 5.12. Table 5.28 also reports propionic acid as a proportion of total VFA.

At 0400, 0800 and 2000 h there was no difference in the propionic acid concentration in the rumens of the cows on the two treatments ($P > 0.1$). At 1200 and 1600 h the cows on the control treatment had a higher propionic acid concentration than the cows on the high FM treatment ($P < 0.05$) and at 0000 h it tended to be higher in the control than the high FM treatment ($P = 0.06$).

The molar proportion of propionic acid was higher for the cows on the control treatment than the high FM treatment at 0000, 0800, 1200, 1600 and 2000 h ($P < 0.05$ for 0000 and 0800 h and $P < 0.01$ for 1200, 1600 and 2000 h).

Table 5.28 Effect of time of day and fishmeal (FM) supplementation on mean propionic acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing kikuyu (n = 8)

Time (h)	Propionic acid (mmol/L)				Propionic acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	27.4	25.3	0.06	0.20	22.2	20.4	0.02	0.43
0400	21.2	19.6	0.26	0.29	21.2	19.2	0.15	0.85
0800	26.6	23.5	0.24	0.53	22.2	20.2	0.03	0.49
1200	26.8	22.0	0.05	0.43	23.6	19.7	<0.01	0.60
1600	27.7	22.8	0.01	0.29	23.2	20.1	<0.01	0.42
2000	35.8	31.1	0.30	0.95	26.0	21.7	<0.01	0.69

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

The mean daily concentration of propionic acid in the rumen fluid of cows on the control and high FM treatments were 27.6 and 24.0 mmol/L, respectively, tending to be higher for the cows on the control treatment ($P = 0.07$). The molar proportions of propionate were 23.2 and 20.3 mol/100 mol VFA for the control and high FM treatments, respectively, higher for the control than the high FM treatment ($P < 0.01$).

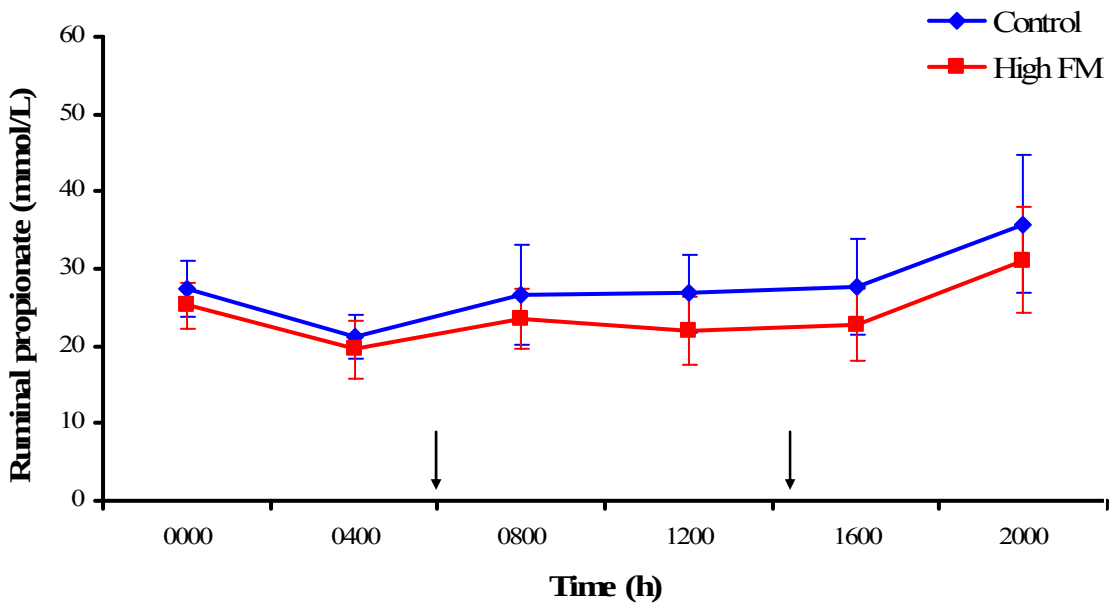

Figure 5.12 Ruminal concentration of propionic acid (mmol/L) of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean acetate: propionate ratio for the control and high FM treatments were 2.88 and 3.37, respectively, greater for the high FM than the control treatment ($P < 0.01$).

The mean concentration of ruminal butyric acid (mmol/L) for the eight cows on each treatment was calculated for each of the six times of the day and is reported in Table 5.29 and shown in Figure 5.13. Table 5.29 also reports butyric acid as a proportion of total VFA.

At 0400 and 0800 h the butyric acid concentration of the cows on the two treatments did not differ from each other ($P > 0.1$). At 1200, 1600 and 2000 h the butyric acid concentration was higher for the cows on the high FM treatment than for the cows on the control treatment ($P < 0.05$). At 1600 h the difference was highly significant ($P < 0.01$). At 0000 h it tended to be higher for the cows on the high FM treatment than the control ($P = 0.06$).

The molar proportion of butyrate was higher for the cows on the high FM treatment ($P < 0.05$). This difference was highly significant at 1600 and 2000 h ($P < 0.01$; Table 5.29).

Table 5.29 Effect of time of day and fishmeal (FM) supplementation on mean butyric acid concentration (mmol/L) and molar proportion (mol/100 mol VFA) in the rumen fluid of cows grazing kikuyu (n = 8)

Time (h)	Butyric acid (mmol/L)				Butyric acid (mol/100 mol)			
	Experimental treatment[1]		P =	SEM[2]	Experimental treatment[1]		P =	SEM[2]
	Control	High FM			Control	High FM		
0000	11.1	12.8	0.06	0.17	9.0	10.1	0.01	0.20
0400	8.9	10.3	0.20	0.23	8.7	10.0	0.04	0.35
0800	11.6	12.9	0.17	0.19	9.8	11.1	0.02	0.27
1200	10.4	12.3	0.03	0.15	9.3	11.0	0.04	0.46
1600	11.6	13.0	<0.01	0.06	9.9	11.5	<0.01	0.19
2000	11.2	14.3	0.01	0.20	8.3	10.1	<0.01	0.29

[1]Control: maize-based supplement containing no FM; High FM: maize-based supplement containing 8 % FM
[2]Standard error of mean

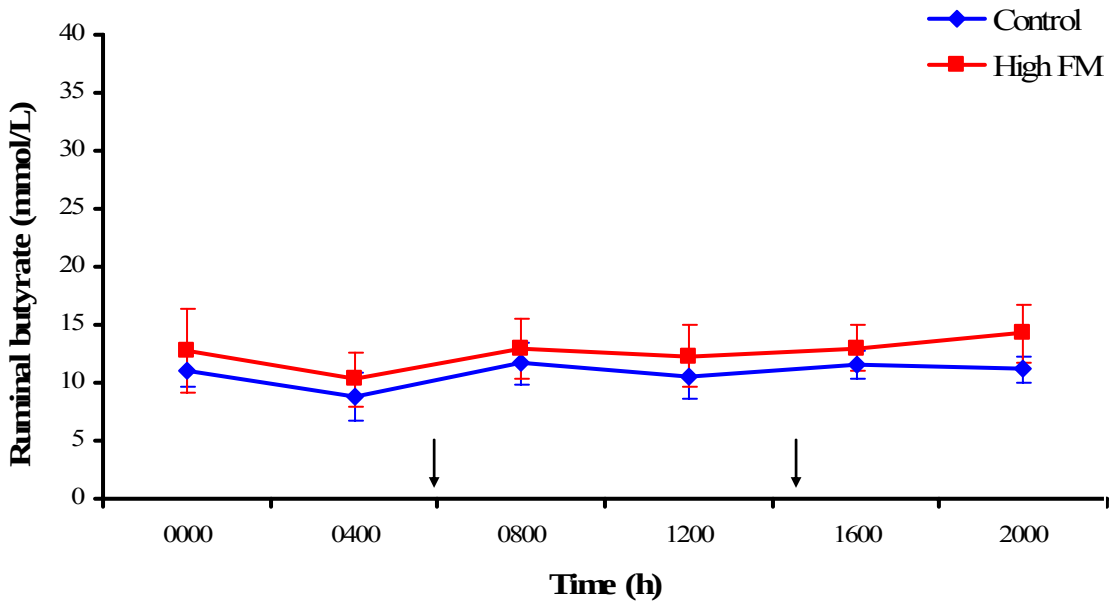

Figure 5.13 Ruminal concentration of butyric acid (mmol/L) of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment) or 8 % FM (High FM treatment). Standard deviation bars are shown. n = 8. Arrows indicate times of concentrate feeding after which fresh pasture was allocated

The mean daily butyric acid concentration in the rumen fluid of the cows on the control and high FM treatments were 10.8 and 12.6 mmol/L, respectively, higher for the high FM than the control treatment ($P < 0.01$). The molar proportions of butyrate were 9.11 and 10.63 mol/100

mol VFA for the control and high FM treatments, respectively, also higher for the high FM than the control treatment ($P < 0.01$).

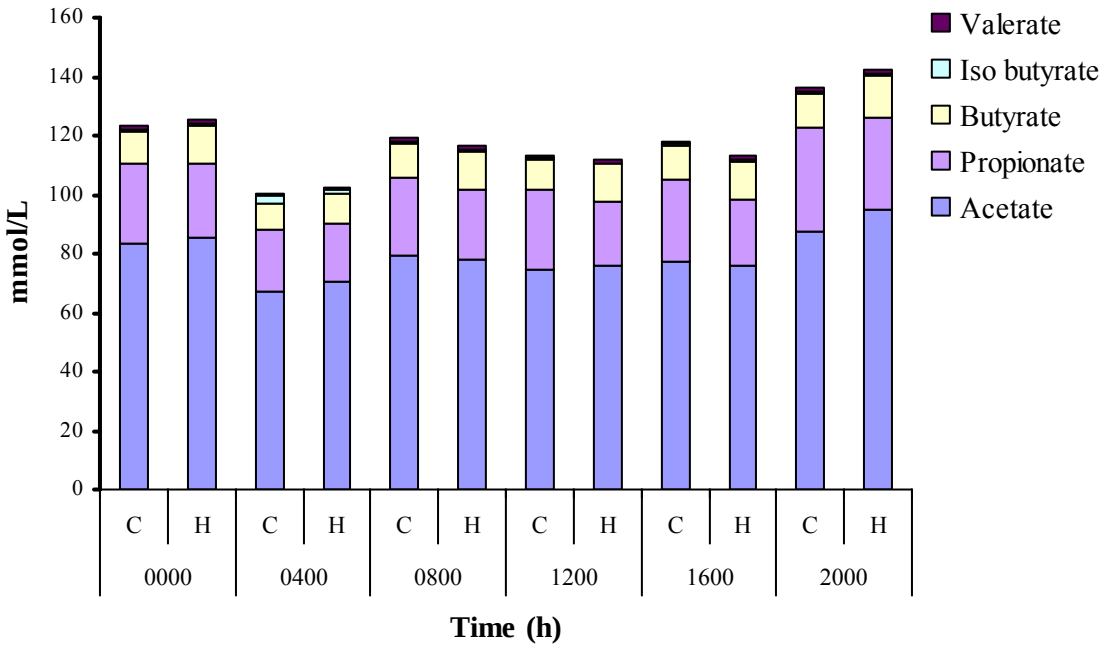

Figure 5.15 Concentrations of individual volatile fatty acids (VFA) making up the total VFA in the rumen fluid of cows grazing kikuyu and receiving 5.5 kg concentrate DM/d containing no fishmeal (FM; Control treatment; C) or 8 % FM (High FM treatment; H)

To summarise: at 0000 h the propionate tended to be higher in the control and the butyrate tended to be higher in the high FM treatment, at 0400 and 0800 h there was no difference between the two treatments, at 1200 and 1600 h the propionate was higher in the control and the butyrate was higher in the high FM treatment and at 2000 h the butyrate was higher in the high FM treatment. Overall the mean daily concentration of butyrate as well as the acetate to propionate ratio were higher in the high FM treatment ($P < 0.05$).

5.2.3 Summary of results

The cows were allowed 13.2 kg DM/cow/d of the kikuyu pasture. The mean intake of this pasture was approximately 6.8 kg DM/cow/d. The chemical composition of the pasture was within the range expected for kikuyu: the mean CP, NDF, ADF, IVOMD and ME were 22.1, 60.3, 30.5, 69.9 % DM and 10.0 MJ/kg DM, respectively.

The main difference between the three experimental treatments was the CP concentration of the supplements: 7.7, 10.1 and 12.7 % for the control, low FM and high FM treatments, respectively. Although the EE rose slightly with the inclusion of FM, the ME of the three concentrates was similar.

The total diets of the cows on all three treatments were adequate in all the main nutrients. There was enough ME to support 19 kg of 4 % FCM/d. The CP (15.6, 16.7 and 17.9 % DM for the control, low FM and high FM diets, respectively) was lower than recommended for the control diet and increased with the inclusion of FM. All three diets were low in RDP as reflected in the low MUN and ruminal NH_3-N values, especially the control diet. Including FM in the concentrate increased both RDP and RUP as well as increasing the Met and Lys levels of the diet.

Cows on the low FM treatment responded by producing 18 % more milk fat, 12 % more 4 % FCM and 11 % more ECM than the cows on the control ($P < 0.05$). Cows on the high FM treatment responded by producing 7 % more milk, 13 % more milk fat (due to the increased milk yield) and 11 % more FCM and ECM than the cows on the control ($P < 0.05$; Table 5.30).

There were no treatment effects on change in BW or BCS ($P > 0.1$).

The starch concentration in the faeces was low, indicating efficient and extensive digestion of starch.

The ruminal pH did not differ between treatments ($P > 0.1$) and, although it varied throughout the day, was never suboptimal (below the 5.8). The ruminal NH_3-N concentration was higher for the cows on the high FM treatment than the control ($P < 0.05$), the latter being below the minimum level of 5 mg/dl for maximum microbial protein synthesis (Satter & Slyter, 1974), indicating the RDP was limiting. This was also reflected in the low MUN levels. Although there was no treatment effect on total VFA concentration ($P > 0.1$) the molar proportions (mol/100 mol total VFA) of acetate and butyrate and the ratio of acetate: propionate were higher for the cows on the high FM treatment than the control ($P < 0.05$ for acetate and $P <$

0.01 for butyrate and acetate: propionate ratio) while the molar proportion of propionate was higher in the control ($P < 0.01$; Table 5.31).

Table 5.30 Effect of fishmeal (FM) supplementation on mean milk yield, milk composition, body weight (BW) and body condition score (BSC)[1] of cows grazing kikuyu pasture and receiving 5.5 kg supplement DM/d (n = 14)

Parameter	Experimental treatment[2]			SEM[3]
	Control	Low FM	High FM	
Milk yield (kg/d)	18.2[a]	18.9[ab]	19.5[b]	0.30
4 % FCM (kg/d)	17.3[a]	19.4[b]	19.2[b]	0.30
Fat (%)	3.71[a]	4.18[b]	3.91[ab]	0.101
Fat yield (kg/d)	0.67[a]	0.79[b]	0.76[b]	0.017
Protein (%)	3.30	3.41	3.34	0.042
Protein yield (kg/d)	0.60[a]	0.64[b]	0.65[b]	0.012
Lactose (%)	4.43[a]	4.60[b]	4.63[b]	0.038
MUN (mg/dl)	9.09[a]	9.44[a]	10.80[b]	0.260
BW beginning (kg)	364	374	352	7.3
BW end (kg)	376	384	360	7.9
BW change (kg)	+12	+10	+8	3.0
BCS beginning	2.2	2.3	2.3	0.08
BCS end	2.2	2.2	2.3	0.05
BCS change	0	-0.1	0	0.08

FCM – fat-corrected milk; MUN – Milk urea N
[1]Five-point system where 1 is thin and 5 is fat (Wildman *et al.*, 1982)
[2]Experimental treatment: Control = supplement containing no FM; Low FM = supplement containing 4 % FM; High FM = supplement containing 8 % FM
[3]Standard error of mean
[a,b]Means in the same row with different superscripts differ ($P < 0.05$)

Table 5.31 Effect of fishmeal (FM) supplementation on mean daily ruminal pH, ammonia-N (NH_3-N) and volatile fatty acid (VFA) concentrations of cows grazing kikuyu pasture and receiving 5.5 kg supplement DM/d (n = 8)

Parameter	Experimental treatment[1]		SEM[2]
	Control	High FM	
pH	6.15	6.15	0.030
NH_3-N (mg/dl)	4.74[a]	6.52[b]	0.294
Total VFA (mmol/L)	118.6	118.5	0.94
Acetate (mol/100 mol)	65.8[a]	67.6[b]	0.36
Propionate (mol/100 mol)	23.2[b]	20.3[a]	0.54
Butyrate (mol/100 mol)	9.1[a]	10.6[b]	0.24
Acetate: propionate	2.88[a]	3.37[b]	0.083

[1]Experimental treatment: Control = supplement containing no FM; High FM = supplement containing 8 % FM
[2]Standard error of mean
[a, b]Means in the same row with different superscripts differ ($P < 0.05$)

5.3 DISCUSSION

5.3.1 Production study

5.3.1.1 Pasture

5.3.1.1.1 Pasture allowance and intake

a) Pasture allowance and intake estimated using the rising plate meter

The pasture height and yield both pre- and post-grazing were higher than in the kikuyu pasture in the trial of Meeske & Van der Merwe (2005) although the amount of pasture removed was similar.

The amount of DM removed off the pasture was higher than in the trial by Fulkerson *et al.* (2005) where cows on kikuyu removed approximately 1000 kg DM/ha or all of the leaf material. Post-grazing residue is directly related to pasture on offer (Fulkerson *et al.*, 2005).

Pasture utilisation was only 45 %, lower than the ryegrass, due to increased residual and stalks in the kikuyu pasture.

The length of the grazing cycle is in agreement with the optimal grazing cycle of 30 days recommended by Henning *et al.* (1995) for cows grazing kikuyu pasture. The average growth rate of the pasture was similar to that of the kikuyu pasture in the trial of Meeske & Van der Merwe (2005).

Once again estimated intake values were not corrected for the growth of pasture between measurements as the post-grazing pasture height was usually measured immediately or within a day after the cows finished grazing that strip. Only one calibration equation was used for pre- and post-grazing pasture, a potential source of inaccuracy.

Fulkerson & Slack (1993) found that for kikuyu pasture, calibrations based on leafy shoot DM were more accurate than if based on total DM for kikuyu pasture. This calibration equation was based on total DM possibly leading inaccurate estimation of kikuyu DMI.

b) Estimation of pasture intake using equations

If the cows consumed 6.8 kg kikuyu DM/d the total DMI would have been 12.3 kg/cow/d which is 3.3 % of BW. This is slightly lower than the average DMI of 3.5% of LW estimated using alkane method by Fulkerson *et al.*, (2005) for cows grazing kikuyu and receiving 3 kg crushed barley/cow/d. The lower DMI for cows grazing kikuyu than ryegrass is to be expected since DMI decreases with temperatures above 20 °C (NRC, 2001).

c) Estimation of pasture intake using the CPM model

As with the cows on the ryegrass trial, the model predicted pasture DMI differed between the three treatments, explainable by the difference in milk production (driving intake) and the difference in BW between the cows.

5.3.1.1.2 Pasture composition

The mean DM concentration of 15.7 ± 2.62 % (n = 8), with a general increase as the season progressed, is within the range of previously reported values for kikuyu pasture of 14.2 % (Botha *et al.*, 2005) to 18.7 % (Meeske *et al.*, 2006).

The mean ash concentration of 11.8 ± 1.58 % DM (n = 8) is slightly higher than the 8.8 ± 2.60 and 9.9 ± 1.65 % reported by Meeske *et al.* (2006) for kikuyu pasture in summer and autumn, respectively. The mean OM concentration (100 – ash) of 88.2 ± 1.58 (n = 8) similar to, although slightly lower, than the OM concentration of 91.4 % DM reported by Hamilton *et al.* (1992) for kikuyu pasture.

The mean IVOMD of 69.9 ± 4.53 % DM (n = 8) is similar to other values for kikuyu of 69.1 % DM (Fulkerson *et al.*, 2005) and 66.2 % DM (Hamilton *et al.*, 1992). The drop in IVOMD from 27 February to 6 March (Table 5.4) could be due to experimental error.

The ME concentration, calculated as 0.82 × GE × IVOMD (Robinson *et al.*, 2004), averaged 10.0 ± 0.28 MJ ME/kg DM (n = 3). Previous studies, Fulkerson *et al.* (1998; 2005), Granzin (2004), Botha *et al.* (2005) and Meeske *et al.* (2006), reported ME values for kikuyu pasture ranging from 8.1 MJ ME/kg DM (Botha *et al.*, 2005) to 10.0 MJ ME/kg DM (Granzin, 2004).

The CP concentration was as expected for kikuyu pasture. It averaged 22.1 ± 3.07 % DM (n = 8) and varied from 18.1 to 26.2 % DM. Hamilton *et al.* (1992), Fulkerson *et al.* (1998;

2005), Granzin (2004), Botha *et al.* (2005) and Meeske *et al.* (2006), reported CP values for kikuyu pasture ranging from 15.6 % DM (Hamilton *et al.*, 1992) to 26.1 % DM (Fulkerson *et al.*, 2005).

On average 41.4 % of this CP was soluble with on average 49.8 % of this being NPN.

The mean NDF concentration was 60.3 ± 4.51 % DM (n = 8), in the range expected for kikuyu, and higher than ryegrass. Fulkerson *et al.* (1998; 2005), Granzin (2004), Botha *et al.* (2005) and Meeske *et al.* (2006) reported NDF values for kikuyu pasture in summer and autumn ranging from 56.8 % (Granzin, 2004) to 68.2 % (Meeske *et al.*, 2006).

The mean ADF concentration of 30.5 ± 3.50 % DM (n = 8) is once again within the expected range. Granzin (2004), Fulkerson *et al.* (2005) and Meeske *et al.* (2006) reported ADF values for kikuyu pasture in summer and autumn ranging from 22.0 % (Granzin, 2004) to 32.2 % (Meeske *et al.*, 2006).

The lignin concentration of the pasture averaged 10.0 ± 0.58 % of NDF (n = 3). The mean NDIP was 38.5 ± 2.26 % of CP and ADIP was 11.8 ± 0.84 % of CP (n = 3). These values were higher than the averages of 2.62 ± 0.313, 30.32 ± 11.771 and 6.04 ± 2.339 (n = 34) for lignin, NDIP and ADIP, respectively, of South African kikuyu samples tested for the AFRGI Animal feeds database (Cronjé, G., personal communication, gert.cronje@afgri.co.za).

The mean EE was 2.1 ± 0.21 % DM (n = 3), lower than the 3.7 % reported by Granzin (2004). The mean calculated NFC concentration was 3.7 ± 0.71 % DM, much lower than the 11.0 % reported by Granzin (2004). The mean starch concentration was 0.3 ± 0.02 % DM.

Kikuyu is known to have a low Ca concentration (Cowan & Lowe, 1998). The mean Ca concentration was 0.37 ± 0.032 % DM (n = 3) and the mean P concentration was 0.35 ± 0.027 (n = 3). The mean Ca to P ratio was 1.08 ± 0.054. Fulkerson *et al.* (1998), Granzin (2004), Botha *et al.* (2005) and Meeske *et al.* (2006) reported Ca values for kikuyu ranging from 0.21 % DM (Granzin, 2004) to 0.43 % DM (Meeske *et al.*, 2006) and P values ranging from 0.28 % DM (Fulkerson *et al.*, 1998) to 0.54 % DM (Botha *et al.*, 2005). Hence the Ca and P levels in the kikuyu pasture were within the expected range.

As the mean post-grazing height was 11 cm, it is possible that the cows selected pasture of higher quality than the samples that were cut to 3 cm above ground level, especially later in the trial when the cows tended to leave more stubble as is a common problem with kikuyu pasture (Fulkerson & Slack, 1993).

The AA levels in the kikuyu (Table 5.6) are in agreement with those in pasture in the study of Jones-Endsley *et al.* (1997) where the Lys and Met concentrations were 6.25 ± 0.095 and 1.16 ± 0.070 g/100 g AA, respectively.

5.3.1.2 Concentrate composition

The chemical composition of the concentrates (Table 5.7) are in agreement with the maize-based concentrate used by Granzin (2004), except that the EE in the present study is higher than the fat concentration of 3 to 4 % DM in the study of Granzin (2004).

The drop in OM and rise in EE and CP as the level of FM increased is due to the high ash, EE and CP concentrations of the FM (Table 5.9). The higher EE in the two FM concentrates is also due to the Megalac. The CP values are lower than in the concentrate used in the ryegrass trial, indicating the variability in raw materials. The maize, and possibly the FM, used in this batch must have had a lower CP content than what was used in the ryegrass trial.

The IVOMD is higher for the low FM than for the other two concentrates, as was found in the concentrates used in the ryegrass trial, and is reflected in the higher ME in the low FM concentrate as IVOMD was used in the equation to calculate ME. This could be due to sampling error or just coincidence.

Once again the ADIP is higher than the NDIP in the control concentrate and Sol CP, ADIP, NDIP and lignin were higher and NPN and EE were lower than what would be expected from the same concentrates based average South African raw materials (see Table 6.3 in section 6.1).

5.3.1.3 Total diet composition

The total diet composition (Table 5.3) can be compared to the recommendations of Erasmus *et al.* (2000) for early lactation cows reported in Table 2.1 of the literature review. The ME concentrations of all three diets (11.6, 11.7 and 11.6 MJ ME/kg) were adequate compared to the recommended level of 11.3 to 11.5 MJ ME/kg DM. However, due to the fact that grazing

cows require 10 to 30 % more ME due to the energy requirements of grazing and walking (Muller & Fales, 1998), the ME concentrations of these diets could be inadequate. At the total DMI of 12.3 kg the diet would have supplied approximately 143 MJ ME/d.

The NDF (39.5, 40.0 and 41.1 % DM) was well above the minimum recommendation of 28 to 32 % while ADF (18.4, 18.3 and 18.4 % DM) was similar to the minimum recommendation of 19 %. The EE (2.2, 2.3 and 2.5 % DM) was below the recommended 5 to 7 %. Calcium (0.76, 0.90 and 1.11 % DM) and P (0.43, 0.47 and 0.56 % DM) were adequate compared to the recommendations of 0.6 to 0.8 % and 0.38 to 0.42 %, respectively.

The CP concentration (15.6, 16.7 and 17.9 % DM) increased with the level of FM in the diet. The CP concentration of the control diet was below the recommended 16 to 18 %. The soluble CP (41.2, 41.8 and 41.2 % CP) was above the recommended 30 to 35 % of CP.

Unfortunately the rumen degradability of the protein was not measured. It can, however, be estimated based on literature values of potential degradability and passage rate, as in section 3.3.1.3.

The passage rate of kikuyu pasture would most likely have been slower than that of ryegrass due to the higher NDF content and the intake being lower. Fractional passage rate from the reticulorumen is slower if DMI is lower (Allen, 1996). In section 3.3.1.3 a passage rate of 7.1 %/h was assumed for ryegrass. If a lower passage rate is assumed for kikuyu (6 %/h; all others values assumed to be the same as for ryegrass) the ruminal escape of protein in the grass would have been 26 % and degradability 74 %, higher than for ryegrass. Kikuyu pasture containing 22.1 % CP of which 74 % is degraded, at a PDMI of 6.8 kg, would have supplied 1112 g RDP and 391 g RUP.

Table 5.32 Approximate daily supply of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) from the three experimental diets of cows grazing kikuyu, calculated based on estimates of ruminal passage rate and protein degradation rate

	Experimental treatment[1]		
	Control	**Low FM**	**High FM**
Total diet			
RDP (g/d)	1364	1407	1447
RUP (g/d)	608	704	801
RDP (% CP)	69	67	64
RUP (% CP)	31	33	36

[1]Control: maize-based supplement containing no fishmeal (FM); Low FM: maize-based supplement containing 4 % FM; High FM: maize-based supplement containing 8 % FM

If the same protein degradability values for the concentrates as calculated in section 3.3.1.3 are assumed then the amount of RDP supplied by the total diets would have been as shown in Table 5.32.

The estimated RDP and RUP supplied by the total diets (Table 5.32) were lower for all three experimental treatments than the recommended requirements of 1730 g RDP and 720 g RUP/d (NRC, 2001; see section 2.2) except that RUP was adequate for the high FM treatment. (These NRC (2001) recommendations are probably overestimates of the requirements for the cows used in this trial that are smaller than the average "small breed cow"; the CP concentration and hence supply, was adequate in the two FM diets.) Increasing the FM content of the diet increased both RDP and RUP supply, the latter more so as a greater proportion of the CP was RUP. The lower RDP supply than in the ryegrass trial is mainly due to lower intake of the pasture as well as lower CP in the kikuyu pasture.

The fact that RDP was low indicates that the response could have been due to increased CP *per se*, in other words it is not clear whether it was due to RDP or RUP or both.

5.3.1.4 Milk production and composition

5.3.1.4.1 Mean for the whole experimental period

a) Milk yield

The milk response is lower than in the ryegrass trial. With maize-based diets the responses to additional AA appear to be higher on diets of higher CP (Rulquin & Vérité, 1993).

Since the diet of each successive FM treatment supplied an additional 100 g RUP/d, the milk response was 0.6 to 0.7 kg milk per 100 g additional RUP supplementation, similar to the average increase in milk production of 0.8 kg/d for each 100 g/d of RUP supplementation reported by Bargo *et al.* (2003).

b) Milk composition

The fact that the milk fat of the cows on the high FM was not higher corresponds with the statement of Schroeder & Gagliostro (2000) that feeding FM could reduce milk fat percentage

mainly due to high concentrations of unsaturated long-chain fatty acids in FM or a reduction in acetate to propionate ratio in ruminal fluid negatively affecting milk fat. The acetate: propionate ratio (Table 5.31) was, however, higher for the cows on the high FM treatment, in agreement with the numerically higher milk fat percentage for the cows on the high FM treatment. In the study of McCormick *et al.* (2001a) milk fat percentage was increased (3.34 vs. 3.11 %) when Holstein cows grazing ryegrass-oat pasture were fed high CP supplement (22.8 %) CP vs. moderate CP supplement (16.6 % CP).

The protein percentage in the milk was lower than the mean of 3.75 % for registered Jerseys in South Africa in 2005/ 2006 (National milk recording scheme, South Africa, Annual Report, 2006, Volume 26, ARC, Livestock Business Division, Animal Production, Irene, 0062). Supplementing rumen protected Lys and Met increases milk protein concentration (Rulquin *et al.*, 1993, Robinson *et al.*, 1995; 1998; 1999; Wu *et al.*, 1997; Xu *et al.*, 1998). The lack of milk protein percentage response in this study indicates that AA *per se* were probably not limiting.

Milk urea N testing can help monitor the efficiency of protein utilisation and the adequacy of dietary fermentable carbohydrates (Muller, 2003b). The mean MUN level of the cows on the high FM treatment was within the target range of 10 to 16 mg/dl suggested by Jonker *et al.* (1999), although on the low side, while it was too low on the other two treatments. These values are much lower than in the trial of Meeske & Van der Merwe (2005) where the MUN was 18.2 mg/dl for Jersey cows producing 14 kg milk/d grazing kikuyu and receiving 3.6 kg/d of concentrate containing 15 % CP and 11.5 MJ ME/kg (as fed) possibly because a higher concentrate level in the present study meant the CP/MJ NE_L (positively related to MUN; Broderick & Clayton, 1997) was lower. The lower MUN was to be expected due to the lower CP in the diet and MUN is closely correlated to dietary CP (Broderick & Clayton, 1997; Bargo *et al.*, 2002b). The low MUN and the increase in MUN with FM supplementation was reflected in the ruminal NH_3-N concentration (Table 5.31). These low MUN and NH_3-N values indicate that RDP and microbial protein synthesis were low (De Villiers *et al.*, 2000).

c) Fat- and energy-corrected milk yield

The response in FCM and ECM of the two FM treatments over the control must have been due to the increased CP (RDP and RUP) of the diet (Table 5.32). Rumen degradable

protein was low in all three diets (see section 5.2.1.3) as reflected in the low MUN values (Table 5.11) and low ruminal NH_3-N (Table 5.31).

The fact that EE and Ca and P increased as the level of FM in the supplement increased could cause confounding effects although the three diets had the similar ME levels.

The lack of additional response to the higher level of FM was probably because ME once again became the first limiting nutrient.

5.3.1.5 Body weight and body condition score

The lack of difference between treatments in terms of change in BW or BCS is in agreement with the studies of Jones-Endsley *et al.* (1997), where the amount of CP in the concentrate was increased, and the study by Hongerholt & Muller (1998), where the RUP in the concentrate was increased, and no difference was found between treatments for BW or BCS.

5.3.1.6 Faeces

The levels of starch in the faeces are much lower than those reported by Granzin (2004) who found faecal starch levels of 2.0 and 2.4 % DM for cows grazing kikuyu (PDMI 9.7 and 8.3 kg DM/cow/d) and receiving 4.5 and 8.1 kg barley-based concentrate, respectively, and faecal starch of 6.1 and 10.9 % DM for cows grazing the same pasture (PDMI 10.0 and 8.2 kg DM/cow/d) and receiving 4.5 and 8.1 kg maize-based concentrate, respectively.

The starch concentrations in the total diets consumed by these animals were 27.39, 25.18 and 24.13 %, for the three experimental treatments respectively, thus the ratio of % starch in faeces to % starch in feed was 0.03, 0.07 and 0.04 for the control, low FM and high FM treatments of this trial, respectively. These were once again lower than those in the trial by Hagg, F. (personal communication, fhagg@kkan.com; see section 3.2.1.6), indicating that starch was digested efficiently and extensively in all three of the experimental treatments.

5.3.1.7 Economics

In order to determine if the inclusion of FM in the supplement would be economical (increase profit) the additional revenue from the milk response would have to be greater than the additional cost.

As an example: replacing 280 g maize (at R1990/ton) with 240 g FM (at R6369/ton) and 40 g Megalac (at R5468/ton) would cost an additional R1.19/cow/d. For the high FM treatment this increased feed cost would be R2.38.

Since milk solids affect milk price, a more direct comparison can be made if FCM is used rather than milk yield *per se*. The cows on the low FM treatment produced 2.1 kg 4 % FCM/d more than the cows on the control treatment. Assuming a milk price of R3.00/kg (for milk with 4 % fat) this would bring an extra income of R6.30/cow/d which would lead to an additional profit of R5.11/cow/d.

Even if the higher FM level was used and additional feed cost doubled (R2.38/cow/d) there would still be additional profit of R3.92/cow/d. If the milk yield instead of 4 % FCM values were used, the increased milk yield of the cows on the high FM treatment relative to the control (1.3 kg/d) would lead to an additional profit of R1.52 per cow per day. If the maize price were very low, such as when the farmer grows his own maize, it is possible that the additional cost of FM would not be covered.

The relative prices of milk, maize and FM would affect the profitability of FM supplementation. In Table 5.33 the FM, Megalac and milk prices are kept constant at R6000/ton, R5500/ton and R3.00/kg, respectively. As the maize price increases the additional profit made from FM supplementation increases due the fact that replacing some of the maize with FM causes a smaller increase in feed cost than if the maize price were lower. If the farmer does not receive a higher price for milk with a higher fat content, the additional income from the milk will only cover the additional feed cost if the price of FM is less than R5400/ton more then the maize price (all except the first row of Table 5.33).

Table 5.33 Effect of changing maize price on additional profit made by replacing 280 g maize in the supplement with 240 g fishmeal (FM) and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing kikuyu, assuming a constant FM price of R6000/ton, Megalac price of R5500/ton and milk price of R3.00/kg

Maize price (R/ton)	Additional cost of low FM diet over control (R/cow/d)	Additional profit from 2.1 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 0.5 kg milk yield/cow/d response (R/cow/d)
500	1.52	4.78	-0.02
1000	1.38	4.92	0.12
1500	1.24	5.06	0.26
2000	1.10	5.20	0.40
2500	0.96	5.34	0.54

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

In Table 5.34 the maize, Megalac and milk prices are kept constant at R2000/ton, R5500/ton and R3.00/kg, respectively. As the FM price increases the additional profit made from FM supplementation decreases due the fact that replacing some of the maize with FM causes a greater increase in feed cost than if the FM price were lower.

Table 5.34 Effect of changing fishmeal (FM) price on additional profit made by replacing 280 g maize in the supplement with 240 g FM and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing kikuyu, assuming a constant maize price of R2000/ton, Megalac price of R5500/ton and milk price of R3.00/kg

FM price (R/ton)	Additional cost of low FM diet over control (R/cow/d)	Additional profit from 2.1 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 0.5 kg milk yield/cow/d response (R/cow/d)
4000	0.62	5.68	0.88
5000	0.86	5.44	0.64
6000	1.10	5.20	0.40
7000	1.34	4.96	0.16

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

In Table 5.35 the feed prices are kept constant and the effect of changing milk price on the profitability of FM supplementation examined. If milk price is not adjusted according to milk composition and the FM price is as much as R4000/ton more than the maize price, FM supplementation to cows on kikuyu pasture will not be profitable unless the milk price is at least

R2.20. The lower the milk price the lower the difference between FM and maize prices would have to be for FM supplementation to be profitable.

Table 5.35 Effect of changing milk price on additional profit made by replacing 280 g maize in the supplement with 240 g fishmeal (FM) and 40 g Megalac[1] per day (low FM treatment vs. control) for cows grazing kikuyu, assuming constant maize, FM and Megalac prices of R2000, R6000 and R5500/ton, respectively

Milk price (R/kg)	Additional income from 2.1 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional profit from 2.1 kg 4 % FCM[2]/cow/d response (R/cow/d)	Additional income from 0.5 kg milk yield/cow/d response (R/cow/d)	Additional profit from 0.5 kg milk yield/cow/d response (R/cow/d)
1.80	3.78	2.68	0.90	-0.20
2.00	4.20	3.10	1.00	-0.10
2.20	4.62	3.52	1.10	0.00
2.40	5.04	3.94	1.20	0.10
2.60	5.46	4.36	1.30	0.20
2.80	5.88	4.78	1.40	0.30
3.00	6.30	5.20	1.50	0.40
3.20	6.72	5.62	1.60	0.50
3.40	7.14	6.04	1.70	0.60
3.60	7.56	6.46	1.80	0.70
3.80	7.98	6.88	1.90	0.80

[1]Rumen protected fat (Church & Dwight Co., Inc., Princeton, NJ)
[2]Fat-corrected milk

Under certain price scenarios it could be possible to make increased profit by including FM in the maize-based supplement of high producing cows in early to mid lactation grazing kikuyu, although the margin is smaller than with cows on ryegrass.

Profitability depends to a large extent on the magnitude of the production response. The quality of the raw materials used in the supplement is important.

5.3.2 Rumen study

5.3.2.1 Ruminal pH

The mean daily ruminal pH was slightly higher than the mean ruminal pH of the cows on ryegrass which is to be expected as the NDF in the kikuyu was higher than the ryegrass and ruminal pH is positively related to NDF content (Kolver & De Veth, 2002). No difference in ruminal pH was expected between the two treatments (Jones-Endsley *et al.*, 1997; Carruthers & Neil, 1997; Schor & Gagliostro, 2001; Bargo *et al.*, 2003a).

The trends in ruminal pH throughout the day were once again consistent with other studies (Carruthers & Neil, 1997; Carruthers *et al.*, 1997; Bargo *et al.*, 2001; 2002a; 2002c; Graf *et al.*, 2005; Bargo & Muller, 2005).

The pH was below 6 from 1430 to 2130 h and from 1530 to 2200 h for the cows on the control and high FM treatments, respectively, in other word for approximately seven to seven and a half hours of the day. It was never below 5.8, the level below which cows would start experiencing sub-clinical acidosis (Graf *et al.*, 2005) and fibre digestion is impaired (De Veth & Kolver, 2001).

5.3.2.2 Ruminal ammonia

The mean ruminal NH_3-N concentration of the cows on the control treatment was below the recommended level for maximum microbial protein synthesis of 5 mg/dl (Satter & Slyter, 1974), indicating that RDP was limiting. Even the cows on the high FM treatment had sub-optimal ruminal NH_3-N concentrations at 1200 h, although the levels were adequate for the rest of the day. These values are below the range of 8.7 to 32.2 mg/dl reported by Bargo *et al.* (2003a) for cows on pasture-concentrate.

The effect of FM supplementation on ruminal NH_3-N concentration was reflected in the MUN content of the milk (Table 5.30) which is to be expected since MUN content is correlated to ruminal NH_3-N (Broderick *et al.*, 1997).

Similar low levels of ruminal NH_3-N were found by Hamilton *et al.* (1992) where cows were grazing kikuyu pasture of 16 % CP. When barley grain was supplemented the NH_3-N concentration was 5.2 mg/dl before feeding, and 4.6, 7.6 and 5.8 mg/dl at 2, 4 and 6 hours after feeding, respectively. When barley plus sunflower meal where supplemented the NH_3-N levels rose to 5.9 mg/dl before feeding, and 10.2, 11.4 and 7.6 mg/dl at 2, 4 and 6 hours after feeding, respectively. When the sunflower meal was treated with formaldehyde the levels were 7.8 mg/dl before feeding and 8.7, 11.2 and 7.8 mg/dl at 2, 4 and 6 hours after feeding.

The daily trend in ruminal NH_3-N contrasts with other studies (Carruthers *et al.*, 1997; Kolver *et al.*, 1998; Bargo *et al.*, 2001; 2002c), probably because RDP was not excessive.

5.3.2.3 Volatile fatty acids

Broderick (1992) also reported no difference in total VFA when cows were supplemented with FM vs. SBM (133.8 vs. 122.2 mmol/L).

The total VFA values are within the range of 90 to 151 mmol/L reported by Bargo *et al.* (2003a) for cows on pasture-concentrate. Carruthers & Neil (1997) reported the total VFA of cows grazing pasture of 18 % CP and receiving NSC supplementation to be 132 mmol/L.

Increasing the amount of CP in the concentrate does not usually affect ruminal VFA concentration (Jones-Endsley *et al.*, 1997; Bargo *et al.*, 2003a). This is in agreement with the studies of Bargo *et al.* (2001) where there was no effect of differing level and source of protein on total VFA or molar proportions of individual VFA and Schor & Gagliostro (2001) who found no difference in total ruminal VFA or molar proportions of individual VFA of cows receiving concentrate with SBM or BM (differing in RUP content).

The molar proportions and concentrations of acetate, propionate and butyrate are within the expected range for highly digestible pasture (Doyle *et al.*, 2005).

The proportions of individual VFA once again agree with the studies of Broderick (1992) and Erasmus *et al.* (1994) where propionate was lower when cows were supplemented with FM (vs. SBM) or BM (vs. sunflower meal). The higher propionate in the control cows makes sense since propionate is the major end product of starch fermentation (Berzaghi *et al.*, 1996). The mean acetate: propionate ratio is also in agreement with the study of Broderick (1992) where

supplementation with FM (vs. SBM) lowered rumen propionate and increased the ratio of acetate: propionate. In the study of Jones-Endsley *et al.* (1997) the acetate: propionate ratio was 2.91 and 3.01 for 12 and 16% CP supplements. The acetate: propionate ratios in this trial are well above the level of 2.2: 1 where milk production starts to be depressed (Emery, 1976).

In the study by Jones-Endsley *et al.* (1997), as in this study, butyrate increased as CP in the supplement increased, possibly because of a lower concentration of NSC in the supplement.

Once again differences in diet composition, DMI and starch intake were probably too small to elicit an effect on rumen parameters. Furthermore any relatively small effects that the experimental treatments could have induced would have been masked by the natural variation between cows.

5.4 CONCLUSIONS

High producing, multiparous Jersey cows in early to mid lactation grazing kikuyu pasture while receiving 6 kg (as is) a day of maize-based supplement, respond, in terms of FCM, to addition of FM in their supplement up to 240 g (as is) FM per day above which there is no additional response. The cows on the low FM and high FM treatments produced 12 and 11 % more 4 % FCM than the cows on the control (19.4 and 19.2 vs. 17.3 kg 4 % FCM/d) due increased milk fat percentage in the former and increased milk yield in the latter.

The magnitude of the production response was not as high as with cows on ryegrass. Due to the milk fat response, FM supplementation can increase profit if milk price is based on milk solids. If not, the milk yield response is only big enough to increase profit if the difference between the maize and FM prices is not too large, depending on the milk price, – for example of the milk price is R3.00/kg, increased profit will not be made if FM costs more than R5400/ton more than maize (an unlikely scenario).

The response is probably due to increased CP in the diets. Milk production was limited by ME to 19 kg 4 % FCM/d. The limiting ME and CP in the diets of these cows was mainly due to the low intake of the kikuyu pasture, probably due to high NDF as well as high temperatures. Focusing on pasture management to stimulate pasture intake might be more rewarding than changing the supplement. The lower CP in the pasture as well as the maize and FM used would

also have contributed to the lower dietary CP. Cows on kikuyu respond to additional CP but RUP and AA are probably less important, suggesting the potential to use cheaper, plant based protein sources. This is an area for future research.

Chapter 6

MODELING THE KIKUYU TRIAL

6.1 MATERIALS AND METHODS

In order to evaluate the usefulness of the CPM Dairy model for pasture-based systems, milk yields of the cows on the control, low FM and high FM treatments were compared with what was predicted by the CPM Dairy model (Version 3.0.7a).

Predictions were based on the average cow of each treatment (Table 6.1). The same environmental and management inputs were used for each of the three treatments (Table 6.2).

Table 6.1 Animal inputs used in the CPM-Dairy model for the cows on the kikuyu control, low FM and high FM treatments

Animal Input	Experimental treatment[1]		
	Control	Low FM	High FM
Lactation	4	5	3
Current age (mo)	66	79	53
First calving age (mo)	24	24	24
Calving interval (mo)	13	13	13
Current weight (kg)	370	379	356
Mature weight (kg)	370	379	356
Calf birth weight (kg)	25	25	25
Days pregnant	4	4	4
BCS	2.2	2.25	2.3
Production (kg)	18.2	18.9	19.5
Fat (%)	3.71	4.18	3.91
Days in milk	94	94	94
Crude Protein (%)	3.30	3.41	3.34

BCS – Body condition score
[1]Kikuyu pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)

The same raw materials as in Table 4.3 were used except that the maize and FM CP were adjusted to 8.5 and 70 % DM so that the CP of the concentrates was closer to the laboratory results (Table 5.7). The CP of the control, low FM and high FM concentrates based on these concentrates would be 7.86, 10.26 and 12.93 % DM, respectively.

Table 6.2 Inputs used in the CPM-Dairy model for environment and management variables for the cows in the kikuyu trial

Environment	
Current temperature (°C)[1]	20
Current RH	85
Previous temperature (°C)	20
Previous RH	85
Wind speed (mps)	0
Hours in sunlight	12
Storm exposure	Yes
Min night temperature (°C)	15
Mud depth (cm)	0
Hair depth (tenths of cm)	0.63
Hair coat	No mud
Management	
Activity	Continuous grazing
Time standing (h/d)	18
Body position changes	6
Distance walked flat (m)	5000
Distance walked sloped (m)	0

Feeds ControlConcK, LowFMConcK and HighFMConcK (Table 6.3) were created using CornGrainGrndFin from the CPM feed library and modifying the nutrients to results of the laboratory analyses (section 5.2.1.2). Once again soluble CP, NPN, ADIP, NDIP, lignin and EE were modified to be closer to what would be expected from these concentrates based on average South African raw materials.

A feed Kikuyu was created using GrssP22Cp48Ndf6Lndf from the CPM feed library and inserting the values from Table 6.3. The model defaults values of GrssP22Cp48Ndf6Lndf were used for AA and nutrients not shown in this table as well as the rates of carbohydrate fermentation in the rumen and protein degradation. The analysed ADIP, NDIP and lignin contents were higher than the average for kikuyu in the AFGRI Animal Feeds database (Cronjé, G., personal communication, gert.cronje@afgri.co.za). Values closer to the latter were used instead.

Concentrate intake was set at 5.5 kg DM and the pasture intake adjusted so that the actual and predicted total DMI were the same. Then the concentrate was replaced with the individual raw materials (from Table 4.3) in the correct proportions.

Table 6.3 Chemical composition of the feeds Kikuyu, ControlConcK, LowFMConcK and HighFMConcK used in the CPM-Dairy model

Parameter	Concentration in raw material			
	Kikuyu	ControlConcK[1]	LowFMConcK[2]	HighFMConcK[3]
DM (%)	15.54	92.44	91.36	91.49
CP (% DM)	22.23	7.74	10.09	12.70
Sol CP (% CP)	41.34	16	17	18
NPN (% SP)	49.81	69	70	71
ADIP (% CP)	6	4	4	4
NDIP (% CP)	30	13	13.5	14
ADF (% DM)	31.34	3.59	3.38	3.57
NDF (% DM)	60.50	13.86	14.93	17.47
Lignin (% NDF)	3	2	2	2
Ash (% DM)	11.83	5.98	7.93	8.65
EE (% DM)	2.13	3	4	5
Ca (% DM)	0.37	1.23	1.53	2.02
P (% DM)	0.35	0.53	0.63	0.81
Met (% RUP)	0.67	3.28	3.22	3.32
Lys (% RUP)	2.83	5.37	6.84	7.69
Arg (% RUP)	2.83	10.74	9.66	9.49
Thr (% RUP)	2.83	7.16	7.04	6.03
Leu (% RUP)	5.49	20.58	14.89	13.71
Ile (% RUP)	2.83	6.56	5.63	6.03
Val (% RUP)	3.83	9.84	8.05	8.14
His (% RUP)	1.00	4.18	4.02	3.92
Phe (% RUP)	3.50	8.65	6.64	6.78

DM – Dry matter; CP – Crude protein, Sol CP – Soluble CP; NPN – Non-protein N; ADIP – Acid detergent insoluble protein; NDIP – Neutral detergent insoluble protein; ADF – Acid detergent fibre; NDF – Neutral detergent fibre; EE – Ether extract;
[1]Control concentrate (no fishmeal)
[2]Low fishmeal concentrate (4 % fishmeal)
[3]High fishmeal concentrate (8 % fishmeal)

6.2 RESULTS

Table 6.4 shows the predictions of the CPM Dairy model (Version 3.0.7a) for the cows on the control, low FM and high FM treatments based on the concentrates from Table 6.3. When the concentrate was replaced with the individual raw materials (from Table 4.3) the model predictions were as shown in Table 6.5.

Table 6.4 The CPM Dairy model predicted outputs from the control, low FM and high FM diets[1] in the kikuyu trial with the analysed concentrates used as raw materials

Parameter	Control	Low FM	High FM
Target Milk (kg/d)	18.2	18.9	19.5
ME allowed milk (kg/d)	18.4	18.2	18.2
MP allowed milk (kg/d)	19.5	20.0	20.1
AA allowed milk (kg/d)	19.6	20.3	21.1
DMI predicted (kg/d)	12.0	12.8	12.3
DMI actual (kg/d)	12.0	12.8	12.3
Pasture DMI (kg/d)	6.5	7.3	6.8
Diet RDP (% CP)	68.4	67.1	66.1
MP from bacteria (g/d)	878	870	806
MP from RUP	436	536	570
Diet CP (% DM)	15.6	17.0	18.0
Diet ME (MJ/kg DM)	11.37	11.11	11.13
Days to lose 1 CS	2440	422[2]	219[2]
Weight change due to reserves (kg/d)	0.03	-0.13	-0.23
Predicted MUN (mg %)	7	9	10

ME – Metabolisable energy; MP – Metabolisable protein; DMI – Dry matter intake; RDP – Rumen-degradable protein; CP – Crude protein; RUP – Rumen-undegradable protein; MUN – Milk urea N; CS – Condition score
[1]Kikuyu pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)
[2]Or decrease milk production -1 kg/d

Table 6.5 The CPM Dairy model predicted outputs from the control, low FM and high FM diets[1] in the kikuyu trial with individual raw materials used to make up the concentrates

Parameter	Control	Low FM	High FM
Target Milk (kg/d)	18.2	18.9	19.5
ME allowed milk (kg/d)	18.6	18.9	19.2
MP allowed milk (kg/d)	20.0	21.8	22.9
AA allowed milk (kg/d)	18.3	19.5	20.2
DMI predicted (kg/d)	12.0	12.8	12.3
DMI actual (kg/d)	12.0	12.8	12.3
Pasture DMI (kg/d)	6.5	7.3	6.8
Diet RDP (% CP)	68.1	65.2	62.6
MP from bacteria (g/d)	896	903	850
MP from RUP (g/d)	439	578	646
Diet CP (% DM)	15.7	17.1	18.0
Diet ME (MJ/kg DM)	11.50	11.39	11.53
Days to lose 1 CS	906	6333	919
Weight change due to reserves (kg/d)	0.07	-0.01	-0.06
Predicted MUN (mg %)	7	9	10

ME – Metabolisable energy; MP – Metabolisable protein; DMI – Dry matter intake; RDP – Rumen-degradable protein; CP – Crude protein; RUP – Rumen-undegradable protein; MUN – Milk urea N; CS – Condition score
[1]Kikuyu pasture + concentrate with no fishmeal (FM; control treatment), 4 % FM (Low FM treatment) or 8 % FM (High FM treatment)

6.3 DISCUSSION

As with the ryegrass trial, the model predicts that AA were more limiting to milk production than MP when individual raw materials are used to make up the concentrates. Predicted AA allowable milk production is higher when the analysed concentrates are used due to high Met. This could be due to analytical error as it is unlikely that all three concentrate would have the same Met content. The MP allowed milk is lower when the analysed concentrates are used in the modelling (Table 6.4 vs. Table.6.5). The MP from bacteria is also lower.

When individual raw materials are used the model predicted ME to be limiting in all three diets except in the control treatment where AA are also limiting.

Unlike with the ryegrass trial, the model accurately predicts the ME allowable milk production (when individual raw materials are used; Table 6.5) for the given daily distance walked.

Once again the predicted dietary RDP concentration is close to that calculated and shown in Table 5.32.

The model accurately predicts MUN except for the control treatment where the predicted MUN is lower than what was observed (Table 5.11).

If PDMI is modified so that ME, MP or AA allowed milk production, whichever is lowest, is equal to actual observed production, the PDMI is 6.4, 7.3 and 6.95 kg/cow/d for the cows on the control, low FM and high FM treatments, respectively. Predicted and actual DMI are very close to each other.

The cows on the control treatment were limited by AA, hence the production response observed. The model could be used to determine under what circumstances ME becomes first limiting, in which case there would be no production response.

If PDMI drops below 6.2 kg DM/cow/d then ME and AA start to become co-limiting and below 5 kg DM/cow/d ME becomes limiting.

If the daily distance walked by the cows is above 6000 m a day then ME starts to become limiting due to the energy required for walking.

If the pasture composition changes AA still limit production more than ME especially when the CP drops to below 19 % DM in which case even MP allowed milk is lower than ME allowed milk. If the pasture CP were to increase and the NDF decrease, ME and AA allowed

milk increase but the former is still higher than the latter since MP from bacteria does not increase.

Milk production is still limited by AA in first lactation cows. Energy starts to limit production at the end of lactation when gestation requirements are high. Production of larger (e.g. 600 kg) cows, with higher PDMI, is still limited by AA more than ME although AA and ME allowed milk production are similar to each other.

The production of the cows on the two FM treatments were limited by ME and would increase if pasture intake could increase, indicating that one of the factors limiting the response to protein supplementation could be the limited capacity for consuming kikuyu pasture.

6.4 CONCLUSION

The CPM Dairy model can predict milk production to within 0.3 kg/d of that actually observed and is useful for predicting pasture DMI. It can also be used to indicate under which circumstances AA limit production. According to the model the kikuyu pasture composition, cow breed and lactation number do not change the fact that AA are first limiting. Cows can respond to AA supplementation if PDMI is greater than 6.2 kg DM/cow/d, the distance walked is less than approximately 6000m a day and the cow is not in late lactation.

Chapter 7

CRITICAL EVALUATION

The results of this trial are directly applicable to farmers in the Southern Cape grazing kikuyu over-sown with annual ryegrass supplemented with only maize and minerals. It tested the principle that addition of a quality protein source to the maize would increase performance of the cows, and demonstrated that the response is greater on ryegrass than kikuyu.

Megalac was used in the concentrates to make the three diets iso-energetic for the experiment. The fact that it is a rumen protected fat means that it is not as fermentable in the rumen as the maize it was substituting. It would have been more desirable to use a carbohydrate (sugar) based energy source.

In practice it would be easier for the farmer to only add FM. A future study could look at addition of FM without additional high-energy raw materials such as Megalac. Fishmeal could replace some of the maize (as was the case in this trial) or it could be added to the concentrate which would dilute other components such as the minerals. The latter would be easier for farmers to implement. An alternative easy system to implement would be to add a protein/ mineral concentrate to the maize instead of only adding minerals.

After the trial was conducted and the feed samples analysed, it was evident that the maize and FM used for the kikuyu trial did not contain as much CP as those used for the ryegrass trial. This partly explains the lower response in the kikuyu trial. Although difficult to implement, it should have been insisted that only high quality, consistent, ingredients be used for the trial. This would have required analysis of the raw materials before the feed was mixed.

It was assumed that the pastures were purely ryegrass or kikuyu. However in practice there are always other plants in the pasture. Determining the botanical composition of the pasture would have given a clearer picture.

A major limitation when analysing the results was the fact that pasture intake, and hence nutrient intake from the diet, was not known. The RPM is a useful tool for managing pastures but is not accurate enough for experimental purposes. The fact that it was based on a regression equation with an R^2 of only 0.4 means the RPM did not accurately estimate the amount of DM available on the pasture.

A more accurate estimate of pasture intake could have indicated fluctuations in intake. For example if it was hot and pasture intake decreased at the time of taking ruminal samples in the kikuyu trial, this could have helped explain the low NH_3-N concentration as low pasture intake would have led to low RDP intake.

The use of inherent plant markers such as alkanes could have been a way of determining DMI. This would have been labour intensive and caused stress on the cows, especially taking faecal samples. Since determining pasture intake was not an aim of the experiment, it was better to interfere with the cows as little as possible and just measure their response to the different concentrates.

Since the aim of the experiment was to determine the response to addition of a quality protein source, estimates of protein degradability would have given more strength to interpretation of the results. *In sacco* studies could have been done with rumen cannulated cows to determine the protein degradability of the pasture and the concentrates. This would have required more cannulated cows (or more time) and would have been more work. There was limited time as well as funding for laboratory analyses.

Analysing the AA composition of the duodenal digesta to see if FM really did increase the EAA passing to the small intestine would have been useful. This would not have been practical as there were no cows with duodenal cannulae on the farm.

In order to compare the results of the trial to the predictions of the CPM Dairy model, many laboratory analyses were required. Some of these were inaccurate and needed to be replaced with long term average values anyway. The CPM Dairy model would only be useful for analysing pasture-based systems in South Africa when there is an extensive database of local pastures and raw materials (such as maize) in the feed library so that only a few basic laboratory analyses are required to be able to assess diet adequacy.

Future studies could look at alternative protein sources such as heat treated SBM. Amino acid profile and digestibility are important but price also plays a role. Studies could be done on different pastures and with different breeds receiving different levels of concentrate supplementation (larger cows receiving more concentrate might respond differently). With fluctuating maize prices, farmers could find themselves using alternative energy sources such as barley. A study could be done using barley instead of maize.

Where there are positive responses, the level of protein source (FM) can be refined. For example in the ryegrass trial it was found that there was no additional response above 240 g FM/cow/d. If the plateau is reached at a lower level of FM it would be more profitable to add less FM. Hence another study could supplement 120, 180 and 240 g FM/cow/d.

A future trial could also be done where cows are receiving silage in addition to the pasture, especially on kikuyu as PDMI is limited.

This trial indicated positive responses in higher producing Jersey cows in early to mid lactation. The question remains as to whether the rest of the herd would also respond. A trial could also be done using only early lactation cows to determine if they respond more.

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APPENDIX A

CLIMATE AND SOIL

Trial 1: Ryegrass

The total precipitation during the measurement period of the ryegrass trial (20 September to 4 November 2005) was 60.6 mm. The total precipitation for August, September, October and November 2005, were 22.6, 36.1, 11.4 and 73.5 mm, respectively, compared to the previous 14 year (1992 to 2005) average of 53.4, 55.4, 82.5 and 95.4 mm for August, September, October and November. The trial was conducted in a time when the rainfall was lower than usual for that area (Figure A1) but this should not have affected the experiment since the pasture was under irrigation.

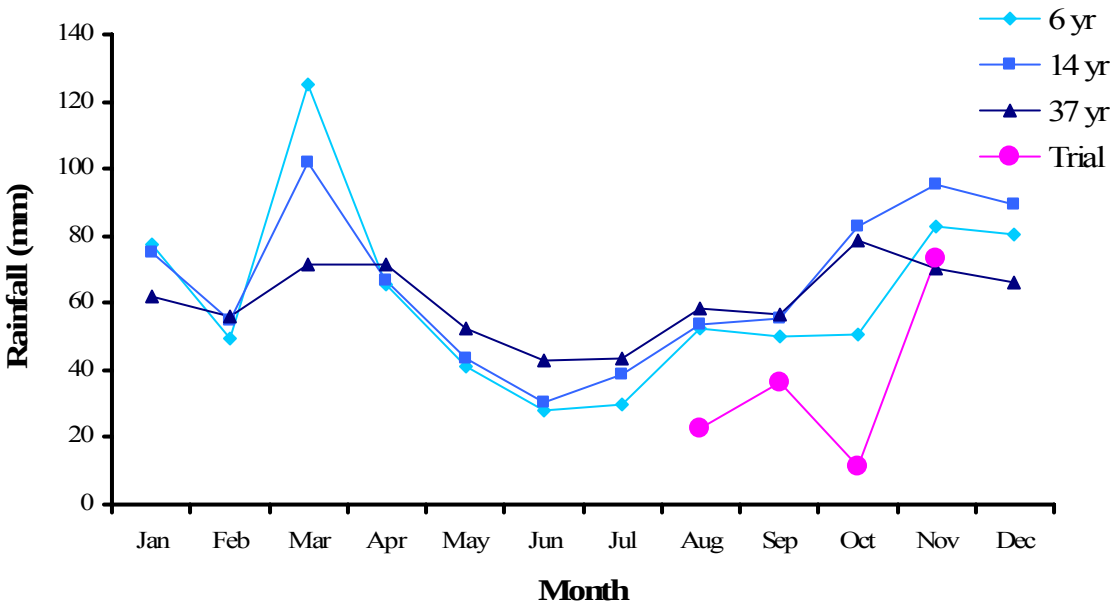

Figure A1 Rainfall (mm) during the months of the ryegrass trial compared to the 6 year (2001 to 2005), 14 year (1992 to 2005) and 37 year average monthly rainfalls

The mean high temperature for the measurement period was 21.1°C and the mean low temperature was 10.6 °C. The mean maximum and minimum temperatures for the respective months, compared to the previous four year (2002 to 2005) average, is shown in Table A1. The mean temperatures during the trial did not deviate significantly from the norm for the area at that time of year.

Table A1 Mean maximum and minimum daily temperatures (°C) for the months during which the ryegrass trial was conducted (2005) compared to the four year (2002 to 2005) mean

Month	Maximum temperature		Minimum temperature	
	Mean 2005	Four year mean	Mean 2005	Four year mean
August	18.2	18.4	7.4	7.8
September	19.8	19.7	8.9	9.4
October	22.2	21.1	10.5	11.8
November	22.3	22.4	12.8	12.8

To summarise: the mean maximum (21.1°C) and minimum (10.6°C) temperatures during the trial were normal for spring in the George area. The rainfall during the trial was lower than usual but should not have affected the experiment since the pasture was under irrigation.

Trial 2: Kikuyu

a) Climate

The total precipitation during the measurement period of the kikuyu trial (19 January to 20 March 2006) was 87.7 mm. The total precipitation for January, February and March 2006 was 61.1, 54.5 and 35.1 mm respectively compared to the previous 14 year (1992 to 2005) average of 75.2, 54.7 and 101.8 mm for January, February and March. This is indicated in Figure A2. The rainfall was normal in January and February but lower than usual in March. This should not have affected the experiment since the pasture was under irrigation.

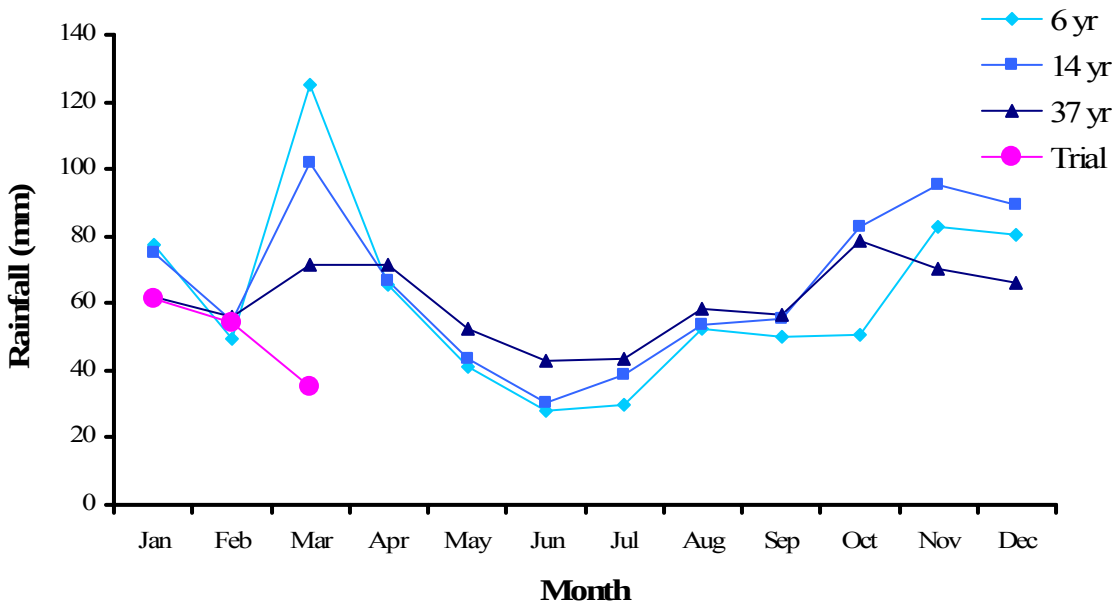

Figure A2 Rainfall (mm) during the months of the kikuyu trial compared to the 6 year (2001 to 2005), 14 year (1992 to 2005) and 37 year average monthly rainfalls

The mean high temperature for the measurement period was 24.5 °C and the mean low temperature was 15.5 °C. The mean maximum and minimum temperatures for the respective months, compared to the previous four year (2002 to 2005) average, is shown in Table A2. The mean temperatures during the trial did not deviate significantly from the norm for the area at that time of year.

Table A2 Mean maximum and minimum daily temperatures (°C) for the months during which the kikuyu trial was conducted (2006) compared to the four year (2002 to 2005) mean

Month	Maximum temperature		Minimum temperature	
	Mean 2006	Four year mean	Mean 2006	Four year mean
January	23.6	24.5	15.8	15.4
February	24.8	24.8	17.1	15.8
March	23.8	24.4	12.9	14.3

b) Soil

The chemical composition of the soil in February 2006 is shown in Table A3 compared to the optimum levels recommended for grass pastures by the Elsenburg Production Technology

Laboratory (Department Agriculture: Western Cape, Private Bag X1, Elsenburg, 7607) who tested the soil sample. Apart from the routine N fertilisation requirement and lime to raise the pH, there were sufficient minerals in the soil.

Table A3 Chemical composition of the soil in which the pasture was grown

Parameter	Level in soil	Optimum level
pH	4.9	5-6
Resistance (ohms)	360	>400
Texture	Sand	-
Acidity (cmol(+)/kg)	1.59	-
Calcium (cmol(+)/kg)	4.10	1-10
Magnesium (cmol(+)/kg)	1.87	0.3-3
Potassium (mg/kg)	127	80-150
Sodium (mg/kg)	99	<100
P (citric acid; mg/kg)	126	50-120
Total cations (cmol(+)/kg)	8.32	-
Copper (mg/kg)	2.04	1-2
Zinc (mg/kg)	19.60	1-2
Manganese (mg/kg)	28.22	10+
Boron (mg/kg)	0.76	0.5-1
Sulphur (mg/kg)	14.65	7+

To summarise: the mean maximum (24.5°C) and minimum (15.5°C) temperatures during the trial were normal for summer in the George area. The rainfall during the trial was lower than usual only towards the end of the trial but should not have affected the experiment since the pasture was under irrigation. There were sufficient minerals in the soil.

APPENDIX B

SELECTION OF THE COWS

Trial 1: Ryegrass

All the lactating cows in the herd at the Outeniqua Experimental Farm were reviewed for selection for use in the trial. All the first lactation cows and all the cows that were further than 140 days into lactation were excluded. The mean milk yield, from 1 to 25 August 2005, for each cow was calculated. A table was compiled showing the cow name, days into lactation and lactation number. In this table the cows were ranked according to milk yield. This table was used to place cows in blocks with the three cows in each block having, as much as possible, matching milk yield and days into lactation. Sixteen blocks were selected. All the cows that did not fit into a block were deleted. Then the cows were ranked according to block number. This is shown in Table B1.

Then the three cows within each block were randomly allocated to a group as shown in Table B2.

The cows were then ranked according to group number and then block. Each group was randomly allocated to a treatment, as shown in Table B3, where C is the control treatment, L is the low FM treatment and H is the high FM treatment.

The name of each cow was replaced with two letters, R for ryegrass trial and C, L or H for treatment, and the number of the block. For example ALET 90 was RL13.

Block 11 was deleted for all purposes of analyzing results since the milk production of cow RH11 dropped near the beginning of the trial and did not pick up again and she had a high milk somatic cell count indicating sub-clinical mastitis. It was preferable to only use results from healthy cows. Without block 11 there were only 45 cows in the ryegrass trial.

Tables B4, B5 and B6 show the cows in the three groups with their mean milk yield from the previous 25 days (1 to 25 August 2005), their days into lactation on 26 August 2005 and lactation number. The mean milk yield of the control, low FM and high FM groups were 21.5, 21.4 and 21.4 kg milk/cow/d respectively. The mean days into lactation of the cows on the day

of selection were 73, 73 and 75 days for the control, low FM and high FM groups respectively and the mean lactation number was 4 for all three groups.

Trial 2: Kikuyu

The selection of the cows for the kikuyu trial was the same as the ryegrass trial. These cows were allocated numbers the same as for the ryegrass trial, except a K (for kikuyu) instead of R (for ryegrass) was used.

Table B7 shows the cows once they have been randomly allocated to the treatments as described above.

Blocks 1 and 11 were deleted for all purposes of analysing results since cows KL1 and KC11 had high milk somatic cell count indicating sub-clinical mastitis and it was preferable to only use results from healthy cows. Without blocks 1 and 11 there were only 42 cows in the kikuyu trial.

Tables B8, B9 and B10 show the cows in the three groups of the kikuyu trial with their mean milk yield from the previous 21 days (27 December 2005 to 16 January 2006), their days into lactation on 16 January 2006 and lactation number. The mean milk yield of the control, low FM and high FM groups were 21.9, 21.9 and 22.0 kg milk/cow/d respectively. On the day of selection of the cows the mean days into lactation were 65, 64 and 64 days for the control, low FM and high FM groups respectively and the mean lactation number 4, 5 and 3 respectively.

Table B1 Blocking of cows for the ryegrass trial

Name	Milk yield	Days into lactation	Lactation number	Block
TAMSA 3	23.9	72	4	1
GERL 14	24.0	76	6	1
TBELL108	24.6	64	4	1
MART117	23.7	81	5	2
GRET 22	23.7	86	5	2
DORA 94	23.9	85	5	2
TALET 71	23.1	66	7	3
IDA 33	23.2	70	4	3
BLON 39	23.5	59	5	3
SYMB 53	22.9	86	6	4
MAGD 71	23.3	98	5	4
ALET 84	23.5	81	5	4
TMAX	22.6	50	5	5
BABS 21	22.6	55	3	5
TBERT 5	22.7	67	4	5
TSUSA	21.5	96	4	6
TELIZE 62	21.5	103	7	6
DORA 85	22.3	117	5	6
TLIZ 6	21.0	74	2	7
BELL102	21.2	80	5	7
THES	21.7	79	3	7
GERL 16	20.9	50	4	8
MART129	21.1	45	3	8
TBERT 20	21.5	50	2	8
TSUSA 1	20.3	22	5	9
FIRE 47	20.5	20	2	9
ALET 82	20.8	36	5	9
SYMB 62	20.3	74	2	10
IDA 34	20.6	64	4	10
TBERT 4	20.8	78	4	10
MART135	20.2	109	2	11
TPANS	20.3	93	4	11
TBERT 6	20.5	111	3	11
TSUSA 11	19.7	26	2	12
TLASS	20.0	22	4	12
MARL 24	20.1	35	8	12
ALET 90	19.2	90	4	13
MARL 47	19.7	103	4	13
TLIZ 8	19.8	100	2	13
TAMSA 5	19.5	104	3	14
BLON 56	19.7	96	2	14
BLON 31	19.7	99	6	14
TARNA 3	18.5	48	2	15
MART137	18.5	59	2	15
TESME 2	19.2	57	2	15
TDORA 83	21.2	116	5	16
TALTA 24	21.2	137	5	16
TLIN	21.3	129	4	16

Table B2 Allocating cows within blocks to groups for the ryegrass trial

Name	Milk yield	Days into lactation	Lactation number	Block	Group
TAMSA 3	23.9	72	4	1	3
GERL 14	24.0	76	6	1	2
TBELL108	24.6	64	4	1	1
MART117	23.7	81	5	2	2
GRET 22	23.7	86	5	2	3
DORA 94	23.9	85	5	2	1
TALET 71	23.1	66	7	3	3
IDA 33	23.2	70	4	3	2
BLON 39	23.5	59	5	3	1
SYMB 53	22.9	86	6	4	2
MAGD 71	23.3	98	5	4	1
ALET 84	23.5	81	5	4	3
TMAX	22.6	50	5	5	2
BABS 21	22.6	55	3	5	3
TBERT 5	22.7	67	4	5	1
TSUSA	21.5	96	4	6	3
TELIZE 62	21.5	103	7	6	1
DORA 85	22.3	117	5	6	2
TLIZ 6	21.0	74	2	7	2
BELL102	21.2	80	5	7	1
THES	21.7	79	3	7	3
GERL 16	20.9	50	4	8	1
MART129	21.1	45	3	8	3
TBERT 20	21.5	50	2	8	2
TSUSA 1	20.3	22	5	9	1
FIRE 47	20.5	20	2	9	2
ALET 82	20.8	36	5	9	3
SYMB 62	20.3	74	2	10	3
IDA 34	20.6	64	4	10	1
TBERT 4	20.8	78	4	10	2
MART135	20.2	109	2	11	2
TPANS	20.3	93	4	11	3
TBERT 6	20.5	111	3	11	1
TSUSA 11	19.7	26	2	12	1
TLASS	20.0	22	4	12	2
MARL 24	20.1	35	8	12	3
ALET 90	19.2	90	4	13	1
MARL 47	19.7	103	4	13	2
TLIZ 8	19.8	100	2	13	3
TAMSA 5	19.5	104	3	14	1
BLON 56	19.7	96	2	14	3
BLON 31	19.7	99	6	14	2
TARNA 3	18.5	48	2	15	1
MART137	18.5	59	2	15	2
TESME 2	19.2	57	2	15	3
TDORA 83	21.2	116	5	16	3
TALTA 24	21.2	137	5	16	2
TLIN	21.3	129	4	16	1

Table B3 Allocating cows to the experimental treatments for the ryegrass trial

Name	Milk yield	Days into lactation	Lactation number	Block	Group	Treatment
TBELL108	24.6	64	4	1	1	L
DORA 94	23.9	85	5	2	1	L
BLON 39	23.5	59	5	3	1	L
MAGD 71	23.3	98	5	4	1	L
TBERT 5	22.7	67	4	5	1	L
TELIZE 62	21.5	103	7	6	1	L
BELL102	21.2	80	5	7	1	L
GERL 16	20.9	50	4	8	1	L
TSUSA 1	20.3	22	5	9	1	L
IDA 34	20.6	64	4	10	1	L
TBERT 6	20.5	111	3	11	1	L
TSUSA 11	19.7	26	2	12	1	L
ALET 90	19.2	90	4	13	1	L
TAMSA 5	19.5	104	3	14	1	L
TARNA 3	18.5	48	2	15	1	L
TLIN	21.3	129	4	16	1	L
GERL 14	24.0	76	6	1	2	H
MART117	23.7	81	5	2	2	H
IDA 33	23.2	70	4	3	2	H
SYMB 53	22.9	86	6	4	2	H
TMAX	22.6	50	5	5	2	H
DORA 85	22.3	117	5	6	2	H
TLIZ 6	21.0	74	2	7	2	H
TBERT 20	21.5	50	2	8	2	H
FIRE 47	20.5	20	2	9	2	H
TBERT 4	20.8	78	4	10	2	H
MART135	20.2	109	2	11	2	H
TLASS	20.0	22	4	12	2	H
MARL 47	19.7	103	4	13	2	H
BLON 31	19.7	99	6	14	2	H
MART137	18.5	59	2	15	2	H
TALTA 24	21.2	137	5	16	2	H
TAMSA 3	23.9	72	4	1	3	C
GRET 22	23.7	86	5	2	3	C
TALET 71	23.1	66	7	3	3	C
ALET 84	23.5	81	5	4	3	C
BABS 21	22.6	55	3	5	3	C
TSUSA	21.5	96	4	6	3	C
THES	21.7	79	3	7	3	C
MART129	21.1	45	3	8	3	C
ALET 82	20.8	36	5	9	3	C
SYMB 62	20.3	74	2	10	3	C
TPANS	20.3	93	4	11	3	C
MARL 24	20.1	35	8	12	3	C
TLIZ 8	19.8	100	2	13	3	C
BLON 56	19.7	96	2	14	3	C
TESME 2	19.2	57	2	15	3	C
TDORA 83	21.2	116	5	16	3	C

Table B4 Cows in the control group at the beginning of the ryegrass trial

Cow	Milk	Days into lactation	Lactation number
RC1	23.9	72	4
RC2	23.7	86	5
RC3	23.1	66	7
RC4	23.5	81	5
RC5	22.6	55	3
RC6	21.5	96	4
RC7	21.7	79	3
RC8	21.1	45	3
RC9	20.8	36	5
RC10	20.3	74	2
RC12	20.1	35	8
RC13	19.8	100	2
RC14	19.7	96	2
RC15	19.2	57	2
RC16	21.2	116	5
Mean	**21.5**	**73**	**4**
SD	**1.56**	**24.1**	**1.9**

Table B5 Cows in the low fishmeal group at the beginning of the ryegrass trial

Cow	Milk	Days into lactation	Lactation number
RL1	24.6	64	4
RL2	23.9	85	5
RL3	23.5	59	5
RL4	23.3	98	5
RL5	22.7	67	4
RL6	21.5	103	7
RL7	21.2	80	5
RL8	20.9	50	4
RL9	20.3	22	5
RL10	20.6	64	4
RL12	19.7	26	2
RL13	19.2	90	4
RL14	19.5	104	3
RL15	18.5	48	2
RL16	21.3	129	4
Mean	**21.4**	**73**	**4**
SD	**1.85**	**29.8**	**1.3**

Table B6 Cows in the high fishmeal group at the beginning of the ryegrass trial

Cow	Milk	Days into lactation	Lactation number
RH1	24.0	76	6
RH2	23.7	81	5
RH3	23.2	70	4
RH4	22.9	86	6
RH5	22.6	50	5
RH6	22.3	117	5
RH7	21.0	74	2
RH8	21.5	50	2
RH9	20.5	20	2
RH10	20.8	78	4
RH12	20.0	22	4
RH13	19.7	103	4
RH14	19.7	99	6
RH15	18.5	59	2
RH16	21.2	137	5
Mean	**21.4**	**75**	**4**
SD	**1.63**	**32.2**	**1.5**

Table B7 Allocating cows to the experimental treatments for the kikuyu trial

Name	Milk yield	Days into lactation	Lactation number	Block	Group	Treatment
DORA 90	26.0	54	5	1	1	C
MART116	24.1	78	5	2	1	C
MART122	23.6	44	4	3	1	C
MARL 49	23.9	69	4	4	1	C
JAPN 57	22.8	43	5	5	1	C
TSUSA 14	22.7	67	2	6	1	C
ALTA 21	22.3	48	6	7	1	C
TDORA 84	22.2	98	6	8	1	C
TTES 1	21.4	79	3	9	1	C
TBERT 13	21.4	61	3	10	1	C
TBERT 7	21.2	71	4	11	1	C
MARL 62	20.9	104	3	12	1	C
TAMSA 1	20.9	74	5	13	1	C
DORA107	20.4	58	2	14	1	C
GRET 33	20.2	68	3	15	1	C
BELL114	20.1	19	4	16	1	C
ELIZE 74	24.9	54	3	1	2	H
TBELL 97	24.4	83	6	2	2	H
MARL 58	23.7	45	3	3	2	H
JAPN 56	23.8	82	4	4	2	H
MART139	23.0	48	2	5	2	H
BLON 47	22.5	58	4	6	2	H
GERL 19	22.4	43	3	7	2	H
TLUA 1	22.4	75	5	8	2	H
MART118	22.0	91	5	9	2	H
TAMSA 11	21.3	54	3	10	2	H
BELL 88	21.2	76	8	11	2	H
BELL 109	20.8	98	4	12	2	H
DORA100	20.6	72	3	13	2	H
TLIN 7	20.9	42	2	14	2	H
BLON 54	20.2	92	2	15	2	H
TAMSA 16	20.4	19	2	16	2	H
TMAGD 72	25.6	81	5	1	3	L
TMARL 31	24.3	71	8	2	3	L
TJAPN 45	24.3	47	7	3	3	L
DORA 82	23.3	64	6	4	3	L
ELIZE 65	22.8	55	6	5	3	L
ELIZE 67	22.7	53	5	6	3	L
JAPN 59	22.1	43	4	7	3	L
MART134	22.0	82	3	8	3	L
TBERT	21.4	82	5	9	3	L
MARL 45	21.3	68	6	10	3	L
JAPN 42	21.2	89	8	11	3	L
TMAX 1	21.2	104	5	12	3	L
TAMSA 10	20.7	71	3	13	3	L
JAPN 44	20.6	51	7	14	3	L
GRET 32	20.3	85	3	15	3	L
DORA 69	20.1	23	6	16	3	L

Table B8 Cows in the control group at the beginning of the kikuyu trial

Cow	Milk	Days into lactation	Lactation number
KC2	24.1	78	5
KC3	23.6	44	4
KC4	23.9	69	4
KC5	22.8	43	5
KC6	22.7	67	2
KC7	22.3	48	6
KC8	22.2	98	6
KC9	21.4	79	3
KC10	21.4	61	3
KC12	20.9	104	3
KC13	20.9	74	5
KC14	20.4	58	2
KC15	20.2	68	3
KC16	20.1	19	4
Mean	**21.9**	**65**	**4**
SD	**1.37**	**22.3**	**1.3**

Table B9 Cows in the low fishmeal group at the beginning of the kikuyu trial

Cow	Milk	Days into lactation	Lactation number
KL2	24.3	71	8
KL3	24.3	47	7
KL4	23.3	64	6
KL5	22.8	55	6
KL6	22.7	53	5
KL7	22.1	43	4
KL8	22.0	82	3
KL9	21.4	82	5
KL10	21.3	68	6
KL12	21.2	104	5
KL13	20.7	71	3
KL14	20.6	51	7
KL15	20.3	85	3
KL16	20.1	23	6
Mean	**21.9**	**64**	**5**
SD	**1.38**	**20.7**	**1.6**

Table B10 Cows in the high fishmeal group at the beginning of the kikuyu trial

Cow	Milk	Days into lactation	Lactation number
KH2	24.4	83	6
KH3	23.7	45	3
KH4	23.8	82	4
KH5	23.0	48	2
KH6	22.5	58	4
KH7	22.4	43	3
KH8	22.4	75	5
KH9	22.0	91	5
KH10	21.3	54	3
KH12	20.8	98	4
KH13	20.6	72	3
KH14	20.9	42	2
KH15	20.2	92	2
KH16	20.4	19	2
Mean	**22.0**	**64**	**3**
SD	**1.39**	**23.6**	**1.3**

APPENDIX C

CALCULATION OF ENERGY REQUIREMENTS OF THE COWS

Trial 1: Ryegrass

Equations from chapter 2 of NRC (2001) were used to calculate the energy requirements of the cows.

The net energy (NE) requirement for maintenance is 0.08 Mcal/kg $BW^{0.75}$. The mean BW of 355 kg was used in the calculation. This can be converted to MJ by multiplying by 4.184 MJ/Mcal, and then converted to ME requirement by dividing by 0.62 since the efficiency of utilisation of NE for maintenance is 0.62 (NRC, 2001).

The NE requirement for lactation (Mcal/kg milk) is 0.360 + [0.0969 (fat %)]. A value of 4 was used for fat % since 4 % FCM was used, thus 0.75 Mcal were required per kg milk which was multiplied by the 4 % FCM production. The NE requirement for lactation can be converted to MJ my multiplying by 4.184 MJ/Mcal and to ME by dividing by 0.64 (the efficiency of utilisation of NE for lactation; NRC, 2001).

Grazing cows also have an energy requirement for activity (walking from pasture to the milking parlour and back). The NE requirement for activity is 0.00045 Mcal of NE/kg BW per km walked + 0.0012 Mcal per kg BW. The cows walked on average 5 km per day. This can be converted to MJ by multiplying by 4.184 MJ/Mcal. A figure was not given in the NRC (2001) for the efficiency of utilisation of NE for activity so it was assumed to be the same as the efficiency of utilisation of ME for maintenance (0.62).

The equation for energy requirements for pregnancy is only for cows 190 to 279 days in gestation. Energy requirement for pregnancy was assumed to be zero for these early lactation cows.

According to table 2-4 of NRC (2001) the NE requirement per kg BW gain is 4.50 and 4.90 Mcal for cows with a condition score of 2.0 and 2.5, respectively. The mean BCS of the cows was between these values so a value of 4.7 Mcal NE/kg BW gain was used. The cows were

gaining on average 0.7 kg per day. The efficiency of converting dietary NE to tissue energy for BW gain is 1.12.

The results of these calculations are shown in Table C1.

Table C1 Energy requirements of cows grazing ryegrass and receiving maize based-concentrate containing either no fishmeal (FM; control), 4 % FM (Low FM) or 8 % FM (High FM)

Maintence requirement		Mean	
NE maint (Mcal/d)		6.5	
NE maint (MJ/d)		27.4	
ME maint (MJ/d)		44.2	
Lactation requirement	Control	Low FM	High FM
4 % FCM production	20.4	24.1	24.2
NE lact (Mcal/d)	15.3	18.0	18.1
NE lact (MJ/d)	63.8	75.4	75.7
ME lact (MJ/d)	99.7	117.8	118.3
Activity requirement		Mean	
NE activity (Mcal/d)		1.2	
NE activity (MJ/d)		5.1	
ME activity (MJ/d)		8.3	
Requirement for BW gain		Mean	
NE BW gain (Mcal/d)		3.3	
NE BW gain (MJ/d)		13.8	
ME BW gain (MJ/d)		12.3	
Total	Control	Low FM	High FM
ME requirement (MJ/d)	164.5	182.6	183.1

Trial 2: Kikuyu

The same methods as for the ryegrass trial were used to calculate the ME requirements of the cows grazing kikuyu. The mean BW of 372 kg and BW gain of 0.18 kg/d were used.

The results of these calculations are shown in Table C2.

Table C2 Energy requirements of cows grazing kikuyu and receiving maize-based concentrate containing either no fishmeal (FM; control), 4 % FM (Low FM) or 8 % FM (High FM)

Maintence requirement		Mean	
NE maint (Mcal/d)		6.8	
NE maint (MJ/d)		28.4	
ME maint (MJ/d)		45.7	
Lactation requirement	Control	Low FM	High FM
4 % FCM production	17.3	19.4	19.2
NE lact (Mcal/d)	12.9	14.5	14.4
NE lact (MJ/d)	54.1	60.7	60.1
ME lact (MJ/d)	84.6	94.8	93.8
Activity requirement		Mean	
NE activity (Mcal/d)		1.3	
NE activity (MJ/d)		5.4	
ME activity (MJ/d)		8.7	
Requirement for BW gain		Mean	
NE BW gain (Mcal/d)		0.8	
NE BW gain (MJ/d)		3.5	
ME BW gain (MJ/d)		3.2	
Total	Control	Low FM	High FM
ME requirement (MJ/d)	142.2	152.4	151.4